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Research article

Soybean (*Glycine max* (L.) Merr.) response to application of mineral nitrogen and bradyrhizobia on Nitisols of Teppi, Southwest Ethiopia

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A R T I C L E I N F O

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A B S T R A C T

Soybean (Glycine max (L.) Merr.), an important crop grown for its protein source for humans and livestock, is widely introduced in different parts of Ethiopia. However, the productivity of the crop is far below its potential in the country due to different factors, among which low soil fertility is a major contributor. Hence, this field experiment was conducted with the objective of determining the optimum rate of starter nitrogen (N) and bradyrhizobium inoculation on yield and yield components of soybean in the 2019 and 2020 cropping seasons. Two levels of bradyrhizobia (inoculated and uninoculated) and six levels of starter nitrogen (0, 9, 18, 27, 36, and 54 kg N ha^{-1}) were arranged in a factorial design. The result showed that soybean grain yield increased by about 60 % with inoculation of bradyrhizobia applied with low rates of starter nitrogen fertilizer, regardless of cropping seasons. Application of a nitrogen rate above 18 kg N ha^{-1} leads to yield decline and has no significant variation from bradyrhizobia inoculation only. Regardless of the cropping seasons, elevated levels of starter nitrogen beyond 27 kg ha^{-1} suppressed nodulation and nodule dry matter. Starter N at a rate of 9 and 18 kg N ha^{-1} improved soybean nodulation by 125–130 % over control and 95 % over bradyrhizobia inoculation alone. Thus, it was recommended to apply bradyrhizobia strains with 9 or 18 kg N ha^{-1} starter nitrogen for better yield of soybean as well as adequate nitrogen fixation in Nitisols having moderate soil nitrogen levels similar to the Teppi areas.

1. Introduction

Soybean (*Glycine* max (L.) Merr.) is one of the most important seed legumes worldwide [1]. Soybean has become a crop grown in several agro-ecological zones of Ethiopia. Currently, soybean production covers a total of 54,543 ha, with a total production of 125, 623 t and a national average productivity of 2.3 t ha^{-1}, which is lower than the world average productivity (3.21 t ha^{-1}) [2]. Despite the growing demand for it as a cash crop in the country, the average productivity of soybean has never reached its maximum yield potential due to improper fertilizer management [3].

Soybean requires large amounts of nitrogen due to its high protein content of about 40 %, in seeds [4], The major source of this element is biological nitrogen fixation, where legumes form a symbiosis with specific bradyrhizobia species in the soil. The use of bradyrhizobium inoculums in the establishment of legumes is widely accepted, particularly in areas where indigenous nodulation has

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been found to be insufficient [5]. The advantages of using bradyrhizobium inoculants show that smallholder farmers can save substantial cash by using quality-tested inoculants on the farm [6]. Bradyrhizobia inoculation of seeds has been extensively researched, and taking advantage of this beneficial nitrogen-fixing root nodule symbiosis is an important part of successfully applied agricultural microbiology [7]. Biological nitrogen fixation is important for crop establishment and yield because no nitrogen fertilizer is used and it meets the majority of the plants' nitrogen requirements [7]. Seed inoculation with Bradyrhizobium has been shown to increase crop plant nodulation, N uptake, growth, and yield response. Under favorable conditions, soybean-bradyrhizobium symbiosis can fix about 300 kg N ha^{-1} [8].

While soybean is a relatively sensitive crop to nitrogen deficiency, both mineral and fixed nitrogen are very essential for the maximum yield and nitrogen content of seeds [9]. This is presumably because N_2 fixation begins only after nodule formation early in the vegetative stage, and nodule senescence, together with competition from seeds for plant assimilates limits fixation during the reproductive stages [8]. Recent works by Ref. [9] have also indicated that nitrogen application has a significant effect on the physiology and seed composition of soybeans, regardless of genotypes and environmental conditions. It was found that using biofertilizers alone, without additive rates from mineral fertilizers at the recommended level of mineral fertilizers, based on soil fertility, was less effective than those with the recommended rates of chemical fertilizers [10].

