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Determination Soil Rhizobium Populations, Intrinsic Antibiotic Resistance, Nodulation and Seed Yield of Faba Bean and Soybean in Western Ethiopia

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Abstract: Legumes like faba bean (*Vicia faba* L.) and soybean (*Glycine max* L.) is able to fix 20–60 kg N ha^{-1} under tropical environments, but these amounts are significant for the succeeding cereal to meet the N requirement for economically attractive mean yields. Density of rhizobium population, intrinsic antibiotic resistance and nodulation and seed yield of faba bean and soybean from soil before and after planting in western Ethiopia was studied. The highest population of rhizobia was found from fields planted with faba bean without rhizobium inoculation in two farm fields and with rhizobium inoculation in one farm fields indicating the variations of effective local rhizobium strains in the soil due to faba bean plantation in the field. Faba bean planted with application of nitrogen fertilizer was produced lower rhizobium population density. Inoculation of different strains of rhizobia was produced considerable variation in population density of rhizobia in soybean. Knowing farm history before planting and selection of available rhizobium for faba bean and soybean was very crucial to use rhizobium strains. The rhizobium population from direct planting was varied among farms indicating planting faba bean and soybean are in the filed past. Mean plant height, seed yield and dry biomass at harvest of faba bean and soybean were significantly varied among farms, with and without rhizobium inoculation. The mean biological nitrogen fixation of faba bean was varied from farm to farm and with and without rhizobium inoculation showing variations of field with farm history with faba bean. Significantly higher nitrogen fixation of soybean was obtained from soybean seed planted without rhizobium strain inoculation. Application of nitrogen fertilizer was gave lower biological nitrogen fixation of faba bean as compared with and without rhizobium strain inoculation. Biological N_2-fixation faba bean could be suppressed by application of nitrogen. The available rhizobium strains of faba bean and soybean (FB-1018, FB-1035 and MAR-1435) were found to be resistant to antibiotics (resistant to Amoxicillin, Ampicillin and Cloxacilline) than the SB-12 strains which are found resistant to Ampicillin and Cloxacilline only. The nutrient concentrations of the soil were varied among farms indicating soil fertility status and management practices variation of the farm field.

Key words: Faba bean · Soybean · *Rhizobia* strains · Intrinsic antibiotic resistance

INTRODUCTION

Agricultural production is constrained by population pressure, declining soil fertility and unpredictable and erratic rainfall. Gradual depletion of N from African soils is posing a serious threat to food production, a symptom for lack of appropriate soil management [1]. Fassil [2] reported that farmers are further hampered by inefficient input supply systems and markets for produce and have limited opportunities to earn off-farm income, have insufficient credit schemes and cash for investment, inadequate extension services, insecure land tenure and

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poor infrastructure. Improved productivity and food self-sufficiency requirement are expected to come as a result of increased farm inputs such as fertilizer [3]. However, the enormous rise in the cost of chemical fertilizers, non-availability of a fertilizer industry in Ethiopia and the health hazards and damage caused though environmental pollution have made the country to search for an alternative source to increase food production [4]. Endalkachew [5] reported soil N deficiency to be common in the tropics and subtropics. Thus, N supply, N management and N-use efficiency will continue to be significant factors in crop production in the region. In Ethiopia, pulses account for about 10.5 per cent of the total food grain production area of the country [6]. Pulse yields are estimated at about 9.6 kg ha^{-1} [7]. The important N_2-fixation crops are food grain legumes, e.g. faba-beans, field peas, chickpeas and lentils, which are cultivated in the highlands and soybean in mid-altitude and lowlands of western Ethiopia. There is an enormous potential in Ethiopia to intensify crop production through legume rotation and intercropping [3].

Currently the cropping pattern is dominated by cereal monocropping, as farmers do not favor legumes due to their lower yields compared to cereals [8] and the need to produce enough cereals for household food security. Fassil [3] reported improvement of nodulation through *Rhizobium* inoculation, increasing biomass production, nitrogen fixation enhancement and the increasing role of useful soil microorganisms. Biological nitrogen fixation (BNF) is an efficient source of nitrogen [9, 10]. About 60% of the total N demand by a nodulating leguminous crop can be supplied through symbiotic N_2 fixation [11]. Legumes are generally contributing a direct agricultural product and are also contributing to the maintenance and restoration of soil fertility by fixing a large proportion of N_2 from the atmosphere in cropping systems [12]. Potentially, this is an important N input into tropical African agro-ecosystems where legumes are a major component of the cropping systems [13].

The input of fixed N from grain legumes may be a significant contributing factor in relation to sustaining productivity in smallholder systems [1, 14, 15] Giller [15] reported that the N_2-fixing potential of soybean is 88-188 kg N/ha/year; between 41-50 kg ha^{-1} [16]; and 31-64% of the crop's requirement [17]. On average, 50 to 60% of soybean N demand was met by biological N_2 fixation [18]. Herridge *et al*. [9] reviewed and estimated that soybeans fixed 16.4 Tg N annually, which represented 77% of the N_2 fixed by crop legumes and 12 to 25 Tg by forage fodder legumes. Bezdicek *et al*. [19] reported that soybeans were capable of fixing over 300 kg N ha^{-1} when the soil was low in available N and when effective strains of bradyrhizobia were supplied in high numbers. Soybean has a superior potential capability to fix atmospheric N_2 in association with these highly specialized soil bacteria [20].

