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Symbiotic Effectiveness of Elite Rhizobia Strains Nodulating Desi Type Chickpea (*Cicer arietinum* L.) Varieties

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Abstract: Chickpea (*Cicer arietinum* L.), is a multi-functional crop with important role in the diet as affordable protein source and in sustaining soil fertility through nitrogen fixation. However, its productivity in Ethiopia of 1.9 t ha^{-1} is lower than its potential of 5 t ha^{-1} under well managed conditions, partly due to soil fertility limitations. Field experiments were conducted to evaluate effectiveness of elite *rhizobia* strains on productivity of chickpea. Four *rhizobial* inoculant treatments and one control with three chickpea varieties were used. Inoculated plants had significantly ($p<0.05$) better performance with most of the symbiotic traits, grain yield and yield related traits than non-inoculated treatments. Shoot nitrogen yield was increased in the range of 13.0 – 31.34% by inoculation with strain ICRE-025 over the two test sites. The highest level of N fixation was achieved in genotype ICC-4918 by inoculation with EAL-029 and ICRE-025. Investigations at both test sites demonstrated that inoculation of chickpea varieties with native *rhizobial* strains were effective and useful for optimized chickpea production.

Keywords: Desi Chickpea, Symbiotic Effectiveness, Nitrogen Fixation, *Rhizobial* Inoculant

1. Introduction

Chickpea (*Cicer arietinum* L.) is a major grain legume cultivated for its edible seeds. Across the world chickpeas are mainly cultivated in the cool, dry season of the semi-arid tropics on residual moisture [1]. In Ethiopia, chickpea is mainly grown in the central, northern and eastern highland areas of the country at an altitude range of 1400-2300 m.a.s.l., where annual rainfall of 500 - 2000 mm is received on vertisols with good water holding capacity [1, 2]. Ethiopian farmers essentially plant chickpea on residual moisture after the end of the main rainy season, usually in late August – October [1].

Chickpea, a multi-functional crop, has an important role in the diet of the Ethiopian small scale farmers' households and also serves as protein source for the rural poor who cannot afford to buy animal products [3]. The crop also serves as a source of cash income and plays a major role in Ethiopia's foreign exchange earnings through export to Asia and Europe [4]. Due to its capacity of biological nitrogen fixation; chickpea can improve the soil fertility status [3, 5]. Recent research shows that chickpea can fix more nitrogen than other pulse crops, thus enhancing soil fertility for subsequent crops [6]. The *Rhizobium* bacteria that are compatible for nodule formation on the roots of chickpea are different from those that nodulate peas and lentils. Thus inoculating chickpea seed with the correct inoculant is critical.

In spite of all these merits and benefits, the productivity of this crop remains low in Ethiopia. While yields of up to 5.5 tons ha^{-1} have been obtained on experimental stations in Ethiopia [7], the latest average national productivity reported is as low as 1.9 tons ha^{-1} [8]. This yield gap between average and potential yield of chickpea could be due to many factors, of which environmental constraint associated with the absence of specific and effective *rhizobia* in the soil is one of the most important [9] that affect the productivity of the crop.

Different reports indicate that chickpea fixes atmospheric nitrogen in the range of 60 - 140kg N ha^{-1} in one cropping season [10, 11, 12]. There is increasing evidence that suggests that more nitrogen can be fixed by existing legume grain crops if they are inoculated more often or with more effective strains of *Rhizobia* [13]. Additionally Ayaz *et al* [14] reported that inoculation of seed with its own specific and suitable *Rhizobium* strain before planting is crucial to fully benefit from grain legume crop in terms of maximum yield and soil improvement.

Ethiopia has a great potential of benefiting from enhanced BNF that may contribute to increased yield and sustained soil fertility. This is because larger area is devoted to grow chickpea as compared to other African counties [8]; there are many *rhizobia* strains that have been identified and proven to be effective and competitive [15]; there are also a number of high yielding released varieties [16]. However, use of high yielding varieties alone may not result in the required level of productivity without involvement of compatible and effective *rhizobia* strains [9, 17].

