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RESEARCH PAPER

Assessment of Indigenous Rhizobia in faba bean (*Vicia faba l.*) Soils of Kersa Malima District Southwest Shoa Zone of Oromia, Ethiopia

Gezahegn Tamiru[1], Achalu chimdi[2*] and Yifru Abera[3]

[1]*Holeta Agricultural Center, Ethiopia;*
[2*] *Ambo University, Department of Natural Resource Management, Ethiopia;*
[3]*Debre Zeit Agricultural Research Center, Ethiopia*
***Corresponding Author:** Email: acahaluchimdi@yahoo.com*

Abstract

*Estimation of the indigenous rhizobia population plays a great role to determine the abundance of rhizobia in the soil and for the need of inoculation. This research paper aimed to assess the population of rhizobia nodulating faba bean in relation to cropping history and soil properties in fababean producing areas of Kersa Malima Districts. Fifteen composite soil samples were collected at the depth of 0-20cm from district`s and the most probable number was used to count rhizobia and plant infection method. The soil rhizobia population was ranged from 0.357*102-9.324*103 cells/g of dry soil. Farm fields with history of legumes cultivation had the highest rhizobia population abundance than cereal-cereal-rotation. Soil pH, organic carbon and available phosphorus influenced soil rhizobia abundance. The study revealed that the land slope influence was not observed at all sites on soil rhizobia population size. Therefore, the present study showed variation in indigenous rhizobia population was observed from soils of Kersa Malima district.*
Keywords: Most probable number, cropping system, rhizobium, Kersa Malima, District

Introduction

Where natural biological N2 fixation is not optimal, inoculation is essential, ensuring that a high and effective rhizobial population is available in the rhizosphere of the plant (Tena et al., 2016). The enumeration of rhizobia is valuable for the assessment of rhizobial population abundance in the soil and how they vary or to assess the number and viability of rhizobia in the soil (Howieson and Dilworth, 2016). Assessment of the symbiotic effectiveness and abundance of soil rhizobia population using the whole soil inoculation technique (Brockwell et al., 1998) can provide a rapid assessment of the entire soil population, so it is more likely to represent the response from a genetically or phenotypically diverse population. The whole soil inoculation (WSI), method, coupled with the most probable number technique, can provide an assessment of the need for inoculation (Denton et al., 2000), as these combined techniques estimate both density and symbiotic effectiveness of the soil population of rhizobia.

Estimating rhizobial population density is a means of predicting whether or not a legume will respond to rhizobium inoculation (Thies et al., 1991). Lindstrom et al. (2010) stated that the population size of native rhizobia plays an important role for BNF (biological nitrogen fixation) to be efficient in fixing nitrogen as well as increasing crop yield. Information on the native rhizobial population is important for understanding the distribution and diversity of the rhizobia population in the soil and to determine the achievement of inoculation (Fening and Danso, 2002). An assessment of the native rhizobial population plays a vital role in a better understanding of soil biodiversity and in improving the contribution of biologically fixed nitrogen to legume production (Blazinkove et al., 2007).

Use of microbial products for improving agricultural productions, and or soil and plant health has been practiced for many years in Ethiopia. However, appropriate scientific information and related knowledge with regard to rhizobia population size is useful for understanding its impact on crop production and commercial rhizobia effectiveness in increasing legumes production. Some literatures indicate that population of indigenous rhizobia influence legumes nitrogen fixation capacity. This assessment will contribute to the body of knowledge and production of best rhizobium strains for legumes crops and of each strain in the region and similar areas. Therefore, this work aimed to assess the size of natural population abundance of faba bean nodulating rhizobia in Kersa Malima district of Southwest Showa Oromia and explore the association of their abundance to cropping systems and soil physicochemical properties.

