International Journal of
Natural Science
(IJNS)

Rhizobium leguminosarum bv.viciae Potential Assessment Using Dosha Faba Bean Isolates from Central and North Gondar, Ethiopia

Emebet Kibkab and Nega Berhane

International Journal of Natural Sciences
ISSN: 2958-9126 (Online)
Vol.4, Issue 1, No.2. pp. 15 - 31, 2024
www.iprjb.org

Rhizobium leguminosarum bv.viciae Potential Assessment Using Dosha Faba Bean Isolates from Central and North Gondar, Ethiopia

Emebet Kibkab[1*] and Nega Berhane[2]
[1*]Department of Agricultural Biotechnology, Institute of Biotechnology, University of Gondar, Gondar, Ethiopia
[2]Institute of Biotechnology, University of Gondar, Gondar, Ethiopia

Article History

Received 19th February 2024

Received in Revised Form 20th March 2024

Accepted 25th April 2024

How to cite in APA format:

Kibkab, E. ., & Berhane, N. (2024). Rhizobium leguminosarum bv.viciae Potential Assessment using Dosha faba Bean Isolates from Central and North Gondar, Ethiopia. *International Journal of Natural Sciences*, *4*(1), 15–31. https://doi.org/10.47604/ijns.2511

Abstract

Purpose: The objective this study was to evaluate nitrogen fixation effectiveness, survival and compatibility of the isolates of on two different soils that came from Dabat and Shentia sites at Gondar, Ethiopia. The author recommends that the isolates test their nitrogen fixing potential under field conditions in slightly acidic and neutral soil before being used as commercial biofertilzer inoculants. The present study provides potential nitrogen fixer input for agricultural research centers.

Methodology: Soil physicochemical analysis and most probable number were done according to their standard procedure. Three top strains were selected as inoculants for faba beans grown on the slightly acidic Shentia soil and the slightly neutral Dabat soil with their control. The symbiotic effectiveness of the strains was evaluated based on plant agronomy and total nitrogen of the plant. The results of the strains analyzed by SPSS version 26.

Findings: The highest rhizobium population size was 5.8 $x10^2$ cells g^{-1} soil, while the lowest was 1.7 $x10^2$ cell g-1 soil observed at the Dabat and Shentia sites, respectively. There is limited nitrogen and phosphorus content other were enough. It was significant difference inter-strain difference in the all agronomic parameter of all treatments in soil compared to each based on agronomic parameter, but across soils there was no significant different except shoot fresh weight. The result of these studies showed that two *Rhizobium leguminosarum bv.viciae* isolates and other combinations of them had considerable effect on agronomic properties.

Unique Contribution to Theory, Practice and Policy: The application of biological nitrogen fixation through *Rhizobium* inoculums is promoted as a solution to the problem of poor soil fertility in areas where legumes are cultivated. In most of the developing countries, including Ethiopia, biological nitrogen fixation technology has not fully flourished, so this study was initiated to evaluate fixation potential of isolates.

Keywords: *Biological Nitrogen Fixation, Inoculums, Rhizobium, Rhizobium leguminosarum, Symbiosis*

INTRODUCTION

Faba bean is the major staple food in eastern, southern Asian, Middle Eastern, European, South American, and central Africa; it is among the most dietary proteins in humans [10]. However, faba bean production in Ethiopia, including Dembiya, Debark, and Dabat woredas, is below its production potential, but the crop is still widely grown in most regions of the country. The production of faba beans decreases from time to time due to poor soil fertility ([8].This is due to the fact that this crop is mostly grown with poor small-scale farmers who are not able to purchase expensive inorganic fertilizer and the negative effects that come by inorganic fertilizer [6].

Currently, more emphasis is placed on the use of BNF technology in agriculture systems in order to overcome the problems associated with depletion of soil fertility as well as the reduction of excessive inorganic nitrogen fertilizer [4]. BNF by *Rhizobium* is considered an inexpensive and environmentally friendly alternative to improve crop yield in comparison to its counterpart of legumes with Rhizobium inoculants. It is the most used BNF technology in agriculture, as evidenced, in previous studies, but in most African countries, including Ethiopia [2].This technology is not well established, so BNF technology may be successful if the isolation and characterization of indigenous *Rhizobium leguminosarum bv.viciae* found in the soil are done properly, as this may foster the discovery of stronger strains for inoculant production. Also, it has been emphasized that prior to the potential assessment of *Rhizobium leguminosarum bv.viciae* isolate inoculants in the pot experiment. This will help to identify the potential *Rhizobium leguminosarum bv.viciae* strain for inoculant production, aiming to enhance faba bean yield and identifying the right places where inoculation is needed.

LITERATURE REVIEW

Rhizobium: Nitrogen Fixing Bacteria

Martinus Willem Beijerinck was the first to isolate and cultivate a microorganism from the nodules of legumes in 1888(27). He named it Bacillus radicicola, which is now placed in Bergey's Manual of Determinative Bacteriology under the genus *Rhizobium*(28).*Rhizobium* forms a symbiotic relationship with certain plants, such as legumes, fixing nitrogen from the air into ammonia, which acts as a natural fertilizer for the plants(29). Current research is being conducted by Agricultural Research Service microbiologists to discover a way to use *Rhizobium's* biological nitrogen fixation(30) **.**The goal of this research is to increase the plants' productivity without using chemical fertilizers.

Rhizobia are one of several groups of bacteria capable of 'fixing' nitrogen i.e., converting dinitrogen gas into ammonia and then into organic molecules such as amino acids because of this ability, nitrogen fixing bacteria are significant conduct between an extremely large pool of nitrogen in the atmosphere and living things that otherwise could only obtain nitrogen by recycling it from existing pools of organic nitrogen (e.g., amino acids, ammonia, nitrate, and nitrite)(31) .Unlike many nitrogen fixing bacteria that can fix nitrogen when 'free-living' (i.e. when not living inside a host plant) ,*Rhizobia* can only fix nitrogen when associated with a plant that provides it with carbohydrates(32) .The carbohydrates provide energy for a process that requires substantial inputs of energy (both ATP and the reducing power of NADH)(33).

