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Effects of Rhizobium, Nitrogen and Phosphorus Fertilizers on Growth, Nodulation, Yield and Yield Attributes of Soybean at Pawe Northwestern Ethiopia

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Abstract: Owing to the rising costs of chemical fertilizers and the growing environmental concerns, there is an ever increasing interest in the role of soil microorganisms in crop nutrition and soil fertility restoration. A field study was therefore conducted to determine the influence *of Bradyrhizobium* inoculation, N and P fertilizers application on nodulation, yield and yield attributes of soybean at Pawe. Three levels of N (0, 11.5 and 23 kg N ha^{-1}); three levels of P (0, 23 and 46 kg P_2O_5 ha^{-1}) with two levels of *Bradyrhizobium* were arranged in RCBD in factorial combinations with three replications. Nodule number, nodule weights, plant height, number of pods and number of seeds, 100 seeds weight and grain yield responded significantly to the interaction effects of *B. japonicum* with N and P fertilizers. Seed yield, biomass yield, and harvest index were significantly affected by the main effects of any one or more of the factors and interaction of any two of the factors. The maximum numbers of nodules of 80.26, fresh and dry weights of 3.77 and 0.99 gm/plant respectively; 100-seed weight of 16.96 gm, number of pods of 80.66 and grain yield of 3151.88 kg/ha were measured by combined effect of 11.5 kg N/ha, 46 kg P_2O_5/ha and *B. japonicum*. The highest plant heights of 79.26 cm, and 100.60 numbers of seeds were measured after applications of 46 kg P_2O_5/ha with *B. japonicum*. Each nodule attributes were significantly and positively correlated each other and with each yield and yield attributes. The results showed that growth and yield potential of soybean and an increase N_2 fixing can be achieved by using inoculation of *B. japonicum* and P application alone or in combination with *B. japonicum*, or P with small dose of N fertilizer. The results obtained in this work might have potential applications for increasing the productivity of soybean and enriching the soil with N.

Keywords: Rhizobium Bacteria, Inoculation, Nitrogen Fixation, Soil Fertility

1. Introduction

There is a general consensus on the need to address the problem of low soil fertility in a given region in order to improve agricultural productivity and thus food security. The augmentation of soil N is generally accomplished by many sources for supplying N to crops [1]. Inorganic N fertilization is needed to alleviate its deficiency. However, it is costly and therefore out of reach of resource poor farmers. In addition, manure obtained from livestock could also be used as a cheap source of nutrients, but nutrient contents are often lower, which requires bulk application to satisfy plant nutrient demand [2]. Biological nitrogen fixation (BNF) by leguminous crops can also supply sufficient N for optimum crop production [3]. The contribution of the fixed N is a key factor in low input agricultural systems to sustain long-term soil fertility. Thus, the significance of BNF as the major mechanism for the recycling of N from the atmosphere to available forms in the biosphere need not be overemphasized.

Most agricultural soils of the tropics, including Ethiopia, are deficient in N and phosphorus (P) nutrients. These two nutrients often limit crop production in Ethiopia [4]. Phosphorus deficiency followed by N is the major constraint in pulse production since it affects growth, nodule formation and development and N-fixation [5]. Phosphorus has important effects on photosynthesis, N fixation, root development, flowering, seed formation, fruiting and improvement of crop quality [6]. Symbiotic N fixation has a high P demand because the process consumes large amounts of energy [7] and energy generating metabolism strongly depends upon the availability of P [8]. Therefore, N2 fixation is very sensitive to P deficiency due to reduction in nodule mass and decreased ureide production [9].

Similarly, mineral N fertilization is a crucial factor in oil seeded legume production [10]. Even though BNF offers an alternative to the use of expensive ammonium based N fertilizer, the high yielding agricultural systems are difficult to sustain solely on BNF. So supplementation with mineral N might then be necessary for maximal yield of grain legumes [11]. The effects of N and P fertilization on growth, yield and yield components and nodulation of legumes are not well investigated. Moreover, no studies have been conducted to find out the effects of N and P application on soybean growth, yield and its nodulation in pawe area soils. Therefore, there is a need to generate such information for improving productivity of soybean in the region. In view of this the present study was conducted with the following specific objectives;

- To examine the effect of *Bradyrhizobium japonicum* inoculation on growth, nodulation, yield and yield components of soybean,
- To evaluate the effects of N and P fertilizer application on the growth, nodulation, yield and yield components of soybean, and
- To examine the interactive effects of *Bradyrhizobium japonicum* with the application of N and P fertilizer.