Inoculation of faba bean cultivars was significant for total biological yield, seed yield and total nitrogen [21]. Therefore, symbiotically effective rhizobia increase nodulation, N-fixation, growth and yields of their host plant [22]. Thus, there is need for faba bean and soybean inoculation and to inventory native rhizobia strains for increased nitrogen fixation under representative field conditions. Walley *et al*.[23] reported that a well-inoculated pulse crops can fix sufficient quantities of N to eliminate the need for N fertilizer inputs. Efforts to develop an innovative utilization of leguminous plants and their plant growth promoting bacteria in sustainable agriculture [5], evaluation of effectiveness of native rhizobia strains resident in soils need to be undertaken for use in faba bean and soybean production. The objectives of this study were to isolate and quantify soil *Rhizobium* populations before and after planting of faba bean and soybean, to assess their ability to tolerate Intrinsic Antibiotic Resistance (IAR), to evaluate their effects on nodulation, nitrogen fixation and seed yields of faba bean and soybean in Western Ethiopia.

MATERIALS AND METHODS

The experiments were conducted in humid highland and mid altitude agro-ecosystems of western Oromia National Regional State, western Ethiopia. They were executed on two farmers' fields around Toke Kutaye and one at Bako Agricultural Research Center in the 2013/14 cropping season. In addition a greenhouse and Microbiology laboratory experiment were executed at Ambo Plant Protection Research Center in 2013. Toke Kutaye lies between 8'9'80"N to 8'9'90 N latitude and 37'56' E to 37°72'E longitude 8.983; 37.85 and at an altitude ranging from 2251 to 2290 meters above sea level, receiving mean annual rainfall of 1045 mm with unimodal distribution [24]. It has a warm sub-humid climate with the mean minimum, mean maximum and average air temperatures of 8.9, 27.4 and 18.1°C, respectively. Bako-Tibe lies at 9°6'N latitude and 37°09'E longitude and at an altitude of the 1650 meter above sea level. The long-term (1961 - 2014) mean annual rainfall at BARC is 1265 mm with unimodal distribution [25]. It has a warm humid climate with the mean minimum, mean maximum and average air temperatures of between 13.4, 28.49 and 20.95°C, respectively. The soil type is a brown clay loam Alfisol [26].

Faba bean seeds (Moti and Tumsa variety) for highland and soybean seed (Jalele) were used for the experiments. Half of the quantities of seeds of both crops were inoculated with rhizobia strains, FB-1035 and SB-12, respectively for faba bean and soybean, which were received from Holetta Agricultural Research Center; the remaining half was without inoculation. Uninoculated and inoculated seeds of faba bean and soybean were planted on oxen-ploughed fields. All management practices were applied as per research recommendations for each crop. All necessary agronomic and soil data collection were done at appropriate crop growth stages following recommended procedures. The collected data were analyzed following standard procedures for rhizobia, soil and plant tissue. *Rhizobium* population densities in soil before and after inoculation were determined using the Most Probable Number (MPN) method and Direct Isolation and Enumeration of Rhizobia from soil samples, respectively.

Most Probable Number Counts: Densities of *Rhizobium* population from soil were studied before planting faba bean and soybean and after planting of both crops with and without rhizobia inoculation. Soil samples were collected from three faba bean and one soybean fields before planting and after planting of the two crops with and without rhizobia. Yeast Extract Mannitol Agar (YEMA) was used to determine the densities of rhizobia populations in the soils. The YEMA was prepared using 10 g manitol, 0.5 g K_2HPO_4, 0.2 g $K_2SO_4.7H_2O$, 0.2g NaCl, 1 g yeast extract, 20g agar and 2.5ml Congo red (1% solution) added to 1000ml distilled water, except for K_2HPO_4, which was dissolved separately in distilled water and mixed in the agar medium. Congo red solution was sterilized separately and added to the medium at the time of pouring into petri dish plates. Ten grams of ground and sieved soil sample was used to make ten-fold serial dilutions up to 10^6 using distilled water. 0.1 ml each of suspension was uniformly spread on the yeast extract mannitol agar medium in petri dishes (Fig 1). The plates were incubated at 28°C for 10 days and colonies counted and converted to colony forming units per g soil (Fig 1) [27]. The procedures were done as described by Olsen *et al* [28].

Direct Isolation and Assessing of Rhizobia from Soils with Plant-infection Technique: The use of host plant for the isolation of rhizobia is an indirect procedure that has the disadvantage to recover only strains that have the ability to compete for nodulating the host plant. Soil samples were collected from farmers' fields before planting of faba bean and soybean. Faba bean seeds (Moti) and soybean (Jalale) were surface sterilized using 95% ethyl alcohol and 0.5% sodium hyphochlorite for three minutes [29-31]. After being washed with several times with distilled sterilized water, five seeds were planted in each pot with soil samples and later thinned to three following germination. After 61 days of growth, plants were carefully uprooted to recover the nodules. The number of nodules plant^{-1} nodule volume and dry weight were determined.