A given legume cultivar nodulated by different strains of the same species of *rhizobium* would fix different amounts of nitrogen. And it is also true that a given strain of *Rhizobium* will nodulate and fix different amount of N in symbiosis with a range of cultivars of the same plant species [9]. Keneni *et al* [18] has also suggested that there may be the need to conduct specific tests for specific breeding materials, strain and environments to improve the selection process to gain host genotype compatible to specific *rhizobia* strain. However, our knowledge on effective rhizobia strains for efficient symbiosis with chickpea varieties is scanty. Besides, the efforts to test and recommend rhizobia inoculums on-farm have been limited. Chickpea farmers therefore traditionally produce the crop without the use of biofertilizers. This study was conducted to evaluate effectiveness of elite *rhizobia* strains on productivity of improved chickpea (*Cicer arietinum* L.) varieties at Debre Zeit and Wolayta Sodo, Ethiopia.

2. Material and Methods

2.1. The Test Environment

The experiment was conducted under field conditions at Debre Zeit (representing the central highlands) and Wolayta Sodo (representing southern region) of Ethiopia during the main cropping season of 2015/2016 (September–January). Soil samples from both locations were collected from the rhizosphere at the depth of 0 to 30 cm for physico-chemical characterization (table 1).

Table 1. *Description of test sites and physico-chemical properties of soil.*

Parameters	Debre Zeit	Wolayta Sodo
Latitude	8.73°N	7.04°N
Longitude	38.97°E	37.2°E
Altitude (m, a. s)	1900	1880
pH (1:1.25 H_2O)	6.61 (Slightly acidic)	6.00 (Slightly acidic)
Organic Carbon (%)[a]	1.2 (Low)	1.67 (medium)
Organic Matter (%)	2.1 (Low)	2.87 (Low)
Total Nitrogen (%)[b]	0.16 (medium)	0.19 (medium)
Available phosphorus (ppm)[c]	73.25 (excess)	9.17 (Low)
Exch K (cmol)/kg[d]	0.40 (High)	0.23 (High)
CEC (cmol)/kg[e]	39.89 (High)	19.70 (Medium)
Moisture content (%)	4.54	1.49
Soil texture[f]		
Clay (%)	61.60	64.6
Silt (%)	31.40	23.4
Sand (%)	7.0	12.0

Method: a = Walklay and Black; b = Kjeldahl; c = Olsen; d &e = Ammonium acetate; f = Hydrometer; ppm = parts per million

2.2. Treatments and Layout

The experiment was conducted using three chickpea verities (Natoli, Teketay and ICC-4918) with four levels of *rhizobia* strains (EAL-029, ICRE-025, ICRE-03 and ICRE-05) and a control (non-inoculated) were used in split plot design with three replications. Chickpea varieties as main plot and the four *rhizobium* inoculants and a control as sub plots were assigned to experimental units randomly. Each of the main plots was divided into five sub plots having plot size of 4m by 1.2m ($4.8m^2$). The spacing of 30 cm and 10 cm was used between and intra row respectively. Accordingly, each sub plots consisted of four rows out of which the outer two rows were used as border rows and in each rows 40 – 45 seeds were planted. Phosphorus fertilizer in the form of TSP was applied to all plots at the rate of 100 kg ha^{-1}. All other crop management and protection practices were applied uniformly to all experimental units as required.

2.3. Inoculants Preparation and Method Used

Three elite *Rhizobia* strains (ICRE-025, ICRE-03 and ICRE-05) proven to be effective during greenhouse and laboratory studies were obtained from the soil microbiology laboratory of the School of Plant and Horticultural Sciences, Hawassa University, Ethiopia and the commercial biofertilizer EAL-029 was obtained from National Soil Laboratory of Ethiopia. All the necessary aseptic precautions were taken when preparing inoculums and inoculation. Accordingly, using peat based inoculation method at the recommended rate of 10g per kilogram of seed, 200g of chickpea seed was soaked in 30 ml of 5% sugar solution. The contents were stirred well. Sugar solution improves the adhesion of inoculant to the seed. Then 2g of inoculant was added on the wetted seed and mixed in a plastic bag until all the seeds were uniformly coated. The whole inoculation procedure was completed in shade as sunlight damages the bacteria. Seed was sown immediately after inoculation.

