SINET: Ethiop. J. Sci., 24(2):197–211, 2001
 ISSN: 0379–2897

STUDIES OF RHIZOBIUM INOCULATION AND FERTILIZER TREATMENT ON GROWTH AND PRODUCTION OF FABA BEAN (*VICIA FABA*) IN SOME 'YIELD-DEPLETED' AND 'YIELD SUSTAINED' REGIONS OF SEMIEN SHEWA

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ABSTRACT: The failure of faba bean production in some regions of Semien Shewa necessitated a research for a possible explanation and solution to reverse the problem. Two regions with a failure in pulse production of faba bean for five to ten years i.e.'yield-depleted' Molale and Mehal Meda, and two regions that are still capable of producing faba bean *i.e.* 'yield-sustained' Ankober and Keyt were selected. Comparative assessments of their nutritional factors, rhizobial type, rhizobial density, and inoculation and fertilizer treatments with respect to their soil types were undertaken. The soil analyses showed that 'yield-depleted' areas were generally characterized by a medium to high concentration of nitrogen (0.1–0.2%), a low content of phosphorus (3–6 ppm) and potassium, a high concentration of calcium and magnesium, and a lower population density of rhizobia (10^1–10^2 g^{-1} soil). The results also showed that shoot length, shoot dry matter, and nodule fresh weight were significantly affected ($p=0.01$) by soil type and different inoculation and fertilizer treatments. Although growth parameters, in general, were improved by different treatments, shoot dry matter and nitrogen contents were not significantly affected in 'yield-sustained' regions as compared to 'yield-depleted' ones. The results also showed that the comparative symbiotic effectiveness of the indigenous strains and the type exotic strain (reference strain) Tal 1397N was not different. It did not also show significant difference on the above mentioned growth parameters with different treatments. This indicates that the indigenous strains are equally effective in nitrogen accumulation provided that the problems of other limiting nutritional factors can be alleviated in the soil. This suggests that combined inoculation and fertilizer treatments can improve growth and reverse production of pulses in 'yield depleted' regions of Semien Shewa.

Key words/phrases: Faba bean, fertilizer and inoculation treatments, growth-related parameters, indigenous and exotic rhizobia, Semien Shewa

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INTRODUCTION

Faba bean (*Vicia faba*), chick pea (*Cicer arietenium*), field pea (*Pisum sativum*) and lentil (*Lens culinaris*) are the major highland pulse crops in Ethiopia, and occupy 12 to 15% of the land under cultivation. They are the major protein source of the majority of the population, and contribute to 4% of the export market of the country (Badege, 1987; Hailu Beyene *et al.*, 1994). These pulses fix up to 80% of their nitrogen requirement in an endosymbiotic relationship with root nodule bacteria, known as *Rhizobium* (Zapta *et al.*, 1987) and are able to accumulate total nitrogen to the tune of 600 kg N ha^{-1} yr^{-1} (Sprent and Bradford, 1977).

Faba bean is the most important of all pulse crops in terms of production achieving high seed yield (7 metric tons/ha) and protein contents (23–32%) (Evans *et al.*, 1972). In Ethiopia, it is most commonly found in the 'Weyna Dega' *i.e.*, between 1800 and 2400 meters elevation (Bond *et al.*, 1985), with greatest concentration in Shewa, Wollo, Tigray, Gojam and Gonder regions (Beniwal, 1987). Historically, Ethiopia is considered as the secondary center of diversity and also one of the nine major agro-geographical production regions of faba bean (Bond *et al.*, 1985; Asfaw Telaye *et al.*, 1994). Consequently, the country is endowed with different land races and farmers' varieties that adapt to different altitudinal and edaphic environments. Although the country is found in one of the major producing regions of faba bean in the world, production is estimated between 6–11 tons/ha (Tesfaye Beshi *et al.*, 1996).

Semien Shewa zone is one of the major highland pulse-producing areas of the country. However, pulse production in the area reduced in recent years. According to information gathered from the farmers, some places such as Molale and Mehal Meda were not able to grow faba bean for the last ten years. However, places that are not distantly located from these regions, such as Ankober and Keyt maintain faba bean production.