Intrinsic Antibiotic Resistance (IAR) of Rhizobia Strains and Survival of Rhizobia on Faba Bean and Soybean: The five isolated strains and standard strains were treated with selected antibiotics to determine their intrinsic antibiotics resistance pattern. The antibiotics (mg l^{-1}) used were: Amoxicillin (8, 16), Ampicillin (2.5, 5), Tetracycline (10, 20), Doxycycline (4, 10), Penicillin (20, 30) Ciprofloxacin (1, 2.5), Norfloxazole (4, 8), Erythromycin (0.1, 2.5), Cloxacilline (2, 5), Chloramphenicol (12, 20) and Ceftriaxone (8, 16) using Standards and Equivalent Minimal Inhibitory Concentration (MIC) as described by CLSI [32]. Stock solution of the antibiotics was prepared immediately before use in sterile distilled. Appropriate quantities of the antibiotic stock solutions mentioned above were added to molten YEMA at 48°C, mixed thoroughly and then poured on petri dishes. Isolates were grown in YEM broth for 48 hours. A portion of each culture was diluted in sterile distilled water to prepare for solutions so that 0.1 ml of each isolate inoculated on a petri dish would finally contain about 10^7 CFU ml^{-1} of the culture. This intrinsic resistance of isolates was determined according to Josey *et al* [33].

Soil and Tissue Sampling and Analysis: Soil samples were collected before treatment application and planting of faba bean and soybean from 10 sites randomly picked and composited. The collected soils were prepared following standard procedures and analyzed at Holleta and Debre Zeit Agricultural Research Center Soil and Plant Analysis Laboratory. Determination of soil particle size distribution was carried out using the hydrometer method [34, 35]. The soil pH was measured with digital pH meter potentiometrically in the supernatant suspension of 1:2.5 soils: distilled water ratio. The exchangeable K and Na were determined by flame photometer. The cation exchange capacity of the soil was determined from 1 *M* NH_4OAC saturated samples [35]. Exchangeable acidity was determined by saturating the soil samples with 1 *M* KCl solution and titrated with sodium hydroxide as described by McLean

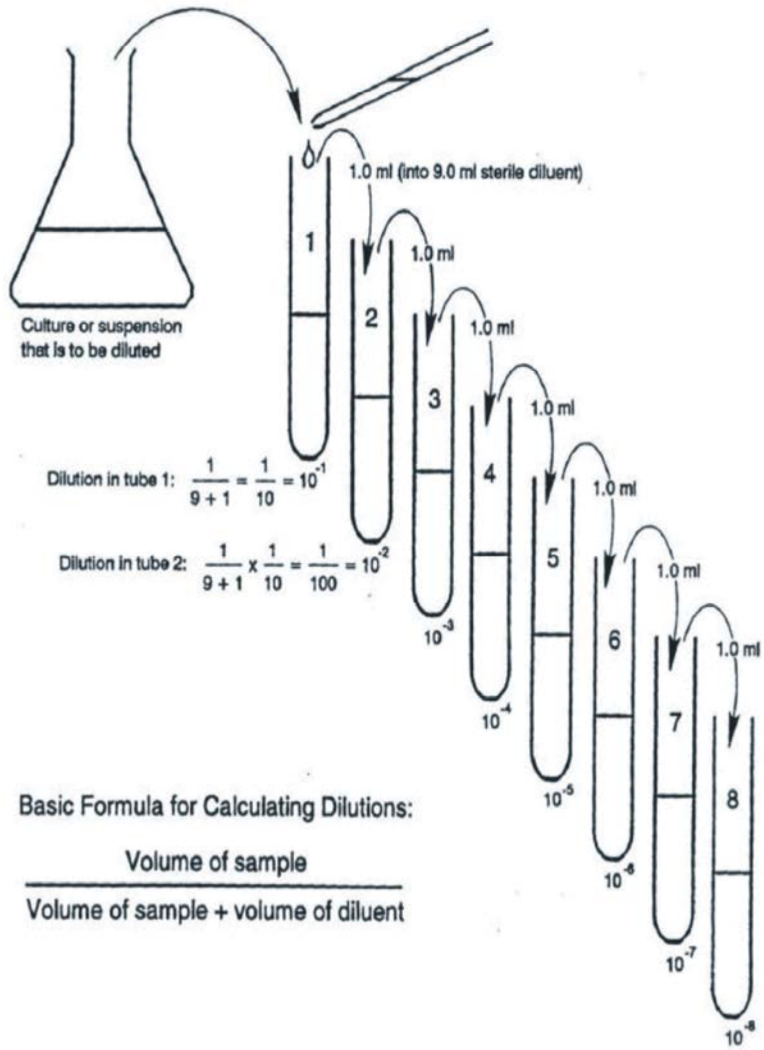

Fig. 1: Procedures for 10-fold serial dilutions for bacterial colony forming unity count.

[37]. Organic carbon was determined following wet digestion method as described by Walkley and Black [38] whereas the Kjeldahl procedure was used for the determination of total nitrogen (N) as described by Jackson[39]. Extractable phosphorus (P) was measured by Olsen method as described by Olsen *et al*.[40]. The steam distillation method was used for determination of NO_3-N and NH_4-N as described by Keeney and Nelson [41].

