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Response of chickpea (*Cicer arietinum* L.) to inoculation with native and exotic *Mesorhizobium* strains in Southern Ethiopia

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A series of pot and two consecutive crop-year field experiments were conducted from 2011 to 2012 in Southern Ethiopia to determine the effectiveness of Mesorhizobium strains on two cultivars of chickpea (Shasho and Nattoli). The eight treatments included: Six rhizobial inoculants, the four best indigenous strains (Cp8, Cp41, Cp97 and Cp105); CpNSTC (National Soils Testing Center inoculant); and CpSK (Canadian inoculant), Nitrogen fertilizer and a control. The results from the field and pot experiments indicated that chickpea crop yield can be improved using proper Mesorhizobium inoculation. Inoculation had a pronounced effect on grain yield, yield component, total N uptake, grain protein content, percentage N derived from the atmosphere (%Ndfa) for the seed, and amount of seed N fixed compared to non-inoculated treatments. In the pot experiment, significant difference was recorded among the mesorhizobial strains used with the indigenous strain Cp41 highly effective in shoot dry weight (41%) mg^{-1} plant, grain yield (50%), total N uptake (117%), and %Ndfa (67.9%) followed by CpSK, Cp8 and Cp97. In the second crop-year field experiment, the indigenous *Mesorhizobium* strain Cp41 also proved highly effective in-nodule dry weight (786%) mg^{-1} plant, grain yield (66%), total N uptake (100%), and %Ndfa (53.7%). The maximum seed protein content was recorded during the second crop-year field experiment in Cp41 (20%), followed by N fertilizer added treatment and CpSK (18%). The chickpea indigenous rhizobial strain Cp41, was superior inoculant for almost all parameters. Thus, there are potential advantages to be gained from using efficient rhizobial inoculants under rain fed conditions in Ethiopia.

Key words: Chickpea, rhizobia inoculants, nodulation, growth, yield.

INTRODUCTION

Ethiopia is the top producer of chickpea in Africa. In Ethiopia, chickpea is the third most important grain legume after faba bean (*Vicia faba*) and common bean (*Phaseolus vulgaris* L.) by volume for small-scale farm

production. Chickpea is an essential pulse crop, providing high-quality protein for human nutrition and a source of cash income for farmers. The national average yield of chickpea is 1.7 t ha^{-1} (CSA, 2013), which is far below the potential yield of 4.5 t ha^{-1}. In Southern Ethiopia, the average yield falls below the national average at 1.1 t ha^{-1}. Among increasing population pressures and soil erosion, soil fertility decline is one of the major factors limiting crop yield in Ethiopia. Long standing use of organic residues for fuel and feed has contributed to this decline. To compensate, commercial fertilizers such as diammonium phosphate (DAP) and urea have been used in some parts of the country for about three decades (FAO, 1984). Chickpea however, is usually grown without fertilizer on marginal land and farmers have a mistaken notion that chickpea, being a legume crop, does not need any nutrient support. Nitrogen (N) is the most commonly deficient soil nutrient in Ethiopia, contributing to reduced agricultural yields throughout the country. The use of inorganic N fertilizer is very low among resource-poor farmers, for whom it is prohibitively expensive. Biological nitrogen fixation (BNF) represents a significant potential source of N input in agricultural soils in the country.

The major N_2-fixing systems, the symbiotic systems, can play a substantial role in improving the fertility and productivity of low-N soils (Abaidoo et al., 1990). Biological N_2 fixation by rhizobia in legume root nodules is one widely studied mechanism by which plants benefit from association with interacting partners. In rhizobium-legume symbiosis, both plant cultivar and *Rhizobium* strain can affect nodulation (Keneni et al., 2012, Mutch et al., 2003). The bacteria benefit the plants by fixing N_2 in exchange for fixed carbon (C), which is either provided directly to the bacteria or indirectly in root exudates. Generally, it is assumed that a pulse crop well inoculated with the bacteria can fix sufficient quantities of N to eliminate the need for N fertilizer inputs in the crop year (Walley et al., 2006). Nitrogen supplied through BNF is less likely to leach or volatilize in pre-cropping or during cropping than N supplied as inorganic fertilizer (Jensen and Hauggaard-Nielsen, 2003). Depending on cultivar, bacterial strain, and environmental factors, the chickpea and *Mesorhizobium* sub sp. *Ciceri* association can produce up to 176 kg N ha^{-1} annually (Beck et al., 1991). Using high yielding varieties of chickpea along with effective rhizobial strains can enhance yields and minimize the need for nitrogenous fertilizer.

Rhizobial species that produce nodules in chickpea are solely specific to chickpea. Therefore, inoculation with effective strains is advised in soils with a weak or nonexistent bacterial presence (Rupela and Saxena, 1987). The isolation and screening of highly efficient and competitive strains of native rhizobial populations for use as inoculants proves very beneficial. Often the most competitive and persistent inoculant strains in a particular field environment are those isolated from similar environments (Chatel and Greenwood, 1973). One year before this study, 42 effective indigenous Meso*rhizobium* strains were isolated and evaluated at the College of Agriculture, Hawassa University, for their ability to enhance nodulation and biomass yield in locally grown chickpea. A total of 4 Meso*rhizobium* strains were selected for pot and field experimentation on the basis of effective nodule number and biomass yield (unpublished). The objective of the study was to evaluate the effect of the selected strains and commercial inoculants on nodulation and yield in two cultivars of chickpea under greenhouse and field conditions at Ele kebele in Southern Ethiopia.

