OPEN ACCESS Freely available online

Journal of Plant Pathology & Microbiology

Research Article

Ecological Competence, Plant Growth Promoting and Symbiotic Characteristics of Different *Mesorhizobium* Strains Nodulating Chickpea (*Cicer arietinum* L.) from Ethiopia

Zehara Mohammed Damtew[1*], Douglas R Cook[3], Alex Greenlon[4], Asnake Fikre[2], Eric J Wettbergg[6], Edward Marques[5], Kassahun Tesfaye[1], Noelia Carrasquilla Garcia[3], Fassil Assefa[1]

[1]College of Natural Sciences, Addis Ababa University, Addis Ababa, Ethiopia; [2]Debre Zeit Agricultural Research Center, Ethiopian Institute for Agricultural Research, Ethiopia; [3]Department of Plant Pathology, University of California, United States; [4]Environmental Science, Policy and Management, University of California, USA; [5]Department of Biological Sciences, Florida International University, Florida, USA; [6]Department of Plant and Soil Science, University of Vermont, Vermont, USA

ABSTRACT

Chickpea provide multiple benefits, due to high nutritive value as well as the ability of the crop to enrich nitrogen poor soils. In spite of its yield potential this legume depends on the rhizobial association. The objective of this study was to identify indigenous promising elite *Mesorhizobium* strains that impart variation eco-physiologically and symbiotically for enhancing nitrogen fixation in chickpea. 20 symbiont strains represented 64 genetically diverse indigenous *Mesorhizobium* species were tested at laboratory and greenhouse. Based on their eco-physiological competence (35%) of the strains grown at 1.5% NaCl, 25% of strain at pH4 and fewer strains (20%) tolerant to 40°C. Most strains (60%) able to utilize D-Sorbitol and D-Glucose carbon substrates and better utilized the amino acids Phenylanine (60%). Most of the *Mesorhizobium* strains exhibited (67%) resistance to antibiotics and up to (83%) heavy metal resistance. Three strains able to release available soluble phosphates from $Ca_3(PO_4)_2$ (118.0 µg/ml) and $FePO_4$ (93.3 µg/ml) after 8 days of incubation and all strains produced the phytohormone ranging from (7.7-28.4 µg/ml). The results highlighted more nodules were recorded from the Natoli variety (32-62 nodules) compared to fewer nodules (31-46) formed on Arerti variety. Moreover, 85% of the strains showed highly effective symbiosis on both Natoli and Arerti varieties. The data provided an important complement to select representative distinct symbiont strains to tested in multi-location field trials for enhance nitrogen fixation activities in chickpea production.

Keywords: Chickpea; *Mesorhizobium*; Eco-physiological; effectiveness; Phosphates

INTRODUCTION

Nitrogen and phosphorus are most commonly limiting nutrient in agricultural crop production in in sub-Saharan Africa including Ethiopia. Chickpea (*Cicer arietinum* L.) like most legume plants access reduced nitrogen from the soil, through their roots, in the forms of nitrate and ammonium [1]. Chickpea is one of the popular pulse crops used for crop rotation for it fixes nitrogen in association with root nodule bacteria from the genus *Mesorhizobium* [2]. Chickpea requires about 13 to 41 kg /ha inputs of nitrogen for growth and development from which it derives 70% of its N through symbiotic N_2 fixation [3,4]. Apart from biological nitrogen fixation, root nodule bacteria are one of the important plant growths promoting rhizobacteria (PGPR) such as Pseudomonas and Bacillus that enhance plant health, productivity and provide addition of fertilizers [5,6]. Indirect act as biocontrol against phytopathogens through various forms of antagonism like competition, production of antibiotics, lytic enzymes and hydrogen cyanide [7]. Thus, it is essential to understand existence of native rhizobia in the soils, genetic variation in bacterial strains and symbiotic response of the cultivar enables to distinguish their

***Correspondence to:** Zehara Mohammed Damtew, Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia, Tel: +251911047518; E-mail: zeharamicro2015@gmail.com

Received date: June 08, 2020; **Accepted date:** August 05, 2020; **Published date:** August 12, 2020

Citation: Damtew ZM, Cook DR, Greenlon A, Fikre A, Wettbergg EJ, Marques E , et al. (2020) Ecological Competence, Plant Growth Promoting and Symbiotic Characteristics of Different *Mesorhizobium* Strains Nodulating Chickpea (*Cicer arietinum* L.) from Ethiopia. J Plant Pathol Microbiol. 11: 508. doi: 10.35248/ 2157-7471.20.11.508

symbiotic efficiency [8]. In Ethiopia [9] isolated 39 root nodule bacteria from chickpea growing areas of the eastern, southeastern and southern parts of the country, of which 23 isolates (59%) were identified as chickpea root nodule bacteria with the same identification methods. However, effective *Mesorhizobium* strains compatible to multiple varieties of the crop inoculation have not been intensively characterized from representative regions of Ethiopia and we lack an understanding of diverse *Mesorhizobium* strain in relation to their eco-physiological competence. Therefore, the purpose of this study is identifying competitive *Mesorhizobium* strains which displayed large spectrum persistence adaptive mechanism with different types of chickpea varieties to addressed developing inoculum towards promising impact on chickpea production.

MATERIALS AND METHODS

Source of strains and growth conditions

Twenty strains were selected from the phylogeny of whole genome sequenced 64 strains belonging to six *Mesorhizobium* spp. which were isolated from root nodules collected from major chickpea growing regions of Ethiopia and compiled with global level population genomics [10]. The strains have been deposited in culture collections at Plant Pathology Laboratory of Davis, University of California, USA and Addis Ababa University. NCIB assembly accession links presented in (Dataset S1) accompanying studies by Greenlon et al. [10] (Table 1).

Table 1: List of *Mesorhizobium* strains and origin of culture collection for strains used in Phenotypic and symbiotic characterization.