Nitrogen Fixation Process

Nitrogen is provided to agricultural lands by the application of urea and ammonium nitrate chemical fertilizers, but the utilization of inorganic fertilizer is not eco-friendly for the environment, which leads to pollution of water bodies, air, and soil (27).The harmful effects of these chemical inputs have encouraged researchers to develop sustainable agriculture practices, for example, by applying biological fertilizers (28).As a result, nitrogen is often a critical limiting element for plant growth and development in agriculture production (26).Nitrogen is a vital element in plant growth that is usually absorbed as nitrate or ammonium, taking part in major components of proteins, enzymes, and chlorophyll, the most important pigment needed for photosynthesis(24).It is also found in other important biomolecules, such as ATP and nucleic acids(30). Even though it is one of the most abundant elements (predominately in the form of nitrogen gas (N2) in the Earth's atmosphere), plants can only utilize reduced forms of this element (33) Plants acquire these forms of "combined" nitrogen through the fixation process: (i) the addition of ammonia and/or nitrate fertilizer (from the Haber-Bosch process) to soil; (ii) the release of these compounds during organic matter decomposition;(iii) the conversion of atmospheric nitrogen into the compounds by natural processes, such as lightning; and iv) biological nitrogen fixation (32).

Significance of Biological Nitrogen Fixation to Soil Fertility

Agricultural soils are replenished periodically in order to maintain an adequate level for crop production through mineral fertilizer or biological nitrogen fixation systems(20).The use of mineral nitrogen fertilizer sources has been employed for decades and has a big impact on the environment(21) .Biological nitrogen fixation is the major means of recycling nitrogen in the biosphere, is an economically justifiable and ecologically safe nitrogen source for agriculture, and plays a key role in land remediation(22) .There is severe of land degradation worldwide, and it is time to stop the destructive uses of land and to institute a serious reversal of land degradation (32). According to(22)biological nitrogen fixation is a relatively low-cost source of nitrogen for small-holder farmers in developing countries where chemical nitrogen input is neither available nor affordable(31).It is important to develop sustainable agricultural production and build up soil fertility (31).Legume-*Rhizobium* symbiotic associations are very important both ecologically and agriculturally in soil based ecosystems, supplying about 64% of the biologically fixed nitrogen in terrestrial agricultural systems (19). Among the legumes, such as common bean, faba bean, pea, chick pea, peanut, and soybean, this has tremendous potential for the contribution of nitrogen fixation to soil ecosystems.

Nitrogen Fixing Bacteria with Faba Bean

Legumes are second only to cereals as a source of human food and animal feed. Their importance as food lies primarily in their high protein content, fat content, and carbohydrates. They are also essential for good health due to their contents of bone building minerals, and vitamins (21).Legume crops such as *faba bean,* are an annual legume botanically known as *Viciae faba* L. (33) are decrease their growth time to time. The crop is known by many names, most of which refer to a particular subgroup rather than the whole species (29). It is among the oldest crops in the world, worldwide, it is the third most important feed grain legume after soybean (Glycine max L.) and

International Journal of Natural Sciences
ISSN: 2958-9126 (Online)
Vol.4, Issue 1, No.2. pp. 15 - 31, 2024
www.iprjb.org

pea (Pisum sativum L.) area and production (33).Its green pod is mainly used as vegetables, dry cotyledons are one of the excellent and cheap sources of lysine rich protein for the poor's (19). Flowers are large, white with dark purple markings, borne on short pedicels in clusters of 1-5 on each axillary raceme, usually between the 5 and 10th node; 1-4 pods develop from each flower cluster, and growth is indeterminate, though determinate mutants are available (32). About 30% of the plants in a population are cross-fertilized, and the main insect pollinators are bumblebees. There is a robust tap root with profusely branched secondary roots. It is one of the best crops that can be used as green manure and one of the best bio factors of nitrogen by fixing 130 to 160 kg N/ha (24). Faba bean cannot only be grown in diver's agro-climatic conditions successfully, but it can also be produced on residual soil moisture, making it relatively more tolerant to biotic and abiotic stress, with minimum input (33).

MATERIALS AND METHODS

Description of Study Sites

The pot experiment's soil was taken from Shinta, the Central Gondar zone, and Dabat, the North Gondar zone, Amhara regional state, Ethiopia. The soil collected sites were located at geographical coordinates of 12.5894° N latitude and Longitude 37.4442° E, at an altitude of 2133mm above sea level at Shentia site, College of Agriculture, University of Gondar, and latitude of 12° 59' 1.54"N and longitude 37° 45' 55.66"E, at an altitude ranging from about 1000 metres to over 2500 metres above sea level at Dabat site, Gondar agricultural research Centre in 2015.

Study Design and Study Period

A randomized complete design (CRD) was used to conduct this study .The study was conducted from May to July 2023 at Collage Agriculture, University of Gondar, Gondar ,Ethiopia.

Analysis of a Soil Sample

In this study, physicochemical analysis of the study site soil samples, such as pH, organic carbon, cation exchange capacity, exchangeable bases (K, Ca, and Mg), and total nitrogen from the representative bulk soil sample before planting were analyzed. Soil analysis for the aforementioned physicochemical parameters was done at the Gondar Agricultural Research Centre .Soil sample $P^{H,}$ CEC (cation exchange capacity), and EC (electrical conductivity) analysis were done according to [9].The availability of phosphorus, potassium, and total nitrogen [7]. Organic matter [12] sodium, magnesium, and calcium were analyzed based on the standard soil analysis method.

Estimation of Native *Rhizobium*, Inoculum Preparation and Pot Experiments

Estimation of Native *Rhizobium* Nodulation Faba Bean

The plant infection count, also known as most probable number (MPN) counts, were used to determine the number of viable and infective Rhizobium following the producer's stated by [13]. Ten gram of soil sample was diluted in aseptic condition in 90ml sterilized distilled water up to $_{10}{}^{-6}$ and was used to inoculate a faba bean seedling adequately grow in acidic treated and sterilized sand using plastic cup in four replication [13] and used to inoculate a faba bean seedling adequately grow in acidic treated and sterilized sand using plastic cup in four replications [13]. Nodule observation was made 30 days after inoculation, positive and negative nodulation of growth units was recorded for all dilutions and converted to number of rhizobia g-1 soil the using most probable table [1].