MATERIALS AND METHODS

A series of greenhouse or pot experiment was conducted in 2011 and two consecutive crop-year field experiments were conducted from mid-August to December in 2011 and 2012 under rain fed conditions in Southern Ethiopia. The experiment was to test the efficacy of *Mesorhizobium* strains inoculation in improving growth, yield, and nodulation of chickpea. The pot experiment and soil chemical analyses were carried out at the soil microbiology laboratory of Hawassa University while the field experiments were conducted at Ele kebele on farmer's field. The plant and seed analyses were done at the Soil Science Department of the University of Saskatchewan (U of S), Canada.

Estimation of indigenous rhizobia nodulating chickpea

Soil samples were collected in the dry season of June 2011 at Ele and Jole Andegna kebeles (Meskan woreda), Huletegna Choroko kebele (Alaba special woreda), and Taba kebele (Damot Gale woreda). The plant infection count or the most probable number (MPN) count was used to determine the number of viable and infective rhizobia (Somasegaran and Hoben, 1994). Ten grams of soil sample were diluted in aseptic conditions in 90 ml sterilized distilled water. One milliliter of the diluted solution was transferred into 9 ml sterilized distilled water up to 10^{-10} and was used to inoculate chickpea seedlings adequately grown in acid-treated and sterilized sand using plastic cups in four replications. Nodule observations were made 45 days after inoculation. Positive and negative nodulation of growth unit were recorded for all dilutions and converted into number of rhizobia g^{-1} of soil using a MPN table.

The enumeration of indigenous rhizobial population by MPN method (Vincent, 1970) revealed that the population size of indigenous rhizobia compatible to this crop varied at different locations, ranging from 0 at Ele and Huletegna Choroko, to 5.8×10^{2} at Taba, and 3.1×10^{4} cell g^{-1} of soils at Jole. With the need for

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Table 1. Average rainfall, maximum and minimum temperature during 2011 and 2012 growing seasons, annual and long-term average (2003-2012) at Butajira, nearby metrological station of the study area.

Year		Rainfall (mm)	[a]Max. T (°C)	[b]Min. T (°C)
2011	July	168.3	23.1	12.0
	August	133.6	23.0	11.5
	September	88.1	24.3	11.9
	October	47.6	25.4	10.7
2012	July	262.0	24.4	12.6
	Annual	981.3	26.2	11.1
	August	236.1	23.9	12.7
	September	217.8	25.8	12.4
	October	98.6	25.5	10.1
	Annual	1523.2	25.6	10.3
10 years (2003-2012)	Annual Average	1146.7	26.1	11.2
Altitude	m above sea level		1950	

[a]Maximum temperature; [b] Minimum temperature.

Table 2. Soil physicochemical characteristics of the 0 to 20 cm soil layer of the experimental sites in Ele at the initiation of the experiments in 2011 and 2012.

Soil characteristic	2011	2012
Texture class [a]	Clay	Clay
pH-H2O (1:2.5)	6.2	6.3
EC ($ms \cdot cm^{-1}$) (1:2.5)	0.078	0.069
Organic carbon (%) [b]	0.74	0.71
Total nitrogen (%) [c]	0.067	0.061
Available P ($mg \cdot P \cdot kg^{-1}$ soil) [d]	2.8	2.6
CEC ($cmolc \cdot kg^{-1}$ soil)[e]	45.1	44.6
Exchangeable bases [f]		
Na ($cmolc \cdot kg^{-1}$ soil)	0.34	0.36
K ($cmolc \cdot kg^{-1}$ soil)	1.29	1.27
Mg ($cmolc \cdot kg^{-1}$ soil)	3.34	3.25
Ca ($cmolc \cdot kg^{-1}$ soil)	22.6	21.8
Micronutrients[g]		
Cu ($mg \cdot kg^{-1}$ soil)	1.33	1.32
Fe ($mg \cdot kg^{-1}$ soil)	8.69	10.32
Mn ($mg \cdot kg^{-1}$ soil)	40.68	41.32
Zn ($mg \cdot kg^{-1}$ soil)	0.89	0.91

[a]Hydrometer; [b]Walklay and Black; [c]Kjeldahl; [d] Olsen; [e&f]Ammonium acetate; [g]diethylene triamine pentaacetic acid (DTPA).

inoculation indicated at three of the locations studied, Ele kebele was selected for the pot and field experimental sites.

Description of the study area

The study was conducted at Ele kebele, about 7 km east of Butajira town and with latitude 08° 12′ N, longitude 38° 27′ E and altitude of 1950 masl. The average annual rainfall from 2003 to 2012 at Butajira, the nearby metrological station is 1146 mm having minimum and maximum temperature of 11.2 and 26.1°C, respectively (Table 1). Before sowing, soil samples were taken from representative points at 0-20 cm depth to make one composite surface soil sample for analysis of soil texture and some chemical properties as results depicted in Table 2, estimated according to the methods described by Van Reeuwijk (2002). A representative soil

sample was collected from this site for pot experiment during field preparation for the 2011 field trial.

