

Research Article

Response of Soybean (*Glycine max* L. (Merrill)) to *Bradyrhizobium* Inoculation, Lime, and Phosphorus Applications at Bako, Western Ethiopia

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Soil acidity and poor soil fertility are the major soil chemical constraints which limit crop productivity in western Ethiopia. In leguminous crops, low productivity is not only a result of declining soil fertility but also reduced N₂ fixation due to biological and environmental factors. Thus, this study was carried out to determine the influence of lime, *Bradyrhizobium* inoculation, and phosphorus fertilizer on soybean yield components and yields and to identify economically feasible treatments that can maximize the productivity of soybean. Factorial combinations of three *Bradyrhizobium* strains (uninoculated, TAL379, and Legumefix), two lime levels (0 and 3.12 t ha⁻¹), and four P levels (0, 23, 46, and 69 kg P₂O₅ ha⁻¹) were laid out in RCBD with three replications. The results showed that the application of lime (3.12 t ha⁻¹) significantly increased soil pH (5.6), plant height (77.2 cm), number of primary branches per plant (6.6), 100-seed weight (17.5 g), grain yield (3431 kg ha⁻¹), and harvest index (41%). Similarly, significantly higher grain yield (3228 kg ha⁻¹) and harvest index (41%) were obtained with inoculation of TAL379 whereas Legumefix inoculation recorded the highest number of primary branches (6.7). The effect of P at 69 kg P₂O₅ ha⁻¹ also gave significantly higher plant height (75.5 cm), number of primary branches (6.6), grain yield (3277 kg ha⁻¹), and harvest index (43%). The interaction of P and *Bradyrhizobium* inoculation significantly influenced days to physiological maturity and number of pods per plant. Similarly, the interaction of phosphorus and lime significantly influenced days to 50% flowering. Likewise, the combination of lime (3.12 t ha⁻¹) with TAL379 inoculation gave the highest aboveground biomass. On the other hand, the interaction of *Bradyrhizobium* × lime × phosphorus revealed that application of 69 kg P₂O₅ ha⁻¹ without TAL379 inoculation under limed condition significantly resulted in the highest number of nodules per plant (79.4) and number of effective nodules (67.9). Thus, it can be concluded that, particularly in the western part of Ethiopia where soil acidity is a major problem, application of phosphorus with *Bradyrhizobium* and lime is an alternative option to enhance biological nitrogen fixation and grain yield of soybean in smallholder farming system.

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is a legume native to East Asia perhaps in North and Central China [1] and it is grown for edible bean, oil, and protein around the world. Soybean is a promising pulse crop proposed for the alleviation of acute shortage of protein and oil worldwide [2]. Soybean is rich in nutritional value due to its high protein and oil content as

well as aspects of its functional composition, such as isoflavones [3].

In Ethiopia, soybean is a multipurpose crop, used for a variety of purposes including preparation of different kinds of soybean foods, animal feed, and soy milk [4]. Currently, there are also factories producing oil from soybean, indicating the increasing importance of the crop in the country. It also countereffects depletion of plant nutrients especially

nitrogen in the soil resulting from continuous monocropping of cereals, especially maize and sorghum, thereby contributing to increasing soil fertility [4]. According to Central Statistical Authority (CSA) [5] report, soybean was produced on about 38,166.04 ha of land and 81241.833 tons was produced in the 2015/16 main cropping season with the productivity of 2.1 t ha^{-1} , which is slightly lower than the world average of 2.6 t ha^{-1} . This low yield may be attributed to a combination of several production constraints among which low soil fertility, periodic moisture stress, diseases and insect pests, weeds, and poor crop management practices play a major role [6].

Soil acidity has become a serious threat to crop production in most highlands of Ethiopia in general and in the south, southwestern, and western part of the country in particular. About 41% of the potential arable land of Ethiopia is acidic [7]. Currently, it is estimated that about 67% of the total arable land of Wollega is affected by soil acidity [8]. Soil acidity constrains symbiotic N_2 fixation [9], limiting *Rhizobium* survival and persistence in soils and reducing nodulation, and causes nutrient imbalance [10, 11]. Wood et al. [12] indicated that multiplication of *Rhizobium* in the rhizosphere and nodulation were inhibited at a pH of 4.3. Increased soil acidity may lead to reduced yields, poor plant vigour, and nodulation of legumes. In acid soils with a high level of Al, Fe, and Mn, the P fixation occurs in the form of Al-P, Fe-P, and Mn-P which is poorly soluble and causes P to be unavailable to plants. Thus, liming acid soils could make the soil environment better for leguminous plants and associated microorganisms as well as increase the concentration of essential nutrients by raising its pH and precipitating exchangeable aluminium [13].

Mesfin et al. [14] indicated that as lime and P application to acid soils increased, plant-available Fe, Mn, Zn, and Cu content of soil decreased, whereas pH, Ca, Mg, and available P increased which in turn improves crop performance. Given severe yield reductions, smallholder farmers in western and eastern Wollega Zones have been abandoning their land temporarily (fallowing) or even permanently in some areas due to soil acidity and low soil fertility [15]. However, owing to the increasing population pressure, abandoning farmland temporarily or permanently has become untenable option [16]. Therefore, the farmers are now opting to managing soil fertility to sustain productivity. Acid infertile soils may be corrected through liming or the use of organic fertilizers [17]. Therefore, the approaches for increasing soybean yield in Ethiopia could start by reducing soil acidity to a level at which crop produce its potential, followed by increase and maintenance of soil fertility through applications of fertilizers, mainly N and P. However, recommendation of high rate of mineral N and P fertilizers is not a viable option for smallholder farmers because most of them lack financial resources [18]. Thus, to reduce the amount of mineral fertilizers required, there is a need to adopt Integrated Soil Fertility Management (ISFM) technologies which combine organic fertilizers with small amounts of mineral fertilizers and build up soil conditions to enhance soil microbial activity, biological N fixation, and soybean yields.