Preparation of Inoculums

A single pure colony was transferred into YEM broth medium and kept at 30 °C for 3 days on a rotary shaker at 120 rpm [1].One ml of inoculum add when plant has three leafe.

Pot Experiment

The symbiotic effectiveness of the new *Rhizobium leguminosarum bv.vicea* strains was evaluated based on their potential in a pot experiment. To perform this research, it needs soil. The soils found in Shinta are located at the border of the Tewodross campus, University of Gondar, and the Dabat site, Dabat woreda, North Gondar Zone, Ethiopia. Plastic pots were bought from the local market. The seed of the Dosha faba bean variety was collected from the Amhara Regional Research Institute, Gondar. Regarding treatment ,isolates with their (GR1E1), and (WK1E1) and other combinations of them were isolated from the microbial and molecular biology laboratory , institute of biotechnology , University of Gondar ,Gondar ,Ethiopia and Standard *Rhizobium* inoculants "EAL110" as a positive control obtained from Menagsha biofertilizer producing plc, Addis Ababa, Ethiopia, and urea obtained from Amhara regional Research Institute, Gondar, and others were left non- inoculated as a negative control.

Assessment of N2 -fixing potential: after strains have been screened for symbiotic effectiveness under sterile conditions, their performance can be assessed in sterile soils in the greenhouse. Soil chemical status such as pH, CEC, TN, and EC can provide useful information about soil. The highly effective isolates were selected, and their symbiotic effectiveness was further determined through a pot soil experiment in a greenhouse. The sterile soil was properly mixed, sieved, and air–dried. A 3 kg of soil was distributed in plastic pots [14]. The Dosha Faba bean variety, which was obtained from Gondar Agricultural Research Centre, was surface sterilized using 95 % ethanol for 10 min and 3 % sodium hypochlorite for 3 min and rinsed with sterile water (Somasegaren and Hoben 1994). Five ungerminated seeds were sown in each pot and later thinned down to three after germination for one week. After a week, each seedling was inoculated with 1 ml of each isolate grown for 3 days in YEM broth on a rotary shaker at 120 rpm.The experiment was set up in triplicate under a greenhouse [13].

The pots were arranged in a complete random design (CRD), with each consisting of a negative control (without nitrogen and inoculate) and a positive control (standard *Rhizobium* and urea). The nitrogen fertilizer was given at a concentration of 0.05 g /l per week until the plants were harvested. All the pots were watered every two days.

After 45 days of growth, the whole plant was carefully uprooted to determine the nodulation number, nodule colour, nodule dry weight, nodule fresh weight, shoot length, shoot fresh weight, and shoot dry weight according to [14]and the analysis of plant nitrogen by the kehjahd method. The effectiveness of the isolates was selected based on accumulating plant record agronomy data, as described in [3].

Data Management and Analysis

The data generated was subjected to statistical analysis to determine the mean variations between the treatments. The analysis of variance (ANOVA) and Tukey's HSD at $p < 0.05$ were considered significant for all analyses by using SPSS software version 26. Symbiotic effectiveness parameters such as shoot length, shoot dry weight, nodule fresh weight ,nodule dry weight, and nodule number data were analyzed by two-way analyzed of variance using SPSS software.

RESULTS AND DISCUSSIONS

Result

Soil Analysis Results

The physiochemical analysis of soil samples revealed that the two study site soils have, as shown in (Table 1).The P^H analysis indicated that the pH values of soil samples were 6.5 and 7.42 recorded from the Shinta and Dabat sites, respectively. Similarly, electrical conductivity, (EC) analysis showed that soil samples have significantly different electrical conductivity with the highest value of 0.094 obtained from the Dabat soil sample and a lower value of 0.073read from the Shinta soil sample.

Table 1: Physicochemical Analysis of Soil Sample

Soil site	Soil analysis parameter										
	Depth of soil /cm	P^H	EC	OM%	Available P/ppm	TN%	CEC	Ca++	K++	Na+	Mg+
Shinta site	10-`15	6.5	0.073	1.741	35.675	0.164	71.587	49.681	0.865	1.172	17.869
Dabat site	10-`15	7.45	0.094	2.273	16.899	0.199	56.862	32.063	0.225	1.280	22.256

Key: EC= Electrical conductivity, OM=organic matter, TN=Total Nitrogen, CEC =Cation exchange capacity

Regarding macro-elements and organic matter analysis, soil samples have different levels of organic matter and microelements. The organic matter content ranged between 2.273 and 1.741% recorded from the Dabat and Shentia site soil samples, respectively. Likewise, the concentration of macro-elements such as phosphorus, calcium, potassium, sodium, and magnesium is significantly different. Whereas the amount of total nitrogen was recorded from 0.199 Dabat and 0.164 Shentia site soil samples, as shown in Table 1.

Determining the Population of Indigenous Rhizobia in the Soil

The most likely number of rhizobia specific to faba bean was calculated by the standard method. The MPN result showed that both the highest and lowest population sizes of Indogioues *Rhizobium* were observed at the Dabat and Shentia sites, respectively. The lowest *Rhizobium* population size was 1.7 $x10^2$ cells $g\text{-}^1$ soil, while the highest was 5.8 $x10^2$ cells g-1 soil observed at the Dabat and Shinta sites, respectively. Using this method, a rhizobial population of colony-forming units per gram of soil was estimated, indicating a low number of native rhizobia able to nodulate the faba bean. Also, there were no significant differences in the population size of Indogioues *Rhizobium*

International Journal of Natural Sciences
ISSN: 2958-9126 (Online)
Vol.4, Issue 1, No.2. pp. 15 - 31, 2024

between the two altitudes of the study site. In addition, the soil analysis showed that the study area has a high deficiency in nitrogen and phosphorus.