Treatments and experimental design

A series of pot experiment and two consecutive crop-year field experiments were conducted from mid-August to December in 2011 and 2012 under rain fed conditions in Ele Kebele. Two chickpea cultivars (Shasho and Nattoli) were used. The eight treatments includes: six rhizobial inoculants, the four best indigenous strains Cp08 (isolated from Mesqan District, Southern Ethiopia), Cp41 (isolated from Bodity District, Southern Ethiopia), Cp97 (isolated from Akaki District, Central Ethiopia), Cp105 (isolated from Dukem District, East Shao, Ethiopia); CpNSTC (National Soils Testing Center inoculant); and CpSK (Canadian inoculant), N fertilizer (20 kg N ha^{-1}, no inoculant) and a control (no fertilizer or inoculation). All plots received the equivalent of 46 kg P_2O_5 kg ha^{-1} as TSP (100 kg TSP). The field and the pot experiments were laid out in complete randomized block design (CRBD) and complete randomized design (CRD) with three replications, respectively. The size of each experimental plot was 4 m × 3 m (12 m^2) with a total of 48 plots. Spacing between chickpea plants, rows, plots, and blocks was 10, 30, 0.5 and 1 m, respectively. For pot experiments, each pot was filled with 4 kg of soil, planted with five seeds per pot, and thinned to three plants at the two leaf stage. All other treatments and management practices were similar to those of the field experiments. Non-nodulating reference chickpea genotype PM233 cultivar from the International Center for Agricultural Research in the Dry Area (ICARDA) (received from the Ethiopian Institute of Agriculture Research, Holleta Research Center) as a reference crop was planted adjacent to the experimental site and in separate pots for assessing percentage N derived from the atmosphere (%Ndfa). Inoculants were prepared from fully grown broth by mixing with ignite based carrier. Seeds were inoculated with the respective rhizobial strains just before planting and kept in shade to maintain the viability of the cells. Seeds were allowed to air dry for a few minutes before planting.

Data collection

The data on nodulation parameters were taken at the mid-flowering stage. Five plants were randomly taken from second border rows on each side of the plot. Nodule number, nodule dry weight, and shoot dry weight were taken from five representative plants per plot. At physiological maturity, plants from the central 6 rows were manually harvested close to the ground surface. Five plants were randomly selected from the central rows of each plot and the number of pods and branches per plant were recorded. The harvested plants were weighed to determine the biomass yield and threshed to determine the grain yield of each plot. Hundred grains were counted to determine 100-grain weight per treatment.

Plant and seed analysis

At physiological maturity, five non-border plants from each plot and non-fixing chickpea cultivar were harvested and separated into straw and grain. These samples were used to determine seed N, straw N, and %Ndfa. The sample materials were oven dried at 70°C to a constant weight and ground to pass through a 2 mm sieve. Nitrogen accumulation in the plant tissue (percent N) was determined using a LECOCNS-2000 carbon, nitrogen, and sulfur analyzer. Ground seed samples were further pulverized to a fine powder in a ball mill and very small portions (approximately 3 mg each sample) of the fine ground samples were pelleted into 6 × 8 mm tin caps. Samples were then analyzed using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V mass spectrometer with a ConFlo IV interface (Thermo Scientific, Bremen, Germany), at the Stable Isotope Facilities, Department of Soil Science, University of Saskatchewan. The total protein content was computed by N content multiplied by a factor of 6.25, in accord with Jackson (1962). Total N per plant (shoot dry weight × N content + grain yield × N seed content) was calculated. The amount of seed N fixed was calculated as (%Ndfa × seed yield × seed N concentration)/100 (Peoples et al., 1995). Percent N derived from the atmosphere (%Ndfa) based on ^{15}N Natural Abundance Method was calculated using the following equation (Unkovich et al., 2008; Bremer and van Kessel, 1990):

$$\%Ndfa = \frac{\delta^{15}N \text{ of reference plant} - \delta^{15}N \text{ of } N_2 \text{ fixing legume}}{\delta^{15}N \text{ of reference plant - B}} \times 100 \quad (1)$$

Where, "δ15N" is:

$$\delta^{15}N = \frac{\text{Atom \% } ^{15}\text{N sample - atom \% } ^{15}\text{N atmosphere}}{\text{Atom \% } ^{15}\text{N atmosphere}} \times 100 \quad (2)$$

Where, the standard was atmospheric N_2 (0.3663 atom % ^{15}N). B is the $\delta^{15}N$ of the N_2-fixing plant grown in N-free medium. The value of B for chickpea was assumed to be −0.7644 (Kyei-Boahen, 2002).

Statistical analysis

Treatment effects were analyzed using the General Linear Model (GLM) procedure of the SAS computer software package (SAS/STAT, version 9.3). Mean values were separated according to Duncan's multiple range test (DMRT) at P = 0.05 (SAS Institute, 2012).

RESULTS AND DISCUSSION

The effect of N fertilization and rhizobial inoculation on nodulation, grain yield, and yield component of chickpea under the pot and field experiments are presented in Tables 3 to 5. The results obtained from the analysis of variance indicated that N fertilization and rhizobial inoculation had significant effects on all studied traits except on plant height.

Nodulation test

The data in Table 3 indicate that inoculation by different strains gave a significantly (P<.001) higher nodule number, nodule dry weight, and shoot dry weight compared to non-inoculated plants of the pot and the two season field experiments. Nodulation was not observed on non-inoculated and N fertilized treatments during the

Table 3. Effect of rhizobial inoculation and N fertilizer application on nodule number (NN), nodule dry weight (NDW), and shoot dry weight (SDW) at 50% flowering stage of chickpea varieties.

