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Influence of *Bradyrhizobia* inoculation on growth, nodulation and yield performance of cowpea varieties

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Cowpea (*Vigna unguiculata* L. Walp) has a major role in daily diet of the rural community and poor urban population, serving as a source of energy, protein and minerals, in developing countries. Its straw used for animal feed, and the crop also improves soil fertility by fixing the atmospheric nitrogen. However, its productivity is constrained by lack of improved varieties and poor agronomic practices. A field experiment was conducted at Ziway, central rift valley of Ethiopia, to evaluate the response of cowpea varieties to Bradyrhizobia inoculation. The treatment consisted of four Bradyrhizobia strains (control, GN-100, GN-102 and MB-140) and five cowpea varieties (Bole, Black eye-bean, TVU1977.0D1, Assebot and White Wonder). The experiment was carried out using a randomized complete block design with three replications. The results revealed marked varietal differences in plant growth, nodulation, yield and yield components. Of the five cowpea varieties studied, Black eye-bean generally showed superior performance in most measured parameters. *Bradyrhizobium* inoculation significantly ($p \leq 0.05$) increased plant growth, nodulation, yield and yield components. The interaction effect of variety and *Bradyrhizobium* caused significant variations in the number of nodules, number of seeds, hundred seed weight and seed yield. The highest grain yield was recorded from Black eye-bean variety (3.08 t/ha) and *Bradyrhizobium* strain GN-102 (3.11 t/ha) inoculation. It could, thus, be deduced that the use of strain GN-102 and variety Black-eye bean markedly increases the productivity of the crop in the region.

Key words: *Bradyrhizobia,* cowpea, nodulation, growth, yield components.

INTRODUCTION

Grain legumes play a vital role in the lives of millions of people in developing countries to achieve food and nutritional security. They complement staple low-protein cereal crops as a source of protein and minerals (Gharti et al., 2014). Legume crops are also valued for their ability to fix atmospheric nitrogen into the soil and play an important role as a rotation crop with cereals and vegetable crops (Jensen et al., 2012; Biswas and Gresshoff, 2014; Stagnari et al., 2017). Legumes also serve as a feed crop in many farming systems and are also grown to supplement farmers' incomes (Muli and Saha, 2002; Voisin et al., 2013). The important and

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diverse role played by food legumes in the farming systems and in diets of poor people, makes them ideal crops for achieving the developmental goals to reduce poverty and hunger, improving human health and nutrition, and enhancing ecosystem resilience.

Cowpea (*Vigna unguiculata* L. Walp) being a legume is an important source of food, income and livestock feed and forms a major component of tropical farming systems because of its ability to improve the fertility status of marginal lands through nitrogen fixation (Timko and Singh, 2008). It has considerable adaptation to high temperatures and drought compared to other crop species, making it suitable for cultivation in semiarid areas (Hall, 2004; Tekle, 2014).

Despite its role in improving soil fertility and serving as a food security crop, little efforts are made to know the response of cowpea varieties to *Bradyrhizobia* inoculation in Ethiopia. Furthermore, *Bradyrhizobia* inoculation of cowpea is not a common agronomic practice among smallholder farmers. Thus, the development of new cultural practice, *Bradyrhizobium* inoculation as N source, which enhance yield and mineral nutrition of grain is imperative to achieve food and nutritional security in the country. Therefore, the aim of this study was to evaluate the effect of *Bradyrhizobium* inoculation on plant growth, nodulation and yield of cowpea varieties grown at Ziway, central rift valley of Ethiopia.

MATERIALS AND METHODS

Description of study site

The experiment was conducted at Ziway, in the Agricultural Research Station of Hawassa University, in 2012 cropping season. The site is located on latitudes of 8°00’ N and longitudes of 38° 45’ E, with an elevation of 1645 m above sea level. Average rainfall during the growing season of five months was 763.0 mm, which is more or less similar to the five years average rain fall (760.9 mm). The mean maximum and minimum temperatures were 27 and 12.7°C, respectively. The area is characterized by bimodal rainfall pattern with a short rainy season from February to April and a long rainy season from June to September with a peak in August (NMA, 2012).