Pot Experiment

Evaluation of the Symbiotic Effectiveness of the Strains on Different Soils

The findings of these studies showed that *Rhizobium leguminosarum bv.vicea* isolates had a considerable effect on agronomic properties such as plant height, nodulation number, nodule color, nodule dry weight, nodule fresh weight, shoot length, shoot fresh weight ,shoot dry weight ,and symbiotic effectiveness. Three top strains were selected as inoculants for faba beans grown on the slightly acidic Shinta soil and the slightly neutral Dabat soil. The symbiotic effectiveness of the strains was evaluated based on nodule color, number of nodules, nodule fresh weight, nodule dry weight, shoot dry mass, and total nitrogen of the plant. The results of the strains showed that they vary significantly in shoot dry mass productions ($p < 0.05$), as shown in Table 2. The results show Table 2, All strains accumulated 83-89 mg/Pl dry matter on Dabat soil and 80-85 mg/Pl on Shentia soil. There was a significant inter-strain difference in all agronomic parameters of all treatments in soil compared to each on the agronomic parameter obtained and also with their standard *Rhizobium* and negative control, but not across soils except shoot fresh weight.

The nodule number of Dabat soil records ranged from 190, and 233 for isolates for WK1E1, and co-inoculant, respectively .163 for isolate WK1E1 to 193 for isolates co-inoculate for Shinta soil. Similarly, nodules dry mass recorded between 15.3 mg/Pl for isolate WK1E1, and 17.8 mg/Pl for isolates co-inoculate in Dabat soil.13.0mg/p for isolate GR1E1 and 14.1 for isolate co-inoculant for Shinta soil. The maximum mean shoot dry mass (90mg/p) was scored by the positive nitrogen control and the minimum (304mg/p) by the negative nitrogen control for Dabat. The maximum mean shoot dry mass (87mg/p) was scored by the positive nitrogen control and the minimum (30mg/p) by the negative nitrogen control for Shinta soil. For Dabat soil, the relative effectiveness expressed as a percentage of shoot dry mass of inoculants over TN control showed that 92.2%, 94%, and 98.8% and Shentia soil the relative effectiveness expressed as percentage of shoot dry mass of inoculants over TN control, showed that 91.9%, 95.4%, and 97.7% of the isolates were found of the isolates were found to be highly effective (80-100%), effective (50-80%), lowly effective (35-50%) and ineffective (< 35%)(Table 2).The highest scores of 80-100% effectiveness of symbiotic nitrogen fixation were displayed by all isolate by WK1E1, GR1E1 and co-inoculant with shoot dry mass.Shoot dry mass >83mg/pl for Dabat soil and >80mg/Pl for Shinta soil (Table2).To be highly effective (80-100%), effective (50-80%), lowly effective(35-50%) and ineffective (< 35%)(Table 1). The highest scores of 80-100% effectiveness of symbiotic nitrogen fixation. Plant total nitrogen ranges from 3.0 for isolate GR1E1 to 3.2 for isolate co-inoculant, and the mean plant total nitrogen of isolates was 3.0 for Dabat soil (Table 1). Plant total nitrogen ranges from 3.1 for isolate WK1E1 to 3.4 for isolate co-inoculant, and the mean plant total nitrogen of isolates was 3.2.

Table 2: Comparative Effectiveness of Selected Strains of *Rhizobium Leguminosarum Bv. Viciae* from North and South Gondar on the Growth and Nitrogen Fixation of Dosha Faba Bean on Soil Culture

	Dabat								Shinta							
Treatment	**NN**	**NFW/mg**	**NDW/mg**	**SL/cm**	**SFW/mg**	**SDW/mg**	**SE (%)**	**TN**	**NN**	**NFW/mg**	**NDW/mg**	**SL/cm**	**SFW/mg**	**SDW/mg**	**SE (%)**	**TN**
WK1E1	190 ±3.6^{ab}	249±3.5^{a}	15.3±1.0	49.0±2.6^{abcd}	270± 5.6^{abc}	83.0±4.4^{abc}	92.2	3.1	163±1.0^{a}	235±2.6^{a}	13.0±1.0^{a}	48.0± 3.0^{abc}	225± 5.9^{ab}	80± 3.6^{abcd}	91.9	3.1
GR1E1	200± 10^{a}	267±3.0	16.2±3.0	50±2.0^{aefg}	278±3.0^{ad}	85.0±2.0^{ad}	94	3.0	170±2.0	248±2.6	13.6±2.0^{b}	50.3± 2.0^{adef}	227± 2.0^{ac}	83± 2.0^{aefg}	95.4	3.3
Co-inoculate	233 ±_2.0	285±2.6	17.8±1.5	52.5±3.9^{behi}	282± 3.0^{bde}	89± 2.0^{be}	98.8	3.2	193±2.0	283±2.6	14.1±3.6^{b}	54.3± 1.5^{dg}	238± 2.6^{d}	85± 1.0^{behi}	97.7	3.4
-VE	0.0 ± 0.0^{d}	0.0±0.0^{b}	0.0±0.0^{a}	32±1.0	175± 1.0	30.4±0.6	33.3	2.9	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{c}	29.9± 0.7	165±2.5	30± 1.0	34.4	3.0
SR	183 ± 2.0^{b}	242±2.0^{a}	14.4±1.7	47±1.0^{cfhj}	262± 6.3^{c}	82.0± 1.0^{cd}	91.1	3.0	162±3.6^{a}	229±4.6^{a}	12.8±3.0^{a}	48.8± 2.6^{beh}	220±5.6^{bc}	79± 1.5^{cfhj}	90	3.1
UREA	0. 0 ± 0.0^{d}	0.0±0.0^{b}	0.0±0.0^{a}	52.0±2.6^{dgij}	291± 5.3^{e}	90.0± 3.0^{e}	100	3.1	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{c}	49.9± 0.05^{cfgh}	241± 2.6^{d}	87± 5.6^{dgij}	100	3.1

NB: values are mean ± SD of 3 three replicates. Mean followed by the same Small letter with column denotes the mean difference is not significant by (Tukey's HSD test) test with $p < 0.05$ alpha level

Figure 1: Showing the Shoot Height of the Different Treatment Inoculant with Two Positive (Urea and Standard Rhizobium) and Negative Control (Without Any Treatment) on Shinta and Dabat Soil Culture

Correlation

The correlation of the result the present study showed that *Rhizobium leguminosarum bv.viciae* inoculation significantly $p < 0.05$ increased all investigated parameters, such as nodulation number, nodule dry weight, nodule fresh weight, shoot length, shoot fresh weight , and the shoot dry weight. This result could be an indication of an effective symbiotic association between the most tested *Rhizobium leguminosarum bv.vicea* with faba bean. Nodulation showed a positive correlation with all measured parameters.

