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Response of soybean to *Rhizobial* inoculation and starter N fertilizer on Nitisols of Assosa and Begi areas, Western Ethiopia

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Abstract

Background: For manipulating N₂ fixation and improve the N benefits to agricultural legumes, there is a need to reduce the inhibitory effect of applied N on *Rhizobia* inoculant. The optimum level of inorganic N as starter fertilizer with inoculation of *Rhizobia* is essential to increase the production of legumes in smallholder farmers. However, the effect of inorganic N level which should be used by farmers with an inoculation to increase the production of soybean is not yet well known in soils of western Ethiopia. Therefore, the study conducted to determine the amount of starter N application which could enhance N₂ fixation and soybean seed yield using *Rhizobial* inoculation and different nitrogen doses.

Methods: Six levels of nitrogen fertilizer (0, 9, 18, 27, 36, and 54 kg N ha⁻¹) and two levels of inoculation (inoculated (1) and un-inoculated (0)) were arranged using split-plot RCBD with three replications.

Result: Seed inoculation with *Rhizobia* was significantly superior over un-inoculated treatments. Both rhizobia inoculation and N rates applied alone increased nodulation and yield of soybean, however, their combination further increased nodulation, growth performance, and seed yield and yield traits of soybean. Although nodulation parameters showed inconsistent results across cropping seasons, N application improved nodulation and shoot dry weight and interacted positively with inoculation across locations. Increasing rates of N from zero up to 18 kg ha⁻¹ increased nodule number and dry weight but further increase of N reduced nodulation. Application of N at 18 kg ha⁻¹ increased nodule number and dry weight by 98.3 and 115.0%, respectively over un-fertilized control as well as by 61.0 and 58.0%, respectively over the greatest N rate (54 kg N ha⁻¹). The highest seed yield of soybean was obtained when inoculation applied with N at 18 and 27 kg ha⁻¹ in 2016/17 and 2017/18, respectively across locations. However, seed yield in the 2016/17 cropping season was much higher than 2017/18. In 2016/17 and 2017/18, inoculation together with N at 18 kg ha⁻¹ increased grain yield by 75.0 and 76.8% compared with for the control plants, 50.7 and 31.6% compared with inoculation alone, respectively. In both cropping seasons, the un-inoculated plants had shown a trend that an increase in seed yield with increased N rate.

Conclusion: Application of fertilizer N up to 18 kg ha⁻¹ may be an optimum rate for effective nodulation and enhanced N₂ fixation and thereby improved soybean yield. The present results demonstrate the potential of improving soybean nodulation, growth, and seed yield profitability using *Rhizobia* inoculant together with starter N at low N Nitisols soils of Western Ethiopia.

Keywords: *Rhizobial* inoculation, N fertilizer, Soybean, Nodulation, Nitisols

Background

Legume crops rank second after cereals, with their 12% contribution to national food production and occupy 18% of the total cultivated area in Ethiopia. In recent

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years, the production of haricot bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) has increased as they are exportable and cash earning commodities (Girma et al. 2013). N₂ fixing soybean crop is of considerable interest for more sustainable agriculture and particularly in organic farming systems (Cazzato et al. 2012). In view of this, biological nitrogen fixation (BNF), a renewable N fertilizer source, holds great promise for smallholder farmers. But the use of *Rhizobium* inoculants for improvement in N-fixation and productivity of grain legumes is still in the developing stage in most parts of sub-Saharan Africa (Abdullahi et al. 2013), including Ethiopia. In Ethiopia, particularly in the western part, soybean is growing in smallholder farmer fields where the use of fertilizer is limited and inoculation of soybean with *Rhizobial* inoculants almost not a common practice (Getachew et al. 2016). But inoculation of effective strain could provide an option to increase grain yields of soybean in low nitrogen soils (Muleta et al. 2017). Due to *Rhizobial* inoculation, the higher amount of net N added to the soil system from the inoculated legumes in the form of Uride (Franche et al. 2009). Through nitrogen fixation, therefore, the use of costly inorganic fertilizer might be reduced and improved grain legume production in resource-poor farmer's field could be robust. This is especially true in the tropics where nitrogen is the most limiting soil nutrient, and where subsistence farmers cannot afford the cost of fertilizers. Furthermore, biological nitrogen (N₂) fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity.

Nowadays, inoculation with efficient strains allows the soybean plant to obtain its entire N needs from BNF (Hungria et al. 2006), which holds great promise for smallholder farmers in sub-Saharan Africa and is a natural process of significant importance in world agriculture (Herridge et al. 2008). In addition, in Ethiopia, several field demonstrations have confirmed that leguminous crops such as soybean, common bean, chickpea, and groundnut show remarkable growth and yield response to rhizobia inoculations in different agro-ecologies (EIAR 2014; Argaw 2014). However, concern has been expressed in terms of whether BNF is capable of meeting increased N needs and or capable of allowing maximum grain yields (Hungria et al. 2006). In addition, the amount of available soil N and applied N is among principal factors that primarily control the amount of N₂ fixation. Furthermore, inoculation alone would be insufficient to provide all the nitrogen required for optimal soybean yields especially in N deficient soils (Mendes et al. 2003).