Source of planting material and Bradyrhizobium strains

Seeds of *Vigna unguiculata* (L) Walp varieties (Bole, Black eye bean, TVU1977.0D1, Assebot and White Wonder) were obtained from the Melkassa Agriculture Research Center, Melkassa, Ethiopia. The varieties were chosen based on their high grain yield and acceptability by farmers. Strains of *Bradyrhizobial* sp. (GN-100, GN-102, MB-140) were obtained from NUFU-Hawassa University collaborative project (NUFUPRO-2007/10144) authenticated collections and which have been biochemically characterized (Negash, 2010; Zikie, 2010).

Experimental design and treatments

Seeds were planted in a factorial randomized complete block design which had a total of 20 treatment combinations with three replicate plots for each treatment. The treatments consisted of four Bradyrhizobia inoculation (control, strains: GN-100, GN-102 and MB-140) and five cowpea varieties (Bole, Black eye-bean, TVU1977.0D1, Assebot and White Wonder).

Cultural practices

Seed inoculation was done under shade in the field to reduce the bacterial cell death. Inoculated seeds were allowed to air-dry for a few minutes before planting. Planting was done using a spacing of 40 cm between rows and 20 cm between plants. Each experimental plot measures 2.2 m × 3.0 m (6.6 m^2). Two seeds were sown in each hole for both inoculated and uninoculated treatments. To avoid cross contamination, the uninoculated seeds were always planted first, followed by inoculated treatment. Soil ridges were made to separate inoculated and uninoculated treatments from each other in order to prevent cross contamination through rainwater movement. After sowing, the seeds were immediately covered with moist soil to avoid rhizobial cell death from desiccation. Weeding was done manually by hoe at two weeks after seedling emergence, and three weeks from the first weeding. To avoid cross contamination, weeding was done in the uninoculated plots first.

Data collection

Plant height

Five plants from the central rows of each plot were randomly selected for measuring plant height. Then the average values of these plants were recorded as plant height of the crop.

Nodule number/plant

Nodulation assessment was undertaken at mid (50%) flowering stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoot and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to drain water from their surface. Nodules were counted and their average was taken for plots as nodule number/plant. Then after, the nodules were oven-dried at 70°C for 48 h for nodule dry weight determination.

Yield and yield components

Yield and yield components at harvesting time for the determination of yield components such as number of pods/plant, number of seeds/pod, hundred seed weight and grain yield, ten randomly picked plants were used. Seed weight was determined by randomly taking 100 seeds of the ten sample plants and weighing it with sensitive balance after oven drying to constant weight.

Soil sampling and analysis

Before planting, 20 soil samples were randomly taken from the experimental field at a depth of 0 to 30 cm using an auger and the samples were mixed thoroughly to produce one representative composite sample of 1 kg. The composite soil sample was air-dried and ground to pass through 2 and 0.5 mm (for total N) sieves. Soil analysis was done following standard laboratory procedures as outlined by Sahlemedhin and Taye (2000). Soil pH was measured in the supernatant suspension of a 1: 2.5 soil to water ratio using a standard glass electrode pH meter (Rhoades, 1982). The Walkley and Black (1934) method was used to determine the organic carbon (%). Total N was determined using Kjedahl method as described by Bremner and Mulvaney (1982). Available P (mg kg^{-1}) was determined by employing the Olsen et al. (1954) method using ascorbic acid as the reducing agent. The soil particle size distribution was determined using the Bouyoucos hydrometer method (Bouyoucos, 1951).

Statistical analysis

The collected data was subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System software (SAS, 2002) version 9.0. Mean separation was done using Least Significant Difference (LSD) test at 5% probability level.

RESULTS AND DISCUSSION

Physicochemical properties of the soil before planting

The result of soil analysis (Table 1) indicated that texture of the soil was clay loam and pH of the soil was alkaline in reaction with a pH value of 8.2. Different crops have different pH requirements. Accordingly, the optimum pH range for cowpea is 6.8 to 8.5 (Onyibe et al., 2006). Therefore, the pH of experimental soil is suitable for cowpea. According to Jackson (1958) and Herrera (2005), soil organic carbon content was rated as very low (< 2%), low (2 to 4%), medium (4 to 10%), high (10 to 20%) and very high (>20%) and thus the soil at the experimental site has low organic carbon content 2.65%. According to Olsen et al. (1954), P content (mg kg^{-1}) <5 is very low, 5 to 15 is low, 15 to 25 is medium and >25 is high. Therefore, the available P in soil at the experimental site (22.00 mg/kg) was medium range. Total nitrogen in the experimental soil was 0.13%. Total N is rated as very low (<0.1), low (0.1 - 0.15), medium (0.15 - 0.25) and high (>0.25) (Havlin et al., 1999). Hence, total N of the soil of the experimental field was in the low range. This shows that the soil requires external application of N fertilizer if satisfactory level of crop production is to be achieved and also most Ethiopia soils, similar to the agricultural soils in other tropical countries, are reported to be generally low in N content (Asgelil, 2000). In general for soils low in mineral nutrients, effective Bradyrhizobia strains which able to contribute N to the soil can substantially contribute to the replenishment of soil N for improved crop yield.

