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Yield, Yield Attributes, Nodulation and Protein Content of Chickpea as Influenced by Variety and Inoculation with Rhizobium Strains

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Abstract
Field experiment was conducted on Arba Minch University research farm during 2015 evaluate the effect of inoculation with different Rhizobium strains on growth, yield, nodulation ability and protein content of chickpea varieties. The treatments consisted of six varieties of chickpea (two desi types: Fetenech and Kutaye; four kabuli types: Harbu, Kasech, Kobo and Yelb) and four levels of inoculation (uninoculated, inoculated with CP-M1, inoculated with CP-EAL 018, and inoculated with CP-EAL 029) arranged in randomized complete block design with three replications. Growth, nodulation, yield and yield attributes of chickpea were substantially affected by the main effects of variety whereas the effect inoculation was significantly only on plant height, nodule number, nodule dry weight per plant (mg) and seed yield. Variation in seed yield and protein content due to variety effect ranged from 13.3 to 24.86qha^{-1} and 27.99 to 36.57% over control treatment, respectively. Rhizobium inoculation also showed 8.34 and 5.36% increment in seed yield and crude protein content, respectively. However, the differences among strains were not significant. The highest significant yield was obtained from Yelbe (24.86qha^{-1}) followed by Kobo (22.7 qha^{-1}) while the lowest was recorded due to Fetenech (13.37 qha^{-1}). On the other hand, Kobo produced the highest crude protein (36.57%) followed by Fetenech (35.96%) and Yelbe (33.12%) whereas the lowest crude protein was obtained from Kutaye (27.99%). This could indicate that variety consideration and inoculation with rhizobium may improve the productivity and quality of chickpea.
Keywords: nodulation, chickpea varieties, rhizobium strain, crude protein content

Introduction

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. Its straw is also used for animal feed and due to its capacity of biological nitrogen fixation; chickpea can improve the soil fertility status (Pundir and Mengesha, 1995).

Chickpea can fix up to 140 kg N ha^{-1} in a growing season (Kumar and Abbo, 2001) and its inclusion in crop rotations can reduce disease severity of non-legume crops.

According Adjei and Chambeiss (2002), soil is the natural habitat of N fixing bacteria but too often our soils do not have either the proper kind of nodule forming bacteria or enough of them to really bring about good legume growth. In this regard, *Rhizobium* inoculation of chickpea seed may substitute costly N fertilizers in chickpea production (Kosgey 1994).

Over the past 38 years, Ethiopian chickpea research has focused mainly on breeding and selection of improved cultivars with better yield and disease resistance. However, this did not result in the desired level of productivity as the average yield is still below 1.6 ton ha^{-1}. Variety development can be seen as a component of a package through which crop yield can be improved and it has to be supported by appropriate agronomic management.

Therefore, one way of improving yield of leguminous crops is inoculation of their seeds with *Rhizobium* bacteria that has already shown remarkable result in other African countries (Woomer, 2012). Therefore, this study was conducted with the objective to evaluate agronomic performance and quality of chickpea varieties under rhizobium inoculation.

MATERIALS AND METHODS

Description of the Study Area

The research was conducted in Southern Nations, Nationalities and Peoples` Regional (SNNPR) State on Arba Minch university research farm. The study area has an altitude around 1290 with total precipitation of 830.7 mm per annum and average temperatures 20.6 ^{0}c.

Table 1: Some selected properties of soil of the study site

CEC and Exchangeable Bases (cmol (+) Kg^{-1} soil					EC (dSm^{-1})	pH_{H2O} 1:2.5
CEC	Ca	Mg	Na	K		
77.39	57.49	12.03	0.21	3.06	0.62	7.53
Av.P (mg/Kg)	%OC	%TN	Texture			
			% Clay	% Silt	% Sand	Soil Class
23.09	2.67	0.24	32	44	24	Clay loam

Procedure, Experimental Treatments and Design

Procedures

Land preparation was done similar to farmers' practices and then the prepared land was divided into three blocks each with twenty four plots. After land preparation, seeds of each variety were inoculated with three strains of rhizobium (CP-MB1, CP-EAL 018 and CP-EAL 029) under shade to avoid to direct sunlight. All seeds were sown on the same day at spacing 40cm x 10cm. Noninoculated seeds were sown first and followed by the inoculated seeds.