2.4. Soil Characteristics of the Study Sites

The physico-chemical properties of the soils from the two test locations, Debre Zeit and Wolayta Sodo, showed medium levels of nitrogen contents (0.16 and 0.19%) but high levels of K. The levels of CEC (39.89 cmol kg^{-1}) was high at Debre Zeit and medium (19.70 cmol kg^{-1}) at Wolayta Sodo with pH values slightly acidic ranging from 6.0 to 6.61 which is within the optimum pH range for crop production (6.0 - 8.2) [20]. It was also found to be favorable for chickpea infective strains of *Rhizobia* [21]. The level of soil phosphorus was excessively high at Debre Zeit (73.25 ppm) and low at Wolayta Sodo (9.17 ppm) (Table 1). Generally soils at the two experimental sites showed minor differences in their content of major nutrients and characteristics, with Debre Zeit soil somewhat more fertile than Wolayta Soda soil.

The amount of rainfall received by the two test sites was nearly the same within the growing season of chickpea. Debre Zeit received 228 mm while Wolayta Sodo received 187 mm rainfall from August to December 2015 (figure 1). However, it was much lower than the minimum requirement (500 mm) reported by Bejiga and van der Maesen [1]. During the study period the weather variables recorded deviated much from the long-term trends at both locations, causing irregular distribution and low amount of rainfall. The growing season was generally characterized by erratic form of rainfall and hence growth of chickpea and activity of micro organisms in the soil to some extent might be adversely affected.

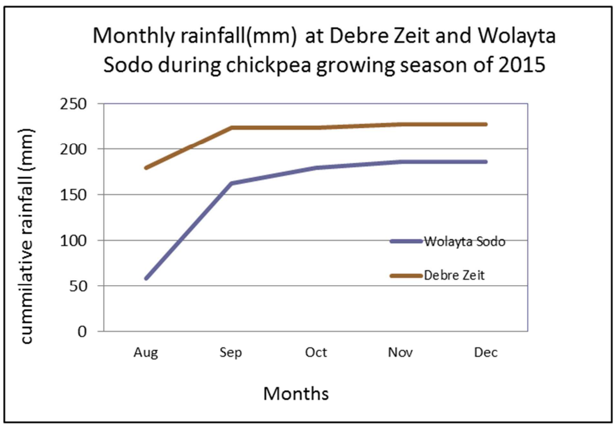

Figure 1. *Graph of monthly rainfall (mm) during 2015 chickpea growing season at Debre Zeit and Wolayta Sodo.*

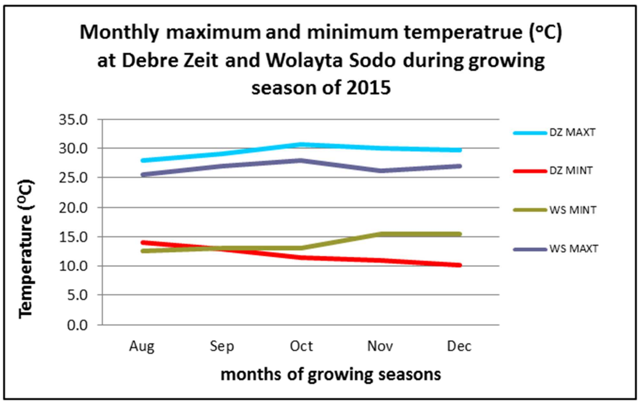

Figure 1. *Graph of monthly maximum and minimum temperature (°C) during 2015 chickpea growing season at Debre Zeit and Wolayta Sodo.*

NB: DZ MAXT= Debre Zeit max. temperature, DZ MINT= Debre Zeit min. temperature,
WS MAXT= Wolayta Sodo max. temperature, WS MINT= Wolayta Sodo min. temperature