Materials and methods

Geographical locations and areas coverage: Kersa Malima district is located at 60 km west of Addis Ababa within Southwest Shoa Zone, Oromia Regional State. It has a total area of about 59,904 ha and 100,820 human populations. Out of this human population, 51,470 are male and 49,350 are female. Chromic Vertisol is the dominant soil type in the district. The major crops that are grown in the area are wheat, faba bean, teff, and chickpea (Kersa Malima Woreda Agricultural Office, 2021).

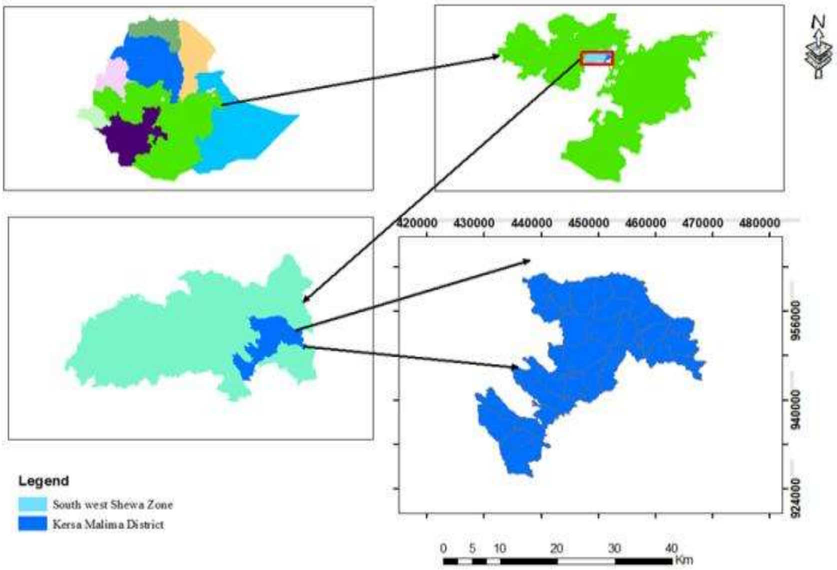

Figure 1: Map of the study area Kersa Malima district

Climate and topography: Kersa Malima district receives an annual maximum rainfall of 1400 mm and minimum rainfall of 900 mm per year and average maximum and minimum temperature of 25 °C and 12 °C respectively. Kersa Malima district is situated at an altitude of 2123m above sea level and the rainfall distribution pattern is very high at upland areas and lower lands during summer (Kersa Malima District Agricultural Office, 2021).

Population, land uses and farming system: The district has a total population of 100,822 out of this 51,470 are male while 49,352 are females. The dominant land use type in the district is cropland (55%), forest land (11%), pasture/grazing land (19%), and others account for 15% of the total land area. The land cover is dominated by farmlands, plantations and grasses. A mixed farming system that combines crops and livestock husbandry characterizes the district. The major crops commonly grown in the district are tef, wheat, fababean, Chickpea and barley. Cattle, sheep and goat are the most common livestock reared by the farmers of the district (Kersa Malima District Agricultural office, 2021).

Site selection, soil sampling and sample preparation: For this study, the sites were selected based on agro-climatic conditions and prevalence of faba bean production. Accessible and potential faba bean growing sites were systematically selected for the study by doing reconnaissance survey. Each Kebele was selected based on the crop rotation that prevails in those areas. The category of each topography was stratified into a relatively homogeneous biophysical feature as flat and gentle. Information regarding cropping systems in the farms consisted of faba bean, wheat, and fallow land. Cropping and inoculation history of fields were collected through interviewing farmers who stayed for long period of time in that area.

Each soil sample was carefully collected to avoid cross contamination between different sampling points. About one kilogram of composite soil sample was collected from at least three representative sub samples from each unit areas at the depth of 0-20 centimeter during June to March 2021 cropping season. The collected soil samples were bagged, labeled and submitted to Holetta Agricultural Research Center Laboratory. The collected soil samples were divided into two parts, one part was used for serial dilution to determine population size of native rhizobia and the second part was used for determination of soil physico-chemical properties. For laboratory analyses sufficient amount of composite soil samples were air dried and ground to pass a 2-mm sieve for most of soil chemical parameters, while for organic carbon and total N soil samples were sieved at 0.5 mm sieve. Then, the samples were analyzed following standard laboratory procedures.