Treatment	Pot experiment			2011/2012 Field			2012/2013 Field		
	NN plt^{-1}	NDW mg plt^{-1}	SDW g plt^{-1}	NN plt^{-1}	NDW mg plt^{-1}	SDW g plt^{-1}	NN plt^{-1}	NDW mg plt^{-1}	SDW g plt^{-1}
Strain									
Control	0.0^{c}	0.0^{c}	2.9^{e}	0.0^{f}	0.0^{d}	5.4^{d}	4^{ed}	125^{d}	8^{e}
Nitrogen	0.0^{c}	0.0^{c}	4.6^{a}	0.0^{f}	0.0^{d}	7.6^{a}	3^{e}	37^{e}	13^{cba}
Cp8	69.3^{b}	473. 9^{a}	3.7^{cb}	28.8^{b}	168.6^{b}	6.5^{cba}	20^{c}	263^{c}	12^{dcb}
Cp41	102.9^{a}	497.3^{a}	4.1^{b}	33.7^{a}	233.0^{a}	7.3^{ba}	57^{a}	982^{a}	16^{a}
Cp97	82.5^{b}	438.8^{ba}	3.4^{cd}	14.0^{d}	24.8^{c}	7.1^{cba}	17^{c}	311^{c}	13^{dcba}
Cp105	8.2^{c}	66.3^{c}	3.2^{edc}	6.5^{e}	13.8^{dc}	6.5^{dcba}	10^{d}	304^{c}	12^{edc}
CpSK	68.6^{b}	348.2^{b}	3.7^{cb}	18.5^{c}	26.2^{c}	6.3^{dcb}	28^{b}	584^{b}	14^{ba}
CpNSTC	5.8^{c}	44.2^{c}	3.0^{ed}	6.3^{e}	10.3^{dc}	6.1^{dc}	8^{ed}	151^{d}	9^{ed}
Variety									
Shasho	31.8^{b}	198.3^{b}	3.9^{a}	12.8	56.7	6.9^{a}	16^{b}	322^{b}	15^{a}
Natoli	52.6^{a}	268.9^{a}	3.3^{b}	14.2	62.5	6.3^{b}	20^{a}	368^{a}	10^{b}
Strain	***	***	***	***	***	**	***	***	***
Variety	***	*	***	NS	NS	*	**	*	***
Strain x variety	***	NS	NS	NS	NS	NS	***	***	*
CV	28.21	32.97	10.78	22.97	30.33	13.19	26.72	20.99	20.96

plt, Per plant. Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT (p = 0.05). *,**,***, and N^{S} showed significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively.

pot and the 2011/2012 study season field experiments. This result indicates that there had been no history of chickpea production in the experimental field. All tested isolates of chickpea rhizobia showed great variation in their capacity to induce the formation of nodules on host plant roots under lath house and field conditions. The mean nodule number per plant obtained from CpNSTC and Cp41 varied from 5.8 to 102.9, 6.3 to 33.7, and 8 to 57 under pot, first-year field, and second-year field experiments, respectively (Table 3). Similarly, the mean nodule dry weight per plant induced by CpNSTC and Cp41 varied from 44.2 to 497.3, 10.3 to 233, and 151 to 982 mg under pot, first-year field, and second-year field experiments, respectively. Significant differences were observed between varieties in terms of nodule number, nodule dry weight, and shoot dry weight under pot and field experiments. Higher nodule number and nodule dry weight was observed in Nattoli (desi type) than in Shasho (kabuli type) chickpea varieties. In terms of shoot dry weight, however, Shasho recorded higher than Nattoli. This finding agrees with that of Keneni et al. (2012), who reported that the Ethiopian and introduced chickpea germplasm were high in genetic diversity for both symbiotic and agronomic characters.

Maximum shoot dry weight (mg^{-1}plant) in pot experiments were recorded when N fertilizer was applied, with a 59% increase over the uninoculated control. Inoculation with Cp41 showed an increase of 41% in shoot dry weight (mg^{-1}plant) over the uninoculated control. Maximum shoot dry weight (mg^{-1}plant), however, was recorded during the second-year field experiment. When Cp41 was applied; there was a 100% increase over the uninoculated control. Inoculation with CpSK ranked second, with an increase of 75% in shoot dry weight (mg^{-1}plant) over the uninoculated control. There was significant interaction between variety and strains during the 2012/2013 year field experiment. This result is in line with Birhanu and Pant (2012), who reported that chickpea inoculation with *Mesorhizobium* strains gave higher nodule number, nodule dry weight, and biological yield compared to uninoculated plants in Shoa-Robit, Ethiopia.

Grain yield and yield components

Number of pods and branches per plant is an important yield determinant in pulse crops. Number of branches

Table 4. Effect of rhizobial inoculation and N fertilizer application on number of branch (NB), number of pod (NP), and 100-grain weight (100GW) of chickpea varieties.

