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Response of Chickpea (*Cicer arietinum L.*) to Indigenous Rhizobial Isolates Inoculation on Vertisol of Central Ethiopian Highland

Abere Mnalku and Getahun Mitiku

Ethiopian Institute of Agricultural Research, Holetta Agricultural Research Center, P.O.Box: 2003, Addis Ababa, Ethiopia; Corresponding e-mail: aberemnalku1972@gmail.com

አህፅሮት

እንደሽምብራ የመሳሰሉትን የጥራጥሬ ሰብሎችን በሃገር-በቀል ልይት ራይዞቢያ መከተብ ምርታማና ትርፋማ ያደርጋል፡፡ ምዕራብ ሸዋ፣ ደንዲ ወረዳ ውስጥ ጊንጪ ላይ የተለያዩ ሃገር-በቀል ልይት ራይዞቢያዎች በሽምብራ ምርትና አባላተ-ምርት ላይ የሚኖራቸውን ተጽዕኖ ለማጥናት በ2008 እና 2009 የምርት ዘመናት የመስክ ሙከራ ተካሂዷል፡፡ ለጥናቱም ስምንት ልይት ራይዞቢያዎች ማለትም CP-5፣ CP-7፣ CP-9፣ CP-10፣ CP-11፣ CP-17፣ CP-100 እና EAL-004 ከ 18 ኪ.ግ. ናይትሮጅን በሄክታር እና ናይትሮጅን-አልባ ማወዳደሪያዎች ጋር ተወዳድረዋል፡፡ ጥናቱም በንስብ ምሉዕ ብሎክ ንድፍ መሰረት መሬት ላይ ያረፈ ሲሆን እያንዳንዱ ትሪትመንት ሶስት ድግግሞሽ ነበረው፡፡ ውጤቱም አዎንታዊ የሽምብራ ምርት ምላሽ እንዳለ ያመለክታል፡፡ ዝንቡጠ-ተክል ቁጥር፣ ገጸ-ምድር ደረቅ ክቡደ-ህይወት እና እህል ምርት በራይዞቢያ በአዎንታዊነት አጥጋቢ ጭማሪ አሳይተዋል፡፡ በመሆኑም CP-17 ልይት ራይዞቢያ ከ18 ኪ.ግ. ናይትሮጅን በሄክታር እና ከናይትሮጅን-አልባ ጋር ሲወዳደር በ5 እና 10 በመቶ፣በቅ/ተከተል የእህል ምርት ብልጫ አሳይቷል፡፡ የከፊል ባጀት ትንተና ውጤት የሚያሳየውም ይሄው ልይት ራይዞቢያ ሽምብራ ዘር ላይ ሲከተብ የምጣኔ-ሀዳግ ትርፉ በአንጻራዊነት ከፍተኛ መሆኑን ነው፡፡ ስለሆነም CP-17 ልይት ራይዞቢያ የማረጋገጫ ስራ ተሰርቶለት በደንዲ ወረዳ አካባቢ ጥቅም ላይ እንዲውል ይመከራል፡፡

Abstract

A field experiment was conducted at Vertisol of Ginchi, Dandi district, West Shewa, Ethiopia during 2015/16 and 2016/17 cropping seasons to study the effect of different indigenous rhizobial isolates on the yield and yield components of chickpea. Eight rhizobial isolates (CP-5, CP-7, CP-9, CP-10, CP-11, CP-17, CP-100 and EAL-004), one positive control (18 kg N ha^{-1}) and one negative control (no fertilizer source) were the treatments. The treatments were laid in randomized complete block design with three replications. Results indicated a positive response of chickpea grain yield to rhizobial isolate inoculations. Number of pod per plant, above ground dry biomass and grain yield were positively ($P \leq 0.05$) altered by the inoculation. Inoculation of rhizobial isolate CP-17 showed a 5 and 10% grain yield increment over 18 kg N/ha and negative control treatments, respectively. The partial budget analysis result showed that application of CP-17 gave the highest marginal rate of return (MRR). Therefore, the rhizobial inoculant CP-17 can be verified and recommended for wider use of chickpea production at Dandi area.