In addition to efforts made in this research domain, there are debates and varying results whether use of mineral nitrogen addition to inoculated soybeans improves yield and biological nitrogen fixation. Some researchers reported that application of mineral nitrogen as a starter or top dressing at flowering stages negatively affected biological nitrogen fixation and no effect on grain yield [11]. On the other hand [12,13], reported that applying a substantial amount of starter nitrogen could enhance soybean yield and yield components, in addition to supporting biological nitrogen fixation along with bradyrhizobia inoculation. This experiment was conducted to clearly determine the cutting point for starter N dose at which soybean yields and nitrogen fixation are not compromised. Even though there is high potential for soybean production, no previous study has investigated the effects of starter N addition and inoculation to determine the optimum dose of starter nitrogen for soybean production in specific area. Thus, it was hypothesized that combined application of different doses of starter nitrogen with bradyrhizobium inoculation had an influence on the growth and grain yield of soybean over farmer practice (no starter nitrogen and no inoculation). In this context, this study was aimed at determining optimum rate of Starter N application and bradyrhizobium inoculation for improved productivity of soybean in Teppi area, southwest Ethiopia.

2. Material and methods

2.1. Site description

The study was conducted at Teppi Agricultural Research Center experimental site in southwestern Ethiopian people's region. It was found 611 km from the capital Addis Ababa at *(07° 11′ 05″ N, 35° 25′ 08″ E)* with an altitude of 1200 m above sea level. The area's mean annual rainfall is 1559 mm, and it has a hot to moderately humid lowland agro-ecology with maximum and minimum average temperatures of 29.7 °C and 15.5 °C, respectively. Major soils in the study area were dominated by dystric nitisol [14]. Experimental soil contains a pH of 6.6, 0.3 % total N, 18.4 mg kg^{-1} available P, 836 cmol $(^{+})$ kg^{-1} K, 2.9 % organic carbon (OC), *30.*2 cmol $(^{+})$ kg^{-1} cation exchange capacity (CEC), with *3820 and 514.*6 cmol $(^{+})$ kg^{-1} of Ca and Mg respectively. It also has clay textural class with 18 %, 58 % and 24 % of sand, clay and silt respectively.

2.2. Experimental design and setup

The experiment was conducted for two consecutive years, in 2019 and 2020, during main seasons. It consisted of two bradyrhizobium inoculations (inoculated and uninoculated) and six mineral nitrogen levels (0, 9, 18, 27, 36, and 54 N kg ha^{-1}) combined in factorial design and randomized in a RCB design with three replications across the year. Soybean variety Belesa-95 was used as a test crop while urea (46 N) was applied at planting starter nitrogen. The Bradyrhzobium inoculant *MAR-1495* used in the study was taken from Holeta agricultural research center microbiology laboratory. Inoculant with a bacterial population of (10^6 CFU g^{-1}) was applied at a rate of 250 g per hectare. A solution of 10 % sugar was prepared to ensure complete attachment of strains to seeds soon before planting and seeds were soaked in the solution. To prevent contamination, plots with no bradyrhizobia were planted first. Phosphorus was applied basal at planting in the form of triple superphosphate (TSP) at the rate of 23 kg P_2O_5 ha^{-1} to all plots equally. Seeds were planted five cm between plants and 40 cm between each row. All agronomic practices except nitrogen and inoculation were equally treated for every experimental unit.

2.3. Data collection

At the 50 % flowering stage, ten border plants were carefully uprooted, and soils in the rhizosphere were taken with a spade to ensure the entire nodule was included. To remove soil from nodules, the root was washed with tap water on a sieve. The total number of nodules per plant was immediately counted and placed in the oven to dry at 70 °C for 48 h before the nodule dry weight of each plant was measured on a sensitive balance (0.001g). The same plants sampled for nodule parameters were oven dried at 70 °C for 48 h, and shoot dry weight was recorded (g $plant^{-1}$). At maturity, ten plants were randomly selected from the harvestable row, and the number of pods was counted. Three pods from the bottom, middle, and upper were counted to determine the number of seeds per pod, and average data was recorded. The net harvestable area of plots was completely harvested and threshed, and dried seed was weighed and adjusted to a moisture content of 12.5 % to determine grain yield (kg ha^{-1}).