No.	Sample strains	Latitude	Longitude	Elevation	Bio sample
1	*M. genospecies* 7A (27P3S2)	10° 24' 41.7"N	38° 10' 8.4"E	2429	SAMN09232638
2	*M. genospecies* 9A (2P3S1-b)	8° 49' 31.7"N	38° 59' 25.4"E	1944	SAMN09232642
3	*M. genospecies* 3A (80P4S2)	12° 20' 56.9"N	38° 3' 35.4"E	1906	SAMN09232671
4	*M. genospecies* 3A (10P4S2)	8° 53' 46.3"N	39° 23' 56.5"E	1815	SAMN09232619
5	*M. genospecies* 4B (19P3S1)	8° 39' 20.4"N	38° 28' 57.5"E	2192	SAMN09232629
6	*M. genospecies* 4B (ET20)	12° 15' 16.9"N	37° 15' 51.5"E	1849	SAMN09232935
7	*M. genospecies* 1B (45P4S1)	12° 26' 43.8"N	37° 20' 48.3"E	1934	SAMN09232659
8	*M. genospecies* 2A (46P3S2)	12° 21' 18.9"N	37° 15' 31.4"E	1873	SAMN09232661
9	*M. genospecies* 2A (29P5S1)	10° 42' 47.3"N	38° 10' 30.6"E	2541	SAMN09232641
10	*M. genospecies* 2A (43P2S1)	12° 27' 43.6"N	37° 21' 29.7"E	1960	SAMN09232656
11	*M. genospecies* 8A (ET1)	11° 27' 58.3"N	38° 12' 46.6"E	2795	SAMN09232926
12	*M. genospecies* 8A (ET4)	9° 53' 46.8"N	38° 21' 29.6"E	2567	SAMN09232940
13	*M. genospecies* 8A (23P2S2)	9° 59' 51.2"N	38° 14' 42.9"E	2122	SAMN09232634
14	*M. genospecies* 4A (ET26)	12° 27' 34.8"N	7° 48' 23.0"E	1841	SAMN09232937
15	*M. genospecies* 4A (90P4S2)	8° 36' 3.4"N	38° 16' 0.8"E	2209	SAMN09232678
16	*M. genospecies* 4A (22P5S2)	10° 24' 56.2"N	38° 10' 35.9"E	2429	SAMN09232633
17	*M. genospecies* 1D (36P3S1)	11° 13' 22.3"N	37° 35' 42.7"E	2261	SAMN09232644
18	*M. genospecies* 1A (ET24)	12° 27' 48.3"N	37° 49' 53.2"E	1754	SAMN09232936
19	*M. genospecies* 1E (38P4S2)	12° 1' 54.3"N	37° 43' 49.3"E	1809	SAMN09232650
20	10P3S1 (unidentified)	8° 53' 46.3"	39° 23' 56.5"	1815	-
21	EAL029 (Reference)	Ethiopia	-	-	-

22	Ha. Ata (Reference)	Tunisia	-	-	-
23	USDA-3383 (Reference)	UcDavis	-	-	-

Eco-physiological characteristics

Tolerance of the *Mesorhizobia* strains to salinity were tested on YAM medium supplemented with, 2, 3 and 4% (w/v) NaCl and tolerance to acidity/alkalinity on the same medium adjusted at pH of 4%, 5% and 10% using 1N HCl and NaOH before autoclaving. They were also grown on the same medium and incubated at 35°C, 37°C and 40°C to evaluate their tolerance to heat stress [11]. The intrinsic antibiotic resistance (IAR) of *Mesorhizobium* strains was performed according [12]. Various filter sterilized antibiotics were prepared at different concentrations (µg/ml); Chloramphenicol (10), Streptomycin (50), Nalidixic acid (10), Erythromycin (10), Neomycin (10) and tetracycline (10). Resistance to heavy metal was tested on YEMA medium containg; $CoCl_2$ 25, $CuCl_2$ 50, $ZnCl_2$ 50, $AlCl_3$ 250, Pb $(CH_3COOH)_2$ 250 and $NiSO_4$ 100 (µg/ml) of water. Resistance was recorded when visible growth was detected on the medium.

Carbon and nitrogen substrates utilization

The ability of strains to utilize different carbohydrates (1% (w/v) as the sole carbon source was tested on basal media contains (g/l); K_2HPO_4 (1), KH_2PO_4 (1), $FeCl_3.6H_2$ (0.01), $MgSO_4.7H_2O$ (0.2), $CaCl_2$ (0.1; $(NH_4)2SO_4$ (1) and agar (15). Carbon sources included Sucrose, α-cellulose, Trehalose, D-Galactose, D-Xylose and D-Sorbitol. Likewise, the ability of the strains to utilize nitrogen substrates such as; L-lysine, L-Phenylanine, L- Tryptophan, L- Leucine, L- Argenine and Glycin was tested on the same basal medium after replacing ammonium sulfate (1 g/l) and reducing mannitol to a final concentration of 0.5% (w/v) according to Amarger et al. [13].

Plant growth promoting properties of strains

Indole acetic acid (IAA) production: Strains were allowed to grow on YMA broth supplemented with filter sterilized L-tryptophan (2 g/l) and grown on orbital shaker at 200 rpm at room temperature for 4 days to test their ability to produce IAA [14]. The cultures were centrifuged at 10000 rpm for 15 min from which 2 ml of the supernatant was mixed with 100 µl of 10 mM orthophosphoric acid supplemented with Salkowaski reagent (1 m of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) in a ratio of 2:1. Samples turned pink color were considered positive indicator for IAA and the absorbance was measured using spectrophotometer at 530 nm and the amount of IAA produced was calculated by comparing with standard curve constructed from different concentrations of IAA.

Production of hydrogen cyanide (HCN): Strains were inoculated in YMA Plates supplemented with 4.4 g/l of glycine to test their ability produce hydrogen cyanide [15]. A Whatman filter paper no.1 moisturized in picric acid solution (2.5 of picric acid and 12.5 of Na_2CO_3 (g/l) of water dissolved reagents) was placed in the upper lids of the Petri plate. The plates were sealed with parafilm and incubated at 28°C. Change in colour from yellow to light brown was considered as weak, brown as moderate and reddish brown as strong was recorded to show the levels of hydrogen cyanide production.