Analysis of selected soil physical and chemical properties: Particle size distribution was determined by the hydrometer method (Day, 1965) and the soil is assigned to a textural class as described by (Rowell, 1994). The most important parameters that were determined are soil pH, total nitrogen (%), available P (ppm), exchangeable acidity, organic carbon (%) and CEC (meq/100g), based on the standard procedures. The pH of the soil was measured from suspension of 1:2.5 (weight/ volume) soil to water ratio using a glass electrode attached to digital pH meter (McLean, 1982). Organic carbon content was determined using the wet digestion method Walkley and Black (1934). Soil organic matter was calculated from organic carbon (OM=1.724 * %OC). Total nitrogen content of soil was determined by the Kjeldahl digestion (Bremner and Mulvancy, 1982). The available phosphorus was determined using Bray-II method (Bray and Kurtz, 1945). Exchangeable acidity of the soil was determined by leaching-titration with 1N KCl as described by Van Reeuwijk (1992). CEC was determined using ammonium acetate method (Black, 1965) at soil and plant analysis laboratory of Holetta.

Table1: Kersa Malima District assessed kebele's

Sample number	Sites/kebele's	Cropping system	Slope
1	BiynaGiche	C-L-C	Gentle
2	Mazora	C-L-C	Flat
3	Mazora	C-L-C	Flat
4	Adadi	C-L-C	Gentle
5	Mazora	C-L-C	Flat
6	KersaWarko	C-L-C	Gentle
7	Muti-Alibo	C-L-C	Flat
8	KersaWerko	C-L-C	Flat
9	BiynaGiche	C-L-C	Gentle
10	BiynaGiche	C-L-C	Flat
11	MutiDayu	C-C-C	Flat
12	MutiAlibo	C-C-C	Gentle
13	MutiDayu	Fallow->4	Flat
14	GodetiWenberi	Fallow-1yr	Flat
15	GodetiWenberi	Fallow-3yrs	Gentle

C-L-C=Cereal-legume-cereal, fall=fallowing

Determination of rhizobia population density: A total of fifteen soil samples were collected from Kersa Malima district and isolated at Holetta soil microbiology laboratory. Each sample had a different cropping history and geographic coordinate. Abundance of rhizobia was determined using the most probable number techniques adapted from Andrade and Hamakawa (1994). The most probable number determination experiment was conducted in the National Agricultural Biotechnology Research Center green house at Holetta.

The plants were frequently inspected and water was provided periodically. The plants were watered with sufficient N – free nutrient solution when necessary (Somasegaran and Hoben, 1994). Each pot was inoculated with 1.0 ml of the diluent replicated four times using different pipette tips to prevent contamination. Soil suspension and inoculation was carried out aseptically. Soil samples one gram were suspended in 9 ml of sterilized saline solution (0.89% NaCl) and mixed on a shaker at 150 rpm for 25 minutes. Each of the soil suspensions was diluted through a six-step tenfold series (10-1 to 10-6), transferring 5 ml of suspension to 20 ml of sterile saline solution at each step (Woomer, 1994).

Un-inoculated seedlings with or without nitrogen were used to serve as controls. After inoculation, the pots were laid down in Completely Randomized Design (CRD). All solutions were sterilized by autoclaving at 1210C for 15 minutes. After 30 days, plants were carefully uprooted and the numbers of nodulated pots were recorded. To determine the MPN, roots were assessed for the presence or absence of nodules. The presence of a single nodule in pots was considered a positive score. The Most Probable Number technique was used to determine rhizobia cells per gram of dry soil, Positive results were compared with a standard most probable number (Andrade and Hamakawa, 1994).