Many factors may be attributed to the failure of pulse production. One of the plausible explanations for this failure may be a decline in soil fertility. Although faba bean can fix nitrogen and improve soil fertility, accumulation of enough nitrogen by the plant depends on the number and compatibility of rhizobia with the host (Sprent and Bradford, 1977; Sorwill and Mytton, 1986), and mineral constraints that affect nodulation and nitrogen fixation (O'Hara *et al.*, 1988).

In order to find which nutrient-related factors are incriminated with the failure of pulse production, an investigation was made in some traditional faba bean growing areas of Semien Shewa. Soils from those that failed to

produce faba bean for several years ('yield-depleted'), and those that continue to grow faba bean ('yield-sustained') were taken. They were compared with one another on the basis of nutrient status, type, number, and effectiveness of rhizobia in nitrogen fixation. Growth of faba bean was monitored on both soils with nitrogen and phosphorus treatments in pot experiments under greenhouse conditions.

MATERIALS AND METHODS

The rhizobium isolation, plant and soil chemical analyses and the pot experiments were carried out at the National Soil Laboratory, the Ethiopian Agricultural Research Organization (EARO). The strain identification test was done at the Department of Biology, Addis Ababa University.

Soil sites and sampling

Sampling areas were; 'yield-depleted' Molale and Mehal Meda; and 'yield-sustained' Ankober and Keyt. These regions are found in Semien Shewa Zone with classical 'Weina Dega' (subtropical) climate characterized by; 1500–2300 altitude, dry subhumid highland forest vegetation, with six (long and short) rainy months, with annual rainfall (800–1000mm), average annual temperature (13–16.3), and evapotranspiration (110–125cm). Composite soil samples of 0–20 cm depth, each from representative fields were collected in the dry season of November 1995. Physical and chemical analyses were done and recorded (Table 1).

Rhizobium isolation

Rhizobia were isolated from soils separately using the 'plant trap method' (Bergerson, 1980) Faba bean seeds, from improved variety Bulga 70, were surface-sterilized for three minutes each with 95% ethyl alcohol and 0.2% acidified mercuric chloride solutions, according to Vincent (1970). After washing with several changes of sterilized distilled water, five seeds were planted on each pot, which were later thinned down to three after germination. After 45 days of growth, plants were uprooted, nodules were picked, surface-sterilized and macerated. Then they were cultivated on yeast extract mannitol agar (YEMA). The ingredients of YEMA (g/l) were: Magnesium sulphate, 0.5g; Sodium chloride, 0.2g; Yeast extract, 0.1g; Mannitol, 10g; Agar, 15. The isolates were purified by repeated streaking and stored on YEMA slants containing calcium carbonate (3g/l). These strains were characterized on the basis of morphological and physiological characters according to Jordan (1984).

Rhizobial status (population density) determination

Rhizobial population in the soils was estimated by plant infection count method (Vincent, 1970), by planting faba bean seeds in sterilizable poly carbonyl plastic pouches using modified Jensen's nitrogen free medium; The medium contains the following g/l: $CaHPO_4$, 1.0g; K_2HPO_4, 0.2g; $MgSO_4.7H_2O$, 0.2g; NaCl, 0.2g; $FeCl_3$, 0.1g; TE (trace element), 1 ml; TE g/l H_3BO_3 2.86g; $MnSO_4.4H_2O$, 2.03g; $ZnSO_4.7H_2O$, 0.22g; $CuSO_4.5H_2O$, 0.08g; $Na_2MoO_42H_2O$, 0.14g. The faba bean seeds were surface-sterilized, as described before, and they were allowed to germinate on petridishes until hyla and radicles were properly seen. Healthy seedlings were transferred into previously sterilized pouches filled with Jensen's nitrogen free medium and folded with blotting paper immersed in water.