Effect of cowpea variety and *Bradyrhizobia* inoculation on growth and nodulation

Plant height (cm)

As indicated in Table 2, varieties showed significant differences (P<0.001) in plant height. However, the response of plant height to Bradyrhizobia inoculation was not significant (P> 0.05). Regarding variety effect, the highest value for plant height was recorded with Black eye-bean variety (68.1 cm) followed by var. Wonderer. Whereas, the lowest value of plant height was recorded from Assebot variety (31.0 cm) which was statistically at par with varieties Bole and TUV. The observed difference in plant height among cowpea varieties might be attributed to inherent genotypic difference (Magani and Kuchinda, 2009; Nwofia et al., 2015). This is in agreement with findings of Karikari et al. (2015) who reported variation on plant height among cowpea cultivars.

Shoot dry weight (g/plant)

Shoot dry weight was significantly (P<0.001) affected by cowpea variety and Bradyrhizobia inoculation (Table 2). The highest (31.64 g/plant) and lowest (22.04 g/plant) shoot dry weight was recorded from vars. Bole and Wonderer, respectively. This is in line with finding of Singh et al. (2011) that some varieties have the ability to out yield the other varieties and exhibit superior plant growth. Addo-Quaye et al. (2011) also found that cowpea varieties have different capacities for dry matter accumulation. The highest (31.2 g/plant) and lowest shoot dry weight (21.46 g/plant) was recorded from *Bradyrhizobium* strain GN-102 inoculation and the control treatment, respectively. The increased shoot dry weight due to inoculation can be attributed to the effectiveness of the Bradyrhizobia inoculants. A similar result was reported by Salih (2002) who observed increased shoot dry weight in soybean plants inoculated with *Bradyrhizobium* strains.

Nodule number per plant

Nodule number was significantly (P<0.001) affected by variety, *Bradyrhizobium* inoculation and their interaction (Table 2 and Figure 1A). Regarding the main effect, the highest and lowest nodule number was recorded from Black eye-bean (15) and Wonderer (10), respectively. The marked variation in nodule number per plant among the varieties could be attributed to difference in the genetic makeup of the individual varieties (Ayodele and Oso, 2014). Likewise, the maximum (15.6) and minimum (5.1) values of this parameter was obtained from *Bradyrhizobium* strain GN-102 and the control treatment, respectively. The enhancing effects of inoculation on nodule number per plant was also supported by the finding of Manish et al. (2011), which showed rhizobial inoculation increased number of root nodules as compared with uninoculated treatments. Thus, the selection of highly effective and competitive strain as

Table 1. Selected physicochemical properties of the soil before planting.

Particle size distribution (%)			Texture	pH (1:2.5 H_2O)	Av. P (mg/kg)	Total N (%)	OC (%)
Clay	Silt	Sand					
28	28	44	Sandy loam	8.20	22.00	0.13	2.65

Table 2. Effect of *Bradyrhizobium* strains on growth and nodulation performance of cowpea varieties.

Treatments	Plant height (cm)	Shoot dry weight (g)	Nodule number per plant	Nodule dry weight (g)
Variety				
Bole	32.5^{c}	31.6^{a}	13.6^{b}	0.14^{a}
Black eye-bean	68.1^{a}	28.6^{ab}	15.0^{a}	0.14^{a}
TUV1977.0D1	33.7^{c}	23.7^{c}d	9.9d	0.12^{bc}
Assebot	31.0^{c}	26.2^{bc}	11.6^{c}	0.13^{ab}
Wonderer	46.6^{b}	22.0d	10.0d	0.10^{c}
LSD (5%)	10.3	3.7	1.1	0.02
Bradyrhizobium				
Control	39.0	21.5^{c}	5.1d	0.09^{c}
GN-100	42.9	28.2^{ab}	14.4^{b}	0.14^{ab}
GN-102	44.5	31.2^{a}	15.6^{a}	0.16^{a}
MB-140	43.1	25.0^{b}	13.0^{c}	0.13^{c}
LSD (5%)	NS	3.3	1.0	0.02
CV%	29.4	17.0	11.3	22.5.
F-value				
Variety (V)	18.9***	8.7***	182.4***	11.4***
Bradyrhizobium (Br)	0.5NS	13.0***	32.8***	4.1**
V * Br	0.5NS	1.7NS	4.5***	0.53^{NS}