Treatment	Pot experiment			2011/2012 Field			2012/2013 Field		
	NB plt^{-1}	NP plt^{-1}	100GW (g)	NB plt^{-1}	NP plt^{-1}	100GW (g)	NB plt^{-1}	NP plt^{-1}	100GW (g)
Strain									
Control	6.1	10.7^{c}	23.9^{c}	5.5	33.0^{d}	25.0	6.3^{c}	46^{c}	30.0
Nitrogen	6.8	13.8^{ba}	27.2^{ba}	6.1	63.2^{a}	26.0	10.7^{a}	58^{ba}	30.0
Cp8	5.4	13.9^{ba}	28.1^{a}	5.7	51.0^{cba}	26.8	8.7^{cba}	53^{bc}	29.8
Cp41	6.1	15.8^{a}	28.9^{a}	7.0	56.6^{ba}	26.6	9.7^{a}	68^{a}	30.2
Cp97	5.4	12.6^{bc}	27.2^{ba}	5.8	41.7^{dc}	24.5	9.5^{ba}	63^{ba}	30.4
Cp105	5.9	11.9^{bc}	24.2^{bc}	5.3	40.4^{dc}	24.8	7.1^{cb}	55^{bc}	30.2
CpSK	6.0	12.9^{bc}	28.0^{a}	6.7	49.3^{cba}	25.0	8.5^{cba}	60^{ba}	30.6
CpNSTC	5.7	11.3^{c}	24.7^{bc}	6.2	43.3^{dcb}	24.6	6.8^{c}	54^{bc}	30.3
Variety									
Shasho	7.8^{a}	13.8^{a}	24.1^{b}	5.6^{b}	42.1^{b}	23.2^{b}	9.3^{a}	67^{a}	31^{a}
Natoli	4.0^{b}	12.0^{b}	28.9^{a}	6.4^{a}	52.5^{a}	27.6^{a}	7.5^{b}	47^{b}	29.6^{b}
Strain	NS	***	**	NS	**	NS	**	**	NS
Variety	***	**	***	*	**	***	**	***	***
Strain × variety	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV	14.35	13.38	8.85	17.04	24.05	7.08	23.26	14.77	3.67

plt, Per plant. Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT (p= 0.05). *,**,***,and N^{S} showed significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively.

plant^{-1} was not significant under pot experiment and during the 2011/2012 study season field experiment, but during the 2012/2013 study season, field experiment there were significant (P<0.01) differences between treatments (Table 4). The maximum number of branches plant^{-1} (10.7) was recorded in N fertilized treatment followed by Cp41 strain inoculated treatment (9.7), with N fertilizer added and Cp41 strain inoculated treatments yielding an increase of 70 and 54%, respectively, in number of pods plant^{-1} over the uninoculated control.

The application of N fertilizer and rhizobial inoculants significantly (P<0.01) enhanced number of pods plant^{-1} of chickpea under pot and field studies. Number of pods plant^{-1} was increased by 48, 30 and 20% over the control treatments by inoculation of Cp41, Cp8 and CpSK, respectively in pot experiments. Similarly, number of pod plant^{-1} was increased by 48, 37 and 30% over the control treatments by inoculation with Cp41, Cp97 and CpSK, respectively, during the 2012/2013 field experiment. These results are in line with Yadav et al. (2011), who reported that inoculation of seed with *Rhizobium* enhanced nodulation, growth, and yield responses of legumes. The effects of seed inoculation on increasing number of pod plant^{-1} were also observed by Ali et al. (2003). Hundred-grain weight was not significant during the two crop-year field experiments; but in pot experiments, there were significant (P<0.01) differences between treatments. Hundred-grain weight was increased by 21, 18, and 17% over the control treatments by inoculation with Cp41, Cp8, and CpSK, respectively, under pot experimentation.

The straw and grain yield data of chickpea showed that rhizobial inoculation as well as N application significantly (P<0.001) increased the straw and grain yield of the crop in both pot and second-crop year field experiments (Table 5). Grain yields were increased by 50, 28 and 33% over the control treatments by inoculation with Cp41, CpSK, and Cp8, respectively, in the pot experiment. Similarly, grain yields were increased by 66, 53 and 49% over the control treatments by inoculation with Cp41, CpSK and Cp8, respectively, during the 2012/2013 crop-year field experiment. Unexpected drought during the 2011/2012 growing season resulted in no significant difference between treatments on straw and grain yield. These results are in line with Kyei-Boahen et al. (2005), who reported that soil generally increased seed yield over the uninoculated control but the magnitude varied over different seasons depending on the prevailing climatic condition. Increase in straw and grain yield of chickpea with effective *Rhizobium* inoculation has also been reported (Romdhane et al., 2007; Bhuiyan et al., 1998; Gupta and Namdeo, 1996).

Branch and pod number per plant were significantly higher in the Shasho variety than in Nattoli. Although in

Table 5. Effect of rhizobial inoculation and N fertilizer application on straw, grain yield (GY), and plant height (PH) of chickpea varieties.

Treatment	Pot experiment			2011/2012 Field			2012/2013 Field		
	Straw (g/pot)	GY (g/pot)	PH (cm)	Straw (kg/ha)	GY (kg/ha)	PH (cm)	Straw (kg/ha)	GY (kg/ha)	PH (cm)
Strain									
Control	10.4^{d}	8.8^{e}	35.5	2100.0	2167.2	32.5	2625^{c}	1720^{d}	43.6
Nitrogen	17.9^{a}	12.2^{ba}	37.8	2473.4	2207.4	34.5	3385^{a}	2794^{a}	46
Cp8	12.4^{dc}	11.7^{ba}	38.4	2617.6	2543.9	34.7	3171^{ba}	2570^{ba}	44
Cp41	14.9^{b}	13.2^{a}	39.0	2993.5	2597.0	34.6	3382^{a}	2866^{a}	47.4
Cp97	10.8^{d}	10.5^{edcb}	38.3	2425.3	1916.1	32.5	2825^{bc}	2316^{bc}	44.3
Cp105	10.7^{d}	9.6^{ed}	36.7	2691.4	2265.8	35.1	2872^{bc}	2187^{c}	44.1
CpSK	12.9^{c}	11.3^{dcb}	37.3	2438.3	2135.3	34.4	3121^{ba}	2632^{a}	45.4
CpNSTC	10.6^{d}	9.9^{edc}	35.9	2458.7	2177.9	34.6	2568^{c}	2102^{c}	45.6
Variety									
Shasho	14.1^{a}	10.6	40.4^{a}	2215.3^{b}	1722.0^{b}	32.1^{b}	3766^{a}	2430^{a}	45
Nattoli	11.0^{b}	11.1	34.3^{b}	2834.3^{a}	2775.6^{a}	36.1^{a}	2222^{b}	2217^{b}	45
Strain	***	***	NS	NS	NS	NS	***	***	NS
Variety	***	NS	***	**	***	***	***	**	NS
Strain x variety	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV	12.83	13.29	6.32	25.40	25.65	9.75	11.76	10.63	9.28

Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT (p= 0.05). *,**,***,and NS showed significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively.

terms of hundred-grain weight, Nattoli recorded significantly higher under pot experimentation (28.9) and during the 2011/2012 study season field experiment (27.6), Shasho yielded significantly higher hundred-grain weight (31) during the 2012/2013 study season field experiment (Table 4).

In terms of grain and straw yield (kg per ha), Nattoli recorded significantly higher during the 2011/2012 study season field experiment, but during the 2012/2013 study season field experiment Shasho yielded significantly higher grain and straw yield as compared to Nattoli (Table 5). This was because during the 2012/2013 study season, there was good rainfall distribution throughout the growing season. These results reveal that the two cultivars differed in their morphology and growth period. Nattoli was an early maturing variety whereas Shasho was a late maturing variety. This indicated that Nattoli would be a suitable cultivar for a short rainy season, whereas Shasho would produce higher grain yields if there is a longer rainy season.

Total N uptake and seed protein content

Total N accumulation in legume plants is one of the best parameters to measure N fixation under experimental conditions. Results on effects of rhizobial inoculation and N fertilizer application on total N uptake (kg/ha) and seed protein content (%) have been presented in Table 6. The rhizobial inoculation and N application significantly (P<.001) increased total N uptake and seed protein concentration in both pot and second-year field experiments. Compared to uninoculated control treatment, total N uptake was increased by 135, 117, 69 and 22% by inoculation with Cp41, CpSK, and CpNSTC, respectively, in the pot experiment. Similarly, total N uptake was increased by 100, 72, 49 and 24% by Cp41, CpSK, N added treatment, and CpNSTC respectively, over uninoculated control treatments during the 2012/2013 field experiment. The maximum seed protein content (23.3%) was recorded in N fertilizer added treatment, followed by Cp41 strain inoculated treatment (23.2%) under pot experimentation. The maximum seed protein content (20%) was recorded in Cp41 strain inoculated treatment, followed by N fertilizer added treatment and CpSK (18%), during the second-year field experiment. Our findings are supported by Aslam et al. (2010), who also reported that inoculants significantly increased grain protein content.

N derived from the atmosphere and N fixed

Results showing effects of rhizobial inoculation and N

Table 6. Effect of rhizobial inoculation and N fertilizer application on total N uptake (TNU), seed protein concentration (SPC), percentage N derived from the atmosphere (%Ndfa) for the seed, and amount of seed N fixed for chickpea cultivars.

Treatment	Pot experiment				Field experiment (2012/2013)			
	TNU (mg/pot)	SPC (%)	Ndfa (%)	N_2 fixed (mg/pot)	TNU (kg/ha)	SPC (%)	Ndfa (%)	N_2 fixed (kg/ha)
Strain								
Control	300.6^{d}	15.5^{c}	-	-	57^{d}	16^{d}	-	-
Nitrogen	708.2^{a}	23.3^{a}	-	-	85^{cb}	18^{b}	-	-
Cp8	429.8^{cb}	17.3^{bc}	62.0^{bc}	128^{bc}	85^{cb}	17^{cd}	40.5^{ab}	41.7^{bcd}
Cp41	654.4^{a}	23.2^{a}	67.9^{a}	261^{a}	114^{a}	20^{a}	53.7^{a}	62.1^{a}
Cp97	445.9^{cb}	20.7^{ba}	56.7^{ab}	161^{cd}	78^{c}	17^{cd}	45.8^{bc}	34.9^{bc}
Cp105	351.1^{cd}	17.3^{bc}	55.7^{bc}	102^{d}	72^{cd}	17^{cd}	37.9^{bc}	32.1^{cd}
CpSK	507.1^{b}	20.5^{ba}	64.5^{ab}	179^{b}	98^{b}	18^{b}	46.9^{ab}	48.8^{b}
CpNSTC	366.2^{cd}	17.3^{bc}	51.5^{c}	89^{d}	71^{cd}	17^{cd}	29.8^{c}	29.1^{d}
Variety								
Shasho	471.2	19.2	59.5	144	89^{a}	17^{b}	41.3	42.1
Nattoli	469.6	19.6	59.9	162	76^{b}	17.9^{a}	43.6	40.8
Strain	***	***	**	***	***	***	**	***
Variety	NS	NS	NS	NS	**	**	NS	NS
Strain x variety	NS	NS	*	NS	NS	*	NS	NS
CV	17.43	16.13	20.53	31.2	14.73	7.27	11.27	18.18

Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT ($p = 0.05$). *,**,***,and N^{S} showed significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively.