2. Materials and Methods

2.1. Description of Experimental Area

The experiment was conducted at Pawe the Agricultural Research Center (PARC) main station during the 2010 main cropping season. The PARC is found in Pawe district which is situated in Metekel Zone of Benishangul-Gumuz National Regional State in northwestern part of Ethiopia. Geographically, PARC is located at 11^0 09” N latitude and 36^0 03” E longitudes. It is 575 km in the Northwest direction of Addis Ababa. The area is characterized with an average annual rainfall of 1300 mm and monomodal rainfall distribution of intense storms that extend from May to October or November [12]. The mean annual maximum and minimum temperatures are 32 and 16°C, respectively.

The trends of monthly mean minimum and maximum temperatures and rainfall of the 2010 cropping season are shown in Figure 1. From Figure 1, it can be seen that the rainfall increases sharply from the end of May to August. Maximum rainfall was recorded in July and August, which indicates that the crop was free of water deficit stress. But the rainfall then declined and ceased in October, which was at the physiological maturity of the crop. Therefore, the development or full growth period of the crop was not faced with any water deficit.

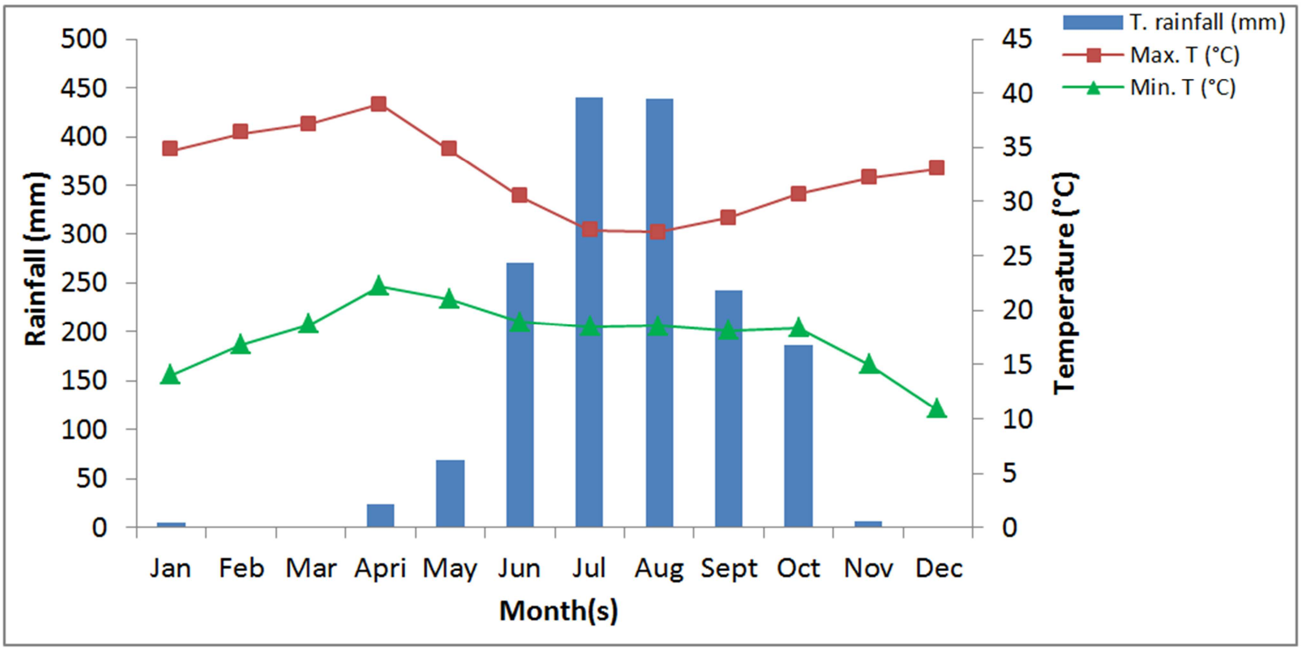

Figure 1. *Monthly mean minimum and maximum temperatures and total rainfall of the experimental station (2010).*

2.2. Experimental Treatments, Design and Procedures

The treatments were arranged in randomized complete block design (RCBD) in factorial combinations with three replications. The treatments were three levels of N fertilizer (0, 11.5 and 23 kg N ha^{-1}), three levels of P fertilizer (0, 23 and 46 kg P_2O_5 ha^{-1}) and two levels of *Bradyrhizobium japonicum* (TAL-379) inoculation (un-inoculated and inoculated). A total of 18 treatments were used in the experiment.

The size of each plot was 4 m × 2.4 m (9.6 m^2). The space between plots was 1m and the space between the blocks was 2m. Each plot contained 4 planting rows and the space between rows was 60 cm. At planting, two soybean seeds were seeded at 5 cm distance within a row and at 4 cm depth. Plant population was maintained by thinning at four to six leaf stages. The middle two rows were used for data collection and plant sampling for tissue analysis. Seeds were hand planted in rows on June 18. All standard cultural practices were applied throughout the growth period. Urea was used as N source while P was applied as triple super phosphate (TSP).