Results and Discussion

The highest rhizobia population abundance was observed at cereal-legumes-cereals rotation cropping system having flat and gentle slopes (Table 2). At sits 1, 2, 3, 4, 5, 6, 7, 9 and 10 indigenous rhizobia population abundance was ranged from 1.469 *103-9.324*103 cells/g of dry soil of Kersa Malima district vertisol (Table 2), This is confirmed the idea of (Woomer et al., 1988) who stated that the importance of the appropriate host legume on occurrence and proliferation of a population of a particular rhizobia species in soils. The highest rhizobia population abundance of 9.324*103cells/g of dry soil observed at site two might be due to the presence of trees in the field.

The lowest rhizobia population abundance 0.917 *102 cells /g of dry soil were observed at site eight were might due to the absence of host crop. The cropping history of farm field was cereal-legumes-cereal, the legumes type /host plant was chickpea cultivated for many years, native rhizobia nodulating faba bean is below 102 cell /g of dry soil of Kersa Malima district Vertisol (Table-2). During the rainy season, the farm field has draining problem, the slope of the farm field was very flat, this is associated with concept of that rhizobia require sufficient oxygen to survive, also rhizobia prefer soils that are moist, but not waterlogged. The most problematic environments for rhizobia are marginal lands with low rainfall, extreme temperature, acidic soils of low nutrient status and poor water holding capacity (Bottomley et al., 1991).

The lowest rhizobia abundance was observed at cropping system cereal-cereal –cereal and fallowing 1-3 years having 0.357*102-0.917*102cells/ g of dry soil of Vertisol at Kersa Malima District which has no legumes cultivation history for many years (Table-2). Also, at site thirteen Vertisol, farm field that were fallowed above 4 years and cultivated cereals for many years low abundance of rhizobia population observed 0.357*102 rhizobia cells/ g of dry soils (Table-2). Likewise, Chemining'wa and Vessey (2006) reported higher rhizobia population and nodulation in fields previously grown with legumes crop than farm field without legumes cultivation history. Legumes create new available niches for a wide range of rhizobia to colonize (Dinnage et al., 2019).

Burghardt (2019), assisting the maintenance of high rhizobial abundance in local plant populations. This may be particularly relevant in perennial legumes.

Table 2: Determination of rhizobia population abundance in soil of Kersa Malima district

Sample No.	Sites	Positivity	CS	Slope	R-cells/g of soil
1	BiynaGiche	2-2-1	C-L-C	Gentle	$2.763*10^3$
2	Mazora	3-2-0	C-L-C	Flat	$9.324*10^3$
3	Mazora	2-3-0	C-L-C	Flat	$2.86 *10^3$
4	Adadi	2-1-0	C-L-C	Gentle	$1.47 *10^3$
5	Mazora	3-1-0	C-L-C	Flat	$4.272 *10^3$
6	KersaWarko	2-1-0	C-L-C	Gentle	$1.47 *10^3$
7	Muti-Alibo	2-1-0	C-L-C	Flat	$1.47 *10^3$
8	KersaWerko	2-0-0	C-L-C	Flat	$0.917 *10^2$
9	BiynaGiche	2-1-0	C-L-C	Gentle	$1.469 *10^3$
10	BiynaGiche	3-0-0	C-L-C	Flat	$2.312 *10^2$
11	MutiDayu	1-0-0	C-C-C	Flat	$0.357*10^2$
12	MutiAlibo	2-0-0	C-C-C	Gentle	$0.917 *10^2$
13	MutiDayu	1-0-0	Fall->4	Flat	$0.357 *10^2$
14	GodetiWenberi	2-0-0	Fall-1yr	Flat	$0.917 *10^2$
15	GodetiWenberi	1-0-0	Fall-3yrs	Gentle	$0.357 *10^2$

Note: CS= Cropping system C-C-C=Cereal-cereal-cereal, C-L-C=Cereal-legume-cereal, fall=Fallowing