Phosphate solubilization on solid medium: Qualitative estimation of phosphate solubilization was performed using three inorganic phosphate sources, tricalcium phosphate $Ca_3(PO_4)_2$, Aluminum phosphate ($AlPO_4$) and Iron phosphate ($FePO_4$) plate assay. Active culture suspension of 10 µl (~10^8 cells/ml) of each strain was spot inoculated on Pikovskaya's agar medium to assess tricalcium phosphate solubilization potential. The medium consisting of ingredients in g/l; glucose (10), $Ca_3(PO_4)_2$ (5), $(NH_4)_2SO_4$ (0.5), $MgSO_4.7H_2O$ (0.1), yeast extract (0.5), NaCl (0.2), $MnSO_4.2H_2O$ (0.002), $FeSO_4.7H_2O$ (0.002) and agar (15) according to Pikovskaya. Simultaneously, the strains were inoculated National Botanical Research Institute's Phosphate medium to assess their solubilization ability of Aluminum phosphate and iron phosphate. The medium contained the following ingredients in g/l: glucose (10), $AlPO_4$ or $FePO_4$) (5), $MgCl_2.6H_2O$ (5), $(NH_4)_2SO_4$ (0.1), KCl (0.2), $MgSO_4.7H_2O$ (0.25) and agar (15) according to Perez et al., [16]. The formation of clear halo zone around colonies and the solubilization index (SI)=(colony + halo zone) to the colony diameter in mm was recorded.

Phosphate solubilization on liquid medium: Based on their solubilization index, strains were selected to perform quantitative estimation of phosphate solubilization in PKV ($Ca_3(PO_4)_2$) and NBRIP ($AlPO_4$ and $FePO_4$) broth. The assay was carried out by inoculating active culture suspension of 100 µl (~10^8 cells/ml) of the respective strains using 100 ml of the respective broth in 250 ml Erlenmeyer flasks. Then the flasks were incubated on a rotary shaker at 200 rpm at room temperature for 8 days. Five ml of the supernatant was taken on the 4th, 8th day to measure pH with pH meter the amount of phosphate released using phosphomolybdate method [17] standard method. For the latter, the supernatant was centrifuged at 14,000 rpm for 15 minutes and the amount of phosphate in the clear culture supernatant as well in control (without inoculation) was measured using spectrophotometer (540 nm). The amount of solubilized P (µg/ml) was quantified against a standard curve constructed from known concentratins of Potassium dihydrogen phosphate (KH_2PO_4).

Symbiotic effectiveness screening at greenhouse

The experiment was conducted in pot sand culture in the greenhouse at Debre Zeit Center, Ethiopian Institute of Agricultural Research (EIAR). Natoli and Arerti Chickpea seeds were treated with 70% ethanol (30 sec), surface sterilized in 2% sodium hypochlorite (3 min) and rinsed five times with sterile water. Seeds were germinated on 1% (w/v) water agar at 28°C and transplanted into surface sterilized 3-kg capacity pots filled with acid washed and sterilized white sand [18]. Each seedling

was inoculated with 1 ml liquid inoculum (~10^9 cells/ml) of each test *Mesorhizobium* strain and 2 reference strains (Ethiopian commercial strain EAL 029, Tunisia strain Ha. Ata). The experiment was done in Complete Randomized design with three replications. The seedlings were provided weekly with N-free nutrient solution and the N-fertilized pot at a rate of 70 µg N ml^{-1} KNO_3 solution once a week [19].

Data scoring and plant sample analysis

Plants were uprooted after 45 days of planting to record number of nodules, nodule dry weight and shoot dry weight. Relative effectiveness of strains was calculated using the formula, RE=(inoculated plant shoots dry weight/shoot dry weight of nitrogen supplemented plant) × 100 [20]. Nitrogen fixing effectiveness was classified as highly effective >80%; effective 50 to 80%; low effective 35 to 50% and ineffective < 35%.

Data analysis

Values were given as means for triplicate samples. Analysis of variance (ANOVA) for comparison between the treatments for shoot dry weight, nodule number and nodule dry weight was performed using the statistical software SAS version 9.3. The difference among treatment means was compared by high range statistical domain (HSD) using Tukey test at 5% probability level.

RESULTS AND DISCUSSION

Physiological and biochemical characteristics

Almost all strains (91%) were able to grow at 1% NaCl concentration, whereas 50% and 35% of the strains were tolerant to 3% and 4% NaCl, respectively (Table 2). Among the groups, *M. genospecies* 7A strain 27P3S2 affinity to *M. ciceri*, *M. genospecies* 1B strain 45P4S1 closest relative to *M. loti*, *M. genospecies* 2A strain 43P2S1 and *M. genospecies* 2A strain 46P3S2 affinity to *M. plurifarium* showed broad range salt tolerance to different NaCl concentrations. This might be due to induced overproduction of low molecular weight proteins which help the cells to osmotic adjustment to intracellular water. Chickpea isolates showed a better growth with 1.5% NaCl [21], whereas the bacterial growth severely affected at 3% NaCl concentrations. Previous studies in Ethiopia [22] showed 11% of the tested chickpea isolates tolerated 5% NaCl.

Table 2: Eco-physiological characteristics of chickpea nodulating *Mesorhizobium* strains.

Sample strains	NaCl	pH	T (°C)	IAR	HMR
M. genospecies 7A (27P3S2)	4	10	35, 37	-	Co, Cu, Zn, Ni
M. genospecies 9A (2P3S1-b)	3	10	35, 37	Ery, Str, Neo	Co, Cu, Zn, Pb, Ni
M. genospecies 3A (80P4S2)	3	5, 10	35, 37, 40	Nal	Co, Cu, Zn, Ni
M. genospecies 3A (10P4S2)	2	10	35	Chl, Ery, Tet	-
M. genospecies 4B (19P3S1)	-	10	-	Ery	Co, Cu
M. genospecies 4B (ET20)	2	0	35, 37	Ery, Str, Nal	Co, Al
M. genospecies 1B (45P4S1)	4	4, 5, 10	35, 37, 40	Ery, Nal, Neo	Cu
M. genospecies 2A (46P3S2)	4	5, 10	35, 37	Ery, Str, Nal	Co, Cu
M. genospecies 2A (29P5S1)	-	10	35, 37	Str, Nal	-
M. genospecies 2A (43P2S1)	4	4, 5	35, 37, 40	Ery, Nal, Neo	Co
M. genospecies 8A (ET1)	-	10	35	Str	-
M. genospecies 8A (ET4)	3	10	-	Str, Nal	-
M. genospecies 8A (23P2S2)	4	10	35, 37	Nal	Co
M. genospecies 4A (ET26)	4	4, 5, 10	35, 37, 40	Ery, Str, Nal, Neo	Zn
M. genospecies 4A (90P4S2)	-	10	35, 37	Ery, Neo	Co, Cu Zn, Ni
M. genospecies 4A (22P5S2)	2	4, 5, 10	35, 37	Chl, Nal	Co, Ni
M. genospecies 1D (36P3S1)	2	-	-	Ery, Str, Nal	Co
M. genospecies 1A (ET24)	3	10	-	Ery, Str, Nal	Cu, Al
M. genospecies 1E (38P4S2)	4	4, 5, 10	-	Ery, Nal, Neo	Co, Cu, Zn, Ni
10P3S1 (unidentified)	-	10	35, 37	-	-