Accordingly, the amount of starter N for the N₂ fixation capacity of *Rhizobial* strains and the efficiency of symbiosis could be an option for optimum growth and seed

yield. When the plant had supplemented with N fertilizer, N₂ fixation by *Rhizobium* suppressed even at low rates of N (Da Silva et al. 1993). For manipulating N₂ fixation and improve the N benefits to agricultural legumes, there is a need to reduce the inhibitory effect of applied N on *rhizobia* inoculant. Because N fertilizer has the potential to change the number of effective rhizobia and influence legume biomass (Peoples et al. 1995). When inoculated plants are supplemented with a small quantity of N fertilizer, the small fraction of N may increase the strains efficiency on N₂ fixation thereby improve seed yield. Even though the current farmer's practice of soybean production using inoculation of effective strain with N fertilizer brought economic benefit in Ethiopia (Muleta et al. 2017), the optimum level of N as starter fertilizer should be used by farmers with an inoculation to increase the productivity of legumes. Therefore, the objective of the present study was to determine the amount of starter N application which could enhance N₂ fixation and soybean seed yield using *Rhizobial* inoculation and different nitrogen doses.

Materials and methods

Description of the study area

Field experiments were conducted during the 2016/17 and 2017/18 cropping seasons at two locations, Assosa and Begi areas, western Ethiopia. Assosa is the capital city of Benishangul Gumuz regional state in western Ethiopia and lies on an altitude of 1480 m above sea level, and located at 09°58'41.7" N, 034°38'09.5" E coordinates. According to the FAO/UNESCO soil classification, the major soil type at the Assosa area is Nitisols, but soils at Begi site expected to be also Nitisols. The dominant soil types in Assosa Area are sandy clay loam and clay loam, which are poor to medium in organic matter and poor nutrients because of the long cropping history without replenishment of nutrients.

Soil physico-chemical analysis

Ten soil samples (0 to 20 cm) were randomly taken using a soil auger from each site 2 weeks before planting. The 10 samples from each site were combined into a composite sample and two sub-samples of the composite from each site were taken to the laboratory for chemical analysis and averaged for all parameters (Table 1). The determination of particle size distribution was carried out by the Bouyoucos hydrometer method (Bouyoucos 1962). Soil pH was measured in the supernatant suspension of 1:2.5 soil and water mixture by using a pH meter. Soil organic carbon was determined by using the Walkley and Black method (Walkley and Black 1934). Total N of the soils was determined through digestion, distillation, and titration

Table 1 Some selected physical and chemical properties of soils for the experimental sites before planting

Type of soil analysis	Location	
	Assosa	Begi
Soil particle size (Texture)	Silt clay loam	Clay loam
pH	5.52 ± 0.04	6.16 ± 0.06
OC (%)	2.01 ± 0.12	2.30 ± 0.30
Total N (%)	0.14 ± 0.15	0.25 ± 0.22
Bray-II available P (mg kg ⁻¹)	14.50 ± 0.65	26.00 ± 1.52
Exchangeable K (g kg ⁻¹)	5.60 ± 0.55	19.40 ± 0.30
CEC (meq 100 g ⁻¹)	18.40 ± 0.38	27.30 ± 0.78

procedures of the Micro-Kjeldahl method as described by (Nelson and Sommers 1973). Available phosphorus was determined by the Bray II method (Bray and Kurtz 1945). Cation Exchange Capacity (CEC) was determined by leaching the soil with neutral 1 N ammonium acetate (pH = 7) (Van Reeuwijk 2002) and exchangeable K extracted by ammonium acetate method and was determined using a flame photometer.

Experimental set up

Soybean variety Belessa-95 was used in the experiment with a known commercial rhizobial inoculant MAR-1495. The experiment was designed in a split-plot with three replications and treatments tested were two inoculation levels (inoculated (1) and non-inoculated (0)) as the main plot and six nitrogen rates (0, 9, 18, 27, 36 and 54 kg N ha⁻¹) as the subplot treatments. Phosphorus from TSP was applied as a basal for all plots at 46 kg P₂O₅ ha⁻¹. Plot sizes were 3.6 m*4.0 m (14.4 m²) consisted of six rows, two rows for nodulation, and shoot dry weight parameters and the other rows excluding borders were harvestable rows. The plots were kept 1.0 m apart with 1 m spacing between blocks. Canals were prepared around each plot making the plots beds. Carrier-based inoculant was applied at the rate of 123 g inoculant for 15 kg seed (used to sow quarter ha). To ensure that all the applied inoculum stick to the seed, the required quantity of inoculant was suspended in 1:1 ratio in a 10% sugar solution. The thick slurry of the inoculant was mixed gently with dry seed so that all the seeds received a thin coating of the inoculant. All inoculations were done just before planting under shade to maintain the viability of bacterial cells. The inoculated and un-inoculated seeds were then planted at a spacing of 10 cm between plants and 60 cm between rows making six rows per plot. Seeds were covered immediately with soil after sowing to avoid the death of cells due to the sun's radiation.

Nodulation and shoot dry weight

Ten plants were sampled randomly from the second border rows of each plot at mid flowering (50% flowering). The whole plant was uprooted carefully using a spade to obtain intact roots and nodules for nodulation parameters and dry weight of plants. Uprooting was done by exposing the whole-root system to avoid loss of nodules. The adhering soil was removed by washing the roots with intact nodules gently with water over a metal sieve. The same ten plants from each plot were used to rate nodulation and to the record number of nodules per plant and nodule dry weight. After nodules were taken, three plants from each plot were cut at the ground level for shoot dry weight determination. Total fresh shoot weight was measured using an electronic balance. Sub-samples of shoots were oven-dried at 70 °C for 48 h using brown envelopes. The dry materials were weighed and shoot dry weight recorded averaged as per plant.