Introduction

Chickpeas are grown with the residual end of season soil moisture in Vertisol areas where water-logging hinders agricultural practices at the height of the rainy season (Getachew and Woldeyesus, 2012). The crop is traditionally grown with minimal external inputs and does not require thorough land preparation like for cereals. Given the high cost of fertilizers, cereal rotations with nitrogen-fixing chickpeas have traditionally been used for improving the productivity of the following cereal crop (MoARD, 2003; Getachew and Woldeyesus, 2012).

Biological nitrogen fixation offers a natural means of providing nitrogen for plants. It is a critical component of many aquatic, as well as terrestrial ecosystems across our biosphere (Wagner, 2011). Rhizobium symbiosis with legume species is of special importance, producing 50% of 175 million tons of total biological N_2 fixation annually worldwide (Sarıoğlu *et al*., 1993). Chickpea (*Cicer arietinum* L.) and *Rhizobium leguminosarum* bv. ciceri association annually produce up to 176 kg N ha^{-1} depending on cultivar, bacterial strain, and environmental factors (Beck *et al*., 1991).

Chickpea has highly specific symbiotic association, with a unique group of rhizobia necessary for formation of nodules and nitrogen fixation. Absence of suitable strains, small population size, and poor survival of rhizobia cause problems in nodules formation (Kantar *et al*., 2007). To avoid uncertainty about natural inoculation, legume seed should be inoculated every time. According to Romdhane *et al*. (2009) chickpea yield can be enhanced by inoculation with competitive rhizobia.

Artificial seed inoculation of chickpea particularly in soils lacking native effective rhizobia is a very useful practice for improving root nodulation and yield of the crop (Muhammad *et al*., 2010). Inoculation increases soil nitrogen along with the increase in root and shoot nitrogen (Ahmed *et al*., 2008). In light of the above and existing situation, the research work was planned to assess the performances of native chickpea rhizobial isolates nodulation potential, yield improving capacity and economic feasibility.

Material and Methods

Experimental site

The study was conducted for two consecutive main growing seasons at rain-fed condition on Vertisol of Dendi district, Ethiopia during 2015/16-2016/17 on nearby sites of no inoculation history in the last five years. The sites are located between 09°02'N latitude and 38°12'E longitude at an altitude of 2200 meters above sea level at 65 km West of Addis Ababa.

Soil sampling and analysis

Five representative soil sub-samples were composited to one at each trial site to carry out proper soil test and interpretation. The chemical properties of soils in the experimental fields taken before planting Table (1) were determined using the standard analytical methods in the Soil and Plant Analysis Laboratory of Holetta Agricultural Research Center.

Inoculants preparation and use

Chickpea inoculant, which was prepared by mixing 20 ml broth culture with 100 g lignite carrier, was stirred well, transferred to the seed lot, and uniformly coated under the shade. Coated seeds were sown immediately after inoculation. All rhizobial isolates were evaluated for number of pod $plant^{-1}$, above ground biomass yield and grain yield $plant^{-1}$ in both years.

Treatment, design, and management

Eight symbiotically effective indigenous rhizobial isolates namely CP-5, CP-7, CP-9, CP-10, CP-11, CP-17, CP100 and EAL-004 were evaluated for their nodulation, above ground biomass and seed yield increasing characteristics at on-farm in comparison to negative and positive controls. The experimental design used was randomized complete block design (RCBD) with three replications. The variety used for the experiment was *Arerty* at a seed rate of 140 kg ha^{-1} on flat bed. Phosphorus was applied at a rate of forty-six (46) kg P_2O_5 ha^{-1} uniformly to all plots in the form of TSP during planting time. Other management practices including weeding, hoeing, pesticide spraying, thinning etc. were uniform to all experimental plots.