2.4. Statistical analysis

The analysis of variance was carried out using the two-way ANOVA procedure provided by R software 4.2.3. The assumption of ANOVA was first checked before data analysis. LeveneTest package for homogeneity of variance and Shapiro test for normality were performed from the Car R package [15]. Data for each year was then analyzed separately across cropping seasons. An analysis of variance was performed to evaluate the effects of treatment factors (N rates and inoculation) on growth, and grain yield. Means were separated using the LSD procedure ($p < 0.05$) whenever there was a significant difference among treatments. Principal component analysis (PCA) was analyzed from the combined mean of two years data to find out the characters that accounted for the most of the total variation. The function core packages "facto extra" and "ggbiplot" of R software were used to generate PCA. The eigenvalues greater than one were used as a cutoff point to determine the PCs. The biplot analysis and its graphical output were then used to interpret the relationship and association between the treatments and the observed quantitative traits [15].

3. Results

3.1. Grain yield

Soybean grain yield was significantly ($p<0.001$) affected by the application of starter nitrogen and bradyrhizobia inoculation throughout both growing years. As indicated in Figs. 1 and 2, there was a positive yield response for soybeans across growing seasons with co-application of mineral nitrogen and bradyrhizobia inoculation. Besides the yield increase due to starter nitrogen, the result was consistent only up to 18 kg N ha^{-1} after which yield tends to decrease. In contrast with inoculated plots, there was a slight yield increment observed among uninoculated plots with an increasing rate of nitrogen fertilizer. Regardless of cropping year, the highest yield (3637 kg ha^{-1} and 3541 kg ha^{-1}) was obtained from the plot treated with inoculation and 18 kg N ha^{-1} during 2019 and 2020, respectively (Figs. 1 and 2). It is also elucidated that this combination of nitrogen and bradyrhizobia improved soybean yield by about 60 % over control in each year.

3.2. Yield components

The effect of co-application of bradyrhizobia with different rates of nitrogen on soybean yield components like the number of pods and seeds was presented (Table 1). Pod number, which is major yield component in soybeans, showed non-significant ($p > 0.05$) variation among treatments in 2019, whereas a significant difference ($p < 0.05$) was observed during second year of experiment. In 2020, besides its non-linearity, inoculation and nitrogen application increased the number of pods, ranging from 20 to 70 % over control. The highest pod number was recorded under bradyrhizobium with 18 kg N ha^{-1} while the lowest was recorded under the control plot with mean pod numbers of 63 and 29 respectively. On the other hand, seed number per pod (Table 1) was not significantly ($p > 0.05$) affected by treatments, which might be attributed to variety.

3.3. Nodulation and shoot growth

Analysis of variance showed that nodulation was significantly ($p<0.01$) affected by the combined application of bradyrhizobia and mineral nitrogen addition (Table 2). In 2019, the highest value (68.7) of nodule number recorded under inoculated plot and nitrogen level of 18 kg N was followed by 9 kg N, which gave 59.7 nodulation which is about 113 % and 103 % improvement over control. In the second season, a similar trend was observed under the application of 9 and 18 kg N ha^{-1}. In both cropping years, the lowest nodule number was recorded under the control plot without bradyrhizobia and nitrogen addition. In addition, high dose application of nitrogen fertilizer to soybeans negatively affected nitrogen fixation since nodulation was impaired under nitrogen rates above 18 kg N ha^{-1}. Nodule dry weight (NDW) was also significantly ($p<0.05$) affected by bradyrhizobia and mineral nitrogen addition throughout cropping seasons (Table 2). Regardless of cropping season, the highest and lowest nodule dry weight was recorded under co-