Statistical analysis

The analysis of variance was carried using the general linear model (GLM) procedure provided by SAS statistical software version 9.20 (SAS 2008). Combined analysis across locations and cropping seasons were performed with seasons (years) considered random for estimating the variability of treatments for different locations across seasons. Hence, location, N rates, and inoculation levels were considered fixed, whereas cropping season and blocks nested within locations were considered random effects. Data for each cropping season was then analyzed separately since across cropping seasons. Analysis of variance was performed to evaluate treatment factors (N rates and inoculation levels) and their interactions on nodulation, growth, and seed yield. Means were separated using the LSD procedure ($p < 0.05$).

Results

Soil analysis for experimental sites

The soil of the experimental site (Assosa) was sandy clay loam in texture, while for Begi site it was clay loam. The average soil pH for Assosa was 5.52, which is strongly acidic. The pH of this site is characteristic of weathered soils which may be problematic for growing crops and need management for the production of most field crops. Based on the results obtained for soil analysis, the average organic carbon (OC) and total nitrogen (N) were 2.01 % and 0.14 %, respectively. Thus, according to the ratings suggested by Tekalign et al. (1991) (%OC between 1.5 to 3.0% as well as %N between 0.12 to 0.25%, have moderate range) both parameter's value falls in the moderate range for Assosa site. In addition, soil available P (Bray-II) was less than 20 ppm and rating low in accordance with

Horneck et al. (2011) as well as Tekalign et al. (1991). Cation Exchange Capacity was < 20 meq 100g⁻¹ soil then rating low according to Horneck et al. (2011) and Tekalign et al. (1991), but moderate according to Hazelton and Murphy (2007). The exchangeable K was low according to Horneck et al (2011) and very low according to Tekalign et al. (1991). The average total nitrogen (N) and available phosphorus (P) were low and maybe not optimal for crop production. The total N and available P contents of the soil for Assosa might be attributed to the relatively higher OC contents. However, the soil physico-chemical status of the Assosa site was characterized as poor compared to Begi site which had ideal soil characteristics for the production of most field crops (Table 1).

The effects of year and location X year (L × Y) influenced most of the parameters evaluated. In addition, year X Nitrogen rate and treatment interactions (I × N) significantly affected the seed yield of soybean (Table 2). However, the effect of the year was more dominant than all the other factors. Moreover, the interaction of N application together with the inoculation level had a significant effect across seasons, but the interaction was not significant or had similar effects for most of the parameters across locations (Table 2). Therefore, data were analyzed and reported for each experimental season across locations. The total rainfall for Assosa varied between the two cropping seasons and was relatively good in 2016/17 compared to in 2017/18. Most of the rainfall in 2016/17 occurred in June and August but

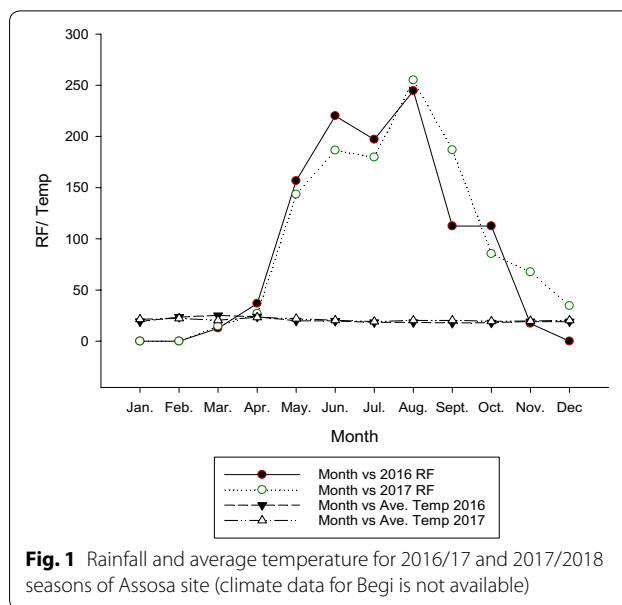


Fig. 1 Rainfall and average temperature for 2016/17 and 2017/2018 seasons of Assosa site (climate data for Begi is not available)

the peak rainfall in 2017/18 in August had an effect on the growing plants that caused temporary water lodged conditions, which affected seedling emergence and crop establishment. Temperatures for the two cropping seasons were not different (Fig. 1).

Table 2 Combined ANOVA for Effect of starter N and *rhizobial* inoculation on nodulation, growth and yield of soybean at Assosa and Begi in 2016/17 and 2017/2018 cropping seasons