Faba bean and soybean tissue and seed samples were collected at 50% flowering and at the harvesting stage. The tissue samples were dried in an oven at 70°C for 24 hours. The collected tissue samples were chopped, dried, ground and ashed for analysis. The seed samples were prepared standard procedures for analysis. The plant tissues were subjected to wet digestion. The N content of the plant tissue was determined by Kjeldahl procedure, whereas the P content was determined by colorimeterically according to Murphy and Riley [42]. Barium-based titrometeric methods were used for SO_4 quantification/ total sulfur determination [43, 44]. The total nutrient uptake was calculated as the sum of seed nutrient uptake and biomass nutrient uptake. Total N concentration (% total N in dry matter) of the whole plant was used to calculate seed nutrient uptake and biomass nutrient uptake by multiplying the N concentration by dry biomass per hectare of faba bean or soybean. The total nitrogen fixation of faba bean and soybean were determined using the N difference method (Ndfa) Munro and Davis [45], using the formula: Ndfa (kg ha^{-1}) = Total N (fixing crop) – total N (non-fixing crop).

RESULTS AND DISCUSSION

Some Soil Physical and Chemical Properties: The soil physical and chemical properties varied among the farm sites. The soil texture of four farm sites was clay to clay loam. The soil pH of different farm sites ranged from 4.36 to 4.95 (pH in water at soil: liquid ratio of 1:2.5) (Table 1). Thus, the soil reaction of the four farm sites was extremely acidic to very strongly acidic based on this pH in H_2O [46]. 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 organic carbon and organic matter were recorded from four farm sites and varied from 2.14 to 4.28% which are in the very low range [46, 47]. The relatively lower soil organic carbon and organic matter of Alfisol could be attributed to the continuous cropping and cultivation, intensive tillage practice and heavy rainfall in the area. The extractable phosphorus concentration of the four farm sites ranged between 5.43 to 10.87 ppm, which are in the medium range [46, 47]. Higher phosphorus concentration was obtained from Bako soybean field. The total N concentration of the four farm sites was ranged from 0.16 to 0.25% (Table 1), which are in the low to medium range [46, 47], thus requiring use of N fertilizers to optimize crop yields. The soil CEC of four farm sites was ranged from 18.5 to 27.56 $cmol^{+}kg^{-1}$, which is medium according to London [46] and FAO [47]. The potassium concentration of the four farm sites was low, medium to high, ranging from 0.14 to 1.13% (Table 1) [46]. The exchangeable sodium (Na) ranged from 1.44 to 2.4%, which is high [46].

The total nitrogen concentration of the different soils varied from 0.16 to 0.25% (Table 1), which was in the low to medium range [46, 47]. The total N concentrations mean that the four farms need different amounts of nitrogen to sustain crop production and productivity. The soil $N0_3$-N concentration of the four farm sites ranged from 2.43 to 55.64 ppm, which were very low, medium, high to very high [48, 49], or low, high to excessive [50]. The soil NH_4-N concentration of 2.92 to 23.43 ppm was found to be in the optimum range (Table 2). Marx *et al*. [50] and Horneck *et al*. [51] reported ammonium-nitrogen concentrations of 2–10 ppm in soils to be typical. Total nitrogen does *not* indicate plant-available N and is not the sum of NH_4-N + NO_3-N. Total N is not used for fertilizer recommendations too. Therefore, nitrogen fertilizer recommendations need to be experimentally determined for crop production.

Rhizobium Populations in the Soils: The mean populations of rhizobia before and after faba bean and soybean planting are indicated in Table 3. The rhizobia varied among farms and within farms due to seed inoculation with rhizobia. Higher rhizobia population densities were obtained from faba bean planted without rhizobia inoculation as compared to those inoculated and before planting of faba bean rhizobia differed between farm 1 and farm 2 (Table 3). This indicates the presence of indigenous rhizobia strains in the field due to a long history of planting of faba bean in those fields. The larger populations without inoculation indicated that the indigenous rhizobia strains were more competitive than the inoculants ones. In farm 3, higher numbers of rhizobia were observed from soil where faba bean was planted with

Table 1: Texture and some soil nutrient concentrations of the study site before planting of faba bean and soybean in 2013 cropping season

Farms	pH 1:2.5	OC	OM	P (ppm)	CEC (Meq/100g)	Total N (%)	Texture
	------------------	------------------	%	------------------	------------------	------------------	
Farm 1	4.36	2.49	4.28	6.69	19.44	0.16	Clay
Farm 2	4.40	2.42	4.16	5.43	18.5	0.25	Clay loam
Farm 3	4.95	2.14	3.68	6.27	27.56	0.2	Clay
Bako (soybean)	4.54	2.46	4.23	10.87	21.72	0.21	Clay

Farm 1-3= farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma)

Table 2: Some soil chemical properties of the soil in the study site before planting of faba bean and soybean in 2013 cropping season

Farms	K (Meq/100g)	Na (Meq/100 g soil)	Exchangeable Acidity	$N0_{3-N}$ (ppm)	NH_4-N (PPM)
Farm 1	0.14	1.44	0.42	2.43	2.92
Farm 2	0.28	1.92	0.35	55.64	23.43
Farm 3	0.42	2.16	0.18	26.85	8.94
Bako (soybean)	1.13	2.40	0.18	44.01	17.60

Farm 1-3= farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma)

Table 3: Soil rhizobia population densities before and after faba bean and soybean planting from study site in 2013 cropping season