Stand for; NaCl=Salt Tolerance, Ph=Acidity or Alkalinity Tolerance, T=Temperature Tolerance; IAR=Intrinsic Antibiotic Resistance; Chl=Chloramphenicol, Ery=Erythromycin, Str=Streptomycin, Nal=Nalidixic Acid, Tet=Tetracycline, Ne=Neomycin; HMR=Heavy Metal Resistance; Co=Cobalt, Cu=Copper, Zn=Zinc, Pb=Lead, Ni=Nickel, Al=Aluminium

Many strains were grown more at pH 10 (85%) than pH 5 (60%), but fewer strains were tolerant to pH 4 (25%) (Table 2). Five strains from *M. genospecies* 1B strain 45P4S1, *M. genospecies* 2A, *M. genospecies* 4A strain ET26, strain 43P2S1, *M. genospecies* 4A strain 22P5S2 and *M. genospecies* 1E strain 38P4S2 affinity to

M. *loti* and were tolerant to pH 4. Previous studies in Ethiopia showed that chickpea rhizobial isolates grew well in moderately acidic pH5 to alkaline pH 9; while sensitive acidity pH 4 [22].

The strains showed growth at optimum temperature 35 up to 37°C, but fewer strains (20%) were tolerant to 40°C (Table 2). M. *genospecies* 1B strain 45P4S1, M. *genospecies* 4A strain ET26 and M. *genospecies* 4B strain 80P4S2 resemble to M. *amorphae* and M. *genospecies* 2Astrain 43P2S1showed rigorous growth on extreme temperature (40°C). Concurrently, Tassew Sirage et al. reported that 50% of the chickpea rhizobia strains were tolerant to 40°C isolated from Ethiopia [23].

The majority of the *Mesorhizobium* strains (60-90%) were resistant to lower concentrations of antibiotics, except tetracycline. Most strains exhibited high resistance to high concentrations of erythromycin (60%), nalidixic acid (65%) and streptomycin (50%) (Table 3). M. *genospecies* 8A strain ET1, M. *genospecies* 9A strain 2P3S1-b and M. *plurifarium* affinity strains group were more tolerant to high concentrations of the tested antibiotics (33-44%) than M. *amorphae* affiliated strains (11-33%). Several studies showed chickpea isolates were resistant to nalidixic acid and erythromycin [24,25].

Most of the *Mesorhizobium* strains exhibited (60%) resistance to the heavy metal $CoCl_2$, but fewer isolates were tolerant $CuCl_2$ (45%), followed by $ZnCl_2$ (30%) and highly sensitive to Al (10%) and Pb (5%). M. *genospecies* 9A strain 2P3S1-b, M. *genospecies* 7A strain 27P3S2 strains were highly resistant to most heavy metals (Table 3). The resistance of chickpea rhizobial isolates to Co and Cu was previously reported [24,26].

The pattern of utilization of carbon substrates showed that most strains (60%) were able to grow using D-Sorbitol and D-Glucose as carbon sources; followed by a number of strains (50%, 45%) utilizing Sucrose and Trehalose respectively and none of the strains did not utilize α-cellulose (Table 3). Earlier study indicated that chickpea rhizobia strains were more known in utilizing the carbohydrates [24,27].

Table 3: Nutritional versatility, intrinsic antibiotic resistance and heavy metals pattern of different *Mesorhizobium* species group.

Sample strains	Closest relative	Carbohydrate	Amino acid
M. *genospecies* 7A (27P3S2)	M. *ciceri*	Sor, Glu	Phe, Try, Arg
M. *genospecies* 9A (2P3S1-b)	M. *sp.* LSJ280B00	Sor, Glu, Suc, Tre	Lys, Phe, Try, Leu, Arg, Gly
M. *genospecies* 3A (80P4S2)	M. *amorphae*	Sor, Tre	Lys, Phe, Try, Leu, Arg
M. *genospecies* 3A (10P4S2)	M. *amorphae*	-	-
M. *genospecies* 4B (19P3S1)	M. *amorphae*	Suc	Phe, Leu
M. *genospecies* 4B (ET20)	M. *amorphae*	Suc	-
M. *genospecies* 1B (45P4S1)	M. *loti*	Glu, Suc	Lys, Arg, Gly
M. *genospecies* 2A (46P3S2)	M. *plurifarium*	Sor, Glu, Suc	Gly
M. *genospecies* 2A (29P5S1)	M. *plurifarium*	Sor, Suc, Tre	Phe, Leu
M. *genospecies* 2A (43P2S1)	M. *plurifarium*	Sor, Glu, Xyl, Tre	Lys, Phe, Gly
M. *genospecies* 8A (ET1)	M. *australicum*	Glu, Suc	-
M. *genospecies* 8A (ET4)	M. *australicum*	Sor, Glu, Suc, Xyl, Tre	Phe, Leu
M. *genospecies* 8A (23P2S2)	M. *australicum*	Sor, Glu, Xyl	Gly
M. *genospecies* 4A (ET26)	M. *amorphae*	Sor, Glu, Tre	Phe, Leu, Arg
M. *genospecies* 4A (90P4S2)	M. *amorphae*	Xyl	Try, Arg
M. *genospecies* 4A (22P5S2)	M. *amorphae*	Glu	Gly
M. *genospecies* 1D (36P3S1)	M. *amorphae*	Sor, Glu, Suc, Xyl, Tre	Phe, Leu
M. *genospecies* 1A (ET24)	M. *amorphae*	Sor, Xyl, Tre	Phe

M. genospecies 1E (38P4S2)	*M. loti*	Sor, Glu, Suc, Xyl	Phe
10P3S1 (unidentified)	*M. loti*	Xyl, Tre	Phe, Try, Leu, Arg

Sor=Sorbitol, Glu=Glucose, Cel=cellulose; Suc=Sucrose, Xyl=Xylose, Tre=Trehalose, lys=Lysine, Phe=Phenylanine, Try=Tryptophan, Leu=Leucine, Arg=Argenine, Gly=Glycine

Similarly, the *Mesorhizobium* strains better utilized the amino acids Phenylanine (60%), Leucine (40%) and Argenine (35%). Few strains such as M. *genospecies* 9A strain 2P3S1-b resemble to M. *sp.* LSJC280B00, M. *genospecies* 4B strain 80P4S2 and M. *genospecies* 2A strain 43P2S1 utilized lysine. Relative strains to M. *plurifarium*, M. *sp.* LSJC280B00 and M. *australicum* utilized Glycine. Earlier study on chickpea isolates from Ethiopian soils showed no growth of isolates on glycine were reported [22], whereas Kucuk et al. observed that few isolates utilized glycine [26].

