Effect of *Bradyrhizobium Japonicum* (TAL 379) on nodulation and grain yield of Soybean

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Abstract
The experiment was conducted at Finoteselam sub center on station during 2006 and 2007 on red soil in a randomized complete block design with three replications to study the effect of Bradyrhizobium japonicum (TAL 379) on nodulation and yield of soybean. Seeds of soybean var. cherry was sprinkled by lukewarm water to adhere the inocula on its surface and inoculated with the strain (TAL 379) at a rate of 10 gm per kg of seed and immediately sown in rows. Significant difference (P<0.05) was recorded for plant height, grain yield, nodule number and nodule volume. There was no nodule in the un inoculated plots implying that the strain is exotic and not found in the soil of Finoteselam. Maximum grain yield was recorded by 23 kg N + TAL 379 (2.17 t ha^{-1}) and TAL 379 alone (2.15 t ha^{-1}). The strain alone has significantly increased the grain yield by 19.3% (347 kg) over the control combined over years. The maximum nodule number per plant was recorded by 46 kg N + 46 kg P_2O_5 + TAL 379 (71.5) and by TAL 379 alone (69). However, effective nodules were observed in the plots inoculated only by the strain in the absence of N fertilizers. The over all result indicates that the strain (TAL 379) has good effect on nodulation and grain yield of soybean. Therefore, for soybean to nodulate and fix atmospheric nitrogen and produce high grain yield, the seed should be inoculated with the Bradyrhizobium japonicum (TAL 379). Facilities to maintain and multiply the strain should be in place to help soybean growing commercial farmers and to initiate other farmer to produce the crop for commercial use with reasonable cost.

Key words: *Bradyrhizobium japonicum*, grain yield, inoculation, nodule, soybean,

Introduction

Fertilization of intensively managed crop systems is essential in maintaining or increasing world food production which is heavily dependant on nitrogen input to maximize yield potential. Nitrogen is the most limiting nutrient for plant growth. Approximately 85 million metric tones of nitrogenous fertilizers are added to soil worldwide annually up from 1.3 million tones in 1930s. (Frink *et al*., 1999). For productivity to be simply sustained at current levels, let alone improved in the future, the N removed in agricultural produce or lost from the system must be replaced by N derived either from nitrogenous fertilizer or biological nitrogen fixation (Peoples *et al*., 1995).

Nitrogen is one of the key elements required for growth and productivity of crops. It is abundant in atmosphere (80%), but can't be utilized by plants as such. It has to be converted to nitrate or ammonium form either by chemical or biological process to be used by plants.

Chemical synthesis of N-fertilizer by industry means is energy intensive and costly (Singh, 1998). However, the same process is also carried out enzymatically by cyanobacteria (blue green-algae) and certain species of bacteria (Singh, 1998).

The contribution of biological nitrogen fixation (BNF) to the N cycle on the other hand can be controlled by manipulating various physical, environmental & biological factors (Hansen, 1994) and may therefore be more open to managing than applying fertilizer N. There is growing international concern about issues of global warming, environmental degradation and loss of natural resources.

Soybean is a grain legume cultivated in many areas in the world from tropics to temperate regions with a seed yield of 1.4 -2 t ha^{-1}. The seed (bean) contains about 18% oil and 38% protein and the extraction residue represents more than 40% of the utilization value of the plant (Asiedu, 1989). Soybean fixes up to 200 kg N ha^{-1} $year^{-1}$ when in symbiotic association with *B. japonicum* (Zhange et al., 2002) reducing the need for expressive and potentially environmental damaging N fertilizer (Asiedu, 1989).

Soybean is a recently introduced crop to Ethiopia. According to CSA (2001/02), the area covered by soybean in the country was 1,769 ha of which 251 ha was in the Amhara region with a total yield of 1621 t and 207 t respectively. The average yield per hectare is by far below the world’s average. To give high yield and use its potential the crop needs association with *Bradyrhizobium japonicum* (the only rhizobial symbiont) to fix atmospheric nitrogen. There is no information in the region regarding the symbiotic effect of soybean and *Bradyrhizobium japonicum*. Therefore, the present work was initiated to study the effect of *Bradyrhizobium japonicum* (TAL 379) on nodulation and yield of soybean.

Materials and methods

The experiment was conducted at Finoteselam on station on a randomized complete block design with three replications. *Bradyrhizobium japonicum* (TAL 379) strain was received from National Soil Research Center Microbiology section (Addis Ababa) whereas soybean seed (Cherry) was received from pulse crops breeding program of the Center. The seeds were measured for each plot and sprinkled by lukewarm water to moisten the seed and adhere the strains on its surface. Immediately, the seeds for inoculation were inoculated with the strain at the rate of 10 g per kg under shade and sown to their respective plots at 40 cm distance between rows and 10 cm distance between plants on 2x4 m^2 plot size.