Farm	Situation	Rhizobia population density (CFU/g soil)
Farm 1 (FB)	Before planting	34 x 10^6
	With rhizobia inoculation	32 x 10^6
	Without rhizobia inoculation	53 x 10^6
Farm 2 (FB)	Before planting	45 x 10^6
	With rhizobia	48 x 10^6
	Without rhizobia	67 x 10^6
Farm 3 (FB)	Before planting	104 x 10^6
	With fertilizer	22 x 10^6
	With rhizobia	137 x 10^6
	Without rhizobia	114 x 10^6
Soybean (Bako)	Before planting	79 x 10^6
	With rhizobia (strain MAR—1435)	56 x 10^6
	With rhizobia (strain SB-12)	118 x 10^6
	Without rhizobium	67 x 106

Farm 1-3= Farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma), CUF= Colony forming unit, FB= faba bean

Table 4: Number of nodule plant^{-1} volume of nodule plant^{-1} and dry biomass of nodule from faba bean and soybean with direct isolation from greenhouse pot experiment

Farms	Number of nodules plant^{-1}	Volume of nodules plant (ml)	Dry biomass nodules (g)
Farm 1 (FB)	22	0.44	0.07
Farm 2 (FB)	37	0.74	0.12
Farm 3 (FB)	31	0.62	0.09
Soybean	37	0.73	0.11

Farm 1-3= farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma) *FB=Faba bean

rhizobia inoculation as compared to without inoculation and before planting. This revealed that in farm 3 the inoculant strain was more competitive than the indigenous local strain found in the soil. A significantly lower rhizobia population density of 22 x 10^6 was observed in soil where faba bean was planted with nitrogen fertilizer as compared with populations both with and without rhizobia inoculated faba bean (Table 3). Applications of nitrogen fertilizer to faba bean significantly reduced the rhizobia population densities in farm 3. This showed faba bean does not engage in symbiotic nitrogen fixation in the presence available nitrogen fertilizers. Small variations of rhizobia population densities were observed from before planting and with rhizobia inoculation in soils of farm 1 and farm 2. This implies that the local rhizobia strains were more competitive than the introduced rhizobia strain. El Behidi *et al*. [52] reported that high rates of available soil nitrogen reduced nodulation and biological nitrogen fixation since plants did not require undertaking symbiosis with nodule bacteria.

The rhizobia population densities of soybean were higher with soybean planted with rhizobium strain (SB-12) inoculation as compared to with the other rhizobia strain or without rhizobia inoculated soybean (Table 3). Soybean planted with (MAR-1435) was gave the lowest rhizobia population densities as compared to before planting and without rhizobia inoculation. This indicates the MAR-1435 rhizobia strain was less competitive and effective for soybean production in the area. The presence of local rhizobia strains in the soil due to planting soybean in the field previously outcompeted the nodulation abilities the exotic rhizobia strain. Therefore, knowledge of the farm history is very crucial in considerations for use of rhizobia inoculation for sustainable faba bean and/or soybean production.

Number of Nodules Plant^{-1} Volume of Nodules Plant^{-1} and Dry Biomass of Nodule from Faba Bean and Soybean: The number of nodules plant^{-1}, volume of nodules plant^{-1} and dry biomass of nodules from faba bean and soybean before planting with direct isolation technique from pot are indicated in Table 4, showing that they varied among farms. Higher numbers of nodules plant^{-1}, volume of nodules plant^{-1} and dry biomass of nodules from faba bean were observed from farm 2, followed by farm 3. The lowest values were observed from farm 1. This indicates that farm 1 was planted to faba bean longer as compared to farm 2 and 3. Nodules were also found on soybean plants (Table 4). Therefore, this affirms that the four soils were planted to faba bean and/or soybean some years back.

Table 5: Number of nodules plant^{-1}, volume of nodule plant^{-1} and dry biomass of nodule and plant from faba bean precursor crop after uprooting from the field in 2013 cropping season

Farms	Faba bean	Number of nodules plant^{-1}	Volume of nodules plant (ml)	Dry biomass nodules (g)	Dry biomass plant^{-1}(g)
Farm 1	With rhizobium	727	10	1.68	120
	Without rhizobium	534	10	1.25	118
Farm 2	With rhizobium	592	8	1.85	176
	Without rhizobium	985	11	2.4	182
Farm 3	With fertilizer	575	13	1.9	171
	With rhizobium	689	14	2.42	174
	Without rhizobium	500	10	1.57	194
Soybean	With rhizobium (MAR-145)	62	20	22	133
	With rhizobium (SB-12)	62	22	20	107
	Without rhizobium	93	24	24	94

Farm 1-3= farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma)

The number of nodules plant^{-1}, volume of nodules plant^{-1} dry biomass of nodules and dry biomass of faba bean and soybean after planting are indicated in Table 5. Higher numbers of nodules plant^{-1}, volumes of nodules plant^{-1} and dry biomass of nodules of faba bean were measured from faba bean planted without rhizobia inoculation as compared with rhizobia inoculated faba bean in farm 2. This indicates that the available local strain in farm 2 was more competitive and effective as compared to the introduced rhizobia strains. Continuous cultivation may be necessary to help the build-up of rhizobia in soil, resulting in increases in nodulation [53]. In farms 1 and 3, higher numbers of nodules plant^{-1}, volumes of nodules plant^{-1}, dry and biomass of nodules of faba bean were harvested from faba bean planted with rhizobia inoculation as compared with without inoculation. This revealed that the introduced rhizobia strain was more effective as compared to the local strains available in the soil. Higher mean dry biomass plant^{-1} was obtained from faba bean planted without inoculation as compared with inoculation in farms 2 and 3 (Table 5). Therefore, the nodulation potential and biological N_2-fixation by different introduced rhizobia strains for sustainable faba bean and soybean production will be influenced by the characteristics of native strains.