Recently, soybean crop production has been promoted in western and eastern Wollega zones, but the average yield is below the potential yield of the crop [19]. Some studies were conducted on the response of soybean to *Rhizobium* inoculation, phosphorus, or lime applications in western Ethiopia [19,20]. However, studies on the combination of *Rhizobium*, phosphorus, and lime application on soil acidity amelioration in smallholder cropping system have not been conducted in western Ethiopia. Thus, based on the fact that inoculation of rhizobia, liming, and supplementation of phosphorus increases nitrogen and phosphorus levels in the soil, their combined application may play a key role in yield and economic benefits of soybean grown in highly depleted acidic soils of western Ethiopia. Therefore, the objectives of the study were to assess the effect of *Bradyrhizobium* inoculants, lime, and phosphorus fertilizer application on nodulation, yield components, and yield of soybean.

2. Materials and Methods

2.1. Description of the Study Area. The experiment was carried out during the main rainy season (April to October) for two consecutive years (2016 and 2017) at Bako Agricultural Research Center (BARC) which is located in Oromia Regional State, East Wollega Zone, Anno district, at about 250 km away from the capital city Addis Ababa on the way to Nekemte town. BARC is located at an altitude of 1650 m above sea level $09^{\circ} 6'00''$ N latitude and $37^{\circ} 09'00''$ E longitude. Figure 1 presents climatic data of rainfall and temperature during the 2016 and 2017 growing seasons. The area has a warm humid climate with annual mean minimum and maximum temperatures of 10.6 and 34.6°C , respectively. The area receives an annual rainfall of 1317 mm mainly from April to October with maximum precipitation in the month of May to September (meteorological station of the center). The predominant soil type of the area is Nitosols. The area is known for its mixed crop-livestock farming system in which cultivation of maize (*Zea mays* L.), hot pepper (*Capsicum annum* L.), soybean (*Glycine max* L.), common bean (*Phaseolus vulgaris* L.), mango (*Mangifera indica* L.), banana (*Mussa* spp), and sugar cane (*Saccharum officinarum* L.) are the major cropping activities.

2.2. Experimental Materials. Improved soybean variety (Dhiddessa) was used as a test crop. The variety was released by Bako Agricultural Research Center (BARC) in 2008. The variety is characterized by the medium maturity group (135–145 days to maturity) having indeterminate growth habit and a yield potential of 2–3.3 ton ha^{-1} at the research station [21]. It is highly adaptable to areas of mid- and low altitudes. Triple superphosphate (TSP) containing 46% P_2O_5 was applied in the row as per the treatment and mixed with soil just at the time of planting. Calcium carbonate (CaCO_3) was used as a source of lime. The liming material used in this study had a purity of 75% CaCO_3 . Carrier-based *Bradyrhizobium* strains, namely, TAL379 (isolated from the soil of the tropics) and Legumefix (isolated from temperate soil)

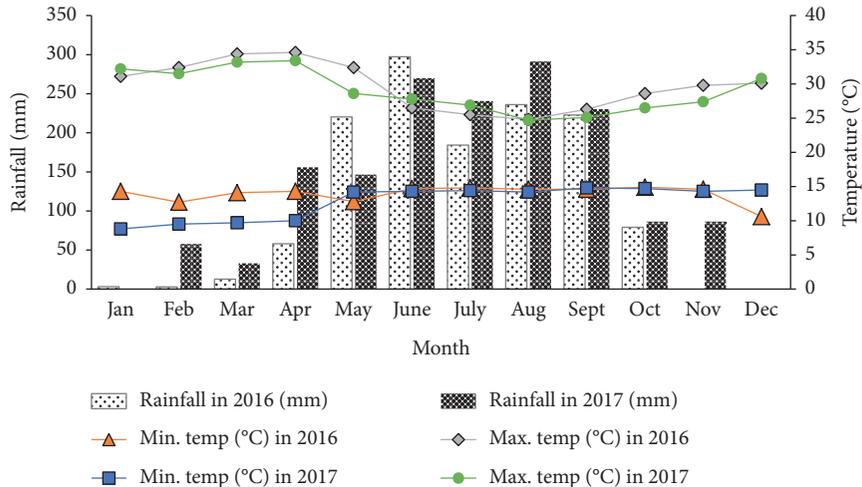


FIGURE 1: Monthly total rainfall (mm) and mean minimum and maximum temperatures (°C) of the experimental station in 2016 and 2017.

were obtained from Managasha Biotechnology Private Limited Company, Addis Ababa, Ethiopia.

2.3. Soil Sampling and Analysis. A representative soil sample was taken using a cylindrical auger at a depth of 0–20 cm randomly in zigzag pattern from the whole experimental field prior to lime application from 15 spots. Finally, composite sample was prepared for analysis to determine the physicochemical properties of the soil of the experimental site. The collected soil samples were air-dried, ground, and sieved using a 2 mm mesh size sieve for the analysis of total N, soil pH, organic carbon, available phosphorus, exchangeable bases (Mg, Ca, and K), cation exchange capacity (CEC), exchangeable acidity, exchangeable Al, and texture. The selected soil physicochemical properties were analyzed at Bako Agricultural Research Center Soil Laboratory.

Soil pH was determined potentiometrically using a pH meter with a combined glass electrode in a 1:2.5 soil-to-water supernatant suspension [22]. Walkely and Black [23] method was used to determine the organic carbon content. The base titration method which involves saturation of the soil sample with 1 M KCl solution and titrating with sodium hydroxide was employed to determine exchangeable acidity. Soil total nitrogen was determined by the Kjeldahl method using micro-Kjeldahl distillation unit and Kjeldahl digestion stand as described by Jackson [24].

Available soil phosphorus was extracted by the Bray II procedure [25] and determined colorimetrically by spectrophotometer. Cation exchange capacity (CEC) of the soil was determined by 1 M ammonium acetate (NH_4OAc) saturated sample at pH 7 where the standard paste was distilled to estimate the ammonium liberated by titration with acid [26]. Exchangeable Mg and Ca were determined in the extract with atomic absorption spectrophotometer while the exchangeable K was determined by flame photometer [27]. Particle size distribution was done by the Bouyoucos hydrometer method [28].

2.4. Treatments and Experimental Design. The treatment comprised three factors, namely, phosphorus fertilizer rates (0, 23, 46, and 69 P_2O_5 kg ha^{-1}), three levels of inoculation (uninoculated, Legumefix, and TAL379) and lime rates (0 and 3.12 t ha^{-1}) as CaCO_3 . The calcium carbonate (CaCO_3) at the rate of 3.12 t ha^{-1} which was recommended by Bako Agricultural Research Center for soybean was used [29]. The treatment was arranged as $4 \times 3 \times 2$ in factorial combinations in RCBD with three replications. The plot size was 8.4 m^2 , comprising 7 planting rows of 3 meters long, separated by 0.4 m between them. One last row in each side of the plot was used as border row, and one row next to the border row on one side of the plot was used for destructive sampling. The remaining 4 rows in the central of the plot were used as net plot for data collection.