Means with the same letter(s) within a column are not significantly different at p< 0.05.

inoculum is very important to increase nodulation and hence the amount of N fixed and yields of the crop. Similar results were obtained by Sharma and Kumawat (2011), who reported increased nodulation by inoculating soybean varieties with Bradyrhizobia strains. Less number of nodule also reported in soybean and chick pea without *Bradyrhizobium* inoculation (Elkoca et al., 2008).

Nodule dry weight/plant

Nodule dry weight was significantly (P<0.001) affected by cowpea variety and Bradyrhizobia inoculation (Table 2). The highest nodule dry weight (0.14 g/plant) was recorded from vars. Bole and Black eye-bean, while the lowest (0.10 g/plant) nodule dry weight was recorded from vars. Wonderer reflecting inherent genetic differences among the cultivars for nodule dry weight. Similar results have been reported by Karadavut and Özdemir (2001) where the effects of cultivars were statistically significant on the nodule dry weight. On the other hand, maximum nodule dry weight (0.16 g/plant) was recorded from *Bradyrhizobium* strain GN-102 followed by strain GN-100, while the minimum nodule dry weight (0.09 g/plant) was recorded from the control treatment. The difference between the nodule dry weight obtained from inoculated plants and uninoculated plants may be attributed to the size of the nodules. Inoculated plants formed bigger nodules than uninoculated plants due to the effectiveness of the introduced rhizobia strain to initiate nodulation with cowpea roots which agrees with the report of Chiamaka (2014). A similar promoting effect of seed inoculation on dry weight of nodules per plant has also been reported by Bejandi et al. (2011) and Nyoki and Ndakidemi (2014).

Effect of varieties and Bradyrhizobia inoculation on yield and yield components

Number of pods/plant

The means of statistical analysis indicates that pods/plant was significantly affected by the treatments (Table 3). Maximum pods/plant (40.3) was achieved by var. Bole, while minimum pods per plant (30.0) was observed in