Significantly higher numbers of nodules plant^{-1}, volumes of nodules plant^{-1} and dry biomass of nodules were observed from soybean planted without rhizobia inoculation as compared to those planted with rhizobia inoculation (Table 5). Non- response to added inoculant was due to the presence of effective indigenous rhizobia [54]. Mungai and Karubiu [55] stated that presence of high numbers of indigenous rhizobia may have limited nodule formation by introduced strains. In addition, availability soil nitrogen also reduced the extent of nodulation. 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 [56, 57]. Similarly, Zahran [58] reported high soil N levels, applied or residual, to reduce nodulation and N_2-fixation, as was similarly reported by Cruz [59]. High soil N can result in a reduction in the number of nodules that form [60]. Soybean planted with rhizobia inoculation (MAR-1425 and SB-12) produced equal numbers of nodules plant^{-1} (Table 5). Higher nodule dry biomass and dry biomass plant^{-1} were produced from soybean planted with rhizobia (strain MAR-1425) inoculation as compared to SB-12 strain soybean or without inoculation. Higher dry biomass plant^{-1} of 133 g followed by 107 and 94 g were produced from soybean planted with rhizobia strain MAR-1425 and SB-12 inoculation and without inoculation. Guo *et al*. [61] reported rhizobia inoculation of legumes usually stimulates plant growth through effects on nodulation and biological N2-fixation. Optimum growth of leguminous plants is usually dependent on symbiotic relationships with N2-fixing bacteria [62]. Therefore, inoculation faba bean and soybean seed with rhizobia where farm history showed no similar crops before was very crucial for economically feasible and sustainable production of faba bean and soybean.

Effects of Inoculation on Plant Height, Number of Pods Plant^{-1}, Number of Seed Pods^{-1}, Seed Yield, 1000 Seed Weight and Dry Biomass: Mean plant height, number of pods plant^{-1} and seed pods^{-1} were varied among farms (Table 6). Higher plant heights of faba bean in farm 1 were observed from faba bean planted with rhizobia inoculation as compared with without inoculation (Table 6). In farms 2 and 3, higher plant height of faba bean were recorded from faba bean planted without rhizobia inoculation as compared to with inoculation. The number of pods plant^{-1} was higher for faba bean planted on farm 2 as compared to farms 1 and 3, indicating better fertility status of farm 2

Table 6: Plant height, number of pods $plant^{-1}$, number of seeds pod^{-1}, seed yields, thousand seed weight and dry biomass of faba bean and soybean on different farmers' fields in Toke Kutaye and BARC, western Ethiopia

Farms	Faba bean	Plant height (cm)	Number of pods $plant^{-1}$	Number of seeds $pods^{-1}$	Seed yield (kg ha^{-1})	1000 seeds weight (g)	Dry biomass (kg ha^{-1})
F-1	With rhizobia inoculation	130	10	2	1258	683	15000
	Without rhizobia inoculation	109	9	2	1058	702	14750
	Maize	204			3040	366	15745
F-2	With rhizobia inoculation	142	15	3	1563	759	22,000
	Without rhizobia inoculation	172	22	2	1791	770	22750
	Maize	214			3138	404	17458
F-3	With fertilizer inoculation	165	13	2	1514	670	21375
	With rhizobia inoculation	154	17	2	1394	685	21750
	Without rhizobia inoculation	166	16	2	1154	640	24250
	Maize	238			4045	366	14614
SB	With rhizobia inoculation	62	8	2	2520	330	5600
	Without rhizobia inoculation	59	8	2	2940	360	6400
	Maize	260			2223	365	12523

F 1-3= farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma), SB= soybean

compared to the other farms. Higher seed yield of 1677 kg ha^{-1} followed by 1354 and 1158 kg ha^{-1} were recorded from farm 2, farm 3 and farm 1, respectively (Table 6). This indicates the three farms were different in soil fertility status. Faba bean planted with rhizobia inoculation produced higher seed yield in farm 1 and farm 3, while in farm 2 it was the vice versa (Table 6). Adamu *et al*. [63] reported significant variations in shoot length, dry matter and nodule fresh weights of faba bean when fertilized and inoculated in various soil types. Dry matter and grain yields of faba bean were significantly different among the treatments with various concentrations of fertilizers in the field conditions [64]. Mean thousand seed weight of 764 g followed by 693 and 665 g were obtained from farm 2, farm 1 and farm 3, respectively (Table 6), indicating variations among farms. This might be due to differential soil fertility status of the different farms. Mean dry biomass of 22458 kg ha^{-1} followed by 22375 and 14875 kg ha^{-1} were obtained from farm 3, farm 2 and farm 1, respectively (Table 6), again indicating variations among farms. An increase of 13 to 24% of shoot dry matter was obtained by inoculating with effective rhizobia and fertilizer use [65]. Therefore, the different farms presently varied with respect to soil fertility status and management systems applied.