Treatments and experimental design

The treatment consisted of six varieties of chickpea (two desi types: Kutaye, Fetench and four kabuli typs namely: Kasech, Kobo, Yelbe and Harbu) and four levels of rhizobium inoculation (uninoculated, inoculated with CP-MB1, inoculated with CP-EAL 018 and inoculated with CP-EAL 029). The treatments were arranged factorial in factorial completely randomized block Design (RCBD) with three replications. The size of each plot was 2.4m x 2m and five rows of chick pea were sown on each plot. The distance between blocks and plots was maintained to 2m and 1m, respectively.

Data collection and analysis

Data were collected on the following parameters

Plant height (cm), the number of branches plant, , days to 50% flowering, days to maturity, number of nodules/plant, nodule dry weight per plant (mg), number of pods per plant, , number of seeds per pod, biological yield (qha^{-1}), 1000-seed weight (g), seed yield (qha^{-1}) and harvest index. Grain protein content will be measured by Kjeldahl method (Maff, 1984).

Data Analysis and Interpretation Techniques

The crop data collected were subjected to analysis of variance using SAS version 9.1. LSD was used to separate the means when the analysis of variance indicated the presence of significant difference (Gomez and Gomez, 1984). Simple linear correlation analysis was also done to determine the association of various agronomic characters.

Results

Effect of variety

All parameters which were measured under this experiment were significantly ($P<0.05$) affected by the main effect of variety. Highly significant ($P<0.001$) variation was observed in plant height (PHT), number of pods per plant (NNPP), number of seeds per pod (NSPP), number of nodules per plant (NNPP), nodule dry weight (NDWT), 1000 seed weight (TSW), harvest index (HI), biological yield (BY) and seed yield due to variety effect. Fetenech gave the shortest significant days to 50% flowering (32.75) and days to maturity (71.87) (Table 3). The variation in days to 50% flowering for varieties Harbu, Kasech, Kobo and Yelbe was observed to be statistically not different. Maturity durations of Harbu, Kobo and Yelbe varieties were also not significantly different (Table 2).

The shortest significant plant height was recorded due to varieties Fetenech (35.5) and Kutaye (33.7cm) while the highest value of this parameter was obtained from Harbu (43.12cm) Kobo (39.27) which was not statistically different from Yelbe (42.6cm) Kasech (40.5cm). Kasech produced the lowest branch (10) number while maximum value was obtained from Fetenech (13.57). Yelbe (31.97), Kutaye (32.65) and Fetenech (31.5) produced maximum significant pod number $plant^{-1}$ whereas the minimum significant values were recorded due to Harbu (24.37) and Kasech (22.42). Main effect of variety also produced significant differences in seed number pod^{-1}, nodule number $plant^{-1}$ and nodule dry weight (g) ranging from 1.18 (Kutaye and Kasech), to 1.31 (Kobo and Yelbe), 13.75 (Kasech) to 20.92 (Kobo) and 22.47 (kasech) to 34.48 (Kobo), respectively. The highest significant 1000 seed weight was produced by Kasech (410.5g) followed by Harbu (352.9g), Kobo (343.5) and Yelbe (332.2). On the other hand, Fetenech gave the lowest significant value of 1000 seed weight (191.57g). Desi types gave the lowest significant values of harvest index and biological yield whereas Yelbe gave the highest value of harvest index (0.45) and biological yield (54.99qha^{-1}) followed by Kobo. Seed yield ranged from 13.37qha^{-1} (Fetenech) to 24.48 13.37qha^{-1} (Yelbe) due to variety effect. Kobo also showed the highest significant value of seed yield (22.713.37qha^{-1}) next to Yelbe (Table 2).