Serial dilutions up to 10^{-8} of each soil were made, and from each dilution, 1ml aliquot was used to inoculate the seedlings. After 45 days of growth, the plants were taken out of the pouches, nodules counted and rhizobial density was estimated according to Somasegaran and Hoben (1994).

Sand culture (hydroponic) experiment

To compare the relative nitrogen fixing capacity of isolates, a separate experiment was done. One effective strain from each of the four fields was selected based upon the previous 'plant trap method'. The strains were the following; Ankober isolate (A1), Molale isolate (M1), Keyt isolate (K1), and Mehal Meda isolate (MM1), and one international reference strain (TAL 1397N). For this purpose 2 kg of imported acid-washed sand was taken and repeatedly washed and filled into previously surface-sterilized plastic pots. Faba bean seeds, surface-sterilized as described earlier, were allowed to germinate after which three seedlings were transferred in each pot. Culture suspensions of each of the five rhizobial strains were prepared by growing in YEMA broth for four days and their number was determined and adjusted to 10^9 cell/ml (Somasagaran and Hoben, 1994). Then 1ml of culture suspension was taken and dispensed on to each seedling. Finally, all pots were covered by acid-washed gravel to avoid probable contamination from the air and nearby pots. Plants were irrigated by Jensen's nitrogen free media, except the positive and negative control pots that were supplied with 0.05%KNO_3 and with neither inoculated nor supplied with KNO_3, respectively. Thirty-five days after planting (DAP), plants were uprooted and measurements for shoot length, shoot dry weight, nodule fresh weight and plant total nitrogen content were recorded.

Pot experiment

Three kilograms of unsterilized soil samples were taken from each sampling area, and separately filled with surface-sterilized (briefly with

95% alcohol) plastic pots (19 cm by 19 cm). Variety Bulga 70 faba bean seeds were randomly selected and surface-sterilized as described previously, and four seeds were planted per pot. The four selected isolates and the exotic strain Tal 1397 N were prepared by growing them in YEM nutrient broth media for four days. The rhizobial number was adjusted to 10^9 cells/ml, and 4 ml of the culture suspension was taken from each strain and inoculated on the pots in the following combination: Ankober isolate (A1) (yield-sustained) on Molale soil (yield-depleted); Molale isolate (M1) (yield-depleted) on Ankober soil (yield-sustained) Keyt isolate (K1) (yield-sustained) on Mehal Meda soil (yield-depleted); Mehal Meda isolate (M-M1) (yield depleted) on Keyt soil (yield-sustained).

In addition to inoculating with rhizobial isolates, the soils were also treated with fertilizer after planting. Nitrogen fertilizer as urea and phosphorus fertilizer as DAP (diammonium phosphate) were applied, respectively. The seven treatments for each of the four soils are given below:

i. $N_{46}+P_{20} = T_1$
ii. local rhizobial isolate = T_2
iii. exotic strain (TAL 1397N) = T_3
iv. $N_{23}+P_{20} = T_4$
v. $N_{23}+P_{20}$+exotic strain (TAL 1397N) = T_5
vi. $N_{23}+P_{20}$+ local rhizoibal isolate = T_6
vii. Soil only (control) = T_7

In order to apply nitrogen and phosphorus at 46 Kg ha^{-1} and 20 Kg ha^{-1} on 3 Kg soil (per pot), 0.072g urea and 0.15g DAP were used. To apply $N_{23}P_{20}$ rate 0.04g urea and 0.15g DAP were taken. Pots were watered every week until saturation. The pot experiment was carried out under greenhouse conditions at the National Soil Laboratory between February and April 1996 under natural illumination with minimum and maximum temperatures of 19° C and 35° C.

The pots were randomized, and placed in the greenhouse according to 2x7 factorial experiment (Somasegaran and Hoben, 1994). When seedlings were visible above the surface of the soil, thinning was done leaving three plants per pot.

After 70 days of growth, pots were washed down to uproot the root of the plants from soil and measurements of plant shoot length, shoot dry weight, nodule fresh weight were recorded. Plant total nitrogen of samples were analyzed by the National Soil Laboratory, Ethiopian Agricultural Research Organization.