2.5. Experimental Procedure and Field Management. The land was ploughed by tractor, disked, and harrowed. The lime was evenly spread and incorporated up to 20 cm depth by using a hand hoe one month before planting of soybean. The seeds were planted at a spacing of 40 cm and 10 cm between rows and within rows, respectively. Nitrogen fertilizer in the form of urea (46% N) was applied as a starter dose at the rate of 18 kg N ha^{-1} . The spacing between blocks and plots were 1 m and 0.5 m, respectively. Two seeds were sown per hill and then thinned to one plant after seedling establishment. All other management practices were done as per the recommendations.

Carrier-based inoculants of each strain were applied at the rate of 10 g inoculants per kg of seed [30]. The inoculants were mixed with sugar by the addition of some water in order to facilitate the adhesion of the strain on the seed. To ensure that the applied inoculants stick to the seed, the required quantities of inoculants were suspended in 1:1 ratio in 10% sugar solution. The thick slurry of the inoculants was gently mixed with the dry seeds so that all the seeds received a thin coating of the inoculants. To maintain the

viability of the cells, inoculation was done under the shade and allowed to air dry for 30 minutes and then sown. Seeds were immediately covered with soil after sowing to avoid death of cells due to the sun's radiation. A plot with uninoculated seeds was planted first to avoid contamination.

2.6. Data Collected. Phenological parameters: days to 50% flowering was recorded as the number of days from sowing to the date on which 50% of plants on the net plot produced at least their first flower. Days to physiological maturity was recorded as the number of days from sowing to the stage when 90% of the plants in a plot have reached physiological maturity, i.e., the stage at which pods lost their pigmentation and begin to dry.

Nodulation parameters: total number of nodules per plant was recorded from five plants sampled randomly from the destructive rows of each plot at midflowering. The whole plant was carefully uprooted using a fork so as to obtain intact roots and nodules. Uprooting was done by exposing the whole root system to avoid loss of nodules. The adhering soil was removed by soaking the ball of soil and root in a barrel filled with water and thoroughly rinsed in a separate water-filled barrel. From the same uprooted plants, the number of nodules per plant was recorded by counting the number of nodules from five plants and averaged as per plant whereas for the effective number of nodules the color inside of the nodule was observed by cutting with a sharp blade and a pink to dark-red color was considered as effective whereas a green colored one was identified as noneffective nodules.

Growth parameters: the plant height of ten randomly taken plants from each of the four middle rows was measured in centimeter (cm) from the ground level to the tip of the plant at harvest maturity and expressed as an average of ten plants per plot. The number of primary branches was counted at physiological maturity by taking ten randomly selected plants from four central rows and expressed as an average of ten plants.

Yield and yield components: the number of pods per plant was counted from ten randomly selected plants from four middle rows at harvest maturity and expressed as an average of each plant. The number of seeds per pod was counted from the randomly taken 10 pods from the net plot and was expressed as an average of ten pods. The weight of 100 seeds that were sampled from each plot was weighed using a sensitive balance, and the weight was adjusted at 10% standard moisture content. Since the soybean plants start shedding the leaves at the late pod setting stage, ten plants were tagged from destructive rows at the late pod setting stage, and then, the old leaves, i.e., the leaves that lose their pigmentation were collected each day and stored in polythene bag up to when the crop reaches the exact date of physiological maturity in order to estimate the aboveground dry biomass yield including leave parts. At physiological maturity, the aboveground dry biomass of ten pretagged plants from the destructive rows was measured after oven-drying the harvested produce at constant weight at 70°C for 48 hours. For obtaining the total aboveground dry biomass,

the dry biomass per plant thus obtained was multiplied with the total number of plants in the net plot area and converted to kg ha^{-1} . This was used to calculate the harvest index. Grain yield was measured by harvesting the plants from the net plot area. The harvested produce was sun-dried for seven days and threshed by hitting with sticks and winnowing was done. The moisture content of the grain was adjusted to 10%. Harvest index was calculated by dividing grain yield per plot by the total aboveground dry biomass yield per plot.

2.7. Data Analysis. All collected parameters were subjected to analysis of variance using Gen Stat 18th edition [31]. Whenever the effects of the treatments were found to be significant, the means were compared using Fisher's protected least significant difference (LSD) test at 5% level of significance. The data presented in graph were done using pivot table program in excel.

2.8. Partial Budget Analysis. The economically acceptable treatments were determined by partial budget analysis to estimate the gross value of the grain yield by using the adjusted yield [32] at the market value of the grain and inputs during the cropping period. Only total costs that varied (TCV) were used to compute costs. Current prices of soybean, inoculants, TSP, and application cost of inoculants and TSP were considered as variable with their cost.

To estimate economic parameters, soybean yield was valued at an average open market price of 8.00 Birr/kg. Costs of land preparation, field management, harvest, transportation, and storage were not included in the analysis as they were not variable. The cost of lime was also not considered in the partial budget analysis because the government subsidizes the farmers to boost their crop productivities. To equate the soybean grain yield with what a farmer would get, the obtained yield was adjusted downward by 10%. Both the costs and benefits were converted to monetary values in Ethiopian Birr (ETB) and reported per hectare. Treatments net benefits (NB) and TCV were compared using dominance analysis following the two steps described below.

The first step was the calculation of the NB as shown in the following formula as suggested by CIMMYT [32]:

$$\text{NB} = (\text{GY} \times \text{P}) - \text{TCV}, \quad (1)$$

where $\text{GY} \times \text{P}$ is the gross field benefit (GFB), GY is the adjusted grain yield per hectare, and P is the field price per unit of the crop.