Table 2: Agronomic performance and nodulation of varieties

Measured Parameters	Varieties						LSD	CV
	Fetenech	Harbu	Kasech	Kobo	Kutaye	Yelbe		
DF	32.75[c]	37.7[a]	35.72[ab]	36.8ab	34.85[bc]	37.32[ab]	*	8.5
DM	71.87[c]	80.35[a]	75.15[bc]	77.2ab	75.9[b]	79.65[a]	**	5.76
PHT (cm)	35.5[c]	43.12[a]	40.5[ab]	39.27b	33.7[c]	42.6[a]	***	8.54
NBR	13.57[a]	12.5[abc]	10[c]	11.3b[c]	13.03[ab]	13.12[ab]	*	18.52
NPP	31.5[a]	24.37[c]	22.42[c]	27.55[b]	32.65[a]	31.97[a]	***	13
NSP	1.22[b]	1.29[a]	1.18[b]	1.31[a]	1.18[b]	1.31[a]	***	4.88
NNPP	18.52[b]	14.35[c]	13.75[c]	20.92[a]	10.94[d]	15.49[c]	***	16.59
NDWT(mg/plant)	30.55[b]	24.75[c]	22.47[c]	34.48[a]	18.06[d]	25.30[c]	***	16.82
TSW (g)	191.57[f]	352.9[b]	410.5[a]	343.5[c]	202.85[e]	332.2[d]	***	1.72
HI	0.36[c]	0.42[b]	0.39[bc]	0.47[a]	0.39[bc]	0.45[a]	***	9.8
BY (qha^{-1})	36.41[c]	49.31[b]	48.98[b]	48.3[b]	39.7[c]	54.99[a]	***	8.9
SY (qha^{-1})	13.37[f]	20.59[c]	19.22[d]	22.70[b]	15.78[e]	24.86[a]	***	8.25

Note: *, ** and *** denote significant differences at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively
Within a row values followed by the same letter are not significantly different at $P \leq 0.05$

Rhizobium inoculation Effect

Inoculation significantly ($P<0.001$) improved nodule number and nodule dry weight (Table 3). The lowest significant values of nodule number (6) and nodule dry weight (9.95gm/plant) were obtained from the uninoculated treatment. In contrary, the highest significant values of nodule number (18.53) and nodule dry weight per plant (30.25gm) were recorded due to inoculation with strain CP-EAL 029 strain. Inoculation was also observed to significantly ($P<0.05$) increase plant height ranging from 37.16cm to 39.78cm and seed yield from 18.61 qha^{-1} to 20.16 qha^{-1} (8.3%) due to strains CP-EAL 029 and CP-EAL 018, respectively. However, the variations among strains were insignificant on nodule number, nodule dry weight, plant height and seed yield (Table 3).

Though statistically not significant, inoculation was observed to numerically increase days to 50% flowering, days to maturity, number of branches per plant, number of pods per plant, number of seeds per pod, number of seeds per plant, 1000 seed weight, harvest index and biological yield seed yield over the control/uninoculated treatment (Table 3).

Table 3: Effect of rhizobium inoculation on growth, yield, yield attributes and nodulation

Measured Parameters	uninoculated	CP-MB1	CP-EAL-018	CP-EAL-029	LSD
DF	35.2	35.81	36.15	36.28	ns
DM	75.9	76.5	77.18	77.16	ns
PHT (cm)	37.16[b]	39.01[ab]	40.5[a]	39.78[a]	*
NBR	11.92	12.53	12.68	12.51	ns
NPP	27.117	28.5	28.95	29.083	ns
NSP	1.24	1.25	1.26	1.25	ns
NNPP	6[b]	18.94[a]	19.17[a]	18.53[a]	***
NDWT mg/plant)	9.95[b]	32.26[a]	31.29[a]	30.25[a]	***
TSW (g)	303.167	306.2	306.6	306.45	ns
HI	0.405	0.416	0.42	0.423	ns
BY (qha^{-1})	45.59	46	47.63	45.89	ns
SY (qha^{-1})	18.61[b]	19.31[ab]	20.16[a]	19.6[ab]	*