Seed yield of soybean was different between soybean planted with and without rhizobia inoculation (Table 6). Higher seed yield advantage of 420 kg ha^{-1}, or 16.67%, was produced from soybean planted without rhizobia strain inoculation. This indicates that soybean had been planted in the field previously. Continuous cultivation of legumes may be necessary to help the build-up of high rhizobia populations in soil, resulting in increases in nodulation and yield [53]. The higher thousand seed weight and dry biomass of soybean were from soybean seed planted without inoculation as compared to those inoculated indicates the local rhizobia were more competitive and effective as compared to the introduced strain. Planting of soybean without inoculation was recommended for soybean production where farm history showed that soybean had been grown previously. Furthermore, appropriate site selection is recommended to identify the effectiveness and competitiveness of exotic rhizobium as compared to locally available rhizobia strains in a soil.

Nutrient Concentrations, Nutrient Uptake and Biological N_2-fixation by Faba Bean and Soybean: The plant nutrient concentrations, nutrient uptake and biological N_2-fixation of faba bean and soybean are indicated in Table 7. The nutrient concentrations varied among farms for faba bean. Total phosphorous concentrations of faba bean tissue were higher for faba bean planted without rhizobia inoculation in farm 2 and farm3, but the opposite was true for farm1 with rhizobia inoculated faba bean. This might be due to soil fertility status variation of the different farm fields.

The total nitrogen concentration of faba bean tissue was higher for faba bean seed planted with inoculation for farm 1 and farm 2, but higher without inoculation for farm 3 (Table 7). Keneni *et al*. [66] found the native rhizobia strains of the Wollo region to be more competitive than the two exotic rhizobia strains. The highest total nitrogen concentration (2.94%) of the faba bean tissue was obtained from faba bean seed planted without inoculation from farm 3. The total $SO_4^{=}S$

Table 7: Nutrients concentrations, uptake and biological N_2-fixation of faba bean and soybean in Toke Kutaye and BARC, western Ethiopia

		Nutrient concentration		Seed		Nutrient Uptake		
Farms	Faba bean	Total p (%)	Total N (%)	$S0_4$=S (%)	Total N (%)	N (kg ha^{-1})	P (kg ha^{-1})	Ndfa (kg ha^{-1})
Farm 1	With rhizobium	0.16	2.69	0.055	1.00	553.5	240.0	254.34
	Without rhizobium	0.13	2.38	0.031	0.95	491.18	191.8	192.0
	Maize	0.16	1.56	0.039	0.34	299.16	251.9	
Farm 2	With rhizobium	0.20	2.39	0.047	1.06	759.00	440.0	514.59
	Without rhizobium	0.25	2.37	0.032	1.09	787.15	568.8	542.7
	Maize	0.33	1.07	0.047	0.33	244.41	576.1	
Farm 3	With fertilizer	0.16	2.29	0.032	1.09	722.48	342.0	506.2
	With rhizobium	0.2	2.55	0.063	1.09	791.70	435.0	575.4
	Without rhizobium	0.25	2.94	0.063	1.11	982.13	606.3	765.8
	Maize	0.20	1.19	0.031	0.29	216.29	292.3	
Soybean	With rhizobium	0.19	1.12	0.029	1.65	155.12	106.4	111.1
	Without rhizobium	0.15	0.90	0.023	1.66	163.84	96.0	198.3
	Control (maize)	0.17	0.99	0.026	0.16	144.01	212.9	

Farm 1-3= farmers name (Solomon Belete, Gadisa Beksisa, Gutuma Kuma)

(%) concentration of faba bean tissue was higher for faba bean seed planted with rhizobia inoculation as compared to without inoculation for farm 1 and farm 2. The total nitrogen concentration of seed of faba bean varied among farms and between faba bean seed planted with and without rhizobia inoculation (Table 7). The total nitrogen concentration of 1.11% of seeds was obtained from faba bean seed planted without rhizobia inoculation (Table 7).

The total phosphorous and nitrogen uptake by faba bean were obtained from faba bean seeds planted with inoculation for farm 1 (Table 7). For farm 2 and farm 3 higher total phosphorous and nitrogen uptake of faba bean were recorded from faba bean seed planted without inoculation as compared to with inoculation. The highest total phosphorous and nitrogen uptake of 982.1 and 606.3 kg ha^{-1} followed by 787.2 and 568.8 kg ha^{-1} were obtained from faba bean planted without seed inoculation from farm 3 and farm 2 (Table 7).

The biological N_2-fixation varied among farms and between rhizobia inoculations. Higher biological N_2-fixation of 765.8 kg ha^{-1} followed by 542.7 kg ha^{-1} was produced from faba bean planted without seed inoculation from farm 3 and farm 2. Percentage of nitrogen fixation is also higher for native rhizobia strains, with these isolates found to be superior to the exotic ones in stimulating growth, promoting dry matter yield, nodulation and nodule wet weight of faba bean in pouch culture [66]. In farm 1 greater biological N_2-fixation of 254.4 kg N ha^{-1} was harvested from faba bean seed planted with rhizobia inoculation (Table 7). This indicates faba was fixed enormous amounts of N_2 with local rhizobia strains and inoculated with exotic rhizobium strains. McVicar *et al*. [67] reported faba bean to be the most efficient nitrogen fixer of all cool season pulse crops. The amounts of N_2-fixed by faba bean are estimated to be between 240 and 325 kg N ha^{-1} [68], with percentage efficiency (66% Ndfa [Nitrogen derived from the air]) [69] and fulfills 80% of its nitrogen requirements [70]. In farm 3 nitrogen fertilizer lower biological nitrogen fixation by faba bean was recorded as compared to with and without rhizobia inoculation (Table 7). Danso and Eskew [71] reported that the amount of nitrogen actually fixed by a legume depends on types of rhizobia strains, host plant, environment and agricultural practices. They further stated that in legumes grown in soils with high available nitrogen, the nitrogen fixation rate was reduced. High soil N and low pH can depress fixation rates [72].