Source of variation	NN (No/plt.)	NDW (g/plt.)	SDW (g/plt.)	PPP	SPP	SY (kg/ha)
Loc	12.25 ^{NS}	0.03 ^{NS}	0.44 ^{NS}	68.75 ^{NS}	1.21 ^{NS}	10.23 ^{NS}
Year	0.21 ^{NS}	0.61 ^{NS}	37.1 ^{***}	64.1 ^{***}	23.89 ^{**}	314.3 ^{***}
Loc × Year	0.66 ^{NS}	0.36 ^{NS}	29.1 ^{***}	0.03 ^{NS}	46.4 ^{***}	80.36 ^{***}
Inoculation (I)	18.02 ^{NS}	65.6 ^{NS}	30880 ^{**}	0.64 ^{NS}	40.1 ^{NS}	81.45 ^{NS}
Loc × I	2.48 ^{NS}	0.73 ^{NS}	5.70 ^{NS}	0.21 ^{NS}	289.0 [*]	41.82 ^{NS}
Year × I	2.81 ^{NS}	0.43 ^{***}	0.00 ^{NS}	5.73 [*]	0.05 ^{NS}	3.95 ^{NS}
Loc × Year × I	0.03 ^{NS}	3.81 ^{NS}	1.54 ^{NS}	3.21 ^{NS}	0.001 ^{NS}	1.65 ^{NS}
Nitrogen rate (N)	0.44 ^{NS}	2.79 ^{NS}	2.83 ^{NS}	1.42 ^{NS}	0.73 ^{NS}	1.94 ^{NS}
Loc × N	0.17 ^{NS}	3.90 ^{NS}	0.53 ^{NS}	0.64 ^{NS}	1.77 ^{NS}	0.17 ^{NS}
Year × N	3.30 ^{**}	0.29 ^{NS}	2.08 ^{NS}	0.82 ^{**}	1.76 ^{NS}	4.15 ^{***}
Loc × Year × N	2.23 ^{NS}	0.52 ^{NS}	0.83 ^{NS}	1.27 ^{NS}	0.27 ^{NS}	24.3 ^{***}
I × N	0.94 ^{NS}	0.75 ^{NS}	5.73 [*]	5.25 [*]	0.56 ^{NS}	4.91 [*]
Loc × I × N	1.89 ^{NS}	1.83 ^{NS}	0.27 ^{NS}	0.74 ^{NS}	2.25 ^{NS}	2.55 ^{NS}
Year × I × N	4.69 ^{***}	0.82 ^{NS}	1.63 ^{NS}	3.79 ^{**}	6.45 ^{***}	1.05 ^{NS}
Loc × Year × R × V	1.70 ^{NS}	1.32 ^{NS}	2.31 ^{NS}	0.20 ^{NS}	1.26 ^{NS}	1.69 ^{NS}
CV (%)	49.42	59.52	20.21	18.76	6.18	9.50

NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; PPP, pod per plant; SPP, seed per pod; SY, seed yield, NS, non-significant (p > 0.05)

*significant (p < 0.05), **highly significant (p < 0.001), ***extremely significant (p < 0.0001)

Nodulation and shoot dry matter

Analysis of variance has shown that nitrogen application had not affected significantly ($p < 0.05$) nodulation parameters of soybean for the 2016/17 cropping season (Table 3). However, nodule number and dry weight had significantly affected by the N rate in the 2017/18 cropping year. Increasing rates of N from zero up to 18 kg ha⁻¹ increased nodule number but further increase reduced the number of nodules. Application of N at 18 kg ha⁻¹ increased nodule number and dry weight by 98.3 and 115.0%, respectively over un-fertilized control as well as by 61.0 and 58.0%, respectively over the greatest N rate (54 kg N ha⁻¹). Shoot dry weight at half the growth stage was also significantly ($p < 0.05$) influenced by N application in the 2017/18 cropping year. N rate at 18 kg ha⁻¹ significantly enhanced shoot biomass of soybean by 35.6% over non-fertilized treatment. Inoculation significantly ($p < 0.05$) improved nodule number, dry weight and shoot biomass in both cropping seasons

except shoot dry weight in 2017/18 cropping season. Significantly, better nodulation and shoot dry weight had obtained at Begi than the Assosa site. The interaction effects between inoculation level and N rate had no significant ($p > 0.05$) effect on nodulation and shoot dry weight for both cropping seasons except nodule dry weight and number in 2016/17 and 2017/18, respectively.

Seed yield and yield components

Analysis of variance showed N application significantly ($p < 0.05$) affected both pods per plant and seed yield of soybean in 2016/17 cropping year but not affected the number of seed per pod. In the 2017/18 cropping season, nitrogen application significantly ($p < 0.05$) influenced seed per pod and seed yield of soybean but not affected the number of pods obtained (Table 4). Increasing levels of N application from zero to 18 kg ha⁻¹ showed an increased seed yield of soybean in both years. Application of N at 18 kg ha⁻¹ showed significantly higher yields

Table 3 Effect of N rates and *Rhizobial inoculation* on nodulation and shoot dry matter of soybean for 2016/17 and 2017/18 cropping seasons

Treatment	2016/17 cropping season			2017/18 cropping season		
	NN	NDW	SDW	NN	NDW	SDW
Location						
Assosa	29.39	0.203 ^b	17.68	31.10	0.223	9.45 ^b
Begi	19.73	0.398 ^a	16.39	13.72	0.492	15.89 ^a
LSD	NS	0.102	2.47	NS	NS	3.03
N rates (N)						
0	22.69	0.247	14.45 ^b	17.52 ^b	0.191 ^c	9.74 ^{cd}
9	25.70	0.369	17.54 ^a	25.20 ^{ab}	0.380 ^{ab}	14.30 ^{ab}
18	23.39	0.317	16.85 ^{ab}	34.74 ^a	0.410 ^a	15.14 ^a
27	29.68	0.330	18.12 ^a	17.11 ^b	0.303 ^b	12.37 ^{bc}
36	23.18	0.287	16.40 ^{ab}	18.32 ^b	0.283 ^b	11.90 ^c
54	22.73	0.253	18.86 ^a	21.57 ^b	0.259 ^b	12.55 ^{bc}
LSD	NS	NS	2.68	10.27	0.101	2.25
Inoculation level						
Uninoculated (0)	6.40 ^b	0.073 ^b	15.99 ^b	11.17 ^b	0.180 ^b	11.64
Inoculated (1)	42.73 ^a	0.527 ^a	18.08 ^a	33.65 ^a	0.535 ^a	13.70
LSD	12.00	0.102	1.81	19.53	0.312	NS
F-test value						
Loc	4.65 ^{NS}	27.52 ^{**}	1.94 ^{NS}	4.33 ^{NS}	3.64 ^{NS}	34.80 ^{**}
Inoculation level (I)	70.56 ^{**}	150.6 ^{***}	10.20 [*]	10.21 [*]	8.72 [*]	6.67 [*]
Loc × I	0.20 ^{NS}	55.96 ^{**}	1.85 ^{NS}	0.001 ^{NS}	0.02 ^{NS}	7.36 [*]
N rate (N)	0.79 ^{NS}	0.80 ^{NS}	2.69 [*]	3.55 ^{**}	3.17 ^{**}	5.81 ^{***}
Loc × N	1.28 ^{NS}	1.01 ^{NS}	0.44 ^{NS}	1.32 ^{NS}	1.48 ^{NS}	0.91 ^{NS}
I × N	1.14 ^{NS}	1.27 [*]	1.75 ^{NS}	4.79 ^{**}	0.96 ^{NS}	1.33 ^{NS}
CV (%)	43.58	60.75	19.09	55.53	58.26	21.54