The data obtained were analyzed using the statistical software M-stat (version c). Two-way factor randomized complete block design (RCBD) analysis and Dunkans' multiple range tests were used.

RESULTS

Many strains were isolated from each sampling field. The isolates were fast growing, acid producing similar to the type strain *Rhizobium leguminosarum* var *viceae*. They were differentiated from one another on the basis of morphological, cultural, and some important biochemical characters such as carbohydrate utilization, and growth on different concentrations of sodium chloride (Jordan, 1984) (data not shown).

Population density

Estimates of number of rhizobia at each site were found to differ significantly ranging from 2.2×10^2 at Mehal Meda to 1.2×10^6 at Keyt per gram of soil (Table 1). Rhizobial numbers were not correlated with pH but significantly associated with available phosphorus (r=0.75).

Table 1. Soil chemical analyses and population density of rhizobia.

Parameters	Ankober	Keyt	Mehal Meda	Molale
pH	6.400	6.280	5.900	6.540
K meq/100g soil [1]	1.088	1.271	0.510	0.958
Ca meq/100g soil [2]	19.163	18.658	29.861	36.684
Mg meq/100g soil [3]	4.087	5.425	9.695	10.305
T.N. % [4]	0.224	0.194	0.202	0.125
O.C. % [5]	2.086	1.683	1.348	0.934
O.M. % [6]	3.596	2.901	2.324	1.610
C/N [7]	9.000	9.000	7.000	7.000
Av.P.ppm [8]	31.010	51.800	5.700	3.320
Av.K.ppm [9]	307.000	382.000	170.000	205.000
Rhizobial density(g^{-1} soil)	1.2×10^5	1.2×10^6	2.2×10^2	8.8×10^2

1, total potassium; 2, total calcium; 3, total magnesium; 4, total nitrogen; 5, organic carbon; 6, organic matter content of the soil; 7, ratio of carbon to nitrogen content; 8, available phosphorus; 9, available potassium.

Symbiotic effectiveness in sand culture

The relative nitrogen fixation capacity of the five isolates *i.e.*, (Ankober) K1, (Molale)M1, (Mehal Meda) MM1 and (Ankober) A1, and the reference Tal 1397N was measured on the basis of total nitrogen content, total dry matter, shoot length and nodule fresh weight in a hydroponic culture (Table 2). This indirectly indicated symbiotic effectiveness of isolates. The result showed that inoculation of plants with all isolates did not show any significant difference (P=0.05) on parameters of symbiotic effectiveness

among one another and the positive control, except nodule fresh weight (Table 2).

Table 2. The effect of inoculation on growth-parameters in a hydroponic (sand) culture (symbiotic effectiveness).

Treatment	Shoot length (cm)	Total dry matter (gm)	Nodule fresh weight (gm)	Plant total nitrogen content (%)
MM1 isolate	34.9±6.78	1.16±0.10	0.77±0.07 b	2.78±0.19
TAL1397N	28.5±1.81	1.54±0.41	1.07±0.12 a	3.24±0.47
K1 isolate	31.1±2.81	1.08±0.29	0.40±0.16 b	2.43±0.32
M1 isolate	29.9±3.24	1.42±0.26	1.02±0.26 a	2.95±0.35
A1 isolate	34.0±1.63	1.49±0.13	0.50±0.05 b	2.68±0.23
Control	31.7±4.49	1.17±0.10		2.20±0.31
KNO_3	39.1±9.71	1.63±0.16		2.56±0.04

Numbers in the same column followed by different letters are significantly different at 5% level (Duncan's multiple range test). Numbers are means and standard deviations (s.d.) of three replicates (3 plants in each pot). Letters in the columns (a,b) are rank of the means.