2.5. Data Collection

Nodulation was assessed at the mid-flowering stage of chickpea. Five representative plants were randomly taken from the border row on each side of the plot for nodule number and nodule dry weight determination. Crop phenological data including days to 50% flowering (DF) and days to 90% maturity (DTM) were also taken at respective growth stages. Five plants from each plot were sampled at the time of harvest and separated into grain and straw for plant tissue analysis for nitrogen content. The straw part was oven dried at 70°C for 48 hours, milled with mechanical miller and passed through a 1mm sieve. Subsequently, the plant straw was analyzed for total N. The straw total N uptake was calculated as a product of their respective straw yield obtained from five plants and N content obtained from the lab analysis i.e., N uptake (shoot dry weight x N concentration) $plant^{-5}$. Nitrogen fixed was determined using the procedure formulated by Yaman and Cinsoy [19] as follows: N fixed (N uptake in an inoculated variety – N uptake in uninoculated variety) were calculated. Thus, the following data were recorded as: shoot nitrogen content (SHNC), shoot nitrogen uptake in gm $plant^{-5}$ (SHNY). Percent change (increase or decrease) in data of nodulation traits, grain yield and nitrogen uptake for inoculated treatments was obtained by the formula given below.

$$\%\text{Change } (\Delta) = \left[\frac{Mi - Mu}{Mu}\right] x\ 100$$

Where, Mi = Mean of inoculated variety, and Mu = Mean of uninoculated variety

2.6. Data Analysis

Statistical analysis of data collected from the field and laboratory was analyzed using mixed linear model procedure of SAS 9.3 after checking the compliance of the data with the assumptions of the statistical test. Mean separation test was done using LSD at p-value 0.05.

3. Results

3.1. Effect of Inoculation on Nodulation Performance of Chickpea

Inoculation of chickpea varieties by Rhizobial strains affected significantly ($p < 0.05$) nodule count plant-1 (NCPP), nodule dry weight (NDW) at Wolayta Sodo. Inoculation did not affect the nodulation status and nodule dry weight of chickpea at Debre Zeit. However, inoculated varieties produced higher number of nodules over control treatments. In the experiment conducted at Wolayta Sodo, varieties inoculated with strains ICRE-03 produced the highest nodule dry weight (0.29 gm plant-1), followed by those treated with strains EAL-029 (0.28gm plant-1) and ICER-05 (0.28 gm plant-1) (table 3).

3.2. Effect of Inoculation on Symbiotic Nitrogen Fixation of Chickpea

Shoot nitrogen content (SHNC) at Wolayta Sodo and shoot dry weight (SHDW), and shoot nitrogen yield (SHNY) were also affected significantly ($p<=0.05$) at Debre Zeit. Shoot nitrogen content (SHNC) of the chickpea varieties was not affected by inoculation at Debre Zeit. However, inoculated varieties had higher nitrogen content over control treatment. Similarly, in an experiment conducted at Wolayta Sodo the highest shoot nitrogen content was recorded in varieties inoculated with strain ICRE-025 (0.62%) followed by ICRE-03 (0.61%).

Rhizobium inoculation affected shoot dry weigh of chickpea varieties at Debre Zeit and increased by 5.7 -16.0% over control. The highest increment was achieved by inoculation with strain ICRE-03. At Wolayta Sodo inoculation did not affect shoot dry weight significantly but varieties inoculated with Rhizobia strains had 9.4 - 21.6% higher shoot dry weight than the control (Table 3).

Shoot nitrogen yield (SHNY) was also affected by rhizobial inoculation at both test sites. In the experiment conducted at Debre Zeit the highest shoot nitrogen yield (23.7 gm plant-5) was recorded from varieties inoculated with Rhizobia ICRE-03, followed by strain EAL-029 (23.24 gm plant-5). Similarly, at Wolayta Sodo the highest shoot nitrogen yield (32.1 gm plant-5) was recorded from varieties inoculated with rhizobia ICRE-025, followed by strain ICRE-03 (30.8 gm plant-5) (Table 2 and 3)

Table 2. *Effect of Rhizobia inoculation on nodulation and symbiotic nitrogen fixation of chickpea varieties by Rhizobia strains tested at Debre Zeit, Ethiopia.*