Number of nodule was strong positive correlated with the number of nodules fresh weight (r=0.997,p<0.05),and with nodule dry weight (r=0.998,p<0.05), and positive correlated with shoot fresh weight (r =0.500, p<0.05),shoot dry weight(r=0.563,p<0.05) and shoot length(r=0.522,P<0.05)for Dabat soil (Table3), and Shinta soil of Number of nodule was strong positively correlated with number of nodule fresh weight (r=0.999,p<0.05), and Nodules dry weight (r=0.997,p<0.05) and positive shoot fresh weight (r =0.473, p<0.05), with shoot dry weight (r=0.556,p<0.05) and shoot length (r=0.642,P<0.05 and with plant total nitrogen(r =0.683, p<0.05)(Table 4).

Nodules fresh weight strongly positively correlated with nodule dry weight (r=0.999, p<0.05), and positive correlated with shoot fresh weight (r=0.496, p<0.05), shoot dry weight (r=0.561, p<0.05) and shoot length (r=0.516, p<0.05) for Dabat soil. Nodule fresh weight of Shinta soil strong positive correlated with nodule dry weight (r=0.996, p<0.05), and positive correlated with shoot fresh weight (r=0.475, p<0.05), shoot dry weight (r=0.556, p<0.05), shoot length (r=0.644, p<0.05) and plant total nitrogen (r=0.697, p<0.05).

Nodule dry weight positive correlated with shoot dry weight (r=0.560, P<0.05), shoot fresh weight(r=0.496, P<0.05), Positive correlated with shoot length (r=0.522, P<0.05) for Dabat soil. Nodule dry weight of Shinta soil positive correlated with shoot dry weight (r=0.555, P<0.05), shoot fresh weight (r=0.463, P<0.05), Positive correlated with shoot length (r=0.631, P<0.05) and plant total nitrogen ((r=0.649, p<0.05).

International Journal of Natural Sciences
ISSN: 2958-9126 (Online)
Vol.4, Issue 1, No.2. pp. 15 - 31, 2024
www.iprjb.org

Dabat soil of Shoot length strongly positively correlated with shoot dry weight (r=0.965, p<0.05), and with shoot fresh weight (r =0.949, p≤0.05), with (r=0.785, p<0.05) positively correlated with plant total nitrogen and Shinta soil Shoot length strong positive correlated with shoot dry weight (r=0.960, p<0.05), shoot fresh weight (r =0.950, p≤0.05) and plant total nitrogen (r=0.702, p<0.05).

Dabat soil strongly positively correlated shoot fresh weight (r=0.986, p≤0.05) with shoot dry weight, plant total nitrogen (r=0.761, p<0.05) and Shinta soil shoot fresh weight (r=0.975, p≤0.05) with shoot dry weight, and plant total nitrogen(r=0.594, p<0.05).

Shoot dry weight of positively correlated Dabat soil positive correlated with (r=0.574, p<0.05) plant total nitrogen, and shoot dry weight positive correlated with (r=0.574, p<0.05) plant total nitrogen for Shinta soil.

Table 3: Correlation Coefficients among Investigated Parameter in Dosha Faba Bean Variety on Dabat Soil

Correlations							
	NN	**NFW**	**NDW**	**SL**	**SFW**	**SDW**	**TN**
NN	1	.997**	.998**	.522*	.500*	.563*	.457
NFW	.997**	1	.999**	.516*	.496*	.560*	.419
NDW	.998**	.999**	1	.522*	.498*	.561*	.439
SL	.522*	.516*	.522*	1	.949**	.965**	.785**
SFW	.500*	.496*	.498*	.949**	1	.986**	.761**
SDW	.563*	.560*	.561*	.965**	.986**	1	.755**
TN	.457	.419	.439	.785**	.761**	.755**	1
**. Correlation is significant at the 0.01 level.							
*. Correlation is significant at the 0.05 level.							

Table 4: Correlation Coefficients among Investigated Parameters in Dosha Faba Bean Variety on Shinta Soil

Correlations							
	NN	**NFW**	**NDW**	**SL**	**SFW**	**SDW**	**TN**
NN	1	0.998**	.997**	.642**	.473*	.556*	.683**
NFW	0.998**	1	.996**	.644**	.475*	.556*	.697**
NDW	.997**	.996**	1	.631**	.463	.555*	.649**
SL	.642**	.644**	.631**	1	.950**	.960**	.702**
SFW	.473*	.475*	.463	.950**	1	.975**	.594**
SDW	.556*	.556*	.555*	.960**	.975**	1	.574*
TN	.683**	.697**	.649**	.702**	.594**	.574*	1

**. Correlation is significant at the 0.01 level
*. Correlation is significant at the 0.05 level

Discussion

As indicated in Table 2 previously, similar soil parameters were exhibited except for p/ppm and p^H.Sheinta soil has two fold higher availability of p/ppm than Dabat soil. Slightly acidic soil was noted with Shinta soil and slightly neutral for Dabat soil. The most likely number of rhizobia specific to faba bean was calculated using the following method Somasegaran and Hoban 1994.Naturalized soil *Rhizobium* population size is one important factor in determining a response to inoculating with a *Rhizobium* strain [14]. Both Dabat and Shinta soils contained a compatible native rhizobia of faba bean, making them suitable for testing the competitive abilities of selected rhizobia isolates against native pot soil rhizobia. The Shinta soils with a higher number was estimated of rhizobia nodulating faba bean g-1 of soil (5.8×10^2) .Relatively, lower number of *Rhizobium* was estimated in Dabat soil, which was 1.7×10^2. On the other hand, all parameters were significantly improved by the inoculation of *Rhizobium* in Dabat soil at $p \leq 0.05$(Table1).However, both of their soils insignificant native rhizobia, according to [13].