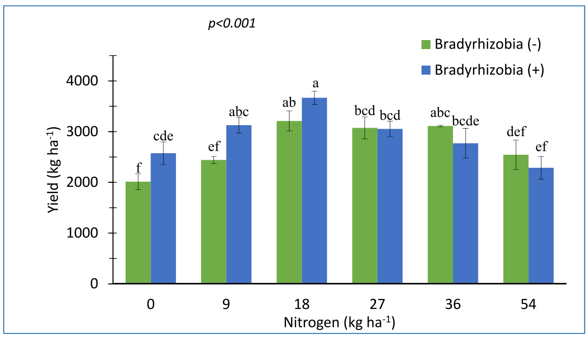

Fig. 1. Effects of different rates of starter N and bradyrhizobia inoculation on grain yield of soybean (kg ha^{-1}) during 2019 cropping seasons.‡ Bars indicates standard deviation of the mean (n = 3), bars capped with same letter(s) are not significantly different at $p<0.05$.

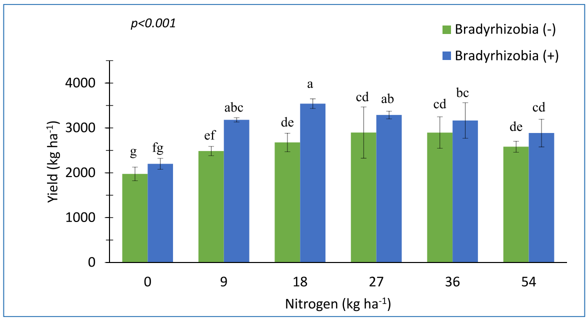

Fig. 2. Effects of different rates of starter N and bradyrhizobia inoculation on grain yield of soybean (kg ha^{-1}) during 2020 cropping seasons.[ł] Bars indicates standard deviation of the mean (n = 3), bars capped with same letter(s) are not significantly different at $p < 0.05$.

Table 1
Effects of starter N rate and inoculation on yield components of soybean (mean ± standard deviation, n = 3) across cropping seasons.

Treatments		2019		2020	
		PPP	SPPo	PPP	SPPo
	N (kg ha^{-1})				
	0	56 ± 14.1	2.47 ± 0.09	28.9 ± 5.05^{d}	1.53 ± 0.03
	9	69 ± 7.7	2.52 ± 0.2	34.6 ± 7.1^{cd}	2.5 ± 0.8
	18	80.7 ± 20.5	2.42 ± 0.02	42 ± 13.4^{bcd}	2.3 ± 0.7
Bradyrhizobia (−)	27	82.2 ± 18.8	2.46 ± 0.05	49.8 ± 20.7^{abc}	2 ± 0.1
	36	75 ± 18.6	2.48 ± 0.05	40 ± 13.7^{bcd}	1.97 ± 0.2
	54	77.8 ± 9.6	2.48 ± 0.15	40.1 ± 6.8^{bcd}	1.63 ± 0.5
	0	90 ± 40.8	2.65 ± 0.1	41.4 ± 10.3^{bcd}	1.83 ± 0.08
	9	76.6 ± 27.6	2.53 ± 0.15	44.2 ± 8.4^{bcd}	1.9 ± 0.4
	18	91.8 ± 21.8	2.47 ± 0.03	63.1 ± 4.4^{a}	2.3 ± 0.1
Bradyrhizobia (+)	27	76 ± 12.3	2.54 ± 0.15	54 ± 2.3^{ab}	2.1 ± 0.2
	36	81 ± 17.9	2.57 ± 0.08	53.7 ± 0.6^{ab}	1.98 ± 0.3
	54	69 ± 21.3	2.61 ± 0.2	47 ± 7.4^{abc}	2 ± 0.3
LSD ($p < 0.05$)		NS	NS	17	NS
CV%		26.7	5.1	22.6	17.9

¥ Where PPP represent pods per plant, and SPPo-represents seed per pods, NS represents non-significant.
*Means followed by same letter(s) within a column are not significantly different at $p < 0.05$.