Variety	*Rhizobia* strain	NCPP	NDW	DTM	SHDW	SHNC	SHNY	N fixed	RE (%)
		--Varieties ----------------------------------							
ICC-4918		12.4	0.26	97.07a	63.6a	0.46	29.4a	3.9	22.8
NATOLI		14.0	0.27	90.6b	54.2ab	0.42	22.6ab	1.9	17.3
TEKETAY		13.1	0.22	89.27b	39.3b	0.37	14.6b	1.6	15.0
		---*Rhizobia* strains -----------------------------------							
	Control	12.27	0.16b	92.22	49.79	0.397	20.24	0.00	0.00
	EAL-029	13.24	0.29a	91.89	51.85	0.439	23.24	3.00	23.88
	ICRE-025	12.71	0.26a	91.78	51.35	0.423	22.42	2.18	13.03
	ICRE-03	13.47	0.26a	93.00	56.46	0.417	23.70	3.46	21.29
	ICRE-05	14.24	0.27a	92.67	52.35	0.397	21.38	1.14	15.18
	Ino-mean	13.42	0.27	92.34	53.00	0.42	22.69	2.45	18.35
P-value (< 0.05)									
	Variety	ns	ns	**	*	ns	*	ns	ns
	Rhizobia	ns	*	ns	ns	ns	Ns	ns	ns
	R x V	ns	ns	ns	ns	ns	Ns	ns	Ns

NCPP = nodule count plant^{-1}, NDW =nodule dry weight plant^{-1}, DTM= days to 90% maturity, SHDW = shoot dry weight gm plant^{-1}, SHNC = shoot nitrogen content (%), SHNY = shoot nitrogen yield gm plant^{-5}, N fixed = nitrogen fixed gm plant^{-5} and RE (%) = Relative effectiveness (%); ** = Significant (P <= 0.01), * = significant (P <= 0.05) and ns=non-significant (P> 0.05), V= Variety, R= *Rhizobia* strain and V*R= the interaction of variety and *Rhizobia* strain, Ino-mean = mean of inoculated treatments. Means with the same letter are not significantly different

Table 3. *Effect of Rhizobia inoculation on nodulation and symbiotic nitrogen fixation of Chickpea varieties tested at Wolayta Sodo, Ethiopia.*

Variety	*Rhizobia* strain	NCPP	NDW	DTM	SHDW	SHNC	SHNY	N fixed	RE (%)
		-- variety ---------------------------------------							
ICC-4918		12.5b	0.25ab	117.3	48.4	0.65	31.4	9.0	3.9
NATOLI		17.7a	0.34a	110.7	54.9	0.57	31.5	4.1	19.0
TEKETAY		07.9c	0.16b	109.9	43.9	0.55	24.0	2.2	10.0
		--------------------------------------- *Rhizobia* strains --------------------------------							
	Control	11.04b	0.14b	112.2	44.11	0.569	25.03c	-	-
	EAL-029	14.13a	0.28a	112.7	47.45	0.576	27.11bc	2.77	15.15
	ICRE-025	12.64ab	0.25a	112.6	50.98	0.621	32.06a	7.02	31.34
	ICRE-03	13.98a	0.29a	113.2	50.18	0.612	30.76ab	5.73	25.84

Variety	*Rhizobia* strain	NCPP	NDW	DTM	SHDW	SHNC	SHNY	N fixed	RE (%)
	ICRE-05	11.60b	0.28a	112.6	52.24	0.569	29.97ab	4.94	22.65
	Ino-mean	13.09	0.28	112.78	50.21	0.59	29.98	5.12	23.75
P-value (< 0.05)									
	Variety	**	*	ns	ns	Ns	ns	ns	ns
	Rhizobia	*	**	ns	ns	Ns	*	ns	ns
	R x V	ns	ns	ns	ns	*	ns	ns	ns

NCPP = nodule count plant^{-1}, NDW =nodule dry weight plant^{-1}, DTM= days to 90% maturity, SHDW = shoot dry weight gm plant^{-1}, SHNC = shoot nitrogen content (%), SHNY = shoot nitrogen yield gm plant^{-5}, N fixed = nitrogen fixed gm plant-5 and RE (%) = Relative effectiveness (%) ** = Significant (P <= 0.01), * = significant (P <= 0.05) and ns=non-significant (P> 0.05), V= Variety, R= *Rhizobia* strain and V*R= the interaction of variety and *Rhizobia* strain, -mean = mean of inoculated treatments, Means with the same letter are not significantly different.