Plant growth promoting properties of *Mesorhizobium* strains

The twenty different *Mesorhizobium* groups were tested for their ability to solubilize various inorganic phosphate sources, produce indole acetic acid and HCN (Table 4). With regard to IAA production, all strains produced the phytohormone ranging from 7.7 (µ g/ml) to 28.4 (µ g/ml) (Table 4). The M. *genospecies* 8A strain ET4 relative to M. *australicum* showed the highest score of IAA (28.4 µ g/ml), indicating a 4-fold difference between the highest IAA and the lowest producer (7.7 µ g/ml) M. *genospecies* 3A strain 10P4S2. Other studies showed M. ciceri and M. *loti* chickpea isolates displayed high IAA production (47.38, 45.2 µ g/ml) by Brigido et al., [21]. Previous chickpea isolate from Ethiopia showed (12.3 and 58.0 µ g/ml) IAA production respectively [22,27]. Three strains (15% of the isolates); M. *genospecies* 3A strain 80P4S2, M. *genospecies* 4A strain 22P5S2 and M. *genospecies* 1B strain 45P4S1 solubilized tricalcium and aluminium phosphates with solubilization indices ranging from 0.34-1.17. No strain solubilized iron phosphate and produced HCN.

Table 4: Plant growth promoting properties of chickpea nodulating *Mesorhizobium* strains.

No	Sample strains	Relative species	Ca_2 (PO_4) (SI)	$AlPO_4$ (SI)	IAA (µ g/ml)
1	M. *genospecies* 7A (27P3S2)	M. *ciceri*			26.13^{abc}
2	M. *genospecies* 9A (2P3S1-b)	M. *sp. LSJ280B00*			15.73^{ij}
3	M. *genospecies* 3A (80P4S2)	M. *amorphae*	1.16	0.34	20.40^{efgh}
4	M. *genospecies* 3A (10P4S2)	M. *amorphae*			7.70^{m}
5	M. *genospecies* 4B (19P3S1)	M. *amorphae*			12.44^{jkl}
6	M. *genospecies* 4B (ET20)	M. *amorphae*			14.74^{ijk}
7	M. *genospecies* 1B (45P4S1)	M. *loti*	1.13	0.7	22.57^{cdef}
8	M. *genospecies* 2A (46P3S2)	M. *plurifarium*			16.30^{ij}
9	M. *genospecies* 2A (29P5S1)	M. *plurifarium*			17.7^{ghi}
10	M. *genospecies* 2A (43P2S1)	M. *plurifarium*			24.87^{abcd}
11	M. *genospecies* 8A (ET1)	M. *australicum*			18.13^{ghi}
12	M. *genospecies* 8A (ET4)	M. *australicum*			28.43^{a}
13	M. *genospecies* 8A (23P2S2)	M. *australicum*			12.36^{jkl}
14	M. *genospecies* 4A (ET26)	M. *amorphae*			23.80^{bcde}
15	M. *genospecies* 4A (90P4S2)	M. *amorphae*			27.0^{ab}

16	*M. genospecies* 4A (22P5S2)	*M. amorphae*	1.14	0.71	21.78^{defg}
17	*M. genospecies* 1D (36P3S1)	*M. amorphae*			23.34^{bcde}
18	*M. genospecies* 1A (ET24)	*M. amorphae*			18.60^{fghi}
19	*M. genospecies* 1E (38P4S2)	*M. loti*			20.43^{efgh}
20	10P3S1 (unidentified)	Isolate			16.41^{hij}

Although the solubilization indices (SI) were comparable (1.12-1.3), the population density of phosphate solubilizing *Mesorhizobium* strains (15%) was much lower than the number (44%) reported by Mulisa Jida et al. and 30% enumerated by Daniel et al. [28]. The SI of isolates in Ethiopia were slightly lower than recorded from SI (1.42) displayed by *Mesorhizobium ciceri* isolated from Iran [29]. The ability to solubilize inorganic phosphate suggesting the ability to solubilize inorganic phosphate may be species related and may constitute an adaptive mechanism against the phosphorous deficiency in acidic and alkaline soils [21].

The amount of phosphorus released by these strains in a liquid culture (Table 5). The data showed, *M. genospecies* 4B strain 80P4S2 was able to release the highest amount of available soluble phosphates from $Ca_3(PO_4)_2$ (118.0 µg/ml) and $FePO_4$ (93.3 µg/ml) after 8 days of incubation. The phosphates released from tricalcium after 8 days of incubation for three strains ranged from (29.0 to 118.0 µg/ml) and at aluminium phosphate (41.67 - 93.3 µg/ml). The *M. genospecies* 4B strain 80P4S2 was found the better strains in solubilization of both inorganic phosphate sources as it released phosphate constantly until 8 days of incubation.

The data revealed a slight decrease in pH as incubation time increased and the maximum pH was observed 6 and the least 4.3. The two strains exhibiting the highest levels of $Ca_3(PO_4)_2$ and $AlPO_4$ solubilization in liquid medium were also shown to produce large solubilization halos when tested in solid medium. The amount of phosphate released in this study was moderate compared to release from tricalcium was ranged (70 to 295 µg/ml) after 8 days of incubation [27].

Table 5: Tricalcium and Aluminium phosphate solubilization efficiency of *Mesorhizobium* strains.