2.3. Bradyrhizobium Japonicum Inoculant Preparation and Seed Inoculation

Seeds were treated with carrier based inoculants containing *Bradyrhizobium japonicum* at the rate of 10 g per kg of seed [13]. In order to ensure that the inoculum sticks to the seed, the required quantity of inoculants were suspended in 1:1 ratio in 10% sucrose solution so that the dry seeds were thoroughly mixed with the thick slurry of sugar solution [14]. After mixing, seeds were air-dried in the shade for 15 minutes and sown within an hour [15].

2.4. Soil Sampling and Preparation for Analysis

About 1 kg pre sowing surface soil sample was collected by means of auger from different spots of the experimental field at the depth of 0-30 cm and bulked together to get a representative composite soil sample based on the procedure outlined in Sahlemedhin and Taye [16]. Then, air-dried and crushed soil samples were thoroughly mixed and packed in a polythene bag, labeled and stored in the laboratory for analysis.

2.5. Chemical and Physical Analysis of Soil Properties

Soil samples were analyzed at the PARC Soil Laboratory. Soil particle size was analyzed using the Bouyoucos hydrometer method [17] and textural class was identified on the basis of the USDA textural triangle. Soil pH was measured potentiometrically in 1:2.5 soil-water suspensions with standard glass electrode pH meter [18]. The Walkley and Black [19] method was used to determine organic carbon content of the soil. The total N content of the soil was determined using the Kjeldhal digestion, distillation and titration procedure [20]. Available P was analyzed based on the Bray II method [21]. The ammonium acetate method was employed to determine the cation exchange capacity of the soil.

2.6. Determination of Shoot Nitrogen and Phosphorus Contents

Five plants were randomly sampled from the middle two rows of each plot at mid-flowering stage. The plant samples were then oven dried at 70°C to a constant weight, and grounded to pass through 1 mm diameter sieve. Total N in shoot was estimated by the Kjeldhal digestion, distillation and titration method [22]. Similarly, P content in plant tissue was estimated by wet digestion with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$). The P content was then determined by the vanadomolybdate yellow color using spectrophotometer at 460 nm [23].

2.7. Data Collection

Five plants were randomly sampled from the two middle rows of each plot at mid-flowering stage of the plants. Then adhering soil particles were removed by washing the roots with their nodules gently with water over a metal sieve. Total number of nodules per plant was counted. The whole nodules from roots were picked; fresh and dry weight and volume of nodules per plant were recorded.

Five plants were randomly taken and plant height was measured with meter on tagged plants. The number of pods was determined at full maturity. Seed yield was harvested after picked up the pods from the randomly taken sampled plants. Five plants were again taken randomly and oven dried at 70°C to constant weight to determine above ground biomass yield. Harvest index was calculated as the ratio of seed yield to above ground biomass yield. Samples of 100 seeds were taken from five randomly taken plants to determine 100 seed weight. Grain yield was recorded from plants harvested at physiological maturity. Grain yield was corrected for 10% moisture content using Draminski moisture meter and converted in to kilogram per hectare.

2.8. Statistical Analysis

The data were then subjected to analysis of variance for factorial experiment in RCBD design using Statistical Analysis System (SAS Version 9.0, 2004) Software [24]. Mean separation was done using Fisher's least significant differences test (LSD) at 5% probability levels. Correlation analysis was also done between nodule characteristics, yield and yield attributes at correlation coefficients(r) value 0.05.

3. Results and Discussion

3.1. Phosphorus and Nitrogen Contents of Tissue

Application of 11.5 and 23 kg ha^{-1} N alone improved the tissue N content by 12.62 and 10.86%, respectively, over the control. This indicates that the use of high dose of starter N suppressed natural biological N fixation, which in turn might have resulted in less N content when artificial inoculation of *B. japonicum* were not made. On the other hand, the combined use of 11.5 and 23 kg ha^{-1} N with *Bradyrhizobium japonicum* improved the tissue N content by 17.82 and 28.27%, respectively compared to the control. Sarr [25] also previously reported significant increases in shoot N of soybean inoculated with *B. japonicum*.

Notwithstanding, remarkable improvements were observed when N and P fertilizers were combined with *B. japonicum*. The highest improvement of 34.77% was obtained when 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 were used in combination with *B. japonicum*. It is well known that *B. japonicum* inoculation and P increase *nitrogenease* activity and nodule mass that ultimately increases tissue N content, while addition of starter N fertilizer fulfills the immediate requirement of N of the plants at germination and these combinations lead to higher availability in soil and uptake of N by the plant.