fertilizer application on N derived from the atmosphere and amount of seed N fixed have been presented in Table 6. Rhizobial inoculation of chickpea showed great variation in N derived from the atmosphere and amount of seed N fixed. The mean percentage N derived from the atmosphere varied from 51.5 to 67.9, induced by isolate Cp41 and CpNSTC, respectively, under pot experiment. Similarly, the highest N derived from the atmosphere (53.7%) was recorded in plants inoculated with strain Cp41, whereas the lowest N derived from the atmosphere (29.8%) was shown by the host inoculated with CpNSTC during the second-year field experiment. Seed inoculation significantly influenced the amount of seed N fixed. It increased from 89 with the CpNSTC inoculant to 261 (mg/pot) with Cp41 inoculant treatments under pot experiments. Similarly, an increase in the amount of seed N fixed, from 29.1 to 62.1 kg/ha was recorded with CpNSTC inoculant and with Cp41 inoculant treatment, respectively during the second-year field experiment. These results were comparable with those of Kyei-Boahen et al. (2005), who reported that the proportion and amount of seed N derived from N_2 fixation were higher for inoculated plants as compared with uninoculated controls across locations. There was no significant difference between varieties regarding N derived from the atmosphere and N fixed. Kyei-Boahen et al. (2002) also found that desi and kabuli chickpea types responded similarly to rhizobial inoculation.

The results of this study indicate that chickpea yield can be improved through proper *Mesorhizobium* inoculation. Inoculation by different strains had a pronounced effect on grain yield, yield component, nodulation, total N uptake, grain protein content, %Ndfa for the seed, and amount of seed N fixed as compared to non-inoculation treatments. Indigenous *Mesorhizobium* strain Cp41 was found to be more significant in its effect for most of the studied parameters, followed by Cp8, CpSK and Cp97, respectively, as compared to the uninoculated control. Indigenous *Mesorhizobium* strain Cp105 and CpNSTC inoculants were found to be ineffective symbiotic nitrogen fixers. This finding agreed with Yadav et al. (2011), in that a *Mesorhizobium* strain indigenous to the growing field locality proved to be a highly effective symbiotic nitrogen fixer for uptake of nutrient content and grain yield of chickpea. Others (Evans, 2005) found indigenous *Rhizobium* strains to be more highly effective symbiotic nitrogen fixers for the uptake of nutrient content and grain yield than introduced commercial inoculants. The current result was also similar with recent report (Tena et al., 2016) that evaluations of rhizobial strains isolated from Ethiopian soils revealed higher rates of N fixation on lentil than the

introduced commercial inoculant. Recently several reports (Chemining'wa et al., 2012; Lamptey et al., 2014; Maleki et al., 2014; Mfiling et al., 2014; Minta and Tsige, 2014; Suryapani et al., 2014) from different countries demonstrated that indigenous rhizobia inoculation improve growth, seed yield, nitrogen fixation and also nutrient up take of legumes. These results indicate that the indigenous chickpea nodulating rhizobial strains used in this study are better adapted to the soil environment and survived in adequate numbers as compared to LtNSTC and LtSK commercial inoculants.

Conclusions

Mesorhizobium strain Cp41, chickpea indigenous rhizobial strain, proved superior in almost all parameters (grain yield, yield component, nodulation, grain protein content, and amount of seed N fixed) as compared to the other inoculants, thus indicating inoculation of chickpea with this particular strain is advantageous in the study area. Chickpea plants that had a longer growth period produced a higher yield (Shasho), compared to plants with a short growth period (Nattoli), when there was adequate moisture during the growing period. This study shows that in locations where rhizobial strains compatible to the target crop are not available, inoculation with appropriate rhizobial inoculants enhance grain yield of the crop. The study, while showing the biodiversity resources available in Ethiopian soils to promote BNF among small-holder producers; it has also indicated the potential that exists to select efficient strains from the indigenous isolates.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Abaidoo RCT, George T, Bohlool BB, Singleton PW (1990). Influence of elevation and applied nitrogen on rhizosphere colonization and competition for nodule occupancy by different rhizobial strains on field-grown soybean and common bean. Can. J. Microbiol. 36:92-96.

Ali A, Ishtiaq M, Jan NE (2003). Effect of Rhizobium Leguminosarum inoculum on the growth and yield of different pea cultivars. Sarhad J. Agric. 19(1):55-59.

Aslam MHKA, Ullah H, Ayaz M, Ahmad E, Sagoo AG, Ullah I, Hussain A, Manzoor M (2010). Nodulation grain yield and grain protein contents as affected by rhizobium inoculation and fertilizer placement in chickpea cultivar bittle-98. Sarhad J. Agric. 26(4):467-474.

Beck DP, Wery J, Saxena MC, Ayadi A (1991). Dinitrogen fixation and nitrogen balance in cool season food legumes. Agron. J. 83:334-341.

Bhuiyan MAH, Khanam D, Khatun MR, Hassan MS (1998). Effect of molybdenum, boron and Rhizobium on nodulation, growth and yield of chickpea. Bull. Inst. Trop. Agric. Kyushu Univ. 21:1-7.

Birhanu M, Pant LM (2012). Effects of Inoculation of Sinorhizobium ciceri and Phosphate Solubilizing Bacteria on Nodulation, Yield and Nitrogen and Phosphorus Uptake of Chickpea (*Cicer arietinum* L.) in Shoa Robit Area. J. Biofertil. Biopestic. 3:129.

Bremer E, van Kessel C (1990). Appraisal of the nitrogen-15 natural abundance method for Quantifying dinitrogen fixation. Soil Sci. Soc. Am. J. 54:404-411.

Chatel DL, Greenwood RM (1973). The location and distribution in soil of rhizobia under senesced annual legume pastures. Soil Biol. Biochem. 5:799-808.