Pot experiment

Variations in shoot length, dry matter, and nodule fresh weight showed that fertilizer and inoculation together with soil type significantly affect these parameters (P=0.01) (Table 3a, 3b, 3c). Total nitrogen content of plants was not significantly affected by either of the two factors, except Molale soil (Table 3d). Comparison of the mean values of shoot dry matter on Keyt and Ankober soils showed no significant difference (P=0.05) between treatments and the control, whereas Molale and Mehal Meda soils responded better to various treatments with this parameter (Table 3b). The increase in shoot dry weight of the highest value of the different treatments was found to be more than 45% of the control (soil only).

Shoot length was found to be significantly improved by various treatments compared with the control, except Molale soil which responded only to the application of high fertilizer treatment (T1) and inoculation of the indigenous rhizobium strain (T2). Inoculation and other treatments were found to increase shoot length between 8% (Keyt soil) and 32% (Mehal Meda soil) as compared to the control.

Nodule fresh weight of the different treatments was found to be more variable than plant height and shoot dry matter. The highest values for nodule fresh weight were recorded from the indigenous strains on Ankober soil (T2), fertilizer treatment with or without inoculation by indigenous and the exotic strains on Keyt soil (T1, T3, T5, T6).

Table 3a. Effect of treatment (fertilizer and/or inoculum) on shoot length of faba bean plants grown in different soil regions.

Treatment/ soil region	T1	T2	T3	T4	T5	T6	T7
Ankober	74.40±0.80a	71.00±1.7ab	68.00±4.32ab	69.30±6.43ab	67.10±3.18b	69.6±1.32ab	67.20±3.18b
Keyt	61.60±0.47a	61.30±1.27a	61.90±1.59a	60.40±3.90ab	58.9±1.48ab	62.20±0.73a	56.90±2.04b
Mehal Meda	52.50±2.90a	43.20±2.70a	41.70±0.69b	52.10±1.55a	53.80±1.63a	53.70±1.38a	39.90±1.79b
Molale	73.10±2.04a	60.00±1.92a	59.40±0.98b	61.90±3.90b	64.30±2.20b	62.30±0.89b	59.30±3.76b

Table 3b. Effect of treatment (fertilizer and/or inoculum) on shoot dry matter of faba bean plants grown in different soil regions.

Treatment/ soil region	T1	T2	T3	T4	T5	T6	T7
Ankober	3.85±0.19a	3.62±0.22a	3.97±0.62a	4.02±0.49a	3.94±0.38a	3.95±0.16a	3.74±0.29a
Keyt	4.14±0.6 a	3.96±0.05 a	3.90±0.23 a	4.06±0.64 a	4.18±0.38 a	4.16±0.36 a	3.94±0.31 a
Mehal Meda	3.30±0.48 ab	2.84±0.34 b	2.56±0.09 b	3.84±0.19 a	3.92±0.30 a	3.70±0.16 a	2.68±0.47 b
Molale	3.46±0.39 a	2.04±0.16 c	2.17±0.26 c	2.85±0.10 ab	2.94±0.10 ab	2.59±0.20 bc	2.36±0.13 bc

Table 3c. Effect of treatment (fertilizer and/or inoculum) on nodule fresh weight of faba bean plants grown in different soil regions.

Treatment/ soil region	T1	T2	T3	T4	T5	T6	T7
Ankober	0.56.±0.04bc	0.96±0.5a	0.64±0.05b	0.63±0.04b	0.50±0.01c	0.63±0.04b	0.67±0.12b
Keyt	1.13±0.03a	0.71±0.02b	1.13±0.12a	0.66±0.09b	1.13±0.04a	1.10±0.14a	0.7±0.08b
Mehal Meda	0.2±0.04d	0.44±0.03bc	0.39±0.05c	0.17±0.08d	0.60±0.04a	0.58±0.02ab	0.16±0.04d
Molale	0.2±0.01cd	0.43±0.04b	0.40±0.05b	0.27±0.04c	0.64±0.03a	0.63±0.07a	0.10±0.01d

Table 3d. Effect of treatment (fertilizer and/or inoculum) on total nitrogen content of faba bean plants grown in different soil regions.