The combined ANOVA analysis showed significant differences ($p \leq 0.05$) among soils for all morphological parameters (Table 2).The effectiveness of the isolate was evaluated on the basis of nodule formation as a result of symbiosis, nodule number, nodule dry weight ,nodule fresh weight ,shoot fresh weight ,shoot dry weight , shoot length , and symbiotic effectiveness. All isolates were form significant number of nodules and exerted a significant effect on plant growth and dry mass.The result of the analyses of variances showed that *Rhizobium leguminosarum bv.vicea* inoculation significantly ($p \leq 0.05$) affected two soils.

Three *Rhizobium leguminosarum bv .viciae* isolates were assessed for their symbiotic effectiveness using sterile soils in a pot experiment under greenhouse conditions (Table 2). [11] Indicated that the ability to form nodules along with the subsequent capacity to fix nitrogen is widely used as a means of evaluating the inherit link between *Rhizobium*, the respective soil, and the host. *Rhizobium leguminosarum bv.viciae* inoculation significantly increased ($p \leq 0.05$) shoot length, number of nodules per plant, nodules dry mass, shoot dry mass, and symbiotic effectiveness as compared to the control treatment (Table 2).Likewise, the managed parameter displayed significant variation among rhizobium isolates treated with Dosha of faba bean plants ($p \leq 0.05$).This finding is similar to [19] showing symbiotic effectiveness of faba bean nodulating on soils culture.

Symbiotic effectiveness (capacity to fix nitrogen), are commonly used to assess the nitrogen fixing potential between *Rhizobium leguminosarum bv.viciae* and their host [1]. Thus, all of the *Rhizobium leguminosarum bv.viciae* isolates assessed in this study showed that the capacity of nitrogen fixation potential in Dabat and Shinta soils with on the host plant, most of its physiological characteristics were found to be similar to the other isolates. Therefore, it is possible to conclude that the isolate may be *Rhizobium* that fix atmospric nitrogen to the plant (13).

The result shown in table 2 suggest that bacterial inoculation's significantly $p < 0.05$ influenced all the parameters investigated compare with control (negative control, standard rhizobium, and urea).Likewise that infective strains of the faba bean rhizobia showed significant difference in shoot dry weight. Several reports showed that shoot dry mass is a good indicator of relative strain effectiveness, and there is a good correlation between shoot dry matter production and nitrogen fixation capacity of legumes [13].

Shoot dry weight is often considered to indirectly measure symbiotic effectiveness when N is the only limiting factor for the growth of the plants (14). Based on the percentage differences of shoot dry weight of inoculated plants as a measure of effectiveness For Dabat soil the symbiotic effectiveness nitrogen fixation scores of 92.2%, 94% and 98% were displayed by WK1E1, GR1E1 and co-inoculate respectively and Shentia soil also 91.1, 95.4 and 97.7 to the above isolate, respectively. The isolates were found to be high effective nitrogen fixers almost performed as excellent as the positive control on the soils culture that were isolated from weken, Guranbia and other combination of this two isolates ,respectively. Generally the result of this study suggests that, the highly performed strains are worthy of further soil on pot in order to select effective and competent strains. This result of this finding is similar with [2].These isolates also showed difference in plant height with Dosha faba bean with each soil this finding similar with [8], 2022.These findings gave hopeful indicator towards production of inoculants for faba bean native to Ethiopia since most of the isolated strains showed ability to induce nodulation on the roots of faba bean. Somasegaran and Hoben 1994 suggested that nodules with a pink color indicates an effective nodules, whereas white and greenish nodules infer symbiosis. This nodule coloration is evidence of the presence of effective N2-fixation an indication of leghemoglobin (4) .Both uninoculated treatments did not form nodules.

The results shown in Table 2 suggest that bacterial inoculation significantly ($p<0.05$) influenced all the parameters investigated compared with control (no inoculation, chemical nitrogen, and standard *Rhizobium*).Likewise, the measured parameter displayed a significantly variable value among *Rhizobium leguminosarum bv.viciae* treated plants at $p<0.05$.The mean nodules number of plants recorded ranges from 200 for isolate WK1E1 to 233 for isolate co-inoculate with Dabat soil. 163 for isolate WK1E1 and 193 for isolates co-inoculate with Shinta. This result is much higher than the previous report of [22]on faba beans from eastern Ethiopia. The mean total NN per plant recorded in this study (was 134 for Dabat and 114.6 for Shinta).

The experiment recorded the mean nodule dry weight between 15.3mg per plant for *Rhizobium leguminosarum bv.viciae* isolate WK1E1, and 17.8 mg per plant for isolates co-inoculate with Dabat soil. The corresponding, ranges were 13.0mg/pl for GR1E1 and 14.1mg for isolates co-inoculate with Shinta soil. Which was greater than that pervious report in Gondar [20].The mean nodule dry weight recorded in this study was 67.5 mg per plant in soil. Therefore, we observed a much higher than 7.8mg per plant, which was reported by [21], but a lower than 145 mg per plant, reported by [15].