Secondly, treatment TCV were listed in increasing order in accordance with dominance analysis. All treatments which had NB less than or equal to treatment with lower TCV were marked with a letter "D" since they were dominated and eliminated from any further analysis. Undominated treatments were subjected to marginal rate of return (MRR) analysis as suggested by CIMMYT [33] in a stepwise manner, moving from lower TCV to the next as shown below:

$$\text{MRR}(\%) = \frac{\text{change in NB (NBb - NBa)}}{\text{change in TCV (TCVb - TCVa)}} \times 100, \quad (2)$$

where NBa = NB with the immediate lower TCV, NBb = NB with the next higher TCV, TCVa = the immediate lower TCV, and TCVb = the next highest TCV.

3. Results

3.1. Selected Physicochemical Properties of the Soil prior to Planting. Laboratory analysis results of selected properties of soil of experimental site before lime application are presented in Table 1. The results showed that the soil of the experimental site is clay in texture. According to the soil analysis, the soil pH of the experimental site was 5.13. The organic carbon content of the experimental soil is medium (1.74%). The analysis further indicated that the total N content of the experimental site was 0.12%. The available P in the experimental soil was 9.34 mg/kg of soil.

3.2. Phenological parameters

3.2.1. Days to 50% Flowering. Significant differences were found among the different levels of phosphorus and lime usage for phenological periods in soybean (Table 2). Increasing the levels of phosphorus without lime significantly shortened the days required to reach 50% flowering compared to lime-treated plots in the same level of phosphorus application rates (Table 2).

3.2.2. Days to Physiological Maturity. The number of days taken by a crop to reach maturity is one of the important factors in determining whether a certain cultivar can be successfully grown in a particular environment and cropping system. Days to physiological maturity was significantly influenced by the interaction effect of *Bradyrhizobium* strains with phosphorus rate application. The longest days (127.5) to physiological maturity was recorded in the plants treated with 23 kg P₂O₅ ha⁻¹ and inoculated with Legumefix and it was statistically at par with both of the inoculation treatments while the shortest days (121.7) to reach physiological maturity was observed in the application of 69 kg P₂O₅ ha⁻¹ without *Bradyrhizobium* inoculation treatments (Table 3).

3.3. Nodulation Parameters

3.3.1. Number of Total and Effective Nodules per Plant. The result in Figures 2(a) and 2(b) showed significant interaction among *Bradyrhizobium* × lime × phosphorus fertilizer applications on number of total and effective nodules per plant. Phosphorus plays a significant role in legume nodulation through its ability to enhance root development and proliferation, thereby affording the rhizobia more sites for infection and initiation of nodule formation. Nodule number was significantly higher in inoculated plants and increased phosphorus application rates under limed condition. The highest number of nodules per plant of 62.3 and

61.3 were recorded at 46 and 69 kg P₂O₅ ha⁻¹, respectively, inoculated with *Bradyrhizobium* strain (TAL379), while the lowest number of nodules per plant (29.7) was recorded in the control treatment (Figure 2(a)). Also, the highest number of effective nodules per plant (67.9 and 69.3) was resulted from the application of 46 and 69 kg P₂O₅ ha⁻¹ and treated with lime inoculated with TAL379 strain while the lowest number of effective nodules per plant (29) was recorded in the control treatment (Figure 2(b)).

3.4. The Main Effect of *Bradyrhizobium*, lime, and Phosphorus on Yield and Yield Components

3.4.1. Plant Height. Plant height was significantly influenced by the main effect of *Bradyrhizobium*, lime, and phosphorus application only. Among various phosphorus levels, the maximum plant height was recorded at the rate of 69 kg P₂O₅ ha⁻¹ while the lowest plant height was recorded at 0 kg P₂O₅ ha⁻¹ (Table 4). This positive growth response of soybean for application of P in acidic soil may be related to better availability of P as the rate of P application increased. Application of lime significantly ($P < 0.01$) improved the plant height of soybean over unlimed control (Table 4). The beneficial effects of liming on plant growth are most likely a result from the enhanced conditions for seedling growth. The result indicated that applying lime to the soil might considerably improve the nutrient availability, particularly phosphorus and calcium, since it improves soil pH under which maximum availability of the nutrient may be obtained.

3.4.2. Number of Primary Branches. Primary branches were significantly influenced by the main effect of *Bradyrhizobium*, lime, and phosphorus application. The highest number of primary branches per plant was obtained from plants inoculated with Legumefix, whereas the lowest number of primary branches per plant was recorded from uninoculated plants (Table 4). The significant improvement in the number of primary branches per plant is an indication of *Bradyrhizobium* inoculation in fixing nitrogen that resulted in improved yield and yield components over the control. Likewise, the number of primary branches per plant in response to phosphorus application at different levels was found to be significant (Table 4). Among the various phosphorus levels, the highest number of primary branches per plant was recorded at the rate of 46 and 69 kg P₂O₅ ha⁻¹ while the lowest number of primary branches per plant was recorded at 0 kg P₂O₅ ha⁻¹. Application of lime also influenced significantly ($P < 0.01$) the number of primary branches per plant. Significantly higher number of primary branches per plant was recorded with liming as compared to without liming (Table 4). This is due to the fact that liming improves the availability of calcium in acid soils, which in turn can increase N₂ fixation.

3.4.3. Number of Pods per Plant. The results in Figure 3 showed a significant interaction between *Bradyrhizobium*

TABLE 1: Selected soil physicochemical properties of the experimental site before planting.

Soil characters	Value	Rating	Reference
1. Particle size distribution			
Sand (%)	33.7		
Silt (%)	7.7		
Clay (%)	58.6		
Textural class	Clay		
2. Chemical analysis			
Soil pH (1 : 2.5 (H ₂ O) suspension)	5.13	Strongly acidic	Takelign [34]
Organic carbon (%)	1.74	Medium	Hazelton and Murphy [35]
Total nitrogen (%)	0.12	Low	Hazelton and Murphy [35]
Available P (mg/kg) soil	9.34	Low	Takelign [34]
Exchangeable Al (meq/100 g) soil	1.23	Medium	Daryl D. Buchholz [36]
Exch. acidity (meq/100 g) soil	1.31	Medium	Daryl D. Buchholz [36]
CEC (meq/100 g) soil	19.78	Medium	Hazelton and Murphy [35]
Exchangeable cations (meq/100 g)			
K ⁺	0.63	Medium	FAO [37]
Ca ²⁺	4.53	Low	FAO [37]
Mg ²⁺	1.40	Medium	FAO [37]

TABLE 2: Interaction effect of phosphorus with lime rates on days to 50% flowering.