Soil pH and Rhizobia Population abundance: The soil pH of Kersa Malima district was ranging from 5.99 - 8.33(Table 3), ranged from moderately acidic to moderately alkaline as rated by Jones (2003), Tekalign (1991) and Murphy 1968). The Highest rhizobia population found in this site was not influenced by soil pH, rather it is associated with the host crop, the filed with agroforestry had highest rhizobia population at Mazora having soil pH 6.86 and rhizobia 9.324*103 cells/g of dry soil Table-2) during dry season due to the shade of tree rhizobia are escape themselves and survive. Drought also affects both the number of rhizobia and N2 fixation rates. Soil pH is the most harmful abiotic constraint to BNF, mainly due to its effect on soil physical and biological characteristics (Zaharan, 1999). At sites of Muti-Dayu, Adedi and Godeti Wenbari soil pH is ranging from 6.86-7.22 and rhizobia population was found in range 0.357 *102 -0.917 *102 cells / g of dry soil (Table-2) the cropping history of the land is cereal-cereal-cereal and have no history of legumes cultivation, therefore at these sites the influence of soil pH on rhizobia abundance is not observed due to neutral pH range. Woomer et al. (1988) also confirmed that the importance of the appropriate host legume on the occurrence and proliferation of a population of a particular rhizobia species in soil.

Relationship of cation exchange capacity (CEC) and rhizobia population abundance: The CEC of the Kersa Malima district is ranged from 26.12-53.22(meq/100g), (Table 3) it is high to very high as rated by Hazelton, and Murphy (2007). The higher rhizobia population abundance 9.324*103 was observed at higher values of CEC 45.48 (meq/100g) and at high CEC value the low rhizobia population abundance was also observed. The CEC value is not less than the values that the soil is suspected to influence the rhizobia population abundance in soil as briefly indicated on (Table-3).

Effect of Phosphorus on rhizobia population abundance: The available soil P of Kersa Malima district is ranged from 2.995-23.18(ppm) (Table 3) it is ranged from low to high as rated by Olsen et al. (1954). Biye Giche, Mazora, Adadi, Kersa Werko and Muti-Alibo sites soil P are higher, and the highest rhizobia population observed at these sites 13.58(ppm), 9.324*103 cells/g of dry soil (Table-3). At Adadi, Muxi-Dayu, Godeti-Wanbari and fallowing 1year and 3 years the field farms cropping history are cereal-cereal, has no legumes cultivated the soil had phosphorus 8.395(ppm) and lowest rhizobia was observed ranging from 0.357*102-0.917 *102 cells/g of dry soil. Abate et al. (2016) also revealed variations in parent material, soil texture, degree of P fixation, soil pH and slope gradient may also contribute to variations in available phosphorus contents amid the land units. Application of chemical fertilizers reduces the number of bacteria associated with the roots that solubilize nutrients such as N, K, P, Fe, and Zn (Reid et al., 2021).

Effect of soil organic carbon on abundance of rhizobia population: The soil organic carbon of Kersa Malima is ranged from very low to medium 0.7-1.95% as rated by Tekalign (1991). The highest rhizobia population abundance 9.324*103 cells / g of dry soil were observed at 1.95 % of organic carbon. At Kersa Werko and Godet-Wenberi sites had three years fallowing and Teff-Chickpea-Teff rotation cropping history had 1.5 and 1.71 % organic carbon and lowest rhizobia population 0.357*102-0.917*102 cells/g of dry soil the reverse of mazora sites, at Organic carbon 1.95% highest rhizobia were observed and it indicates the influence of host crop and present of native rhizobia (Table-3). Graham (2008), Kasper et al. (2019) reported that the population of rhizobia in different soils is heterogeneous and varies quantitatively and qualitatively, responding to different abiotic and biotic factors.

Total nitrogen and rhizobia population abundance: The total nitrogen of Kersa Malima ranged from 0.063- 0.134 % (Table 3), it is low to high as indicated by Murphy (1968). Lower N may be due to low organic carbon of the soil, the highest Rhizobia populations were observed at highest total nitrogen 0.134% having 9.324*103 cells/g of dry soil. The same rhizobia number and the same total N are observed at three sites. The lowest rhizobia numbers are observed at lowest total nitrogen.