4. Discussion

4.1. Effect of Inoculation on Nodulation Performance of Chickpea

In the present study all chickpea varieties showed nodulation in uninoculated treatment, confirming the presence of pre-existing native *Rhizobia* in the soil. Thus significant variation as a result of inoculation was observed among the genotypes for nodulation traits at Wolayta Sodo (Table 3). Though significant variations were not observed for nodule count (NCPP) and nodule dry weight (NDW) at Debre Zeit, inoculated varieties produced higher NCPP, NDW (Table 2). There was a strong genotypic/varietal effect on nodulation and nitrogen fixation in this study. Genotype ICC-4918 was included to the experiment as non nodulating check but was found to be nodulating. Regarding non nodulating genotypes of chickpea, there are recently published reports of findings which are contradicting. Gul et al [22] reported that the four genotypes (ICC 4993, ICC 19183, ICC 4918 and ICC19181) purchased from ICRISAT did not produce any nodule in control as well as inoculated treatment while other treatments produced nodule even without inoculation. On another finding Gul et al [23] also point out that ICC-4918 was late maturing and low yielding genotype being used as non nodulating check. In contrast to this Keneni et al [18] reported that a non nodulating genotype ICC-19180 was found to be nodulating with best performance for grain nitrogen yield and assimilation efficiency of fixed nitrogen and suggested that whether a change in environment alone can induce nodulation of a genotype that is naturally non-nodulating in another environment needs to be investigated in the future.

In the current study the nodules were observed and collected not only from inoculated seed of the genotype ICC-4918 but also was from the uninoculated plots where ICC-4918 was planted at both test sites. The findings from this study supports the suggestion given by Keneni et al [18] but postulates that the reason for ICC-4918 failing to nodulate in earlier studies in India was basically due to lack of compatible Rhizobia strains. It is plausible to conclude here that the efficiency of nodulation and nitrogen fixation depends on the compatibility between the chickpea genotype and the Rhizobia strain and the environmental conditions mostly soil factors.

4.2. Effect of Inoculation on Symbiotic Nitrogen Fixation of Chickpea

Rhizobial inoculation affected significantly (p<= 0.05) shoot dry weight and shoot nitrogen yield both at the two test sites. However, the response to inoculation was higher at Wolayta Sodo for most of measurements of symbiotic performances. For instance mean performance of shoot nitrogen content (0.59 %) was higher than that of SHNC (0.42 %) at Debre Zeit. SHNY (28.99 gm plant^{-1}), amount of nitrogen fixed (5.11 gm plant^{-5}) and relative effectiveness in terms of percentage of fixed nitrogen increased over uninoculated treatment (23.75%) were also higher than that of Debre Zeit (18.35%) (Table 2 and 3). The reason could be due to less fertile soil (table 1) and low presence of indigenous population of *Rhizobia* at Wolayta Sodo [4]. Imran et al [24] also reported that inoculation response was more significant in soils having lower indigenous rhizobial population and fertility. On the other hand Debre Zeit was less responsive to inoculation for symbiotic characters of chickpea. Soil fertility status (table 1) has great impact on the response of chickpea variety to inoculation. In fact, Debre Zeit is the center of chickpea breeding and production for many years and hence there might be high rate of native *rhizobia* population in the soil capable of hindering the development of the new test *Rhizobia* strains used in the present study. There have been several reports by many authors such as [25, 26, 27, 28] that the presence of native *Rhizobia* is a major problem for inoculant performance under field conditions.

Depending on variety and *Rhizobia* strain used, the percentage of fixed nitrogen increased over control (uninoculated treatment) of the two test sites were from 13.01- 31.34% (Table 2 and 3) in chickpea shoot dry weight. The potential of fixation by the genotypes may be generally limited because of a shortage of soil moisture as chickpea crop normally grown on residual moisture [18]. Moreover, the climate of the growing season deviated from the long term trend due to El Niño effect that caused short and erratic rainfall resulting in longer dry periods that might have affected the development of crop and soil micro organisms. Beck & Rupela [29] reported that fixation in winter-sown chickpea reached over 80–81%, whereas spring-sown chickpea, where moisture was a limiting factor, fixed only 8–27%. Additionally, Bergersen [30] reported that the nature of soil *Rhizobial* populations may affect the nitrogen fixation potential of legumes.