Table 2
Effects of starter N rates and inoculation on nodulation and growth of soybean (mean ± standard deviation, n = 3) across cropping season.

Treatments		2019			2020		
		NN	NDW	SDW	NN	NDW	SDW
	N (kg ha^{-1})		(g $plant^{-1}$)			(g $plant^{-1}$)	
	0	19.0 ± 11.4^{f}	0.24 ± 0.12^{e}	45.3 ± 4.9^{f}	13.2 ± 5.9^{d}	0.2 ± 0.01^{e}	42.3 ± 7.2
	9	41 ± 5.6^{de}	0.48 ± 0.08^{cd}	60.8 ± 2.6^{bcd}	41.3 ± 10.7^{b}	0.6 ± 0.2^{bc}	49.4 ± 11
	18	59 ± 17.1^{abc}	0.65 ± 0.17^{b}	63.4 ± 2.6^{abc}	41 ± 10.6^{b}	0.6±0^{bc}	58.8 ± 14
Bradyrhizobia (−)	27	61.3 ± 12.7^{ab}	0.61 ± 0.02^{bc}	64 ± 1.4^{ab}	30 ± 11.5^{bc}	0.6±0^{bc}	70.7 ± 16.8
	36	42 ± 8.2^{cde}	0.46 ± 0.06^{d}	59.3 ± 1.3^{cde}	34.5 ± 14.6^{b}	0.48 ± 0.1^{cd}	38.4 ± 10.8
	54	49.3 ± 9.5^{bcde}	0.56 ± 0.05^{bcd}	56.3 ± 2.4^{de}	29.9 ± 15.3^{bc}	0.50 ± 0.1^{d}	56.8 ± 21
	0	32.3 ± 13^{ef}	0.31 ± 0.09^{e}	55.8 ± 4.4^{e}	20.5 ± 3.9^{cd}	0.33 ± 0.06^{de}	49.8 ± 9.8
	9	59.7 ± 8.6^{ab}	0.57 ± 0.1^{bcd}	61.3 ± 2.4^{bc}	58.3 ± 9.8^{a}	0.78 ± 0.2^{ab}	58.4 ± 15.6
	18	68.7 ± 14.4^{a}	0.8 ± 0.07^{a}	67.9 ± 1.4^{a}	58.1 ± 5.9^{a}	0.8 ± 0.07^{a}	61.3 ± 13.8
Bradyrhizobia(+)	27	55.6 ± 9.3^{abcd}	0.57 ± 0.03^{bcd}	63.9 ± 1.3^{ab}	33.3 ± 3.9^{bc}	0.45 ± 0.09^{cd}	50.4 ± 12.8
	36	52.2 ± 10.8^{abcd}	0.56 ± 0.04^{bcd}	59.5 ± 2.7^{bcde}	37.9 ± 5.5^{b}	0.57 ± 0.15^{c}	60.3 ± 14
	54	52 ± 3.5^{abcd}	0.56 ± 0.09^{bcd}	60.3 ± 1.4^{bcde}	29.9±4^{bc}	0.59 ± 0.13^{c}	65.9 ± 10
LSD ($p < 0.05$)		17.5	0.13	4.7	13.8	0.18	NS
CV%		20.9	14.8	4.65	22.8	19.9	24

¥ Where NN represent nodule number plant-1, NDW-represents nodule dry weight per plant (g) and SDW represents shoot dry weight (g)., NS represents non-significant.
*Means with same letters within a column are not significantly different at $p < 0.05$.

application of bradyrhizobia with 18 kg N ha^{-1} and control plot respectively. Shoot dry matter was significantly ($p < 0.05$) affected by treatments in 2019, whereas in 2020 there is no significant difference observed ($p < 0.05$) among treatments. As indicated in Table 3, even though it was different from the control, inoculation alone could not be efficient enough to promote vigorous nodulation potential as well as shoot growth in soybean. Shoot dry matter has shown a significant difference ($p < 0.05$) only during 2019. The result of shoot dry matter also follows similar trends with nodulation since the highest shoot dry weight (67.9 and 63.9 g $plant^{-1}$) was recorded under inoculated plots combined with 18 and 27 kg N ha^{-1} respectively.