After 60 days, nodule count was made by uprooting five random plants $plot^{-1}$ and nodule dry weight was measured by oven drying at 70^{o}C for 48 hours. Grain yield was measured destructively by excluding outer hills and rows of each plot via moisture adjustment to 12%. All these data were subjected to ANoVA and accompanied mean separation (at 5% probability level) by Statistix 10 software.

Partial budget analysis was made to investigate the economic feasibility of rhizobial isolates inoculation. Partial budget, dominance and marginal analyses were used. The average grain yield was adjusted downward by 15% to reflect what farmers could get under their own management on large plots of land (Wagner, 2011). The two years (2015/16-2016/17) mean farm gate prices for chickpea (ETB 16.50 kg^{-1}) and average man days (ETB 38.00) were used in the analysis. In this study, for a treatment to be considered as a worthwhile option to farmers, the minimum acceptable marginal rate of return (MRR) was considered to be 100% (Gorfu *et al*., 1991).

Results and Discussion

Weather

According to Croser *et al*. (2003), the most common abiotic stresses affecting chickpea production, in the order of importance, are drought, heat and frost. The actual growing period of chickpea in the site is from mid-September to late January. The amount of rain fall received in those months was 257.7 and 204.5 mm, for the respective years (Figure 1). As chickpea is normally grown using the residual moisture after the main rain season, the amount and distribution of rainfall was favorable for its growth and development in both years. Likewise, the average mean monthly temperatures recorded during the growing periods were 17 and 16^{o}C, respectively (Figure 1).

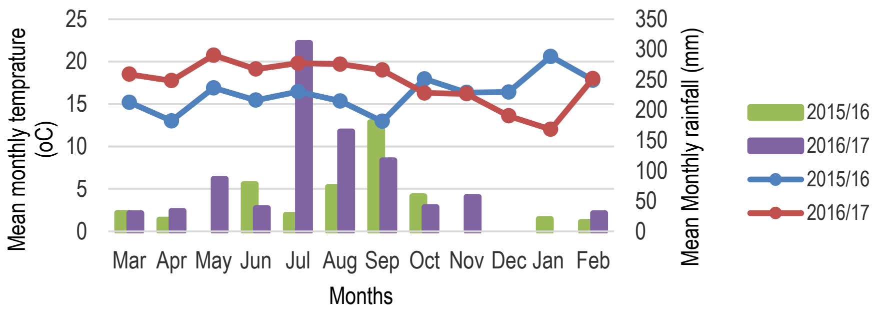

Figure 1.Seasonal temperature and rainfall records (Hint: the bar and lines are to the monthly rainfall and mean monthly temperature, respectively)

The weather condition was, thus, optimal for the growth and production of chickpea (EEPA, 2004). Neither terminal drought nor water logging that would negatively influence chickpea performance was occurred. However, the light rain showered during November 2016/17 might have been critical as the time was the flower initiation period of the crop.

Soil analysis

As it is presented in Table 1, the experimental sites possess clay textured and low total nitrogen soil. The average soil pH of the trial sites was 6.02, which is moderately acidic and ideal for the production of most field crops. The soil test result also displayed that the average available phosphorus (P) was above the critical levels (14.19 ppm). According to Jones (2003), the phosphorus rating will be in the low ranges, which is sub-optimal for chickpea production which actually demands 14 kg ha^{-1} P_2O_5 in 1.5 ton of grain production (Aulakh, 1985).

Table 1. Readings of major soil physicochemical properties of Vertisols of Ginchi

Parameter	Mean	Range	Test Methods
Clay, silt & sand (%)	66,22 & 11		Sedimentation
Total N(%)	0.13	0.09-0.18	Modified Kjeldhal
pH	6.02	5.74-6.17	1:2.5 H_2O
Available P (ppm)	14.19	9.25-20.15	Bray II
OC (%)	1.23	1.05-1.29	Walkley and Black (1934)
Exch. K (meq/100g)	2.02	1.82-2.38	Ammonium Acetate-AAS
Exch. Mg (meq/100g)	16.22	14.67-16.83	Ammonium Acetate-AAS
Exch. Na(meq/100g)	0.22	0.13-0.27	Ammonium Acetate-AAS
Exch.Ca (meq/100g)	39.38	34.6-41.7	Ammonium Acetate-AAS

The average organic carbon content of the testing soil is 1.23%, which is rated as moderate and gives average structural condition and stability to the soil, according to Charman *et al*. (2007). Metson (1961) reported that the exchangeable cations were found at a very high range except sodium.