Treatment/ soil region	T1	T2	T3	T4	T5	T6	T7
Ankober	2.86±0.10a	2.78±0.22a	3.13.±0.80a	2.79±0.03a	3.10±0.05a	3.29±0.24a	3.14±0.16a
Keyt	2.96±0.14a	2.97±0.29a	3.05±0.22a	2.58±0.95a	2.78±0.19a	2.99±0.06a	3.00±0.39a
Mehal Meda	2.83±0.08a	2.94±0.11a	2.75±0.21a	3.15±0.20a	2.95±0.17a	2.71±0.16a	3.07±0.18a
Molale	2.87±0.11b	2.91±0.43b	2.59±0.30b	2.95±0.14b	3.53±0.06a	3.02±0.23b	2.86±0.10b

Numbers in the same column followed by different letters are significantly different at 5% level (Duncan's multiple range test). Numbers are means and standard deviations (s.d.) of three replicates (3 plants in each pot). Letters in the columns (a,b,c,d) are rank of the means.

The highest nodule fresh weight of the 'yield-depleted' Molale and Mehal Meda soils, however, was only recorded from the inoculation of the exotic strain Tal 1397N and the indigenous strains with and without low fertilizer treatments (T5, T6). The inoculation of different soils with local rhizobial inoculum (T2) alone did not show any difference from that of the exotic strain on many of the growth parameters measured in all soils except shoot length on Molale and Mehal Meda soils, and nodule fresh weight on Ankober and Keyt soils. However, inoculation together with nitrogen and

phosphorus fertilizers with exotic strain (T5) and indigenous strain (T6), were found to vary in the different growth parameters on different soils.

The exotic strain together with fertilizer treatment (T5) was found to significantly differ from the dual inoculation and fertilizer treatment of indigenous strains (T6) in shoot length of Keyt soils, shoot dry weight and nitrogen content of Molale soils, and nodule fresh weight of Mehal Meda soils (3a, 3b, 3c, 3d). The latter (T6) showed better result than (T5) in shoot length and nodule fresh weight on Ankober soil. However, in most cases both T5 and T6 were found to significantly improve the growth parameters compared to the reference (soil only treatments). Treatment of the different soils with high ($N_{46}+P_{20}$) (T1) and low ($N_{23}+P_{20}$) (T4) fertilizer showed significant increases on shoot length and shoot dry weight on Ankober and Molale soils, and shoot length and nodule fresh weight on Keyt soil, respectively (3a, 3b, 3c). However, low fertilzer treatment with inoculation (T4) showed better results on nodule fresh weight in Ankober and Molale soils and shoot dry weight in Mehal Meda soils than the T1 treatment. Generally, the results indicated that growth parameters were improved by inoculation and fertilizer treatment of soils of the sampling regions, particularly in the 'yield-depleted' regions of Molale and Mehal Meda.

DISCUSSION

The soil contents of 'yield-depleted' Molale and Mehal Meda, and 'yield-sustained' Keyt and Ankober regions showed that they vary in some of the important bioelements that are vital for growth and production. Based on the classification of Ngeborg (1986), and Desta Beyene and Angaw Tsigie (1986), the 'yield-sustained' Keyt and Ankober regions are in the category of "high nitrogen and high available phosphorus soils" whereas the 'yield-depleted' regions of Molale and Mehal Meda are within "the medium nitrogen and low available soils" (Table 1). The difference in the mineral and organic matter contents between the two areas might be partly due to dung manure application at Ankober and Keyt ('yield-sustained' areas) that is not common at Molale and Mehal Meda ('yield-depleted' areas). The role of manure in the increase of faba bean production was also attested by the work of Yousif (1987) in the Sudan, which is associated with the enrichment of the soil with nutrients and increase in the rhizobial population.