3.4. Principal component analysis

Principal component analysis (PCA) for growth and yield explained the contribution of the most important traits that accounted for the total variation and is presented in Table 3. Principal component analysis resulted in two principal components (PC1 and PC2) with eigenvalues ranging from 1.41 to 3.71, and these components explained 84.3 % of the total variation. PC1 explained 72.8 % of the total variation, while PC2 accounted for 11.5 % of the observed variation among the tested treatment combinations (Fig. 3). The first principal component was mostly related to the nodule dry weight, nodule number, and shoot dry weight of soybeans (Fig. 3). The traits that contributed to PC2 were the number of seeds per pod (SPPo) and grain yield with positive value loading. Pod per plant never contributed to variation in both principal components.

The treatments and the quantitative variables were shown on a biplot of the first two PCAs to clearly visualize their associations and differences (Fig. 4). The first and second PCA biplots explained 84.3 % of the total variation. Biplot displayed that nodule number; grain yield, nodule dry weight, and shoot dry weight were considered the most determining variables (Fig. 4). The highest grain yield and number of seeds per pod characterized the treatments that were positioned in the top right quadrant, while nodule number, nodule dry weight, shoot dry weight, and pod per plant were determined by treatments demarcated in the bottom right quadrant. The treatments concentrated around the origin had similar effects on growth and yield, and those that were found far from the origin are considered unrelated treatments (Fig. 4).

4. Discussion

4.1. Effects of starter nitrogen and bradyrhizobia on soybean grain yield

The hypothesis conceptualized was well met since different doses of starter nitrogen fertilizer along with bradyrhizobium inoculation had brought significant variation to the growth and grain yields of soybeans grown for two years in this experiment. Application of starter N has been reported to have a positive effect on the yield of soybeans, especially when combined with the bradyrhizobia strain [12,16]. The present finding was quite in agreement with the findings of [12], indicating that soybean grain yield was influenced by the interaction of bradyrhizobia and starter nitrogen application. Increased soybean grain yield with application of bradyrhizobium inoculation and a reduced rate of starter nitrogen level could be attributed to improved physiological maturity, chlorophyll content, and grain filling due to improved photosynthetic assimilation[16]. It was also reported in Ref. [17] that there was an increase in soybean yield due to the application of nitrogen fertilizer, though it negatively affected biological nitrogen fixation.

In line with our present finding [18], concluded nitrogen fertilization up to 25 kg N ha^{-1} brought 10–15 % higher grain yield gain compared to crops treated without inoculant under varying inoculation levels. On the contrary [19,20], reported that the application of 100 kg N ha^{-1} enhanced grain yield, which might be due to absence of indigenous rhizobium in the soil and low soil nitrogen levels. The grain yield of soybeans can be increased despite unfavorable climatic and low soil fertility conditions by applying moderate N rates combined with optimum inoculant dosages [11,18]. In contrast with previous studies [10,21,22], who reported soybean grain yield did not positively respond to nitrogen application when biological nitrogen fixation is efficient, the present finding elucidated that nitrogen application increased soybean grain yield across cropping seasons except for high-dose applications. Results from this study proved that excessive or insufficient nitrogen fertilizer was not beneficial to an increase in grain yield of soybean, and intermediate levels of starter nitrogen fertilization (18 kg ha^{-1}) increased grain yield, especially when combined with bradyrhizobia [23]. Our

Table 3
Eigenvalues, the percent of variance and cumulative variance obtained from PCA based on six variables among 12 treatments.