P ₂ O ₅ rates (kg ha ⁻¹)	Lime rate (t ha ⁻¹)	
	0	3.12
0	63.3 ^{abc}	63.9 ^{ab}
23	61.1 ^d	64.8 ^a
46	61.6 ^{cd}	64.1 ^{ab}
69	62.4 ^{bcd}	64.5 ^a
LSD (0.05)	1.2	
CV (%)	2.9	

Means followed by the same letters are not significantly different at 5% level of significance.

TABLE 3: Interaction effect of *Bradyrhizobium* inoculation with phosphorus application rates on days to physiological maturity of soybean.

P ₂ O ₅ rates (kg ha ⁻¹)	<i>Bradyrhizobium</i> inoculation		
	No inoculation	TAL379	Legumefix
0	125.2 ^{abcd}	126.5 ^{ab}	124.8 ^{abcd}
23	124.3 ^{bcde}	122.3 ^{de}	127.5 ^a
46	123.2 ^{cde}	126.0 ^{abc}	125.3 ^{abc}
69	121.7 ^e	124.5 ^{bcde}	125.8 ^{abc}
LSD (0.05)	2.8		
CV (%)	2.0		

Means within columns and rows followed by the same letters are not significantly different at 5% level of significance.

strains and phosphorus levels on the number of pods per plant. The lowest number of pods per plant was recorded in control treatment (0 kg P₂O₅ ha⁻¹ + uninoculated) whereas the highest number of pods per plant was recorded at the rate of 69 kg P₂O₅ ha⁻¹ inoculated with TAL379 strain (Figure 3). The positive effects of the inoculants might be due to better amount of nitrogen rendered through nitrogen fixation which promoted vegetative growth and plant height, thus improving the number of pods per plant. This could

again be attributed to the availability of phosphorus that would have increased the intensity of photosynthesis, nitrogen fixation, root development, flowering, seed formation, and fruiting.

3.4.4. Number of Seeds per Pod and Hundred-Seed Weight.

There was no significant difference in the number of seeds per pod. Most probably, the number of seeds per pod significantly varied between different genotypes; however, the seeds per pod are less affected by external factors like fertilization when a single genotype is considered. Compared with unlimed control, applying lime at 3.12 t ha⁻¹ increased hundred-seed weight by 6.54% (Table 4) which may be due to the ability of this treatment to improve Ca nutrition of the crop.

3.4.5. Grain Yield.

In this study, *Bradyrhizobium* inoculation, lime, and phosphorus applications significantly influence the grain yield of soybean. The highest grain yield of soybean was obtained from plants inoculated with TAL379 whereas the lowest grain yield was recorded from uninoculated plants (Table 4). This was related to the symbiotic relationship between *Bradyrhizobium* and soybean plants, which resulted in the fixation of atmospheric nitrogen into the roots and translocation of amino acids to the shoots, thus leading to increased yield. The maximum grain yield was recorded at the rate of 69 kg P₂O₅ ha⁻¹ while the lowest above grain yield was recorded at no application of phosphorus (Table 4). The increase in grain yield with the increasing rate of phosphorus may be attributed to the better availability of phosphorus for plants as the rate of external phosphorus application increases which in turn is observed on better plant performance. The better performance of the crop with liming may be related to the better nodule development which stimulated effective N₂ fixation, increasing the amount of N available to support growth.

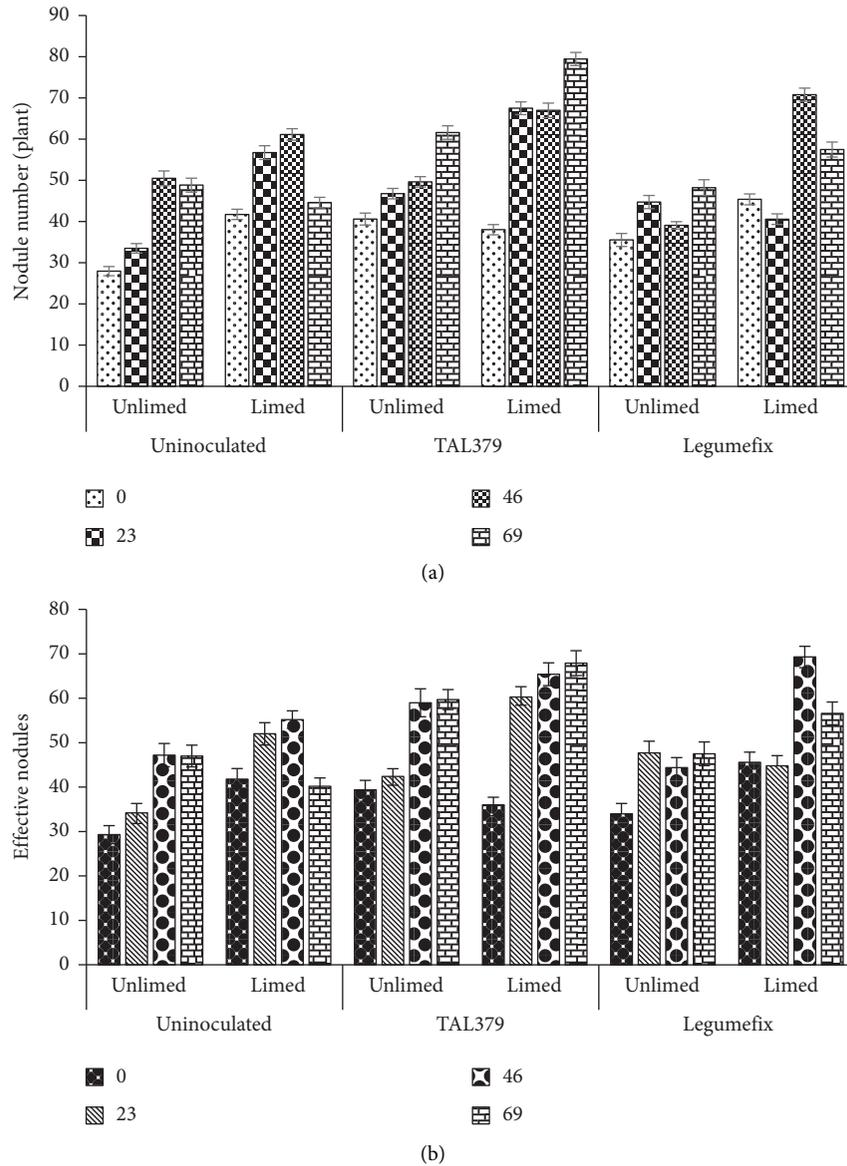


FIGURE 2: Interaction effect of *Bradyrhizobium* × lime × phosphorus application on (a) the number of nodules per plant and (b) the number of effective nodules per plant.