Table 3: Selected soil chemical properties of Kersa Malima District

Sample Nº	Sites	pH	Organic Carbon (%)	Total Nitrogen (%)	Cation Exchange Capacity (meq/100g)	Avail Phosphorus(mg/kg)
1	Biyna Giche	6.93	0.82	0.064	41.6	12.8
2	Mazora	6.86	1.95	0.134	45.48	13.588
3	Mazora	7.16	1.32	0.098	41.62	13.193
4	Adadi	8.33	0.7	0.067	36.92	2.995
5	Mazora	8.15	0.82	0.067	41.2	11.196
6	Kersa Warko	7.3	0.82	0.067	39.54	9.193
7	Muxi-Alibo	7.68	1.05	0.067	32.32	10.00
8	Kersa Warko	7.35	1.71	0.064	53.22	10.787
9	Biyna Giche	5.99	1.6	0.120	52.52	23.18
10	Biyna Giche	8.32	1.05	0.113	46.00	10.393
11	Muti Dayu	7.2	0.97	0.070	41.5	22.93
12	Adadi	6.86	1.05	0.078	37.78	21.554
13	Muti Dayu	7.13	0.74	0.095	40.54	9.999
14	GodetiWanbari	7.22	0.88	0.070	26.12	10.392
15	GodetiWanbari	7.07	1.52	0.063	45.58	8.395

Slopes and rhizobia population abundance

At (Kersa Malima) the highest rhizobia population abundance was observed at the flat slopes. This might be associated with soil fertility depletion and moister losing of the soil is low. At Gentle slope than soil fertility depletion and high soil erosion are significantly influence rhizobia abundance in soil, however, at gentle slopes high rhizobia population abundance were observed to some extents. Therefore, the effects of slopes on rhizobia abundance needs further study, to definitely explain about its influence on rhizobia population abundance clearly.

Table 4: Selected soil physical properties of Kersa Malima and Welmera District

	Kebele's/sites	parameters	Values	units
1	Mazora	Textural class		Clay
		Sandy	15.00	%
		Silt	28.75	%
		Clay	56.25	%
2	Biyna Giche	Textural class		Clay
		Sandy	7.50	%
		Silt	25.25	%
		Clay	67.25	%
3	Kersa Werko	Textural class		Clay
		Sandy	10.00	%
		Silt	38.75	%
		Clay	51.25	%
4	Adadi	Textural class		Clay Loam
		Sandy	12.50	%
		Silt	31.25	%
		Clay	56.25	%
5	Godeti Wenbri	Textural class		Clay Loam
		Sandy	7.50	%
		Silt	31.25	%
		Clay	61.25	%

Conclusions and Recommendation

Determination of indigenous rhizobia population is important to properly design field inoculation and identifying areas that has low and highest rhizobia abundance. The indigenous rhizobia nodulating fababean abundance is influenced by cropping history, especially when legumes are cultivated for many years. Crop rotation is best management practice for enhancing soil indigenous rhizobia population. The rhizobia population significantly increased following legumes crop cultivation at all locations. The Findings provides a basis for selecting appropriate information on the need to inoculate the soil. Eventually to get the best strain that promises for commercial bio-fertilizer production and support the end users. The number of rhizobia population at studies area ranged from 0.357*102- 9.324*103cells/ g of dry soil. The increase of number of rhizobia population was significantly influenced by soil pH, crop rotation and organic carbon. Slope effects on indigenous rhizobia population was not observed at all sites. In conclusion it is recommended that the inoculation of rhizobia strain nodulating host legumes crops should be based on the results of Most Probable Number. Effects of slope on rhizobia population and distribution need further investigation.

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Data Availability

The data used to support the results of this study are included within the manuscript and any further information is available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no competing interest.

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