Variables	PC1	PC2
NN	0.49	−0.11
NDW	0.50	−0.01
SDW	0.49	−0.17
PPP	0.33	−0.50
SPPo	−0.19	−0.70
Yield	0.35	0.48
Eigen Value	3.71	1.41
Variance (%)	72.80	11.50
Cumulative. Variance (%)	72.80	84.30

¥ where, NN = nodule number per plant, NDW = nodule dry weight, SDW = shoot dry weight, PPP = pod per plant, SPPo = seed per pod.

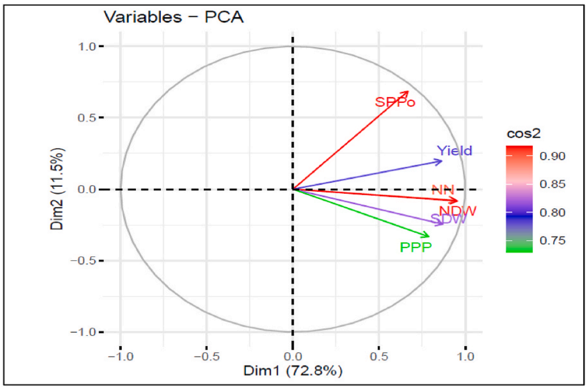

Fig. 3. Contribution of individual variables to the first two dimension. Where, NN = nodule number per plant, NDW = nodule dry weight, SDW = shoot dry weight, PPP = pod per plant, SPPo = seed per pod.

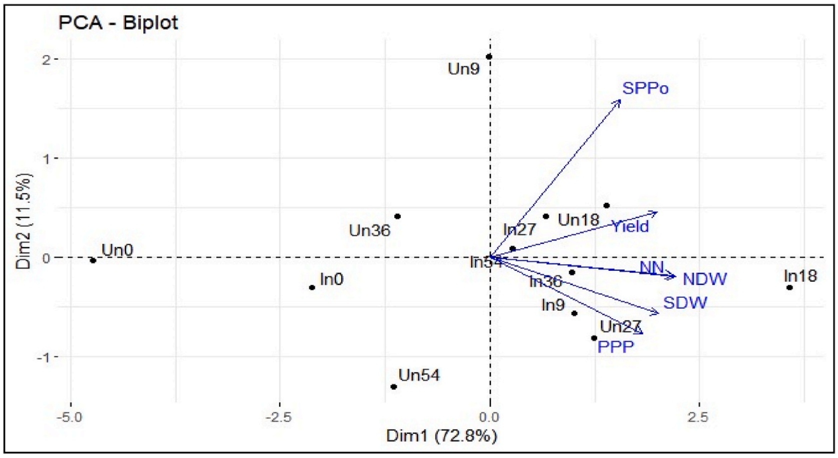

Fig. 4. Biplot of the first two principal components to the total variation for 12 treatments. Where un/in indicates bradyrhizobium uninoculated/inoculated, while number numbers from 0 to 54 represent respective starter nitrogen doses (kg ha-1).

results are in agreement with the work of [23], who pointed out that a high rate of nitrogen application reduced grain yield by suppressing root and root nodule growth in soybeans. Researchers agreed that a small dose of starter nitrogen applied near the seed could improve early growth and soybean grain yield, especially in cases where soil fertility is low [12] or adverse environmental conditions exist [23].

4.2. Effects of starter N rate and inoculation on yield components of soybean

In 2020, inoculation with bradyrhizobia with different levels of inorganic nitrogen enhanced the number of pods per plant. This positive effect of inoculation with bradyrhizobia might be due to its positive effect on fruit setting and/or decreasing abscission of flowers and young pods [10]. Inoculation of bradyrhizobia with lower rates of mineral nitrogen induced an increase in the number of seeds per pod. Inoculation of plants, fertilized with mineral nitrogen by bradyrhizobia improved their productivity. Moreover, the beneficial effect of inoculation with bradyrhizobia on grain yield increased by decreasing the level of applied mineral nitrogen [10,24].