It was also evident that, as compared to the control plants, inoculation induced a significant improvement in the mean plant of shoot height. The height 52.5cm was recorded for the isolate co-inoculate inoculated with Dabat soil and 54.3cm recorded for co-inoculate inoculated with Shinta. The negative control plant has a 32 cm Dabat soil shoot height and a 52cm positive control (N treated plant Dabat soil) .The result of this study is comparable to the results of different studies [20].29.9cm shoot length and 52.3cm positive control (N treated plant Shinta soil). This enhancement of shoot height could be attributed to the fact that *Rhizobium leguminosarum bv.viciae* many agreement plant growth by providing products of N2 fixation .Based on shoot dry weight accumulation in reference to N-fixing and control plants. All inoculated plants were symbiotically effective with Shentia and Dabat soil according to [13].Three isolates were high symbiotic effective in soils. Other two isolates symbiotically effective to soils. This result underline the important for local screening of *Rhizobium leguminosarum bv.viciae* isolates which improve N2 fixation in faba bean. Generally the results of this study indicate that potential of local *Rhizobiu*m isolates. This result is higher in plant height [16].report noted 49.7 maximum height of plant.

Chemical nitrogen fertilizer application did not show any significant effect on nodulation .This result with pervious report of [25].All selected isolate produced higher nodule number and dry weight in soil culture, similar finding was reported previously by (21).

Isolate GR1E1 for 50.3 and co-inoculant 54.3cm produce higher shoot height than nitrogen treated plant 49.9cm on Shinta soil, while co-inoculant isolate 50.3cm over nitrogen treated plant 50cm on Dabat soil. There was a similar report of inoculation of faba bean on soil findings to [22]. Generally, there was a significant difference inter-strain difference in the all agronomic parameters of all treatments for with in soil compared to agronomic parameter obtained from their standard *Rhizobium* and negative control, but across there was a soils, significant different in shoot fresh weight. The parameter of faba bean was better in Shinta soil than Dabat soil for shoot fresh weight. These differences may be due to the natural harbour of higher background of soil fertility [20].

The result of this study is an indication of an effective symbiotic association between the most tested *Rhizobium* and soils (Pimratch *et al*. 2004).Nodulation showed a positively correlated with all measured parameters (Table1).Nodule number Dabat soil strongly positively correlated ($r=0.998$, $p<0.05$) nodule dry weight, and nodule dry weight Shinta soil positive correlated ($r=0.997$, $p<0.05$) with as reported by [20].This result could be an indication of effective symbiotic association between most tested *Rhizobium* with faba bean [18].Nodulation Showed positive correlation with all measured parameter. Indicating the determinate factor of nodulation on nitrogen fixation and efficient symbiosis in faba bean on nodulation was also positively correlated with symbiosis effectiveness as was reported by [19].This is in agreement with previous work on the *Rhizobium* of the faba bean [25].Shoot dry matter is used as an accurate measure of nitrogen fixation in symbiotic nitrogen fixation experiments [20].Similar finding were reported by [26].

Shoot dry weight is positively correlated with ($r=0.561$, $p<0.05$) nodule dry weight for Dabat soil, and shoot dry weight is positively correlated with ($r=0.555$, $p<0.05$) nodule dry weight for Shinta soil. Shoot dry matter is used as an accurate measure of nitrogen fixation in symbiotic nitrogen fixation expermints [14]. Similar finding were reported by [13] which implies their symbiotic effectiveness. Likely many research findings reveal that nodulation status is positively correlated with symbiotic effectiveness and plant dry weight [21].According to [13], reported that the first parameter for a *Rhizobium leguminosarum bv.vicea* used as an inoculants or biofertilizer must be superior and highly effective in symbiotic formation with the host plant, which in turn positively influences nitrogen fixation. The best three isolates showed effectiveness as compared with positive and negative controls in Dosha variety on Dabat and Shinta soil. Therefore, it is to conclude as three best performing *Rhizobium* isolates could be used as bio-fertilizer in host on Dabat and Shinta soil.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The results of this study indicated that isolates scored the highest percentage in the effectiveness of symbiotic nitrogen fixation, which may qualify them as respective candidates for inoculants. The result of these studies showed that two *Rhizobium leguminosarum bv.vicea* isolates and other combinations of them had considerable effect on agronomic properties such as nodulation number, nodule colour, nodule dry weight, nodule fresh weight, shoot length, shoot fresh weight, shoot dry weight, and symbiotic effectiveness .Moreover, their symbiosis efficacy was evaluated using slightly acidic soil of Shentia soil and slightly neutral Dabat soil. This research indicates the potential of these isolates to be effective on two different soils, which could give us an indication for potential nitrogen fixing.

Recommendations

The author recommends that the isolates test their nitrogen fixing potential under field conditions in slightly acidic and neutral soil before being used as commercial biofertilzer inoculants.

Acknowledgement

The authors gratefully acknowledge University of Gondar, Institute of Biotechnology for laboratory facility…

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors…

REFERENCES

1. Brockwell, J. and Bottomley, P., 1995. Recent advances in inoculant technology and prospects for the future. *Soil Biology and Biochemistry*, *27*(4-5), pp.683-697.
2. Chaudhary, P., Singh, S., Chaudhary, A., Sharma, A. and Kumar, G., 2022. Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Frontiers in Plant Science*, *13*, p.930340.
3. Chen, L., Figueredo, A., Villani, H., Michajluk, J. and Hungria, M., 2002. Diversity and symbiotic effectiveness of rhizobia isolated from field-grown soybean nodules in Paraguay. *Biology and Fertility of Soils*, *35*, pp.448-457.
4. Ladha, J.K., Peoples, M.B., Reddy, P.M., Biswas, J.C., Bennett, A., Jat, M.L. and Krupnik, T.J., 2022. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Field Crops Research*, *283*, p.108541.
5. Mínguez, M.I. and Rubiales, D., 2021. Faba bean. In *Crop physiology case histories for major crops* (pp. 452-481). Academic Press.
6. Misra, V. and Mall, A.K., 2023. Biofertilizer: Boon for Sustainable Sugarcane Production. In *Climate-Resilient Agriculture, Vol 1: Crop Responses and Agroecological Perspectives* (pp. 571-585). Cham: Springer International Publishing.
7. Olsen, S. R., Cole, C. V., Watannable, F. S. and Dean, L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular of the United States Department of Agriculture, Washington D. C., United State, 939
8. Rathnathilaka, T., Premarathna, M., Madawala, S., Pathirana, A., Karunaratne, K. and Seneviratne, G., 2023. Biofilm biofertilizer application rapidly increases soil quality and grain yield in large scale conventional rice cultivation: A case study. *Journal of Plant Nutrition*, *46*(7), pp.1220-1230.
9. Richards LA. ,1954 Diagnosis and Improvement of Saline and Alkali Soil USA Handbook No.60, Oxford and IBH Publishing Co. Calcutta; c1954. p. 160.
10. Semba, R.D., Ramsing, R., Rahman, N., Kraemer, K. and Bloem, M.W., 2021. Legumes as a sustainable source of protein in human diets. *Global Food Security*, *28*, p.100520.
11. Sessitsch, A., Howieson, J.G., Perret, X., Antoun, H. and Martínez-Romero, E., 2002. Advances in Rhizobium research. *Critical Reviews in Plant Sciences*, **21**(*4*), pp.323-378.
12. Smith, H.W. and Weldon, M.D., 1941. A comparison of some methods for the determination of soil organic matter. *Soil Science Society of America Journal*, *5*(C), pp.177-182.
13. Somasegaran, P., Hoben, H.J., Somasegaran, P. and Hoben, H.J., 1994. Quantifying the growth of rhizobia. *Handbook for Rhizobia: Methods in Legume-Rhizobium Technology*, pp.47-57.
14. Vincent JM1970Amanual for the practical study of root nodule bacteria.IBP handbook 15 blackwell scientific publications,oxfaord.