Treatment	$Ca_2(PO_4)$				$AlPO_4$			
	4th day		8th day		4th day		8th day	
	pH	P (µg/ml)	pH	P (µg/ml)	pH	P (µg/ml)	pH	P (µg/ml)
M. genospecies 4A (22P5S2)	5	37.47^{b}	4.8	70.47^{b}	70.47^{b}	10.43^{b}	10.43^{b}	43.43^{b}
M. genospecies 1B (45P4S1)	5.4	29.0^{c}	5.8	29.0^{c}	29.0^{c}	8.67^{b}	8.67^{b}	41.67^{b}
M. genospecies 3A (80P4S2)	4.8	85.0^{a}	4.8	118.0^{a}	118.0^{a}	60.33^{a}	60.33^{a}	93.33^{a}
LSD (0.05)		2.24		2.24		3.66		3.66
CV		2.87		1.62		9.99		3.9

Nodulation and symbiotic effectiveness of strains under greenhouse conditions

The inoculants showed significant variation in nodulating the two varieties ranging from (31-62) number of nodules per plant (Table 6). However, more nodules were recorded from the Natoli variety (32-62 nodules) compared to fewer nodules (31-46) formed on Arerti variety (Table 6). This might indicate that the varieties responded differently to individual strains. The strains induced nodule dry weight within the range of 55 mg/plant to 557 mg/plant on two varieties. Earlier study on chickpea isolates from Ethiopian soils showed nodule dry weight variation between 44 mg/plant to 497 mg/plant [30]. Study at Sudan showed that 35% nodules dry weight increased rate over the uninoculated control [31].

The Natoli variety produced shoot dry weight in the range of (0.62-1.38 g) per plant on both varieties. More shoot dry matter accumulation was recorded from the Natoli variety (0.78-138 g) compared to fewer shoot dry matter (0.62-1.18 g) formed on Arerti variety. The shoot dry matter in this study did not exceed the values reported (0.6-1.36 g/plant) for chickpea isolates by Mulisa Jida and Fasil Assefa [22]. Symbiotic effectiveness in relation to shoot dry matter by the inoculated plants in reference to the nitrogen fertilized control, 85% of the strains were highly effective with shoot dry matter accumulation of >80% on both varieties. Equivalently the remaining (30%) were

categorized as effective with shoot dry matter accumulation of 50-80% on Natoli and Arerti varieties. Study at Ethiopia by Wubayehu Gebremedhin et al., [9] showed 125% symbiotic effectiveness and recently Tassew Siraj et al., [23] reported (70%) on Natoli and 21% Arerti symbiotically highly effective and effective isolates. Significant variations were not observed for shoot nitrogen content; *M. genospecies* 2A strain and *M. genospecies* 4A strain ET26 accumulated relative shoot nitrogen content (1.2%) on both respective varieties. Most strains accumulated low nitrogen content compared to shoot nitrogen concentrations in inoculated chickpea plants [27].

Table 6: Inoculation response on different nodulation traits of chickpea varieties at greenhouse.

Sample strains	NN		NDW (mg)		SDW (gm)		
	Natoli	Arerti	Natoli	Arerti	Natoli	Arerti	SN (%)
M. genospecies 7A (27P3S2)	41.83^{a-g}	31.67^{d-h}	100.67^{def}	80.67^{d-g}	1.08^{a-e}	0.87^{a-e}	1.06^{f-j}
M. genospecies 9A (2P3S1-b)	39.50^{a-g}	46.17^{a-g}	84.00^{d-g}	154.33^{cd}	0.78^{b-e}	1.11^{a-d}	0.98^{jk}
M. genospecies 3A (80P4S2)	56.17^{a-d}	23.33^{ghi}	94.67^{def}	104.33^{def}	1.11^{a-e}	1.03^{a-e}	1.18^{a-d}
M. genospecies 3A (10P4S2)	40.50^{a-e}	27.33^{fgh}	123.00^{def}	83.67^{d-g}	1.12^{abcd}	0.69^{cde}	1.14^{a-f}
M. genospecies 4B (19P3S1)	48.83^{a-f}	37.33^{b-h}	91.00^{def}	557.00^{a}	1.00^{a-e}	1.06^{a-e}	1.12^{b-g}
M. genospecies 4B (ET20)	62.00^{a}	45.67^{a-g}	107.67^{def}	106.33^{def}	0.81^{a-e}	0.87^{a-e}	1.10^{c-i}
M. genospecies 1B (45P4S1)	35.17^{b-h}	29.17^{fgh}	361.67^{b}	64.33^{d-g}	1.17^{abc}	0.80^{b-e}	1.08^{e-i}
M. genospecies 2A (46P3S2)	42.50^{a-g}	25.50^{fgh}	137.67^{def}	86.67^{def}	1.10^{a-e}	0.92^{a-e}	1.20^{ab}
M. genospecies 2A (29P5S1)	47.67^{a-g}	26.33^{fgh}	130.00^{def}	104.00^{def}	1.22^{abc}	0.98^{a-e}	1.13^{a-f}
M. genospecies 2A (43P2S1)	59.50^{ab}	33.50^{c-h}	114.33^{def}	89.67^{def}	1.38^{ab}	1.18^{abc}	1.17^{a-e}
M. genospecies 8A (ET1)	46.83^{a-g}	37.17^{b-h}	145.67^{de}	121.00^{def}	1.06^{a-e}	0.94^{a-e}	1.03^{h-k}
M. genospecies 8A (ET4)	46.67^{a-g}	36.83^{b-h}	99.67^{def}	82.00^{d-g}	1.16^{abc}	0.83^{a-e}	0.97^{k}
M. genospecies 8A (23P2S2)	46.33^{a-g}	27.00^{fgh}	108.00^{def}	55.00^{fg}	1.45^{a}	0.62^{cde}	1.21^{a}
M. genospecies 4A (ET26)	39.00^{a-g}	30.50^{e-h}	124.33^{def}	129.33^{def}	1.03^{a-e}	1.12^{a-d}	1.15^{a-e}
M. genospecies 4A (90P4S2)	41.50^{a-g}	32.00^{d-h}	150.67^{cd}	56.67^{fg}	1.18^{abc}	0.93^{a-e}	1.15^{a-e}
M. genospecies 4A (22P5S2)	56.83^{abc}	32.83^{c-h}	153.00^{cd}	234.67^{c}	1.01^{a-e}	0.86^{a-e}	1.19^{abc}
M. genospecies 1D (36P3S1)	34.17^{c-h}	30.83^{e-h}	104.33^{def}	91.33^{def}	1.04^{a-e}	0.79^{b-e}	1.14^{a-f}
M. genospecies 1A (ET24)	32.33^{c-h}	28.67^{fgh}	110.33^{def}	92.33^{def}	1.12^{a-d}	0.95^{a-e}	1.06^{f-j}
M. genospecies 1E (38P4S2)	54.67^{a-e}	39.67^{a-g}	121.00^{def}	109.00^{def}	1.12^{a-d}	1.08^{a-e}	1.04^{g-k}
10P3S1 (unidentified)	54.00^{a-e}	37.67^{a-h}	154.00^{cd}	87.00^{def}	1.18^{abc}	0.69^{cde}	1.09^{d-i}
EAL029 (Reference)	31.50^{e-h}	24.17^{e-h}	101.33^{def}	74.00^{d-g}	0.96^{a-e}	0.78^{b-e}	1.01^{ijk}
Ha. Ata (Reference)	30.50^{e-h}	13.83hi	107.33^{def}	105.67^{def}	0.94^{a-e}	0.48^{de}	1.05^{f-k}
Control (Untreated)	0.00^{i}	0.00^{i}	0.00^{g}	0.00^{g}	0.69^{cde}	0.46^{e}	1.12^{b-h}
Nitrogen (Fertilizer)	0.00^{i}	0.00^{i}	0.00^{g}	0.00^{g}	1.14^{abc}	0.96^{a-e}	1.09^{d-i}