Mean values followed by different letters in a row show statistically significant difference

NS, non-significant ($p > 0.05$)

*significant ($p < 0.05$); **highly significant ($p < 0.001$); ***extremely significant ($p < 0.0001$)

Table 4 Effect of N rates and *Rhizobial* inoculation on yield components and seed yield of soybean for 2016/17 and 2017/18 cropping seasons

Treatment	2016/17 cropping season			2017/18 cropping season		
	PPP	SPP	SY	PPP	SPP	SY
Location						
Assosa	29.80	2.50	2173.2 ^b	17.23	2.12	1292.2 ^b
Begi	31.62	2.03	2823.7 ^a	19.54	2.10	2534.4 ^a
LSD	NS	0.16	144.6	NS	NS	112.53
N rates (N)						
0	28.42 ^{bc}	2.21	2192.8 ^c	17.51	2.08 ^b	1434.9 ^c
9	31.47 ^{abc}	2.31	2519.6 ^a	19.53	2.10 ^b	1891.6 ^b
18	34.03 ^a	2.27	2781.7 ^a	17.56	2.05 ^b	1922.1 ^b
27	29.86 ^{bc}	2.31	2402.3 ^b	19.35	2.11 ^b	2210.3 ^a
36	28.21 ^c	2.26	2348.3 ^{bc}	17.40	2.10 ^b	2087.1 ^a
54	32.29 ^{ab}	2.24	2746.3 ^a	18.97	2.21 ^a	1933.7 ^b
LSD	4.00	NS	204.15	NS	0.099	134.81
Inoculation level						
Uninoculated (0)	28.35	2.29	2218.3 ^b	18.65	2.12	1689.1 ^b
Inoculated (1)	33.07	2.24	2778.6 ^a	18.12	2.09	2137.5 ^a
LSD	NS	NS	73.88	NS	NS	137.4
F-test value						
Loc	0.42 ^{NS}	65.80 ^{**}	155.95 ^{***}	3.34 ^{NS}	0.52 ^{NS}	939 ^{***}
Inoculation level (I)	5.57 ^{NS}	0.84 ^{NS}	443.1 ^{***}	0.34 ^{NS}	1.24 [*]	82.04 ^{***}
Loc × I	2.06 ^{NS}	0.06 ^{NS}	102.7 ^{***}	1.40 ^{NS}	0.14 ^{NS}	15.91 [*]
N rate (N)	2.68 [*]	0.81 ^{NS}	10.48 ^{***}	0.64 ^{NS}	2.61 [*]	31.30 ^{***}
Loc × N	1.50 ^{NS}	0.59 ^{NS}	6.98 ^{***}	0.47 ^{NS}	0.06 ^{NS}	30.93 ^{***}
I × N	6.16 ^{***}	3.70 ^{**}	14.08 ^{***}	0.95 ^{NS}	4.09 ^{**}	51.27 ^{***}
CV (%)	15.81	6.53	9.90	23.60	5.73	8.53

Mean values followed by different letters in a row show statistically significant difference

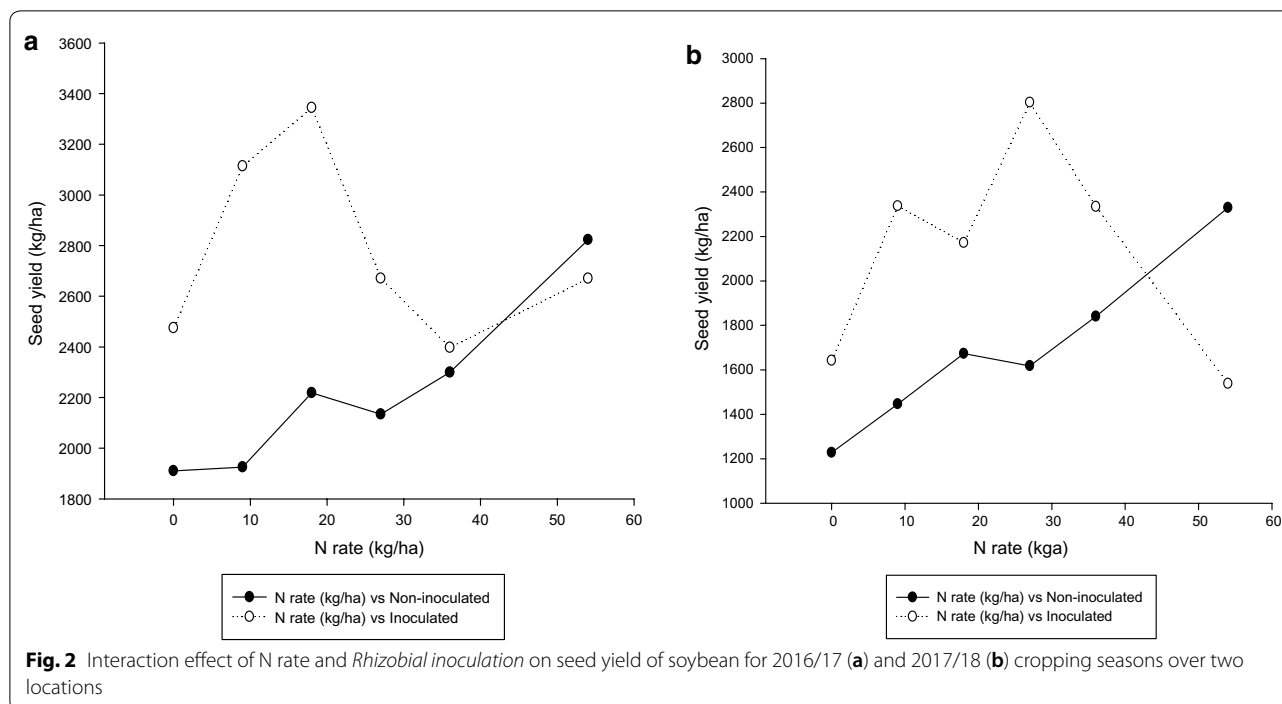
NS, non-significant ($p > 0.05$)