Note: *, *** and ns denote significant difference at $P \leq 0.05$, $P \leq 0.001$ and nonsignificant differences, respectively
Within a row values followed by the same letter are not significantly different at $P \leq 0.05$

Crude protein content

Inoculation showed improvement in crude protein content of chickpea varieties (Table 4). 1.23%, 4.96%, 10.94%, 6.78%, 6.75% and 3.16% increment in crude protein content was recorded due to inoculation with strain CP-MB1 in Fetenech, Harbu, kasech, Kobo, Kutaye and Yelbe, respectively, over uninoculated treatment (Table 4). Similarly, inoculation with strain CP-EAL-29 gave 1.65%, 5.09%, 10.06%, 4.18%, 10.46% and 3.16% increment in crude protein content in Fetenech, Harbu, kasech, Kobo and Kutaye, respectively, when compared with the control. Inoculation with strain CP-EAL-018 improved crude protein content in Kasech, Kobo, Kutaye and Yelbe (Table 4) by 3.54%, 2.40, 7.10% and 3.55%, respectively.

Averaged over inoculation levels, Kutaye gave the lowest value of crude protein content (27.99) followed by kasech (29.11). On the other hand, Kobo produced the highest value of crude protein (36.57%) followed by Fetenech (35.96%) and Yelbe (33.12%) accounting for 30.65%, 28.47% and 18.32% increment over Kutaye, respectively.

Averaged over varieties, the main effect of inoculation showed 5.39%, 2.21% and 4.07% increment in crude protein content for the strains CP-MB1, CP-EAL-018 and CP-EAL-29, respectively, when compared with the control treatment. Overall, the uninoculated treatment gave the lowest value of crude protein content (Table 4).

Table 4: Effect of rhizobium inoculation on crude protein content of varieties (%)

	Varieties						
Inoculation	Fetenech	Harbu	Kasech	Kobo	Kutaye	Yelbe	**Mean**
Uninoculated	35.80	32.25	27.43	35.39	26.38	32.94	31.70
CP-MB1	36.24	33.85	30.43	37.79	28.16	33.98	33.41
CP-EAL-018	35.40	31.98	28.40	36.24	28.27	34.11	32.40
CP-EAL-29	36.39	33.89	30.19	36.87	29.14	31.44	32.99
Mean	35.96	32.99	29.11	36.57	27.99	33.12	

Discussions

Variety effects

The significant variation in most of the parameters measured on varieties under this study might be attributed to genetic differences in genotypes used, hot temperature and water stress at reproductive stage during the experiment.

Varieties which flowered and matured late / kabuli types produced higher amount biological yield (kgha^{-1}) which might resulted from increased rate and duration of total leaf area (Terry et al., 1983) thereby higher interception of solar radiation (Littleton et al., 1979). Similarly, these varieties gave higher grain yield over desi types which can be ascribed to the fact that longer development period provides full use of available resources for plants resulting in higher yields (Thies et al., 1995). Correlation analysis also showed positive and significant relationship of seed yield with days to 50% flowering (r= 0.88***) and days to maturity (r= 0.8***) (Table 5).

Early maturing variety (Fetenech), produced the lowest value of harvest index which is in line the report of Ranju (2005) who reported that lower harvest index is directly related with less plant dry biomass production in desi cultivars and hence with inadequate supply of photosynthate to the developing seeds. This is also further supported by positive and significant correlation of seed yield with 1000 seed weight (r= 0.71***), biological yield (r= 0.94***) and harvest index (r= 0.88***). Plant height also showed significant and positive relationship with the number of seeds per pod (r = 0.59*), 1000 seed weight (0.77***), biological yield (r= 0.84***), harvest index (r=0.52**) and seed yield (r= 0.78***).