3.4.6. *Harvest Index*. Inoculation significantly influenced soybean harvest index where inoculation with *Bradyrhizobium* strains TAL379 and Legumefix improved the harvest index of soybean by 41% and 40%, respectively (Table 4). The increased harvest index with inoculation of *Bradyrhizobium* strains also implies higher partitioning of dry matter into grain. Phosphorus gave the highest harvest index (43%) at the rate of 69 kg P₂O₅ ha⁻¹ whereas the lowest harvest index (37%) was recorded at the rate of 0 kg P₂O₅ ha⁻¹ (Table 4). Application of P at the rate of 69 and 46 kg P₂O₅ ha⁻¹ increased harvest index by 13% and 7.9% relative to 23 kg P₂O₅ ha⁻¹. On the other hand, the application of 23 kg P₂O₅ ha⁻¹ gave statistically at par mean harvest index to that of unfertilized control. The increased mean harvest index with the increase of P fertilizer rate might be due to the influence P for greater fruit and seed setting than the aboveground

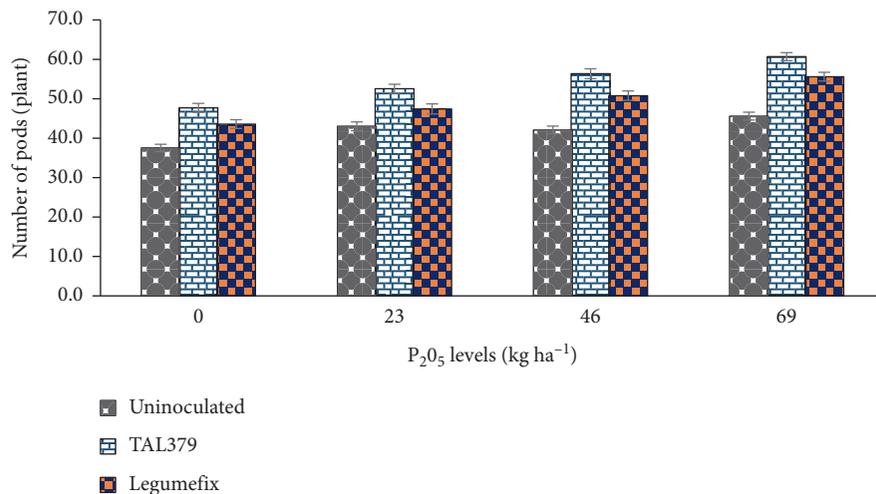
biomass yield. The result also showed that the harvest index was significantly affected by lime application. Lime application at the rate of 3.12 t lime ha⁻¹ produced significantly higher harvest index (41%) than with no lime application (39%) which might be due to the role of lime in neutralizing soil acidity, which in turn increases the availability of P which contributes to flower initiation and seed formation.

3.4.7. *Aboveground Biomass Yield*. The interaction effect of *Bradyrhizobium* and lime rates revealed significant differences in the aboveground biomass yield (Figure 4). The lowest aboveground biomass was recorded in lime untreated plot without rhizobium inoculation in the second cropping season (2017) while the highest aboveground biomass yield was observed in the combined application of lime with

TABLE 4: Main effects of *Bradyrhizobium*, lime, and phosphorus fertilizer rates on grain yield and harvest index of soybean.

Treatment	PH	NPB	NSP	HSW (g)	GY (kg ha ⁻¹)	HI (%)
Inoculation						
Uninoculated	73.0 ^{ab}	6.0 ^b	2.6	16.6	3020 ^b	0.39 ^b
TAL379	74.3 ^a	6.3 ^{ab}	2.6	17.1	3228 ^a	0.41 ^a
Legumefix	71.8 ^b	6.7 ^a	2.6	17.1	3048 ^b	0.40 ^{ab}
LSD (0.05)	1.8	0.42	NS	NS	158.6	0.02
P₂O₅ rate (kg ha⁻¹)						
0	71.7 ^b	5.8 ^b	2.6	16.6	2869 ^c	0.37 ^c
23	70.0 ^b	6.3 ^{ab}	2.6	17.1	3043 ^{bc}	0.38 ^c
46	72.9 ^b	6.6 ^a	2.7	16.8	3206 ^{ab}	0.41 ^b
69	75.5 ^a	6.6 ^a	2.7	17.3	3277 ^a	0.43 ^a
LSD (0.05)	2.1	0.49	NS	NS	183.1	0.021
Lime rate (t ha⁻¹)						
0	68.9 ^b	6.1 ^b	2.6	16.3 ^b	2766 ^b	0.39 ^b
3.12	77.2 ^a	6.6 ^a	2.6	17.5 ^a	3431 ^a	0.41 ^a
LSD (0.05)	1.5	0.34	NS	0.6	129.5	0.015
CV (%)	6.2	11.5	6.3	10.9	12.6	7.8

HSW: hundred-seed weight; NPB: number of primary branches; PH: plant height; NSP: number of seeds per pod. Means with the same factor and column followed by the same letter are not significantly different at 5% level of significance.

FIGURE 3: Interaction of effect of *Bradyrhizobium* strains and phosphorus rates on the number of pods per plant.

Bradyrhizobium inoculation in both years (2016 and 2017 cropping seasons). The increased aboveground biomass under lime application supplied with *Bradyrhizobium* inoculation might be due to the fact that lime improves soil P availability through reduction in its fixation in acid soils as well as increases calcium availability in the soil whereas *Rhizobium* inoculation improves nitrogen fixation which in turn enhance high dry matter production.

3.5. Partial Budget Analysis. Analysis of the net benefits, total costs that vary, and marginal rate of returns are presented in Table 5. Information on the costs and benefits of treatments is a prerequisite for the adoption of technical innovation by farmers. The study assessed the economic benefits of the treatments to help develop recommendation from the agronomic data. This enhances the selection of the right combination of resources by farmers in the study area.