Table 1. *Interaction and main effects of Bradyrhizobium japonicum inoculation and mineral N and P fertilizers on plant tissue N and P contents of soybean at mid-flowering stage.*

Un-inoculated					Inoculated			
Applied N	Applied P (kg P_2O_5 ha^{-1})				Applied P (kg P_2O_5 ha^{-1})			
(kg ha^{-1})	0	23	46	Mean	0	23	46	Mean
Plant tissue N content (%)								
0	3.233	3.655	3.416	3.435	3.514	3.795	3.851	3.72
11.5	3.641	3.373	3.683	3.566	3.809	3.57	4.357	3.912
23	3.584	3.697	3.556	3.612	4.147	3.879	4.006	4.011
Mean	3.486	3.575	3.552	3.538	3.823	3.748	4.071	3.881
Plant tissue P content (%)								
0	0.339	0.471	0.602	0.471	0.527	0.414	0.715	0.552
11.5	0.471	0.565	0.527	0.521	0.339	0.301	0.339	0.326
23	0.565	0.565	0.433	0.521	0.226	0.377	0.659	0.421
Mean	0.458	0.534	0.521	0.504	0.364	0.364	0.571	0.433

The response of P content in tissue with or without inoculation with *B. japoniucm* was higher at the highest level of applied P (Table 1). At the P rate of 46 kg ha^{-1} P_2O_5, inoculation with *B. japonicum* increased the P uptake by 18.78% compared to the un-inoculated one. Application of 23 and 46 kg ha^{-1} P_2O_5 without inoculation of *B. japonicum* increased the P uptake by 38.94 and 77.58%, respectively. Furthermore, Olivera [26] also reported that phosphorus application to legumes increased plant biomass including nodule biomass and shoot P content due to the increased rate of N fixation.

Total P tissue content was further improved to a maximum of 110.9% over the control due to the application of 46 kg ha^{-1} P_2O_5 with *B. japonicum* inoculation followed by 94% through combined application of 23 kg ha^{-1} N + 46 kg ha^{-1} P_2O_5 and inoculation with *B. japonicum*. Increased P contents of straw, seed and P uptake in soybean due to combined application of P and *B. japonicum* inoculation was also reported by Moharram *et al.* (1994).

3.2. Number of Nodules per Plant

The interaction of *Bradyrhizobium japonicum*, N and P had significant ($P < 0.05$) effect on the number of nodules. Application of 46 kg ha^{-1} P_2O_5 and inoculation of *B. japonicum* alone significantly increased number of nodules per plant by 240.7% and 123%, respectively. However, application of N fertilizer alone did not bring about significant effect on the number of nodules (Table 2).

Better nodule number per plant was observed due to the interaction of the three factors. Accordingly, inoculation of *B. japonicum* with combined application of 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5, followed by combined use of B. *japonicum*, 0 kg ha^{-1} N and 46 kg P_2O_5, and 23 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5, significantly increased the mean number of nodules per plant as compared to most other treatments (Table 2). Greater number of nodules due to inoculation and N and P application suggested that there was better combining and symbiotic relationship between introduced *B. japonicum* and soybean. Wall [27] recognized that P in coincidence with the plant demand of N controls the nodule growth and alter the symbiotic processes between the legume and *Rhizobium*. This is in agreement with the findings of [28] and [29] who reported that inoculation with *B. japonicum* significantly, increased the number of nodules per plant of soybean as compared to the control treatments.

3.3. Nodule Fresh and Dry Weight per Plant

The results of analysis of variance indicate that all the three factors and their interactions affected fresh weight of nodules significantly ($P < 0.0001$). Soybean seed inoculation with *B. japonicum* resulted in greater mean nodule fresh weight than the control (Table 2). Like mean nodule number per plant, addition of either 11.5 kg ha^{-1} N or 23 kg ha^{-1} N, showed insignificant change to the mean nodule fresh weight per plant. This can be again attributed to the inhibitory effect of N at higher levels on nodule number and size.

However, the maximum mean nodule fresh weight per plant was obtained by the interaction effect of *B. japonicum*, 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 which was followed by after combined application of *B. japonicum,* 11.5 kg ha^{-1} N and 23 kg ha^{-1} P_2O_5, and 0 kg ha^{-1} N, 46 kg ha^{-1} P_2O_5 and *B. japonicum* (Table 2). Nodule fresh weight was highly correlated with number of nodules indicating that nodule fresh weight increased as the number of nodules increased. Datsenko [31] concluded that number and weights of nodules are commonly used as the criteria of effective complementary interaction between macro and micro symbionts; thereby correlate on the whole with the rate of atmospheric nitrogen fixation. Apparently this was due to the formation of greater number of nodules from *B. japonicum* inoculated treatments. Jalaluddin [32] also observed increment in fresh and dry weights of nodules in soybean in the *B. japonicum* inoculated plants as compared to control.

In line with the other nodulation parameters the nodule dry weight was also significantly affected ($P < 0.0001$) by the interaction of *Bradyrhizobium japonicum*, N and P. Soybean seed inoculation with *B. japonicum* and addition of 46 kg ha^{-1} P_2O_5 increased the mean nodule dry weight per plant than the un-inoculated control (Table 2). However, there were no mean nodule dry weight differences among N fertilizer rates.