Effects of inoculation on number of pod

The study has displayed the presence of statistically significant response of number of pod per plant (NPPP) between the treatments (Table 1) and the years. Accordingly, rhizobial isolate CP-17 gave statistically superior NPPP (83.87) as compared to CP-5, CP-11 and CP-100. Isolate EAL-004 (73.2) and CP-9 (72.83) were the best performing isolates in NPPP in this order next to CP-17. Nevertheless, few rhizobial isolates did perform lower than the negative control, which is often attributed to lower effectiveness of the inoculated isolate than the indigenous rhizobia. Tena *et al*. (2016) also reported a 48% NPPP increment through indigenous rhizobial strain CP-41 inoculation over the uninoculated control. Ali *et al.* (2003) also reported an increased NPPP through seed inoculation and phosphorus fertilizer banding. The increase in nitrogen supply to the crop due to seed inoculation might have ultimately contributed to NPPP increase.

Aboveground biomass response to inoculation

As to the over year or average above ground biomass (AGB) response, similar trend was observed to that of NPPP response; Treatments and year dipicted statistically significant difference (Table 2) for AGB. Accordingly, rhizobial isolate CP-17 gave 4968 kg ha^{-1}, which is solely highly significantly superior to 3918 kg ha^{-1} that belongs to CP-5. EAL-004 was the second best performing isolate (4846 kg ha^{-1}) but lack significant superiority over the rest of the isolates. The results confirm that CP-11 and CP-5 were the highest and lowest aboveground biomass producer isolates, respectively. Ibsa (2013) had similar result that application of inoculants gave a positive and highly significant effect on total biomass of chickpea.

Table 2. Average performance of chickpea to rhizobial inoculation (2015/16-2016/17)

Treatment	NPPP	AGB	GY
CP-5	64.37b	3918.0b	2140.8b
CP-7	70.77ab	4523.2ab	2402.8ab
CP-9	72.83ab	4646ab	2456.9ab
CP-10	69.93ab	4233.3ab	2263.3ab
CP-11	65.87b	4457.3ab	2365.3ab
CP-17	83.87a	4968.0a	2738.4a
CP-100	67.07b	4662.1ab	2489.8ab
EAL-004	73.2ab	4846.0ab	2610.5ab
18 kg N ha^{-1}	83.83ab	4792.3ab	2527.4ab
Negative control	71.27ab	4546.1ab	2478.7ab
CV	19.1	18.71	18.78
LSD (P<= 0.05)			
Treatment	14.51	981	527.96
Year	6.49	438.76	NS

NPPP= No of pod per plant; NSPP= Number of seed pod $^{-1}$AGB= Above ground dry biomass (kg ha^{-1}); GY= Grain Yield(kg ha^{-1})

Effect of inoculation on Grain yield

The average performance of chickpea grain yield, as a reliable parameter to measure the nitrogen fixing capacity of rhizobial isolates inoculation (Patra *et al*., 2012) has depicted significant response among treatments but not between years (Table 2). Accordingly, CP-

11 depicted statistically significant superiority over CP-5 that yielded 2140.8 kg grain yield ha^{-1}. Rhizobial isolate CP-17 which had 2738.4 kg grain yield ha^{-1} was the highest mean grain yield record of the study followed by EAL-004 (2610.5 kg ha^{-1}) and CP-100 (2489.8 kg ha^{-1}) in their order. Proportionally, CP-17 surpassed CP-5 and the negative control by 28, and 11%, respectively. Hence, CP-17 was best performing rhizobial isolate at Dandi Vertisol during the study periods.