To have successful inoculation with an effective isolate that would result in an enhanced nitrogen fixation, one or both of the following conditions should be in place. First, the number of available invasive *Rhizobia* may be insufficient to nodulate the host adequately. Second, the average effectiveness of the population in the soil has to be inadequate to support the host's fixed nitrogen requirements [30]. Difference in method of determination of the amount of nitrogen may also underestimate the amount of nitrogen fixed [31]. *Rhizobial* inoculation also affected other associated characters of symbiotic nitrogen fixation. Chickpea varieties inoculated with strain EAL-029 produced higher nodule number, nodule dry weight and shoot nitrogen content followed by strain ICRE-03 at par for the two test sites. High fixed nitrogen in the shoot part of chickpea and high percentage increase of fixed nitrogen over the control treatment was also obtained from varieties inoculated with ICRE-03 followed by ICRE-025 (Table 2 and 3) at the two test sites with variable magnitude implying that the elite *Rhizobia* strains were performing at equal level with the standard check (EAL-029) even at higher magnitude. Generally chickpea varieties treated with *rhizobial* inoculant produced higher mean value of symbiotic traits and the current results are in agreement with previous reports of Tena *et al* [32], Beshir *et al* [33], Abdula [4] and Argaw [34] that were conducted on chickpea and other grain legumes in which inoculation increased parameters of symbiotic and agronomic traits.

Another unique result of this study regarding non nodulating genotype ICC-4819 was its superior performance in symbiotic trait measurements. The highest shoot dry weight (63.39 gm plant^{-1} at Debre Zeit) and the highest nitrogen concentration (0.46% at Debre Zeit, 0.65% at Wolayta Sodo) were also observed from straw sample of genotype ICC-4918. The possible explanation for high shoot dry weight and high nitrogen concentration in genotype ICC-4918 was its late maturity. In fact DTM for ICC-4918 was 97 days and 117 days at Debre Zeit and Wolayta Sodo respectively (table 2 and 3), that means it was late by 7 days from early maturing variety. According to Bidlack et al [35] findings, late maturing varieties accumulated more biomass in vegetative shoot components (leaf and stem) while early maturing varieties partitioned more photosynthate into reproductive structures (flower, pods, seeds).

Regarding compatibility and effectiveness of elite indigenous rhizobia in the current study, strains ICRE-03, IRCE-025 and ICRE-05 were found to be superior in most symbiotic parameters measured compared to the standard check (EAL-029). For instance comparison of rhizobia strains performance in measurements of shoot dry weight and relative effectiveness (Percentage of nitrogen increased), chickpea varieties inoculated with strain ICRE-03 had the highest shoot dry weight (56.46 gm plant^{-1}) followed by strain ICRE-05 (52.35 gm plant^{-1}) at Debre Zeit. Similarly in the experiment conducted at Wolayta Sodo varieties inoculated with strains ICRE-05 had the highest shoot dry weight (52.24 gm plant^{-1}), followed by strain ICRE-025 (50.98 gm plant^{-1}). Other comparison criteria for effectiveness of rhizobia in symbiotic nitrogen fixation is a parameter known as relative effectiveness (RE) as reported by Imran et al [24] and Maâtallah et al [36]. Thus, chickpea varieties inoculated with strain EAL-029 had the highest (23.88%), relative effectiveness value followed by strain ICRE-03 (21.29%) at Debre Zeit. Similarly, under experiment conducted at Wolayta Sodo varieties inoculated with strain ICRE-025 gave the highest relative effectiveness (31.34%) followed by strain ICRE-03 having relative effectiveness value of 25.84% implying that all the elite rhizobia strains under the study were compatible and effective in symbiotic nitrogen fixation as long as biotic and abiotic factors were kept constant.

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