The maximum mean nodule dry weight per plant was obtained from the interaction effects of *B. japonicum*, 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 which was followed by combined application of

B. japonicum, 23 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5. This reveals that the combination of *B. japonicum* and P along with starter N produced the maximum nodule biomass. The result accords with the findings of [33] that using *B. japonicum* strains with P fertilizer increased the nodule dry weight as compared with the control. Presence of P and small N in the soil with *B. japonicum* strain might have individually or in combination positive effect on nodule weight and nodule volume parameters.

Table 2. *Interaction effect of Bradyrhizobium japonicum, inoculation and mineral N and P fertilizers on nodule characteristics and plant height of soybean at Pawe.*

		No. of nodules	F. weight (g)	D. weight (g)	Plant. Ht. (cm)
Un-inoculated					
N (kg ha^{-1}) x P (P_2O_5 kg ha^{-1})					
0	0	20.33^{j}	0.99^{h}	0.310^{i}	64.69^{h}
	23	32.93^{i}	2.049^{cd}	0.6^{gf}	74.2^{c}
	46	69.26^{bc}	2.33^{c}	0.8^{bc}	76.94^{b}
11.5	0	23.80^{j}	1.70^{def}	0.72^{de}	75.97^{bc}
	23	49.26^{fg}	1.37^{fgh}	0.65^{ef}	66.09^{gh}
	46	68.93^{bcd}	2.06^{cd}	0.82^{bc}	68.46^{ef}
23	0	23.66^{j}	1.65^{ef}	0.68^{de}	69.60^{ef}
	23	34.33^{hi}	1.03^{h}	0.44^{h}	65.46^{h}
	46	60.00^{de}	1.20^{gh}	0.45^{h}	67.74^{fg}
Inoculated					
0	0	45.40^{g}	1.98^{cde}	0.69^{de}	68.90^{ef}
	23	55.33^{ef}	1.56^{fg}	0.49^{h}	76.82^{b}
	46	73.13^{ab}	2.89^{b}	0.76^{bcd}	79.26^{a}
11.5	0	69.53^{bc}	2.28^{c}	0.74^{cd}	70.26^{de}
	23	63.33^{cde}	3.03^{b}	0.65^{ef}	71.96^{d}
	46	80.26^{a}	3.77^{a}	0.99^{a}	75.64^{bc}
23	0	42.06^{gh}	2.17^{c}	0.7^{de}	69.05^{ef}
	23	57.40^{ef}	1.42^{fg}	0.5^{gh}	68.36^{ef}
	46	71.66^{abc}	1.70^{def}	0.84^{b}	74.58^{c}
LSD (0.05)		9.11	0.38	0.08	2.16
CV (%)		10.53	11.95	7.53	1.83
SE (±)		2.61	0.10	0.02	0.61

Means followed by the same letter in a column are not significantly different at P = 0.05; No = Number, F = Fresh, D = Dry, Ht = height, SE = Standard error, CV = Coefficient of variation, LSD = Least significant difference.

3.4. Plant Height

The results of analysis of variance indicate that the interaction of *Bradyrhizobium japonicum*, N and P was significant ($p < 0.0001$) on plant height. As a result, the use of 23 and 46 kg ha^{-1} P_2O_5 alone significantly improved the mean plant height by 14.7 and 18.9%, respectively (Table 2). In consonance with the findings of this study, [29], [34] and [35] also reported an increase in plant height of soybean due to sole application of P. Sole application of 11.5 and 23 kg ha^{-1} N improved the mean plant height by 17.4 and 7.6%, respectively, over the control (Table 2). In line with this, a few studies showed that application of reduced amount of N as starter fertilizer could improve growth by soybean [36]; [37]; [35].

The *B. japonicum* improved the mean plant height of soybean by 6.5% than the un-inoculated plants. However, the maximum mean plant height improvement of 22.5 and 18.8% were obtained due to the interaction effects of *B. japonicum* and 46 kg ha^{-1} P_2O_5, followed by combined application of *B. japonicum* and 23 kg ha^{-1} N respectively, (Table 4). Significant improvement of 16.9% in mean plant height was obtained when 11.5 kg ha^{-1} N, 46 kg ha^{-1} P_2O_5 and *B. japonicum* were used. This was followed by a 15.3% increase due to the combined use of 23 kg ha^{-1} N, 46 kg ha^{-1} P_2O_5 and *B. japonicum* (Table 2). In general, the results indicate that combined use of N and P fertilizers with *B. japonicum* resulted in better mean plant height

3.5. Seed Yield per Plant

The results of analysis of variance indicate that the interaction of all the three factors did not significantly affect the seed yield, plant biomass and harvest index. However, the response of seed yield to seed inoculation of *B. japonicum*, application of starter N, and P and interactions of P by *B. japonicum* was significant. Accordingly, sole application of 11.5 kg ha^{-1} N improved the mean seed yield per plant by 8% over the control but addition of 23 kg ha^{-1} N alone showed the mean seed yield per plant statistically at par with the control (Table 3).