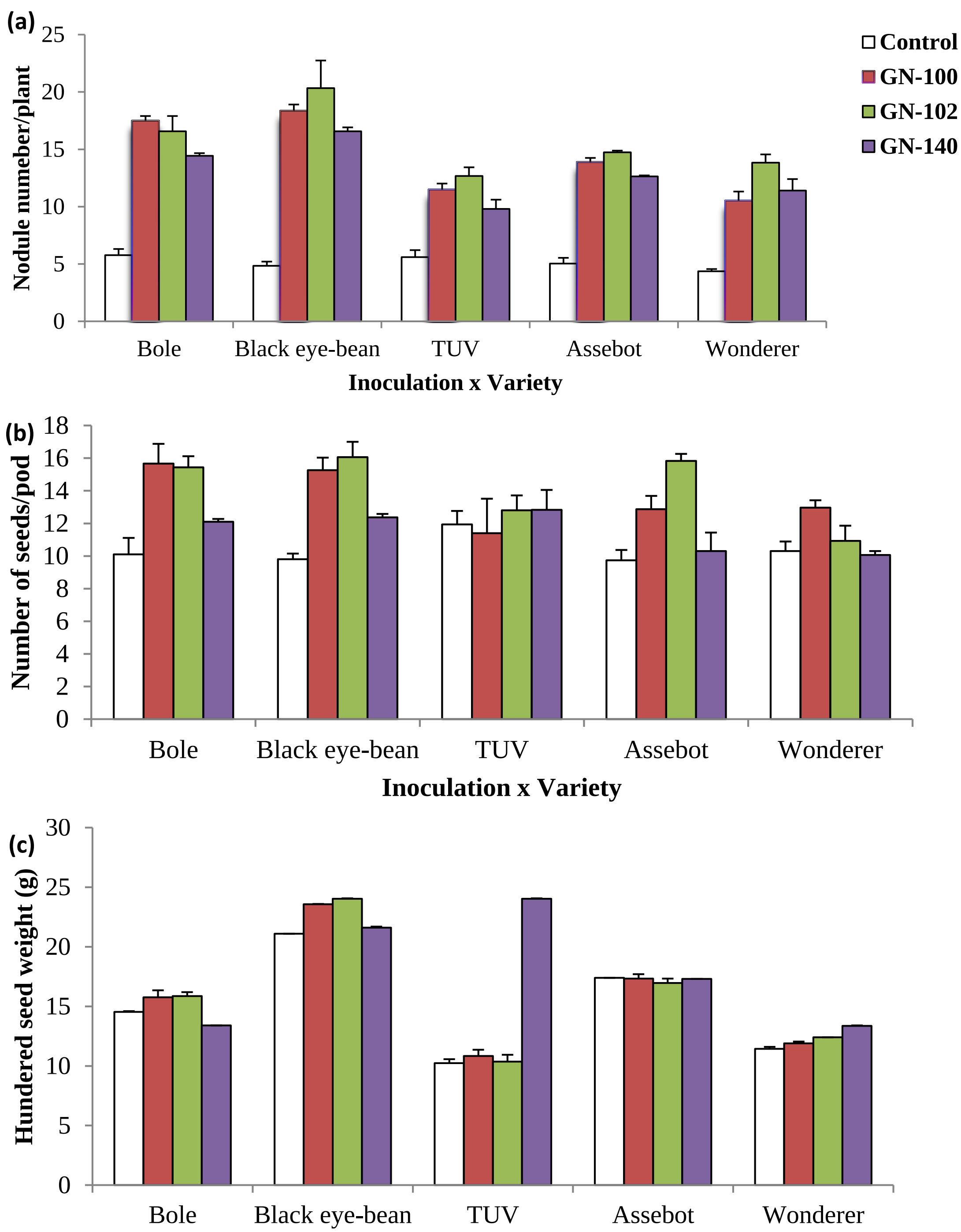

Figure 1. The interactive effect of Bradyrhizobia inoculation x variety on: (A) Nodule number/plant; (B) Number of seeds/pod and (C) Hundred seed weight (g). Vertical lines on bars represent S.E of the statistical means.

var. Wonderer, but, the differences in pods/plant among Wonderer, Assebot, TUV1977.0D1 and Black eye-bean varieties were observed to be statistically at par. The mean values for inoculation also shows significant effect for pods/plant. Maximum pods/plant (40.5) was obtained with *Bradyrhizobium* strain GN-102 inoculated plants, while less pods/plant (22.6) was recorded for the control treatment. This increased pod number with applied inoculants could be associated with enhanced growth and higher assimilate accumulation which resulted from better N nourishment due to symbiotic N fixation. The result is in agreement with the work of Malik et al. (2006)

Table 3. Effect of *Bradyrhizobium* strains on yield and yield components of cowpea varieties.

Treatments	Number of pods per plant	Number of seeds per pods	Hundred seed weight (g)	Grain yield (t/ha)
Variety				
Bole	40.3[a]	13.3[a]	14.9[c]	2.67[b]
Black eye-bean	33.9[b]	13.4[a]	22.8[a]	3.08[a]
TUV1977.0D1	31.8[b]	12.2[ab]	13.9[d]	2.73[b]
Assebot	31.6[b]	12.2[ab]	17.3[b]	2.68[b]
Wonderer	30.0[b]	11.1[b]	12.3[e]	2.38[c]
LSD (5%)	6.1	1.3	0.4	0.3
Bradyrhizobium				
Control	22.6[c]	10.4[c]	14.9[c]	2.34[c]
GN-100	32.2[b]	13.6[a]	15.9[b]	2.76[b]
GN-102	40.5[a]	14.2[a]	15.9[b]	3.11[a]
MB-140	34.8[b]	11.5[b]	17.9[a]	2.60[b]
LSD (5%)	5.4	1.2	0.3	0.2
CV%	21.9	12.6	2.9	11.6
F-value				
Variety (V)	10.6***	19.6***	107.3***	7.36***
Bradyrhizobium (Br)	3.6*	4.5**	867.6***	15.8***
V* Br	0.9[NS]	2.9**	141.8***	0.82[NS]

Means with the same letter(s) within a column are not significantly different at p< 0.05.

and Dereje (2007) which showed increased number of pods per plant with *B. japonicum* inoculation in soybean. Similarly, Argaw (2014) also obtained similar result in soybean, in that number of pods in soybean increased due to *Bradyrhizobium* inoculation. Hoque and Haq (1994) reported that seed inoculation increased number of pods per plant of lentil in Bangladesh. Contrastingly, Yamur and Engin (2004) reported that inoculation did not affect the number of pod per plant.