The results of the present study are consistent with [6,13] reported higher pod number per plant in the plants from seeds inoculated with bradyrhizobium. At low starter nitrogen levels, bradyrhizobia's beneficial influence on pod number improved, and the minimal amount of fertilizer had the greatest impact on soybean growth [10]. There is a negative relationship between increased rates of mineral nitrogen, bradyrhizobium inoculation since excessive biomass occurred, and negatively induced yield components like pod and seed formation [10,13].

4.3. Nodulation and growth of soybean influenced by starter N and bradyrhizobia

The present result concluded that, overall nodulation was enhanced by bradyrhizobia inoculation of soybeans by about 10–20 % over uninoculated treatments across cropping seasons. The highest number of nodules were generated at the lowest nitrogen application rates, demonstrating that the moderate soil nitrogen of the experimental soil and bradyrhizobium association encouraged nodule initiation. Higher nitrogen rates suppressed nodulation in this study, which might be due to the inhibition of infection threads and nodules [25,26]. Similarly, to previous observations [27], the number of nodules and nodule dry weight increased with increasing nitrogen concentration, reaching a peak at 18 kg N ha^{-1}. This study showed that nitrogen enhanced the number of nodules in the concentration range of 0–18 but decreased the number of nodules at 18 kg N ha^{-1}. The present result was in sharp agreement with [12], who reported that applying starter nitrogen levels beyond 18 kg N ha^{-1} leads to decrease in nodule number, nodule dry weight, and shoot growth. The work of [11] concluded that there were significant decreases in traits related to biological nitrogen fixation, like nodulation, and nodule weight, under nitrogen-applied plots, emphasizing the negative effect of mineral nitrogen addition. The reduction in nodulation was more pronounced in the treatment that received mineral N at sowing and as topdressing, demonstrating that the higher the amount of mineral N supplied, the more intense the negative effect on nodulation [6,25], and consequently the biological nitrogen fixation related parameters [12]. All inoculated treatments increased nodule and nodule dry weight over uninoculated treatments as reported in Ref. [21]. However, contrary to these authors, inoculation alone did not show significant efficiency over nitrogen applied plots except for higher doses.

5. Conclusion

Application of bradyrhizobia along with different levels of nitrogen fertilizer at planting showed a significant effect on yield and yield components of soybeans grown for two cropping seasons. Starter nitrogen has shown a potential benefit since soil has a low ability to provide nitrogen at the early stages of soybean growth until plants establish symbiosis with nitrogen fixing bacteria. Grain yield in both years has a single peak trend of initially increasing and then decreasing with increasing starter nitrogen level. Grain yield tends to increase until the nitrogen level reaches 18 kg N ha^{-1} beyond which it starts to decline. Nitrogen application alone was not efficient for yield when compared with their corresponding inoculated plots. Lower levels of starter nitrogen addition promoted nodulation, nodule dry weight, and shoot growth over control plots when applied with bradyrhizobia. Higher doses of nitrogen (above 27 kg N ha^{-1}) suppressed nodule numbers, indicating that an excess amount of nitrogen in the rhizosphere has an inhibitory effect on root infection and nodule formation. Co-application of bradyrhizobium inoculation with an economically and environmentally optimum rate of nitrogen fertilizer is important to meet soybean nitrogen demand and improve yield. Therefore, from this study, it was concluded that application of 9 kg N ha^{-1} or 18 kg N ha^{-1} with bradyrhizobia inoculation was recommended as the optimum starter nitrogen for better yield and biological nitrogen fixation in Yeki area soils having a moderate nitrogen level. The present report only represents specific agroecology and farming system. Future works should focus on examining these effects under varying nitrogen sources and bradyrhizobium species, as well as differing soil nutrient levels.

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Data availability

The data associated with the current study were deposited into a publicly available repository at https://data.mendeley.com/datasets/z4w85dvypn/1.

CRediT authorship contribution statement

Guta Amante: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Conceptualization. **Mulisa Wedajo:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Shiferaw Temteme:** Writing – review & editing, Supervision, Resources, Project administration, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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