Seed inoculation of *B. japonicum* alone significantly increased mean seed yield per plant by 18.7% as compared to the un-inoculated control treatment. The findings of this study are also supported by the findings of [29] who reported an increase in seed yield of soybean due to *B. japonicum* inoculation in Pakistan. The combined use of 46 kg ha^{-1} P_2O_5 and *B. japonicum* significantly maximized the mean seed yield per plant by 59.2% as compared to the control. Furthermore, the combined use of 46 kg ha^{-1} P_2O_5 and *B. japonicum* increased the mean seed yield of soybean by 34.1% over the use of seed inoculation of *B. japonicum* alone. This might be related to the increased nodulation through symbiosis between soybean and *B. japonicum,* which resulted in more N_2-fixation that leads to increased yield parameters.

3.6. Plant Biomass and Harvest Index per Plant

Plant biomass was affected significantly by seed inoculation of *Bradyrhizobium japonicum* and sole application of N. As a result, soybean seed inoculation of *B. japonicum* alone increased the mean plant biomass per plant by 12.8% over the un-inoculated control. The result indicated that N fixation by *B. japonicum* enhanced the vegetative growth of soybean, which resulted in substantial increase in its biomass yield. Tahir [35] also reported increase in plant biomass due to inoculation of *B. japonicum* alone by 62.8% over the control. The maximum mean biomass yield per plant was obtained after application of 23 kg ha^{-1} N. However, application of 11.5 kg ha^{-1} N showed the mean biomass yield per plant statistically at par with the control (Table 3). The increase in biomass yield per plant was possibly because of supply of N with other soil mineral N form that was responsible for the highest vegetative growth of soybean. Mrkovacki [37] reported that maximum results for biomass yield were seen by applying 30 kg ha^{-1} N to inoculated soybean instead of higher rates of N.

Analysis of variance indicated that only independent application of N and P had significant variations on harvest index per plant. But inoculation and their interactions failed to respond significantly. The lowest mean harvest index per plant, even less than the control was recorded from the plots that received 23 kg ha^{-1} N. Although application of 11.5 kg ha^{-1} N showed statistically at par mean harvest index with the control, it resulted in 5.8% increase in mean harvest index per plant (Table 3). The decreased mean harvest index per plant with the increase of N fertilizer might be due to the influence of vegetative growth and increased above ground biomass yield, which reduced the harvest index.

Considering the main effects of P application on harvest index, the maximum mean harvest index per plant was obtained from application of 46 kg ha^{-1} P_2O_5, which resulted in 19.1% increase over the control. The sole application of 23 kg ha^{-1} P_2O_5, however, gave statistically similar mean harvest index per plant to that of the un-inoculated control treatment (Table 3). The increased mean harvest index per plant with the increase of P fertilizer rate might be due to the influence of greater fruit and seed setting than above ground biomass yield. The result found in this study is in agreement with the results of [38] who reported that harvest index was significantly influenced by applied P in soybean crop.

Table 3. *Main effects of Bradyrhizobium japonicum, inoculation and mineral N and P fertilizers on seed yield, plant biomass and harvest index of soybean at Pawe.*

Treatments	Seed yield/plant (gm)	Plant biomass/plant (gm)	Harvest Index
Inoculation			
Un-inoculated	10.16^b	22.50^b	0.46
Inoculated	12.60^a	25.40^a	0.51
LSD (0.05)	1.13	2.3	NS
N (kg/ha)			
0	11.45^{ab}	22.22^b	0.52^a
11.5	12.36^a	22.38^b	0.55^a
23	10.32^b	27.25^a	0.38^b
LSD (0.05)	1.38	2.82	0.07
P (kg P_2O_5/ha)			
0	10.71^b	23.07	0.47^b
23	9.77^b	24.02	0.42^b
46	13.65^a	24.76	0.56^a
LSD (0.05)	1.38	NS	0.07
CV (%)	17.99	17.44	22.09
SE (±)	0.42	0.64	0.02

Means followed by the same letter in a column are not significantly different at P = 0.05; NS = No significance, SE = Standard error, CV = Coefficient of variation, LSD = Least significant difference.

3.7. Number of Pods and Seeds per Plant

The results of analysis of variance indicated that individual application of *Bradyrhizobium japonicum*, N, P and their interactions had significant effect ($P < 0.0001$). Application of 23 and 46 kg ha^{-1} P_2O_5 resulted in the same mean number of pods (Table 4). On the other hand, application of 11.5 kg ha^{-1} N resulted in better mean pod number than the mean pod number obtained by application of 23 kg ha^{-1} N and the control. Inoculation of *B. japonicum* by itself however showed equal mean pod number per plant than un-inoculated treatment (Table 4).