Number of seeds/pod

Number of seeds/pod was markedly affected by variety, *Bradyrhizobium* inoculation and their interaction (Table 3 and Figure 1B). Regarding main effect, the highest and lowest seeds/pod was recorded from Black eye-bean (13.4) and Wonderer (11.1), respectively (Table 3). The differences among Bole, Black eye-bean, TUV and Assebot were statistically at par. The report is in agreement with the findings of Dugje et al. (2009) and Singh et al. (2011), that different cowpea varieties have different genetic makeup as such they have different number of seeds. Similarly, number of seeds/pod was significantly increased with *Bradyrhizobium* inoculation where the highest (14.2 and 13.6) and the lowest (10.4) values of this parameter were obtained from *Bradyrhizobium* strains GN-102 and GN-100 and the control treatment, respectively. This significant difference of number of seeds/pod among inoculants and varieties were in line with the finding of Muhammad (2002) where inoculation with *Bradyrhizobium*, not only increased the shoot growth, but also the seeds/pod.

Hundred seed weight

Hundred seed weight was significantly (P<0.001) affected by variety, inoculation and their interaction (Table 3 and Figure 1C). Regarding main effect, the heaver and lighter seed weight was recorded due to Black eye-bean (22.8 g) and Wonderer (12.3 g), respectively. The significant difference in hundred seed weight among the varieties may be due to the difference in translocation and partitioning efficiency of assimilates from source to sink (El Naim and Jabereldar, 2010). Likewise, hundred seed weight was significantly increased with *Bradyrhizobium* inoculation where the maximum (17.9 g) and the minimum (14.9 g) values of this parameter were obtained from *Bradyrhizobium* strain MB-140 and the control treatment, respectively. Similar, findings were reported by Ali et al. (2004) where inoculation brought a significant effect on seed weight, of chickpea. Kazemi et al. (2005) reported that soybean seed inoculation with bradyrhizobia significantly increased seed weight.

Grain yield

Inoculation of Bradyrhizobia strains significantly affected the grain yield/ha (Table 3). Among the varieties significantly highest grain yield (3.08 t/ha) was recorded for var. Black eye-bean followed by var. Bole and the lowest grain yield (2.38) was obtained from var. Wonderer. The significant variation in grain yield among the varieties is largely due to differences in inherent yielding potential of the varieties. Similarly, Haruna and Usman (2013) observed a significant variation in grain yield of some improved varieties of cowpea at the same location and attributed it to genetic makeup of the varieties examined. The highest grain yield (3.11 t/ha) was recorded from *Bradyrhizobium* strain GN-102 inoculation followed by strain GN-100 and MB-140, whereas the lowest grain yield (2.34 t/ha) was recorded from the control treatment. The increase in grain yield due to Bradyrhizobia inoculation may be attributed to the effectiveness of the inoculant in fixing N thereby meeting the nutrient requirement of the plant (Nyoki and Ndakidemi, 2013). Ulzen et al. (2016) also observed a significant increase in grain yield of cowpea after inoculation with *Bradyrhizobium* inoculant.

Interaction effects of *Bradyrhizobium* inoculation and variety

Among the parameters tested under this study, number of nodules/plant, number of seeds/pod and hundred seed weight were significantly affected by the interaction effect of *Bradyrhizobium* inoculation and variety (Figure 1A to C). Inoculating cowpea seed with Bradyrhizobia strains were observed to be associated with increment in most of these parameters. Generally, the maximum values were obtained from Bradyrhizobia inoculation when compared to uninoculated control.

Conclusion

The results obtained in this study have shown that bradyrhizobia inoculation can improve plant growth, nodulation and grain yield of cowpea varieties grown in the low-N soils of Ethiopia. The higher plant growth and increased nodulation in *Rhizobium*-inoculated plants translated into increased grain yield. Based on the findings of the current study the use of cow pea variety Black-eye bean with *Bradyrhizobium* strain GN-102 could be recommended for enhanced growth and yield performance of cowpea.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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