HSD (5%)	24.52	84.74	0.42	ns
CV_2	20.82	22.08	19.8	3.75

Means followed by the same letter within a column are not significantly different at ((P<0.05) level of probability, NN=Number of nodules per plant, NDW=Nodules dry weight, SDW=Shoot Dry Weight; SN=Shoot Nitrogen (%); CV=Coefficient of variation, HSD=High Range Statistical Domain.

The overall eco-physiological, nutritional PGP and symbiotic tests (Table 7) showed that M. *genospecies* 9A strain 2P3S1-b closest affinity to M. *sp.* LSJC280B00 and M. *genospecies* 4B strain 80P4S2 relative to M. *amorphae* showed wide range of eco-physiological tolerance and versatile substrates utilization (Table 6). Subsequently, M. *genospecies* 1B strain 45P4S1 closest relative to M. *loti*, M. *genospecies* 2A strain 43P2S1 affinity to M. *plurifarium*, M. *genospecies* 4A strain ET26 relative to M. *amorphae* indicating pronounced competitiveness.

Table 7: Overall rating of eco-physiological, nutritional and PGP characteristics of chickpea nodulating *Mesorhizobium* strains.

Sample Strains	Characteristics								Effectiveness (%)		
	NaCl	pH	T °C	IAR	HMR	C	N	PGP	Score	Nat	Are
M. *genospecies* 7A (27P3S2)	3	1	2	0	4	2	3	1	16	95	90
M. *genospecies* 9A (2P3S1-b)	2	2	2	3	5	4	6	1	24	68	115
M. *genospecies* 3A (80P4S2)	2	2	3	1	4	2	5	3	22	98	107
M. *genospecies* 3A (10P4S2)	1	0	2	3	0	0	0	1	7	99	72
M. *genospecies* 4B (19P3S1)	1	1	1	1	2	1	2	1	10	88	111
M. *genospecies* 4B (ET20)	1	1	2	3	2	1	0	1	12	71	90
M. *genospecies* 1B (45P4S1)	3	3	3	3	1	2	3	3	21	103	82
M. *genospecies* 2A (46P3S2)	3	2	2	3	2	3	1	1	17	97	96
M. *genospecies* 2A (29P5S1)	0	1	2	1	0	3	2	1	11	107	102
M. *genospecies* 2A (43P2S1)	3	2	3	3	1	4	3	1	21	122	123
M. *genospecies* 8A (ET1)	0	1	1	1	0	2	0	1	6	93	97
M. *genospecies* 8A (ET4)	2	1	1	2	0	5	2	1	14	102	86
M. *genospecies* 8A (23P2S2)	3	1	2	1	1	3	1	1	13	127	65
M. *genospecies* 4A (ET26)	3	3	3	4	1	3	3	1	21	91	116
M. *genospecies* 4A (90P4S2)	0	1	2	2	4	1	2	1	13	104	96
M. *genospecies* 4A (22P5S2)	1	3	2	2	2	1	1	3	15	76	105
M. *genospecies* 1D (36P3S1)	1	0	0	3	1	5	2	1	13	91	82
M. *genospecies* 1A (ET24)	2	1	0	3	2	3	1	1	13	99	98
M. *genospecies* 1E (38P4S2)	2	3	0	3	4	4	1	1	17	99	112
10P3S1 (unidentified)	0	0	0	0	0	2	4	1	11	104	72

Stand For; Nacl= Salt Tolerance Ph=Acidity Or Alkalinity Tolerance, T=Temperature Tolerance, IAR=Intrinsic Antibiotic Resistance; HMR=Heavy Metal Resistance; C= Carbohydrates Sources, N= Nitrogen Sources, PGP=Plant Growth Promoting; Nat=Natoli, Are=Arerti

CONCLUSION AND RECOMMENDATIONS

The finding reveals that Ethiopian *Mesorhizobium* strains typically have high eco-physiological and nutritional variability that are vital to local adaptation. Three strains have multiple growth promotion properties; solubilization of phosphates from calcium and aluminium phosphates that have the capacity to mobilize phosphorus in the soil. All strains produced the growth development phytohormone IAA. It was interesting to note that more than 90% of the strains showed highly effective symbiosis on both Natoli and Arerti varieties interms of relative shoot dry matter accumulation by the inoculated plants in reference to the nitrogen fertilized control plants. The overall eco-physiological competence and symbiotic effectiveness suggests the potential of these strains to test in multi-location field trials for enhance nitrogen fixation activities in chickpea production.