Also, combined application of 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 and 23 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 with no inoculation produced significantly greater number of mean pod number, improving it by 107.7 and 61.3%, respectively, (Table 4). Application of 23 and 46 kg ha^{-1} P_2O_5 with *B. japonicum* resulted in 59.7 and 164.7% increase in mean pod number, respectively (Table 4). The significant improvements in mean pod number ranged from 33.2% to 110.7% due to application of 23 kg ha^{-1} N and 11.5 kg ha^{-1} N with *B. japonicum*, respectively. The maximum mean number of pods was recorded from plants that received 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 with *B. japonicum* inoculation (Table 4). An investigation conducted by [35] indicated that 94% increase of pod number per plant was recorded where 25 kg ha^{-1} N was combined with P and *B. japonicum* on soybean in Pakistan.

The results of analysis of variance indicate that the interaction of *Bradyrhizobium japonicum*, N and P significantly ($P < 0.0001$) affected the number of seeds per plant. The minimum mean number of seeds per plant, even far less than that of the un-inoculated control, was found by the application of 23 kg ha^{-1} N (Table 4). This could presumably be due to the fact that high rate of N fertilizer application significantly reduced number of nodules and nodule weights (Table 4) and the inhibitory effects of added N fertilizer to nodulation and N fixation lead to poor seed filling. The study by [39] indicated that the dropping percentage in seeds number per plant with high N treatments ranged from 12-46% as compared to the control.

Bradyrhizobium japonicum inoculation alone gave better mean number of seeds count per plant than the control but when it was supplemented with 11.5 kg ha^{-1} N and/or 46 kg ha^{-1} P_2O_5, it increased the mean seed number per plant further (Table 4).

This might be related to better N availability at the early growth stage of soybean, which in turn might have resulted in more nodule mass formation and adequate N fixation during growing season specifically seed setting period; all of these contributing to the observed increased seed yield.

A considerable improvement in seed number per plant was also seen by independent applications of 46 kg ha^{-1} P_2O_5 and 23 kg ha^{-1} P_2O_5 as compared to the control (Table 4). However, the maximum mean number of seed count per plant was recorded by the combined applications of 11.5 kg ha^{-1} N, 46 kg ha^{-1} P_2O_5 and *B. japonicum* inoculation. The result confirms the findings of [40] who also observed significant increment in number of seeds per pod when *B. japonicum* inoculation was combined with different levels of P.

3.8. Hundred Seed Weight

The results of analysis of variance indicate that the main effects of factors under consideration and their interactions showed significant differences on hundred seed weight except for the interaction of N by P. Seed inoculation resulted in higher mean hundred seed weight than un-inoculated treatment (Table 4). It increased hundred seed weight by 10.6% over the un-inoculated treatment. Increased in thousand seed weight resulted from *B. japonicum* inoculation was observed in soybean by [38]. Separate N applications contributed more or less the same output (Table 4). When applying 46 kg ha^{-1} P_2O_5, reasonable hundred seed weight was measured than 23 kg ha^{-1} P_2O_5. The maximum value of hundred seed weight was measured from mixed application of N and P inorganic fertilizers along with *B. japonicum* inoculation. It resulted in 26.9% increase in hundred seed weight followed by 20.8% through *B. japonicum* inoculation with 11.5 kg ha^{-1} N + 46 kg ha^{-1} P_2O_5 and 23 kg ha^{-1} N + 46 kg ha^{-1} P_2O_5, respectively (Table 4).

Table 4. *Interaction effect of Bradyrhizobium japonicum, inoculation and mineral N and P fertilizers on yield and yield attributes of soybean at Pawe.*