Contrary to the reports of previous studies (Mirza et al., 2007; Parveen et al., 1999) who demonstrated positive relationship of branch number and pod number in chickpea with biological yield, negative relationship was observed between these traits under this study. This could be probably high branching might be resulted in production of empty and under sized pods, dropping of pods and leaves due to insufficient translocation of photosynthetates to these parts. The negative and highly significant relationship of 1000 seed weight with branch number (r= -0.73***) and pod number (r = -0.78***) can confirm this statement (Table 5). Additionally, Khanna-Chopra and Sinha (1987) explained that leaf fall can account for almost 20 to 30% of the loss in the total dry matter weight of chickpea.

Inoculation effects on agronomic parameters and protein content

Data from this study revealed that the magnitude of variation in most parameters measured under due to inoculation effects is lower than variation due to varieties. This might be ascribed to relatively higher nitrogen content of the soil of experimental site inhibited nodulation of varieties (Namvar et al., 2011). Similarly, Bhuiyan et al., (2008) reported that inoculation with Rhizobium improved nodulation, nodule dry weight and seed yield whenever nitrogen content is below critical point. The improvement in plant height, number of nodule per plant, nodule dry weight, seed yield and protein content of varieties due to inoculation could be attributed to greater root growth thereby enhanced water and nutrient /nitrogen uptake (Togay et al., 2008; Werner and Newton, 2005; Zai et al., 1999). The variation in protein content of varieties could be related to their genetic potential as well as growing environment. Crude protein content showed positive and significant relationship with seed number pod^{-1}, nodule number plant^{-1} and nodule dry weight (mg plant^{-1}) (Table 5).

Table 5: Correlation between traits

	DF	DM	PHT	NBR	NPP	NSPP	NNPP	NDWT	TSW	HI	BY	SY	CP
DF	1.00	0.93***	0.81**	-0.26ns	-0.32ns	0.64***	0.16ns	0.17ns	0.71***	0.77***	0.85***	0.88***	0.03ns
DM		1.00	0.73***	-0.04ns	-0.16ns	0.65***	0.04ns	0.05ns	0.52**	0.67***	0.78***	0.8***	-0.01ns
PHT			1.00	-0.19ns	-0.45*	0.59*	0.31ns	0.31ns	0.77***	0.52**	0.84***	0.78***	0.20ns
NBR				1.00	0.76***	0.10ns	0.18ns	0.18ns	-0.73***	-0.18ns	-0.36ns	-0.29ns	0.16ns
NPP					1.00	0.08ns	0.16ns	0.15ns	-0.78***	0.02ns	-0.36ns	-0.19ns	0.12ns
NSPP						1.00	0.38ns	0.39ns	0.30ns	0.78***	0.60*	0.75***	0.66**
NNPP							1.00	0.99***	0.04ns	0.33ns	0.05ns	0.19ns	0.58*
NDWT								1.00	0.04ns	0.32ns	0.06ns	0.19ns	0.58*
TSW									1.00	0.45*	0.82***	0.71***	-0.05ns
HI										1.00	0.69***	0.88***	0.24ns
BY											1.00	0.94***	-0.06ns
SY												1.000	0.17ns
CP													1.00

**** - Correlation is significant at 0.001 level
** - Correlation is significant at 0.01 level
* - Correlation is significant at 0.05 level
ns- Correlation is non-significant at 0.05 level

DF-days to 50% flowering, DM- days to maturity, PHT- plant height, NBR- number of branches per plant, NPP- number of pods per plant, NSPP- number of seeds per pod, NSPPL- number of seeds per plant, NNPP- number of nodules per plant, NDWT- nodule dry weight per plant (mg), TSW- 1000 seed weight (g), HI- harvest index, BY- biomass yield (qha^{-1}), SY- seed yield (qha^{-1}) and CP- crude protein content (%).

From this study, it can be concluded that higher yield varieties can give relatively higher protein content and inoculation with rhizobium can improved grain yield and protein content of chickpea. Accordingly, selecting either Yelbe or Kobo variety and inoculating with Rhizobium strain CP-EAL- 018 can be the best option for improving productivity, nodulation capacity and protein content of chickpea.

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