An efficient rhizobia strain is not expected to express its full capacity for nitrogen fixation if limiting factors impose limitations on the vigor of the host legume [73]. Belnap [72] found that phosphorous addition can stimulate fixation rates and effective rhizobia strains may increase rates of fixation. BNF by legumes is a key process in Low External Input Agriculture (LEIA) technologies as it potentially results in a net addition of N to the system [74]. Therefore in legumes production consideration should be given to selection of rhizobium strains, host plant, environment and agricultural practices as options for sustainable productivity.

The total phosphorus, nitrogen and SO_4 concentrations of soybean tissue were obtained from soybean seed planted with rhizobia inoculation as compared to without rhizobia (Table 7). Higher nitrogen uptake and biological N_2-fixation of 163.8 and 198.3 kg ha^{-1} was produced from soybean seed planted without rhizobia inoculation. Better nodulation of soybean was

Table 8: Effects of different concentrations of antibiotics on growth of faba bean and soybean rhizobia strains

	Different concentrations of antibiotics (Mg L^{-1})									
	Amoxicillin		Ampicillin		Tetracycline		Doxycycline		Penicillin	
Isolate	8	16	2.5	5	10	20	4	10	20	30
FB-1018	+	+	+	+	-	-	-	-	-	-
FB-1035	+	+	+	+	-	-	-	-	-	-
MAR-1435	+	+	+	+	-	-	-	-	-	-
Sb-12	-	-	+	+	-	-	-	-	-	-

Table 9: Effects of different concentrations of antibiotics on growth of rhizobia strains

	Different concentrations of antibiotics (Mg L^{-1})											
	Ciprofloxacin		Norfloxazole		Erythromycin		Cloxacilline		Chloramphenicol Succinate 1 g		Ceftriaxone 0.5 g	
Isolate	1	2.5	4	8	0.1	2.5	2	5	12	20	8	16
FB-1018	-	-	-	-	-	-	+	+	-	-	-	-
FB-1035	-	-	-	-	-	-	+	+	-	-	-	-
MAR-1435	-	-	-	-	-	-	+	+	-	-	-	-
Sb-12	-	-	-	-	-	-	+	+	-	-	-	-

obtained from field planted without inoculation with rhizobia. Maximum N_2-fixation in a legume requires that the legume be adequately nodulated [58]. Total phosphorous 106.4 kg ha^{-1} concentration was produced from soybean seed planted with rhizobia inoculation, indicating increased phosphorous uptake of soybean. The presence effective local rhizobia in the soil increased the quantity of N_2 fixed by soybean. Therefore, knowing farm field history is recommended before using the rhizobia inoculation for faba bean and soybean production.

Growth of Rhizobia Strains as Affected by Antibiotics: The effects of different antibiotics concentrations on growth of rhizobia are indicated in Tables 8 and 9. Four rhizobia strains (two for faba bean and two for soybean) revealed differences in survival, persistence and competitiveness in the presence of different antibiotics. All isolates exhibited variations in their intrinsic antibiotic resistance (IAR) to different concentrations and types of antibiotics [66, 75]. Purcino *et al*. [76] reported survival, persistence and competitiveness of the rhizobia strains are the major factors determining their successful use as inoculants. Strains FB-1018, FB-1035 and MAR-1435 were resistant to Amoxicillin, Ampicillin and Cloxacilline (Table 8). The Sb-12 strain was able to resist Ampicillin and Cloxacilline. Similar findings were reported by Zerihun and Fassil [75] on different isolates of *R. leguminosarum* bv. *Vicia*. 100% the tested rhizobium strains were found to be susceptible to Tetracycline, Doxycycline, Penicillin, Ciprofloxacin, Norfloxazole, Erythromycin, Chloramphenicol Succinate 1 g and Ceftriaxone 0.5 g (Tables 8 and 9). The four rhizobia strains showed greater susceptibility to the common antibiotics used for treatment of bacterial diseases.

CONCLUSIONS

The rhizobia population densities varied among faba bean and soybean fields before and after inoculation with rhizobia. Both locally available native as well as introduced rhizobia strains resulted in higher nitrogen fixation. Nodulation, dry matter yield and seed yields of faba bean and soybean were increased with exotic rhizobia strain inoculation and native strains available in the soil. The amounts of total biologically fixed N_2 in inoculated and uninoculated faba bean were higher than the amounts of chemical N fertilizer applied. Increasing and extending the role of biofertilizer such as *Rhizobium* would decrease the need for chemical fertilizers and reduce adverse environmental effects. The results here indicate presence of effective indigenous rhizobia strains in the soil due to history of faba bean and soybean production in the area. Appropriate site selection is recommended to identify the effectiveness and competitiveness of exotic rhizobia as compared to locally available rhizobia strains in the soil for faba bean and soybean production. The four rhizobia strains showed greater susceptibility to the common antibiotics used for treatment of bacterial diseases. Further studies should be carried out to explore the biodiversity of locally available rhizobia strains and to take advantage of the natural biodiversity of this resource resident in its soils for biological N_2-fixation of faba bean and soybean.

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