Rhizobial density of the four sampling regions showed that soil samples of Ankober and Keyt, have a very high number of rhizobia ($>10^2$ g^{-1} soil) where as Mehal Meda and Molale have low rhizobial population ($<10^2$ g^{-1} soil) according to the categorization of Date (1982). Differences in rhizobial

population is mainly governed by different environmental factors the most important of which are; the presence or absence of the homologus host (Date, 1982), failure to grow the host for quite a long time in a fallow system and crop rotation (Loutfi *et al.*, 1980). The other factor which has contributed for the low rhizobial population number in the 'yield depleted' areas, is the low availability of soil nutrients like organic matter, phosphorus, and potassium that affect growth and nitrogen fixation of legumes (Hynman, 1986).

Periodic reconnaissance surveys in the region showed that pulse crops were not grown in some of the farmer's fields in Molale and Mehal Meda, for almost eight years. This may have contributed to the decrease of rhizobial number in these soils. Field experiments on leaving fallow soil without growing the homologous host in Egypt showed a concommitant decrease in rhizobial population of *Rhizobium leguminosarum* and other microorganisms that necessitated inoculation of commercial rhizobia in the field (Loutfi *et al.*, 1980). Failure to grow the host for quite a long time is said to deprive the soil of litter incorporation and the subsequent microbial activities. This would otherwise improve the low availability of organic matter and soil nutrients like phosphorus and potassium, particularly in 'yield-depleted' areas.

Although a large number of diverse rhizobial strains were isolated from all of the sampling regions, preliminary screening for effective strains showed only a few of them were effective in nitrogen accumulation. In a screening experiment of faba bean rhizobia from several pulse growing areas of central Ethiopia, Desta Beyene and Angaw Tsigie (1987) managed to isolate 23 symbiotically effective strains from 108 isolates (11%), signifying that such strains are very small in number in different soils. Variability among strains both in symbiotic effectivity of isolates from *Vicia faba* in Ethiopia (Van Berkum *et al.*, 1995) and other strains of *Rhizobium leguminosarum var viceae* isolated elsewhere (Brockman and Bezdicek, 1989) is a common occurrence along different habitats and geographic regions.

The comparative symbiotic effectiveness of the selected local rhizobia and the exotic strains was not significantly different from one another in a hydroponic culture. However, their inoculation alone or with nitrogen and phosphorus fertilizers was found to significantly increase shoot length in Ankober, Keyt, Molale and Mehal Meda soils. Regarding shoot dry weight, the 'yield-depleted' regions of Molale and Mehal Meda responded well to inoculation and fertilizer treatments. The fact that effective nodulation occurs independently of inoculum addition in Keyt soil by both homologous K1 isolate and M-M1 suggests that the indigenous rhizobia are able to survive and compete in a nutrient rich soil. This indicates that the

selected indigenous rhizobial strains are equally effective and competitive provided that the nutritional limiting factors in the soil are alleviated. From all the strains used for inoculation, the exotic TAL 1397N was found to enhance better growth than the indigenous ones although the difference was not significant. The strain also showed better result in growth with the application of low mineral fertilizer ($N_{23}+P_{20}$) on Molale soil compared with the soils of the other regions. This showed that application of starter dose nitrogen and phosphorus increase the nitrogen fixing ability of the strain.

The variability in shoot length, dry matter, and nodule fresh weight of faba bean showed that growth is affected at a highly significant level ($P=0.01$) by both soil type and seven factorial treatments. Nodule fresh weight was found to be variable with different treatments in the hydroponic and pot experiments as compared to other growth parameters. Significant differences between treatments in 'yield-depleted' and 'yield-sustained' areas on nodule fresh weight showed that yield response to mineral and bacterial treatments are more pronounced in the former than the latter. In general, high values for nodule fresh weight and other plant growth characters in these experiments are manifestations of the availability of sufficient nutrient source for tissue development and the establishment of sufficient rhizobial population in the soil. Apart from nitrogen and phosphorus, different limiting factors such as the concentration of calcium, pH, type and density of rhizobium are also said to govern nodule development (Munns, 1977; Date, 1982). All these factors are manifested differently in 'yield-depleted' and 'yield-sustained' areas.