*significant ($p < 0.05$), **highly significant ($p < 0.001$), ***extremely significant ($p < 0.0001$)

increased pod per plant and seed yield by 16.5 and 21.2% over non-N applied treatments in the 2016/17 cropping season. While in 2017/18 N at 27 kg ha⁻¹ significantly resulted in higher seed yield which increased yield by 35.1% over unfertilized control. In both cropping years, inoculation improved soybean seed yield which increased by 20.2 and 21.0% than non-inoculated plants in 2016/17 and 2017/18 cropping seasons, respectively. Soybean yield had significantly higher at the Begi site than Assosa. Results showed that the interaction effect between inoculation level and N application rates significantly ($p < 0.05$) influenced seed yield and yield components of soybean in both seasons except the number of pod per plant in 2017/18 (Table 4).

The results showed that the interaction effects between N rate and inoculation of strain significant ($p < 0.001$) influenced the seed yield of soybean in 2016/17 (Fig. 2a). Application of N as a starter increased yield of soybean especially an enhanced yield

was obtained in combination with inoculation of strain. The highest seed yield of soybean had obtained when N at 18 kg ha⁻¹ applied with inoculation, while the least seed yield was produced non-inoculated plants. Application of N above 18 kg ha⁻¹ resulted in decreased soybean seed yield for inoculated plants. On the contrary, the seed yield of soybean increased with N fertilizer application for non-inoculated treatment in 2016/17. Similarly, in the 2017/18 cropping season, the interaction effects between N rate and inoculation of strain significant ($p < 0.001$) influenced the seed yield of soybean (Fig. 2b). Application of N as a starter increased yield of soybean with an increased rate of N from zero up to 27 kg ha⁻¹ with inoculation of strain. The highest seed yield of soybean had produced when 27 kg N ha⁻¹ applied with inoculation, while the least seed yield had produced in non-inoculated plants. Application of N above 27 kg ha⁻¹ resulted in decreased soybean seed yield for inoculated plants, but the seed yield of



soybean increased linearly with N application rates in non-inoculated treatments.

Discussion

Legumes like soybean are the most important crops and common hosts for a range of symbiotic bacteria, known as rhizobia that naturally fix atmospheric N (Zahran 1999). In the present study, irrespective of the experimental site, inoculated plants showed a significant improvement in nodulation, growth, and yield of soybean. The ability of inoculation to provide top yields over un-inoculated plants in both seasons was a result of adequate inoculation of efficient rhizobia strain. A significant effect of *Rhizobium* inoculation on soybean seed yield and quality improvement compared to N fertilizer was found by Sogut (2006) with each treatment applied alone. Inoculation of efficient nitrogen-fixing symbioses most directly related to effective nodule formation for enhanced plant growth. However, the population density, effectiveness in forming nodules, and competitive ability are the major factors that determine the degree of inoculation response (Singleton and Tavares 1986). Although the inoculation of soybean showed improved nodulation and growth further improvement can be obtained when a starter N is combined with inoculation of the efficient strain (MAR-1495). Inoculation of effective strain will mostly be insufficient to satisfy the total fixed-N demand of the growing legume, creating a need for effective nodulation (Herridge 2008) or apply a starter amount of

N with inoculation for balancing N budget of the plant (Kubota et al. 2008). Moreover, N fertilization with rhizobia inoculant has been found to increase the crucial role of legumes in agricultural production. It appears that soybean was able to satisfy N requirements when inoculation applied with a small amount of starter N rate to ensure the N requirement for economically maximum seed yield. Benefits of inoculation and N application can be useful if the farmer's field is low in N availability or if the soybean cultivar has large N demand (Kubota et al. 2008). Efficient strains of *Rhizobium* selection which produce symbioses and could achieve greater nitrogen fixation gains by soybeans less affected by external nitrate are suggested by McNeil (1982).