Chemining'wa GN, Ngeno J, Muthomi JW, Shibairo SI (2012). Effectiveness of indigenous pea rhizobia (Rhizobium leguminosarum bv. viciae) in cultivated soils of central Kenya. J. Appl. Biosci. 57:4177- 4185.

CSA (Central Statistical Agency) (2013). Agricultural Sample Survey 2012/2013: Area and Production of Crops (Private Peasant holdings, Meher Season).Statistical Bulletin 532, Addis Ababa, Ethiopia.

Evans J (2005). An evaluation of potential Rhizobium inoculant strains used for pulse production in acidic soils of south-east Australia. Aust. J. Exp. Agric. 45:257-268.

FAO (1984). Ethiopian highlands reclamation study (EHRS). Final report.Vol.1–2. Rome.

Gupta SC, Namdeo SL (1996). Effect of Rhizobium strains on symbiotic traits and grain yield of chickpea. Indian J. Pulses Res. 9(1):94-95.

Jackson ML (1962). Nitrogen determination from soils and plant tissues. In: Soil Chemical Analysis. Constable & Co. Ltd., London. pp. 183-204.

Jensen ES, Hauggaard-Nielsen H (2003). How can increased use of biological N_2 fixation inagriculture benefit the environment? Plant Soil 252:177-186.

Keneni G, Bekele E, Assefa F, Imtiaz M, Debele T, Dagne K, Getu E (2012). Phenotypic diversity for symbio-agronomic characters in Ethiopian chickpea (Cicer arietinum L.) germplasm accessions. Afr. J. Biotechnol. 11(63):12634-12651.

Kyei-Boahen S, Giroux C, Walley FL (2005). Fall vs. spring rhizobial inoculation of chickpea. Can. J. Plant Sci. 85:893-896.

Kyei-Boahen S, Slinkardb AE, Walley FL (2002). Evaluation of Rhizobial Inoculation Methods for Chickpea. Agron. J. 94(4):851-859.

Lamptey S, Ahiabor BDK, Yeboah S, Asamoah C (2014). Response of soybean (Glycine max) to rhizobial inoculation and phosphorus application. J. Exp. Biol. Agric. Sci. 2(1):73-77.

Maleki A, Pournajaf M, Naseri R, Rashunavadi R, Heydari M (2014). The effect of supplemental irrigation, nitrogen levels and inoculation with Rhizobium bacteria on seed quality of chickpea (Cicer arietinum L.) under rainfed conditions. Int. J. Curr. Microbiol. Appl. Sci. 3(6):902-909.

Mfiling A, Mtei K, Ndakidemi P (2014). Effect of Rhizobium Inoculation and Supplementation with Phosphorus and Potassium on Growth and Total Leaf Chlorophyll (Chl) Content of Bush Bean *Phaseolus vulgaris*, L. Agric. Sci. 5:1413-1426.

Minta M, Tsige A (2014). Effect of Rhizobium Inoculation on Herbage Yield, Quality and Nitrogen Fixation of Annual Forage Legumes on Nitisols in Central Highlands of Ethiopia. Acta Adv. Agric. Sci. 2(10):29-48.

Mutch LA, Tamimi SM, Young JPW (2003). Genotypic characterization of rhizobia Nodulating Vicia faba from the soils of Jordon: a

comparison with UK isolates. Soil Biol. Biochem. 35:709-714.
Peoples MB, Herridge DF, Ladha JK (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? Plant Soil 174:328.
Romdhane SB, Tajini F, Trabelsi M, Aouani ME, Mhamdi R (2007). Competition for nodule formation between introduced strains of Mesorhizobium ciceri and the native populations of rhizobia nodulating chickpea (Cicer arietinum) in Tunisia. World J. Microbiol. Biotechnol. 23:1195-1201.
Rupela OP, Saxena MC (1987). Nodulation and Nitrogen Fixation in chickpea. In: M.C. Saxena and K.B. Singh, editors, The Chickpea. CAB International, Wallingford, Oxon. pp. 191-206.
Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey R, Giller K, Alves B, Chalk P (2008). Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph No. 136, 258 p.
SAS Institute, Inc. (2012). *The SAS System for Windows. Release 9.3*; SAS Institute, Inc.: Cary, NC, USA.
Somasegaran P, Hoben H (1994). Hand book for Rhizobia. Springer-Verlang, New York. pp. 1-138.
Suryapani S, Malik AA, Sareer O, Umar S (2014). Potassium and Rhizobium application to improve quantitative and qualitative traits of lentil (Lens culinaris Medik.). Int. J. Agron. Agric. Res. 5(3):7-16.
Tena W, Wolde-Meskel E, Walley F (2016). Symbiotic Efficiency of Native and Exotic Rhizobium Strains Nodulating Lentil (Lens culinaris Medik.) in Soils of Southern Ethiopia. Agronomy 6(11):1-10.
Van Reeuwijk LP (2002). Procedures for soil analysis. International Soil Reference and Information Center, Netherlands. Sixth edition.
Vincent JM (1970). A Manual for the Practical Study of Root Nodule Bacteria. Blackwell, Oxford.
Walley FL, George WC, Perry RM, Patrick MC, Guy PL (2006). Nitrogen Economy of Pulse Crop Production in the Northern Great Plains. Agron. J. 99(6):1710-1718.
Yadav J, Verma JP, Rajak VK, Tiwari KN (2011). Selection of Effective Indigenous Rhizobium Strain for Seed Inoculation of Chickpea (*Cicer aritenium* L.) Production. Bacteriol. J. 1:24-30.