The application of the highest concentration of nitrogen and phosphorus (NP) in this experiment (T1) was found to increase yield as compared to low fertiliser treatment (T4). The latter, however, responded well together with inoculation. This implies that the application of higher concentration of NP or phosphorus together with inoculation of the effective rhizobial isolate increases faba bean growth. Apart from nitrogen accumulation, the increase in some growth-related parameters on different soils with inoculation may also be associated with other factors other than nodulation. Recently, Noel *et al.* (1996) identified *Rhizobium leguminosarum* as a supplementary plant growth promoting rhizobacteria (PGPR) producing cytokinin and indole acetic acid to enhance growth of canola and lettuce.

A positive response of phosphorus application to nitrogen fixation was also recorded from several leguminous crops, such as faba bean (Abdulsalaam and Tahir, 1991), pigeon pea, *Cajanus cajan* (Hernandez and Focht, 1983), and the leguminous tree, *Leuceana leucocephala* (Duguma and Okali, 1987). In Ethiopia, several experiments showed that faba bean cultivars generally

responded to inoculation and NP fertilizers. The response was relatively higher to phosphorus than nitrogen depending upon location and soil types (Desta Beyene, 1986; Desta Beyene and Angaw Tsigie, 1986; Sahle Medhin Sertsu and Desta Beyene, 1986; Balesh Tulema and Asnakew Weldeab, 1993; Tekalign Mamo and Asgelil Dibabe, 1994). The application of phosphorus is also said to be positively interacting with potassium, calcium, and other nutrients to maintain the pH and other physical factors in the soils. This, in turn, creates a suitable environment for the growth and nitrogen fixation of nodulating leguminous plants (Hernadez and Focht, 1983).

Phosphorus deficiency in a tropical soil may be due to its low content or its fixation with the formation of a complex with aluminium and iron in acidic soils (Tekalegn Mamo and Haque, 1991), or with calcium in arid and semi-arid soils (Diez *et al.*, 1992). This is a common character of wet tropical soils. Although attempt has not been made to explain the type of phosphorus deficiency in 'yield-depleted' regions of Semien Shewa, the low soil pH and the difficulty to measure < 0.01g extractable phosphorus per kilogram soil (10ppm) may indicate phosphorus fixation (Hernandez and Focht, 1983). In order to efficiently extract phosphorus and other nutrients from P-deficient soils, the use of microorganisms such as Arbuscular Mycorrhizal Fungi (AMF) and Phosphate Solubilizing Bacteria (PSB) is often recommended in tropical soils (Subarao, 1993). Recently, Wasse Haile *et al.* (1999) evaluated the efficacy of several strains of PSB isolated from phosphorus fixing acidic soils in Ethiopia to improve phosphorus nutrition of faba bean (*Vicia faba*) in soils together with the inoculation of rock phosphate.

Although phosphorus was found to be the most limiting factor to growth in 'yield-depleted' areas, application of nitrogen also appeared to enhance growth in all tested soil types without showing any difference in nitrogen content. This indicated that the effect was indirect, perhaps through stimulation of plant growth. The fact that nodulation appeared in most treatments with nitrogen is similar to, and even better than the control indicated that faba bean was not only inhibited by the application of nitrogen, but was also stimulated by it. Richards and Soper (1979) reported tolerance up to 600 mg^{-1} N pot^{-1} without affecting nitrogen uptake into the faba bean plant. The incorporation of starter nitrogen together with phosphorus, regardless of the nitrogen content in the soil, therefore, stimulates growth and enhances efficient nodulation and assimilation of nitrogen by faba bean (Kumar *et al.*, 1993).

From this study, it can be concluded that failure of pulse production in some parts of Semien Shewa can be reversed through integrated management involving manure application, liming to control soil acidity,

and screening effective rhizobial and other growth promoting rhizosphere microorganisms for inoculation with limited fertilizer application.

ACKNOWLEDGEMENTS

The financial support from the Institute of Biodiversity Conservation and Research (IBCR) is highly acknowledged. We also appreciate the help of the National Soil Laboratory of the Ethiopian Agricultural Research Organization for allowing us to work in their greenhouse and laboratories.

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