Analysis of variance has shown that nitrogen application had not affected significantly ($p \leq 0.05$) nodulation parameters of soybean for the 2016/17 cropping season. However, significant effects had detected due to N rates on nodule number and dry weight as well as shoot dry weight in 2017/18. Even though the application of N fertilizer inhibits nodulation and N_2 fixation, however, the degree of inhibition depends largely on various factors including the host plant, rhizobia strain and rate of N application (Senaratne et al. 1987). Response to added N in 2017/18 indicated that the nodules formed were unable to provide all of the crop's N requirements. Differences due to nitrogen application on the mean number and dry weight of nodules per plant as well as shoot biomass were significant and increasing nitrogen level above

18 kg N ha⁻¹ decreased nodulation and growth of soybean in 2017/18. Diep et al. (2002) also found a reduction in nodulation of vegetable soybean with increased levels of N application. Similar to the present study Senaratne et al. (1987) found a significant increase in the amount of N₂ fixed for inoculated seeds at 20 kg ha⁻¹ compared to the non-nodulating control. Similarly, Yinbo et al. (1997) also found a depression in nodulation of soybean with an addition on N fertilizer, but a starter N resulted in the highest proportion of plant N drove from N₂ fixation. On the other hand, Hungria et al. (2006) found a decrease in the contribution of N₂ fixation at 30 kg N ha⁻¹ supplied at sowing. The application of N fertilizer can suppress the N₂-fixing activity of a plant even at low N rates (Da Silva et al. 1993). The reduction of N₂ fixation by *Rhizobium* supplemented with N source is usually attributed to fertilizer N affects nodulation mainly by destroying idole acetic acid, reducing lectin production by the host as well as root hair formation and curling (Dogra and Dudeja 1993). Although externally applied nitrate generally inhibits nitrogen fixation, McNeil (1982) reported the beneficial effect from starter N applied before the establishment of an effective symbiosis with *Rhizobium* strain. Nodulation was generally higher at 9–18 kg N ha⁻¹ of N fertilizer. Consequently, the number and dry weight of nodules observed with inoculation above 27 kg N ha⁻¹ were smaller in size than nodules produced in smaller N rates.

Most studies on the effect of fertilizer-N on soybean growth and N₂ fixation concluded that fertilization reduces N fixation (Yinbo et al. 1997; Diep et al. 2002; Clayton et al. 2003; Salvagiotti et al. 2009) through a reduction in the number, weight, and activity of nodules (Semu and Hume 1979). However, this effect depended on a variety of factors such as the amount of N applied and type of soil, climate, and farming system. The extent of the reduction in nodulation and growth in the present study was related to the amount of N applied. The soil environment should be appropriate for the exchange of signals that precedes infection during Compatibility between root-nodule bacteria and the host plant (Graham 2008). Among several limiting factors that restricted the success of inoculation, the amount of soil N plays an important role in the control of legume–rhizobia interactions, limiting the symbiotic process (Dogra and Dudeja 1993; Herridge 2008). No response to added N observed in 206/17 for nodulation parameters. Although some variability existed, for both locations there was a tendency for high soil N to depress nodule efficiency in the half growth stage. Our data indicated that an increase in N application limited the ability of the inoculated rhizobia population to effectively nodulate soybean plants. The nodulation that occurred on the un-inoculated check-in

both growing seasons indicates that indigenous rhizobia likely contributed to ineffective nodules formation. These results indicated that few legume rhizobia to cause false nodulation were present in the soil without inoculation. Although soil tests indicated relatively high levels of total N at Begi, rhizosphere levels of N might likely be depleted due to active plant uptake and thus were not inhibitory to nodulation even at N rate 18 kg ha⁻¹. Waterer and Vessey (1993) reported that inhibition of nodulation is dependent on the concentration of N in the soil solution and that depletion of inhibitory concentrations (as might occur in the root zone due to active plant N uptake) could provide a window during which nodulation could occur.

Soybean is grown by smallholder farmers under low inputs agricultural system with little or no fertilizer application associated with subsistence agriculture, characterized by the low use of inputs. Hence, biological nitrogen fixation using effective strains in the traditional cropping system is of vital importance for system sustainability. Besides triggering a significant yield improvement, decrease the use of chemical fertilizers. In both cropping seasons, inoculation resulted in a non-significant effect on pod number and seed per pod of soybean compared to the un-inoculated treatment, but soybean yield was significantly increased by rhizobia inoculation in 2016/17 and 2017/18. Inoculation with N application significantly enhanced seed yield and yield components of soybean in both seasons except the number of pod per plant in 2017/18. The interactions indicate that the response to inoculation and applied N resulted in increased yield traits and ultimately improved plant yield performance of soybean. Besides, the yield was further increased by additions of N indicated the need for small N required for maximum yield. Similarly, a positive effect of fertilizer N application with rhizobia inoculation was found by Kubota et al. (2008). Hence, it may be necessary to apply supplementary N especially to legumes such as soybean to realize maximum yield (Afza et al. 1987).

The interactions in the present study showed that the response to the inoculant level and applied N on yield and its traits varied among the two cropping seasons and results reported for each season. Therefore, in both cropping seasons results showed the use of starter N with inoculation of strain was significant for growth and seed yield of soybean. Even though an appropriate rhizobium strain be inoculated for successful nitrogen fixation, an insufficient amount of N in the soil rhizosphere can hamper nodulation and thereby reduce yield. However, a positive effect of fertilizer N application was detected when seeds had been inoculated with rhizobial strain in both seasons. Grain yield in 2016/17 season was much higher than 2017/18. The highest seed yield of soybean had obtained at 18 kg N ha⁻¹ applied with inoculation

which increased soybean yield by 50.7 and 75.0% over inoculated but unfertilized and un-inoculated and unfertilized (control), respectively. While the least yield was obtained on un-inoculated plants. However, the application of N above 18 kg ha⁻¹ found with the decreased yield for inoculated plants in 2016/17. It may be due to the application of N fertilizer-induced reduction of N₂ fixation and hence contributed substantially to decrease soybean seed yield and yield traits. Simanungkalit et al. (1995) also reported positive responses to starter N rates (25 and 50 kg ha⁻¹) with inoculated soybean in a sandy clay red yellow Podzol in Indonesia. Mendes et al. (2003) also found an increase in soybean seed yield at starter N (50 kg ha⁻¹) with *Rhizobium* inoculation and the authors suggested the N rate was a viable input for increasing grain yield. Recently, Ntambo et al. (2017) also found the application of N at 50 kg ha⁻¹ with rhizobial inoculation increased soybean yield. In contrast, Chemining et al. (Chemining'wa et al. 2007) reported non-significant results either using rhizobial inoculation or starter N application on different grain legumes.