		Number of pods/plant	Number of seed/plant	100 seed Weigh (gm)	Grain yield (kg/ha)
			Un-inoculated		
N (kg ha^{-1}) x P (P_2O_5 kg ha^{-1})					
0	0	27.93^{h}	58.40^{gh}	13.36^{i}	1730.42^{h}
	23	70.13^{b}	83.46^{c}	13.85^{hi}	2511.25^{ef}
	46	68.66^{b}	93.13^{b}	16.02^{bcde}	2977.08^{b}
11.5	0	35.60^{g}	61.73^{efg}	15.34^{def}	2360.00^{f}
	23	29.40^{h}	70.53^{d}	13.74^{hi}	2602.50^{de}
	46	58.00^{c}	65.13^{def}	16.24^{ab}	3060.42^{ab}
23	0	32.80^{h}	54.60^{h}	14.8^{fg}	2171.46^{g}
	23	27.40^{h}	60.13^{fgh}	13.67^{hi}	2396.04^{f}
	46	45.06^{de}	60.86^{fg}	14.40^{gh}	2346.04^{f}
			Inoculated		
0	0	32.46^{gh}	78.13^{c}	14.78^{fg}	2638.33^{de}
	23	44.60^{de}	67.40^{de}	15.14^{fg}	2644.38^{de}
	46	73.93^{b}	100.60^{a}	16.11^{bcd}	3057.92^{ab}
11.5	0	58.86^{c}	91.33^{b}	15.29^{ef}	2763.75^{cd}
	23	48.93^{d}	61.13^{fg}	15.38^{cdef}	2910.21^{bc}
	46	80.66^{a}	120.00^{a}	16.96^{a}	3151.88^{a}
23	0	37.20^{fg}	58.26^{gh}	15.03^{fg}	2608.33^{de}
	23	42.80^{ef}	61.26^{efg}	15.26^{ef}	2622.71^{de}
	46	73.13^{b}	100.00^{b}	16.15^{bc}	2893.96^{bc}
LSD (0.05)		6.05	6.18	0.78	170.83
CV (%)		7.42	5.37	3.15	3.91
SE (±)		2.42	1.88	0.14	49.07

Means followed by the same letter in a column are not significantly different at p = 0.05; SE = standard error, CV = coefficient of variation, LSD = least significant difference.

3.9. Grain Yield per Hectare

The results of analysis of variance showed that the response of grain yield to the interaction effects of *Bradyrhizobium japonicum*, N and P was significant ($P < 0.0001$). Soybean seed inoculation with *B. japonicum* produced higher mean grain yield than that of the un-inoculated control (Table 4). The increase in grain yield could be attributed to increase in yield components of the crop through *B. japonicum* inoculated plots. Egamberdiyeva [41] reported increase in grain yield due to *B. japonicum* inoculation of soybean in Uzbekistan. Great grain yield variation was also observed among the P fertilizer rates. Addition of 46 kg ha^{-1} P_2O_5 alone could yield more grain yield than 23 kg ha^{-1} P_2O_5. However, better grain yield was harvested when 46 kg ha^{-1} P_2O_5 was added with 11.5 kg ha^{-1} N.

The final grain yield of a crop is a function of cumulative contribution of its various growth and yield parameters which are influenced by various agronomic practices. The highest grain yield was recorded when the three factors interacted to each other. As a result interaction of 11.5 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 along with *B. japonicum* inoculation gave the maximum grain yield. This was justified with the finding of [42] who obtained highest grain yield of soybean when the plant was inoculated with *B. japonicum* in combination with N and P fertilizers.

3.10. Correlation of Nodulation and Yield Parameters

Correlation coefficient values exhibited that nodule number, volume, fresh and dry weights were highly significantly and positively correlated with each other. All the nodule characteristics were also correlated significantly and positively with each yield and yield attributes except to the biomass yield per plant. This indicates that the development of effective and promising nodules of the crop due to P supply and *B. japonicum* inoculation could promote N uptake through the process of BNF which ultimately enhances the final grain yield and yield attributes of the crop. The yield of plant is a dependent variable, depends upon all other growth and yield contributing components. Therefore, it is generally correlated with all other components.

4. Conclusions

In general, the response of P content in tissue with or without inoculation with *B. japoniucm* was higher at the higher levels of applied P. Similarly, all the *B. japonicum* treatments used alone or in combination with inorganic fertilizers improved the tissue N content of soybean. Generally, the results of this study revealed that seed inoculation of *B. japonicum* and P application alone or in combination with *B. japonicum*, or P with small dose of N fertilizer could improve nodulation potential of soybean. Higher dose of N applied alone or in combination either with P or *B. japonicum* reduced the efficiency of *B. japonicum* for various nodule characteristics, growth parameters and yield traits of soybean. In general, starter N of 11.5 kg ha^{-1} N with *B. japonicum* and 46 ka ha^{-1} P_2O_5 resulted in better nodulation, growth and development, yield and yield attributes of soybean crop.

The results obtained in this work might have potential applications for increasing the productivity of soybean and enriching the soil with N. However, since the experiment is conducted for one season and at one location, it is difficult to give comprehensive recommendation on best combinations of inorganic fertilizers and *B. japonicum*. It is, therefore, necessary to repeat the experiment under various soil conditions and fertilizers rates with an appropriate symbiont strain. Therefore, it would be worthwhile to conduct a similar study in N depleted fields prevalent in smallholder production systems.

List of Abbreviations

No = Number, F = Fresh, D = Dry, Ht = height, SE = Standard error, CV = Coefficient of variation, LSD = Least significant difference, B. japonicum,=Bradyrhizobium japonica, ANOVA, =Analysis of Variance, BNF= Biological Nitrogen Fixation, RCBD=Randomized Complete Block Design, SAS= Statistical Analysis software, masl= Meters Above Sea Level

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