15. Amanuel, G., Kühne, R.F., Tanner, D.G. and Vlek, P.L.G., 2000. Biological nitrogen fixation in faba bean (Vicia faba L.) in the Ethiopian highlands as affected by P fertilization and inoculation. *Biology and fertility of soils*, *32*, pp.353-359.

16. Anteneh A (2012a). Characterization of Symbiotic Effectiveness of Rhizobia Nodulating Faba bean (Vicia faba L.) Isolated from Central Ethiopia. Research Journal of Microbiology 7(6):280-296.

17. Anteneh A (2012b). Evaluation of symbiotic effectiveness and size of resident R. leguminosarum bv. viciae nodulating lentil (Lens culinaris medic) in some Ethiopian soils. Archives of Agronomy and Soil Science 2012:1-17

18. Ayneabeba Adamu, Fassil Assefa, Asfaw Hailemariam, Endashaw Bekele. 2001. Studies of Rhizobium inoculation and fertilizer treatment on growth and production of faba bean (Viciae faba) in some yield depleted and yield sustained regions of Semien Showa. Ethiopia J Sci. 24:197–211.

19. Dereje T, Fasil A, Heluf G, Gemechu K (2015). Nutritional, ecophysiological and symbiotic characteristics of rhizobia nodulating faba bean (Vicia faba L.) collected from acidic soils of Ethiopia. African Journal of Environmental Science and Technology 9(7):646-654.

20. Fening, J.O. and Danso, S.K.A., 2002. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. *Applied Soil Ecology*, *21*(1), pp.23-29.

21. Girmaye K, Mulissa J, Fasil A (2014). Charcteriazation of phosphate solublizing faba bean (Vicia faba L.) nodulating rhizobia isolated from acidic soils of Wollega. Journal of Science Technology and Extensive Journal of Applied Sciences 1-10 ISSN 2409-9511.

22. Hoque MM, Haq MF. 1994. Rhizobial inoculation and fertilization of faba bean in Bangladesh. Lens Newslett. 21:29–30.

23. Johnston, A.W.B. and Beringer, J.E., 1976. Mixed inoculations with effective and ineffective strains of Rhizobium leguminosarum. *Journal of Applied Microbiology*, *40*(3), pp.375-380.

24. Jordan DC (1984). Family III. Rhizobiaceae. In: Bergey's Manual of Systematic Bacteriology, (Krieg NR, Holt JG eds). The Williams and Wilkins, Baltimore 1:234-254.

25. Minalku A, Gebrekidan H, Assefa F. 2009. Symbiotic effectiveness and characterization of Rhizobium strains of Faba bean (Viciae faba L.) Collected from eastern and Western Harareghe Highlands of Ethiopia. EJAS. **11**(*2*):223–244.

26. Zerihun B, Fassil A (2011). Symbiotic and phenotypic diversity of R. leguminosarum bv. viciae from Northern Gondar, Ethiopia. African Journal of Biotechnology 10(21):4372-4379.

27. Hurst, C.J., 2021. Discovering the Symbiotic Nature of Microbial Life: Summarizing Milestone Publications from 1866 Through 1947. *Microbes: The Foundation Stone of the Biosphere*, pp.213-241.

28. Volpiano, C.G., 2021. Caracterização genômica (taxonomia e simbiose) e fenotípica (controle biológico de fitopatógenos) de bactérias isoladas de feijoeiro da Coleção SEMIA: revisão taxonômica da Ordem Rhizobiales (Hyphomicrobiales).

29. Kumar, N., Srivastava, P., Vishwakarma, K., Kumar, R., Kuppala, H., Maheshwari, S.K. and Vats, S., 2020. The rhizobium–plant symbiosis: state of the art. *Plant microbe symbiosis*, pp.1-20.

30. Soumare, A., Diedhiou, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S. and Kouisni, L., 2020. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. *Plants*, *9*(8), p.1011.

31. Philip, P.S., Karthika, K.S. and Rajimol, R.P., 2021. Functional Nitrogen in Rhizosphere. In *Soil Nitrogen Ecology* (pp. 113-138). Cham: Springer International Publishing.

32. Raza, A., Zahra, N., Hafeez, M.B., Ahmad, M., Iqbal, S., Shaukat, K. and Ahmad, G., 2020. Nitrogen fixation of legumes: Biology and Physiology. *The Plant Family Fabaceae: Biology and Physiological Responses to Environmental Stresses*, pp.43-74.

33. Kopp, D. and Sunna, A., 2020. Alternative carbohydrate pathways–enzymes, functions and engineering. *Critical reviews in biotechnology*, *40*(7), pp.895-912.