The results in this study indicated that the inoculated treatments and lime treatments resulted in higher net benefits than the uninoculated treatments for all P fertilizer treatments (Table 5). The partial budget analysis was done on the basis of the cost of TSP and inoculants due to the fact that lime is supplied to the farmers in the area free of charges and it was difficult to quantify its economic benefit in one year as lime has a long-term effect. Thus, the partial budget analysis was done without considering the costs of lime, but the cost of TSP and inoculants, application cost of fertilizer, and cost of mixing inoculants with seeds were considered.

The partial budget analysis showed that the combined application of 23 kg P₂O₅ ha⁻¹ and 3.12 t lime ha⁻¹ inoculated with TAL379 produced the highest net benefits of 26569.6 Birr ha⁻¹ with the highest marginal rate of return of 784.7% (Table 5). On the other hand, the control treatment produced the lowest net benefit (14983.2 Birr ha⁻¹). This

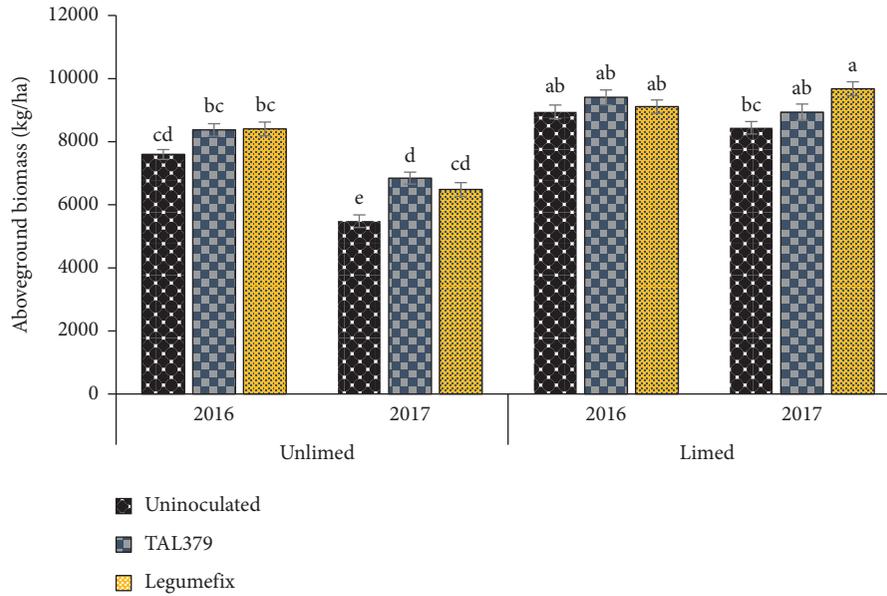


FIGURE 4: Interaction of effect of *Bradyrhizobium* strains and lime rates on the aboveground biomass yield. Bars followed by similar letters are not significantly different to each other at LSD (0.05).

TABLE 5: Partial budget analysis of the effects of *Bradyrhizobium* strains, phosphorus, and lime rates on soybean.

Inoculation	P ₂ O ₅ (kg ha ⁻¹)	Lime (t ha ⁻¹)	Yield (kg ha ⁻¹)	Adjusted yield (kg ha ⁻¹)	Gross return (Birr ha ⁻¹)	TCV (Birr ha ⁻¹)	Net benefit (Birr ha ⁻¹)	MRR (%)
-I	0	0	2081	1872.9	14983.2	0	14983.2	0
-I	0	3.12	2314	2082.6	16660.8	370	16290.8	471
TAL379	0	0	2200	1980	15840	195	15645	563
TAL379	0	3.12	2678	2410.2	19281.6	565	18716.6	738
Legumefix	0	0	2167	1950.3	15602.4	195	15407.4	D
Legumefix	0	3.12	2609	2348.1	18784.8	565	18219.8	D
-I	23	0	2524	2271.6	18172.8	967	17205.8	D
-I	23	3.12	3195	2875.5	23004	1337	21667	532
TAL379	23	0	3075	2767.5	22140	1162	20978	553
TAL379	23	3.12	3903	3512.7	28101.6	1532	26569.6	784.7
Legumefix	23	0	3068	3512.7	22089.6	1162	20927.6	D
Legumefix	23	3.12	3576	3218.4	25747.2	1532	24215.2	D
-I	46	0	2950	2833.2	21240	1864	19376	D
-I	46	3.12	3799	3419.1	27352.8	2234	25118.8	D
TAL379	46	0	3627	3264.3	26114.4	2059	24055.4	D
TAL379	46	3.12	3808	3004.2	27417.6	2429	24988.6	D
Legumefix	46	0	3338	3427.2	24033.6	2059	21974.6	D
Legumefix	46	3.12	3990	3591	28728	2429	26299	D
-I	69	0	3396	3056.4	24451.2	2761	21690.2	D
-I, 69, 3.12	69	3.12	3591	3231.9	25855.2	3131	22724.2	D
TAL379	69	0	3618	3256.2	26049.6	2956	23093.6	D
TAL379	69	3.12	3712	3312	26726.4	3326	23400.4	D
Legumefix	69	0	3680	3312	26496	2956	23540	D
Legumefix	69	3.12	3614	3252.6	26020.8	3326	22694.8	D

-I = without inoculation; 39 Birr = cost of TSP/kg; 100 kg of soybean = 800 Birr; one sachets of inoculant = 40 Birr; TCV = total costs that vary; MRR = marginal rate of return; D = dominated.

implies that farmers would be better of inoculating their soybean in combination with the application of 23 kg P₂O₅ ha⁻¹ and 3.12 t lime ha⁻¹ as these increase soybean yields and thus increase farmer’s income. Thus, application of

23 kg P₂O₅ ha⁻¹ and 3.12 t lime ha⁻¹ inoculated with TAL379 is profitable and recommended for the farmers in the study areas and other areas with similar agroecological conditions.