REFERENCES

1. Xuan W, Beeckman T, Xu G. Plant nitrogen nutrition: sensing and signaling. Current Opinion in Plant Biology. 2017;39:5-65.
2. Nour SM, Fernandez MP, Normand P, Cleyet-Maret JC. Rhizobium ciceri sp. nov. consisting of strains that nodulate chikpea (*Cicer arietinum* L). International Journal of Systematic Bacteriology. 1994;44:511-522.
3. Aslam M, Ahmad HK, Ullah H, Ayaz M, Ahmad E, Sagoo AG, et al. Nodulation grain yield and grain protein contents as affected by rhizobium inoculatioin and fertilizer placement in chickpea cultivar bittle-98. Sarhad J Agri. 2010;26:467-474
4. Khaitov B, Abdiev A. Performance of chickpea (*Cicer arietinum* L.) to biofertilizer and nitrogen application in arid condition. Journal of Plant Nutrition. 2018;41:1980-1987
5. Delic D, Srbinovic OS, Radovic J, Kuzmanovic D, Rasulic N, Aleksandar SA, eta l. Differences in symbiotic N_2 fixation of alfalfa, Medicago sativa L. cultivars and Sinorhizobium spp. strains in field conditions. Rom Biotech Lett. 2013;18:8743-8750
6. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. J Microb Biochem Technol. 2015;7:096-102.
7. Perez-Fernandez M, Alexander V. Enhanced Plant Performance in *Cicer arietinum* L. Due to the Addition of a Combination of Plant Growth-Promoting Bacteria. Agriculture. 2017;7:2-8
8. Ben Romdhane S, Aouani ME Mhamdi. Inefficient nodulation of chickpea (*Cicer arietinum* L.) in the arid and Saharan climates in Tunisia by Sinorhizobium meliloti biovar medicaginis. Annals of Microbiology. 2007;57:15-20
9. Wubayehu G, Fassil A, Thuita M, Masso C. Nutritionally versatile, abiotic stress resistant and symbiotically effective chickpea (*Cicer arietinum* L.) Root nodulating rhizobial isolates from Eastern, Southeastern and Southern Ethiopia. Electron J Biol. 2018;14:87-99
10. Greenlon A, Chang PL, Damtew ZM, Muleta A, Carrasquilla-Garcia N, Kim D, et al. Global-level population genomics reveals differential effects of geography and phylogeny on horizontal gene transfer in soil bacteria. Proceeding of the National Academy of Science of the United states of America. 2019;116:15200-15209
11. Laranjo M, Oliveira S. Tolerance of *Mesorhizobium* type strains to different environmental stresses. Antonie van Leeuwenhoek. 2011;99:651-662.
12. Maatallah J, Berrah EB, Sanjuan J, Lunch C. Phenotypic characterization of Rhizobia isolated from chickpea (*Cicer arietinum*) growing in Moroccan soils. Agronomie. 2002;22:321-329.
13. Amarger N, Macheret V, Laguerre G. *Rhizobium gallicum* sp. nov. and Rhizobium giardinii sp. nov., from Phaseolus vulgaris nodules. Int J Syst Bacteriol. 1997;47:996-1006.
14. Bric JM, Bostoc RM, Silverstone SE. Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ. Microbiol. 1991;57:534-538.
15. Chandra S, Choure K, Dubey RC, Maheshwari, DK. Rhizosphere competent *Mesorhizobium* loti MP6 induces root hair curling, inhibits Sclerotinia sclerotiorum and enhances growth of Indian mustard (Brassica campestris). Braz J Microbiol. 2007;38:124-130.
16. Perez E, Sulbaran M. Ball MM, Yarzabal LA. Isolation and Characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem. 2007;39:2905-2914.
17. Murphy J, Riley JPA. Modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta. 1962;27:31-36.
18. Somasegaren P, Hoben HJ. Hand book for rhizobia. Methods in legume rhizobium technology. Springer-Verlag New York, Inc. 1994.
19. Broughton WJ, Dilworth MJ. Control of Leghaemoglobin Synthesis in Snake beans. Biochem J. 1971;125:1075-1080.
20. Date RA. Assessment of Rhizobial status of the soil. In: Nitrogen Fixation in Legumes, pp. 85-94 (Vincent JM, ed). Academic Press, Sydney. 1993.
21. Brigido C, Alexandre A, Oliveira S. Transcriptional analysis of major chaperone genes in salt-tolerant and salt-sensitive Mesorhizobia. Microbiol Res. 2012;167:623–629.
22. Mulissa J, Fassil A. Phenotypic diversity and plant growth promoting characteristics of *Mesorhizobium* species isolated from chickpea (*Cicer arietinum* L.) growing areas of Ethiopia. Afr J Biotechnol. 2012;11:7483-7493
23. Tassew S, Fassil A. *Mesorhizobium* ciceri and *Mesorhizobium* plurifarium are the dominant symbiotically effective strains on Natoli and Arerti chickpea host varieties. Ethiop J Biol Sci. 2018;17:19-35
24. Maatallah J, Berraho EB, Munoz S, Sanjuan J, Lluch C. Phenotypic and molecular characterization of chickpea rhizobia isolated from different areas of Morocco. J Appl Microbiol. 2002;93:531-540
25. Wondwosen T, Endalkachew W, Fran W. Genetic and phenotypic diversity of rhizobia nodulating chickpea (*Cicer arietinum* L.) in soils from southern and central Ethiopia. Can J Microbiol. 2017;63:690-707.
26. Kuçuk C, Kivanc M. Preliminary characterization of Rhizobium strains isolated from chickpea nodules. Afr J Biotechnol. 2008;7:772-775.
27. Mulissa JM, Fassil A. Phenotypic diversity and plant growth promoting characteristics of *Mesorhizobium* species isolated from chickpea (*Cicer arietinum* L.) growing areas of Ethiopia. Afr J Biotechnol. 2016;11:7483-7493.

28. Daniel M, Fassil A. Screening for symbiotically effective and ecologically competitive chickpea rhizobial inoculants from Ethiopian soils. Ethiop J Biol Sci. 2015;14:1-18.
29. Alikhani HA, Saleh-Rastin N, Antoun H. Phosphate solubilization activity of Rhizobia native to Iranian soils. Plant Soil. 2006;287:35–41.
30. Wondwosen T, Endalkachew W, Walley F. Response of chickpea (*Cicer arietinum* L.) to inoculation with native and exotic *Mesorhizobium* strains in Southern Ethiopia. Afr J Biotechnol. 2016;15:1920-1929
31. Mohamed AA, Hassan MA. Evaluation of two chickpea (*Cicer arietinum* l.) cultivars in response to three Rhizobium strains at River Nile State, Sudan. Merit Res J Agric Sci. 2015;3:062-069.