In 2017/18, August month which to be near the vegetative period of plants had more rainfall. It was likely that more rainfall affected negatively growth of plants, so it resulted in a slightly decreased in yield of soybean compared to in 2017/18. Soybean seed yield in 2017/2018 was generally low compared to 2016/17 but N application and seed inoculation had positive effects, and their combined application at N rate 27 kg ha⁻¹ increased yield by 73.2 and 128.2% improvement over unfertilized but inoculated and over control, respectively. Daba and Haile (2000) suggested that inoculation of strain and starter N (23 kg ha⁻¹) fertilizer gave a significantly higher grain yield, nodule number, and dry matter yield in soils of Eastern Ethiopia. These results are consistent with those of a field study in Brazil Hungria et al. (2006) that reported low rates of symbiotic BNF in soybean with relatively high N fertilizer. They found a decrease in the contribution of N₂ fixation even at the low supply of starter N (30 kg of N ha⁻¹). However, McKenzie et al. (2001) observed that the application of N fertilizer (20, 40, or 60 kg N ha⁻¹) increased pea yield in 24% of the 58 trials conducted by an average of 9% with rhizobia inoculation. Namvar et al. (2011) also reported an increase in yield and its attributes on chickpea due to application N fertilizer with inoculation. They found that the greatest N level (100 kg ha⁻¹) found to increase in chickpea yield in the presence of *Rhizobium* inoculation. However, nodule number and dry weight decreased significantly with increased N rate (0, 50, 75, and 100 kg ha⁻¹). A significant superiority of inoculation with *Rhizobium* over un-inoculated treatments at all rates of N fertilization (50, 75, and 100 kg N ha⁻¹) was also reported by Khaitov and

Abdiev (2018). The authors found N at 75 kg N ha⁻¹ combined with inoculation showed the highest seed yield and quality of chickpea. The greatest N rate found increased yield with inoculation may be due to chickpea fix lower N which could be inadequate to meet its N requirement and hence requires additional high N rate as a starter to form a symbiosis with rhizobia. As Hardarson and Atkins (2003) reported leguminous crops to differ in their ability to fix atmospheric N₂. Hence, soybean requires a lower N rate as a starter during inoculation than a chickpea. An increase in grain yield of common bean with increasing rates of N application in the presence of *Rhizobium* inoculation was also previously observed by Argaw and Akuma (2015) under field conditions in Ethiopia. However, Da Silva et al. (1993) also found that both foliar and soil N application methods inhibited nodulation at high N rates and did not significantly increase common bean yield compared with low N rate (10 kg N ha⁻¹). In both seasons, un-inoculated treatments showed a consistent trend that the seed yield of soybean increased with increased N fertilizer. Growth and yield improvement soybean plants may be attributed to fertilizer use in the absence of inoculation. Similarly, Ntambo et al. (2017) also found increasing nitrogen fertilizer in non-inoculated plants enhanced plant height, pod number, shoot dry weight, and grain yield of soybean. Argaw and Kuma (2015) also found a significant increase in grain yield of common bean with increased rates of N application for un-inoculated treatments.

Conclusion

The effects of Rhizobial inoculation and nitrogen doses had found to be significant on growth and seed yield of soybean, according to combined data over two years for the two locations. In both cropping seasons, the use of starter N with inoculation of strain was significant for growth and seed yield of soybean. Results clearly showed N application at 18 kg ha⁻¹ significantly affected nodulation, growth, and seed yield of soybean compared to 9 and 27 kg N ha⁻¹ rates. Starter N has a potential benefit when soil has low ability to provide N in early season, low residual N as well as low soil organic matter. Therefore, Rhizobial inoculation with starter N application that is adequate to meet the N requirement for economically attractive seed yields are better soybean management practices. To conclude, 18 kg N ha⁻¹ could be the most suitable and can be used as a starter N with inoculation of strain for soybean growth and seed yield in both locations (Assosa and Begi).

Abbreviations

BNF: Biological nitrogen fixation; CEC: Cation Exchange Capacity; EIAR: Ethiopian Institute of Agricultural Research; GLM: General Linear Model; OC: Organic carbon; RCBD: Randomized Complete Block Design.

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Authors' contributions

We have equally likely made our great contribution for the betterment of the research strating from the development of the research idea with soil researchers in the institute and writing the proposal. However, in addition to analyzing the soil samples for the research areas, I participated more on writing and constructing ideas about the manuscript. As a result I am the first author of this manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

Supporting data for the results reported in this article can be found in progress and annual research review reports in Ethiopian Institute of Agricultural Research, Assosa Agricultural Research Center.

Ethics approval and consent to participate

All procedures performed in our study did not involved human participants, human data or human tissue.

Consent for publication

We understand that the information will be published without our/our child or ward's/our relative's (circle as appropriate) name attached, but that full anonymity cannot be guaranteed. We understand that the text and any pictures or videos published in the article will be freely available on the internet and may be seen by the general public.

Competing interests

The authors declare that they have no competing interests.

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