4. Discussion

Preplanting soil physicochemical properties of the experimental site were analyzed to know the fertility status of the experimental plot. The results showed that the soil of the experimental site is clay in texture. Rienke and Joke [33] reported as soybean gave high yield in loamy textured soil, but it can also grow better in clay soils. According to the soil analysis, the soil pH of the experimental site was strongly acidic based on the rating done by Tekalign [34]. Soybean has been found to do well in pH values of 5.5–7 and any pH below these values will affect its growth and needs amendment [38]. The organic carbon content of the experimental soil is medium according to the rating done by Hazelton and Murphy [35]. Organic carbon in soils influences physical, chemical, and biological properties of soils, such as soil structure, water retention, nutrient contents, and retention and microbiological life and activities in the soils. The analysis further indicated that the total N content of the experimental site is low to sustain high crop production [35]. The low total nitrogen might have been caused by soil acidity that tends to reduce microbial mediated processes that results in poor organic matter decomposition, mineralization of nitrogen, N uptake by plants, and denitrification [39]. Phosphorus levels in the soil can be used as a guide to indicate whether phosphate fertilizer is required for plant growth. The low available P content of the soil is probably attributed to high P fixing capacity of the soil at Bako [40].

Application of lime, *Bradyrhizobium* inoculation, and phosphorus significantly influences nodulation, phenological, growth, and yield and yield components of soybean. Increasing the levels of phosphorus without lime significantly shortened the days required to reach 50% flowering compared to lime-treated plots in the same level of phosphorus application rates. This is due to the fact that liming reduces the toxicity effects of soil acidity and improves the growth performance of the crop. Availability of essential nutrients and biological activity in soils are generally greatest at intermediate pH at which organic matter breaks down and releases essential nutrients like N, P, and S. Similar results were also reported by Hirpa et al. [16] who reported that phenology and growth of common bean genotypes were significantly increased by application of lime. This result is in contrast with the work of Mesfin et al. [14] who reported nonsignificant differences due to the interaction effect of lime and phosphorus rates on days to flowering and maturity of common bean.

The delayed maturity with inoculation might be due to the fact that better nitrogen produced by N_2 fixation through inoculation promoted vegetative growth which in turn extended days to maturity while phosphorus fastens days to flowering which in turn shortens the days to reach physiological maturity of the plants. The results are supported by Tairo and Ndakidemi [41] and Tesfaye et al. [42] who reported that soybean inoculation with *Bradyrhizobium* showed extended phenological development compared to uninoculated plants.

The nodule parameters showed a significant interaction between *Bradyrhizobium* × lime × phosphorus fertilizer

applications. Phosphorus plays a significant role in legume nodulation through its ability to enhance root development and proliferation, thereby affording the rhizobia more sites for infection and initiation of nodule formation. The greater number of nodules developed on the root of soybean after inoculation, lime, and phosphorus application showed the importance of lime and phosphorus in enhancing nodulation and symbiotic association between rhizobia and host plant and consequently improved N_2 fixation. Application of phosphorus with *Bradyrhizobium* significantly resulted in improved number of nodules compared with supplying phosphorus without *Bradyrhizobium* [20]. The results of this study also revealed that few nodules were observed on uninoculated plots, which is an indicator of the presence of soybean nodulating indigenous rhizobia bacteria in the experimental soil [43]. The better performance of the crop with liming also related to the better nodule development which stimulated effective N_2 fixation, increasing the amount of N available to support growth.

The results showed a significant interaction between *Bradyrhizobium* strains and phosphorus levels on the number of pods per plant. The positive effects of the inoculants might be due to better amount of nitrogen rendered through nitrogen fixation which promoted vegetative growth and plant height, thus improving the number of pods per plant. This could again be attributed to the availability of phosphorus that would have increased the intensity of photosynthesis, nitrogen fixation, root development, flowering, seed formation, and fruiting. Increased number of pods per plant of soybean was reported due to the combined application of phosphorus and inoculation [43–45].

Plant height, primary branches, grain yield, and harvest index were significantly influenced by the main effect of *Bradyrhizobium*, lime, and phosphorus application only. Among various phosphorus levels, the maximum yield and yield components were recorded at the rate of 69 kg P_2O_5 ha⁻¹. This positive growth response of soybean for application of P may be related to better availability of P as the rate of P application increased [20, 46]. The improvements of soybean yield and yield components were related to the symbiotic relationship between *Bradyrhizobium* and soybean plants, which resulted in the fixation of atmospheric nitrogen into the roots and translocation of amino acids to the shoots, thus leading to increased yield. Such a significant effect of *Bradyrhizobium* inoculation on soybean grain yield has also been reported by other researchers [43, 44, 47].

The beneficial effects of liming on plant growth are most likely a result from the enhanced conditions for seedling growth. The result indicated that applying lime to the soil might considerably improve the nutrient availability, particularly phosphorus and calcium, since it improves soil pH under which maximum availability of the nutrient may be obtained. Another reason could be liming neutralizes acid soil which in turn creates a conducive environment for root growth and also makes native soil P available for plant uptake [48].

The higher yields obtained with inoculation, lime, and phosphorus application indicate that the *Rhizobium* technology and lime application to acidic soil with a reduced rate

of phosphorus are efficient in supplying nitrogen to legumes as inorganic nitrogen fertilizer and it is a better option for resource-poor farmers who cannot afford to purchase expensive inputs. Also, these results suggest that inclusion of *Rhizobium* inoculation in the production package for soybean production in the study area is likely to be cost-effective since the inoculum sachets are fairly affordable.

5. Conclusion

Soil acidity and poor soil fertility are the major soil chemical constraints which limit crop productivity in western Ethiopia. Application of *Bradyrhizobium*, lime, and phosphorus showed a considerable difference in nodulation, phenological, growth, and yield and yield components of soybean. Thus, it can be concluded that use of *Bradyrhizobium* together with lime and phosphorus is an important practice to boost the productivities of soybean crop even in the second year of cropping season on the same plot of land due to the residual effect of *Bradyrhizobium* and lime on the soil and on the crop. However, as this study was done at one location on one soybean variety, the experiment has to be repeated over locations to determine the response of different maturity group of soybean varieties to appropriate rates or combination of *Bradyrhizobium* strains, lime, and phosphorus fertilizers which can maximize the productivity of the crop and reduce soil acidity problem in the study area.

Data Availability

The authors declare that they can submit the data at whatever time based on the request. The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Alemayehu Dabessa initiated the research, wrote the research proposal, conducted the research, did data entry and analysis, and wrote the manuscript. Tamado Tana was involved in analysis, methodology, supervising, and writing, reviewing, and editing of the research proposal and manuscript and also read and approved the final manuscript.

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