The reports of El-Hadi *et al*. (1999) and Kyei-Boahen *et al*. (2002) are in agreement with this finding that *Rhizobium* inoculation to chickpea increased seed/grain yield by 70-72 and 50% over the negative control, respectively. Wolde-Meskel *et al*. (2018) also reported a 38% grain yield increase in applying rhizobial inoculant and phosphorus nutrient over the negative control. The absence of significance difference between years would be attributed to the more or less stability of environmental factors. .

Moreover, Karadavut and Ozdemir (2001) also reported increase of grain yield (20%), above ground biomass and number of pods $plant^{-1}$ on chickpea due to rhizobia inoculation as compared to the control. The grain yield response of rhizobial strains CP-5, CP-7, CP-10 and CP-11 were lower than the negative control. The relative betterment of the control confirms the presence of relatively competent indigenous rhizobia on the area due to a long history of chickpea planting (Abera *et al*., 2015). On the other hand, because of prior colonization of the rhizosphere, plants inoculated with these isolates were not able to benefit from the indigenous rhizobia.

Economic analysis

According to the results of partial budget analysis, the highest net benefit (ETB 37856.06 ha^{-1}) was obtained from the application of 500 g of CP-17 ha^{-1}. The dominance analysis depicted the domination of all treatments other than CP-5 and CP-17. Since no beneficiary will prefer alternatives that gives lower net benefits than net benefit of the alternative with lower total variable costs, the dominated treatments were eliminated from further economic analysis. The marginal rate of return for the non-dominated treatments was found to be 2793.1% (Table 2).

This implies that for each ETB 1.00 investment in chickpea production under inoculation, the producer can get ETB 1.00 and additional ETB 27.93. Since the minimum acceptable rate of return assumed in this experiment was 100%, the treatment with application of CP-17 gave an acceptable marginal rate of return (2793.78%). Therefore, according to the results of the economic analysis, chickpea producers on Dandi Vertisol of the central highlands of Ethiopia could get the highest marginal rate of return with the application of CP-17 rhizobial strain.

Table 3 . Partial budget analysis of rhizobial isolates experiment on chickpea, 2016-2017

Treatment	GY (kg ha^{-1})	Adj. yield - 15% (kg ha^{-1})	Gross benefit (Birr ha^{-1})	TVC (Birr ha $^{-1}$)	Net benefit (Birr ha^{-1})	Dominance (Birr ha^{-1})	MC (Birr ha^{-1})	MNB (Birr ha $^{-1}$)	MRR (%)
CP-5	2140.8	1819.68	30024.72	250.00	29774.72				
CP-17	2738.4	2327.64	38406.06	550.00	37856.06		8381.34	300.00	2793.78
CP-9	2456.9	2088.37	34458.02	250.00	34208.02	Dominated			
CP-10	2263.3	1923.81	31742.78	250.00	31492.78	Dominated			
CP-11	2365.3	2010.51	33173.33	250.00	32923.33	Dominated			
CP-7	2402.8	2042.38	33699.27	250.00	33449.27	Dominated			
CP-100	2489.8	2116.33	34919.45	250.00	34669.45	Dominated			
EAL-004	2610.5	2218.93	36612.26	250.00	36362.26	Dominated			
18 kg N/ha	2527.4	2148.29	35446.79	1462.00	33984.79	Dominated			
Control	2478.7	2106.90	34763.77	0.00	34763.77	Dominated			

TVC = Total variable cost; MC = Marginal cost; MNB = Marginal net benefit; MRR = marginal rate of return

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Conclusion

Despite yield increment due to CP-17 rhizobial isolate, the economic yield obtained from the same isolate was quite promising. The analytical results of the soil were found to be sub-optimal for the production of chickpea except phosphorus. This confirms that producing chickpea using rhizobial isolate CP-17 along with and 46 kg P_2O_5 on Ginchi Vertisol is quite paying in terms of yield and benefit. Therefore, it is suggested that further verification of the isolate should be carried out in replicated condition in similar soil and weather conditions.

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