Treatment combination

Treatment no	Nitrogen (kg ha^{1})	P_2O_5 (kg ha^{1})	Strain (TAL 379)
1	0	0	0
2	0	46	0
3	0	0	TAL
4	0	46	TAL
5	23	0	0
6	23	46	0
7	23	0	TAL
8	23	46	TAL
9	46	0	0
10	46	46	0
11	46	0	TAL
12	46	46	TAL
13	69	0	0
14	69	46	0
15	69	0	TAL
16	69	46	TAL

Data collection

At 50 % flowering three plants were randomly dug out from boarder rows of each plot and nodule number was counted and nodule volume was measured (Fig.1a&b). At maturity five plants were randomly selected from each plot and number of pods per plant was counted. Similarly, five pods per plant were randomly selected from each plot and number of seeds per

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Figure 1. Soybean counted nodules (a) nodule volume (b)

plant was counted. Five representative plants were randomly selected from each plot and plant height was measured using a meter scale. Among 5 rows of soybean per plot, 3 central rows were harvested from each plot and grains and straws were separated and straw and grain yield per plot were measured. For all parameters the mean was recorded

Result and Discussion

Significant differences ($P<0.05$) were observed for plant height, nodule number, nodule volume and grain yield in each year and combined over years. the highest plant height was recorded by 23 kg N + 46 kg P_2O_5 ha^{-1} followed by 46 kg N + 46 kg P_2O_5 ha^{-1} + TAL 379 and 69 kg N + 46 kg P_2O_5 ha^{-1} + TAL 379. The effect of the strain on plant height over the control and other treatment was non significant i.e. the presence or absence of the strain has no effect on plant height but the significant difference among treatments in plant height was due to the applied NP fertilizers (Table 1).

Table1. Effect of *Bradyrhizobium japonicum* on the plant height of soybean

Treatment	*Mean plant height (cm)*		
	2006	2007	Combined
1	50.20	43.60	46.90
2	55.67	45.67	50.67
3	57.53	48.13	52.83
4	53.07	53.40	53.23
5	49.20	48.47	48.83
6	61.27	51.07	56.17
7	60.20	49.60	54.90
8	52.53	53.13	52.83
9	48.93	47.13	48.03
10	58.07	53.27	55.67
11	47.27	53.73	50.50
12	55.13	56.13	55.63
13	48.00	46.60	47.30
14	53.53	52.07	52.80
15	51.67	49.40	50.53
16	54.67	56.07	55.37
LSD (0.05)	8.094	6.417	7.154
C.V (%)	9.06	7.62	8.42

Maximum grain yield was recorded by 23 kg N + TAL 379 and TAL 379 alone followed by 46 kg P_2O_5 + TAL 379. The strain alone has increased grain yield over all treatments equally with 23 kg N ha^{-1}(Table 2). This indicates that, there is no need to apply N to increase grain yield of soybean in inoculated fields. The reason for the reduction of the grain yield and yield components during the second year was terminal moisture stress.

In Jimma and Awassa areas investors who grow soybean have used the strain for better yield advantage. EIAR has found good response of the strain to grain yield and went for scaling up at Jimma during 2006 (masmedia). Therefore, the result is in agreement with those activities.

Table2. Effect of *Bradyrhizobium japonicum* on the grain yield of soybean

Treatment	*Mean grain yield kg ha^{-1}*		
	2006	2007	Combined
1	2054	1550	1802
2	2533	1575	2054
3	2661	1638	2149
4	2364	1714	2039
5	2030	1582	1806
6	2399	1638	2018
7	2665	1665	2165
8	2330	1638	1984
9	2146	1580	1863
10	2431	1616	2024
11	1946	1628	1787
12	2381	1643	2012
13	1834	1566	1700
14	2122	1630	1876
15	2085	1629	1857
16	2191	1716	1953
LSD (0.05)	484.2	58.22	337.8
C.V (%)	12.85	2.15	10.64

Nodule was formed in all inoculated plots (fertilized and unfertilized).The maximum nodule number was recorded by 46 kg N + 46 kg P_2O_5 + TAL 379 (71.5) and TAL 379 alone (69) followed by 23 kg N + 46 kg P_2O_5 + TAL 379(67.5) and 23 kg N ha^{-1} + TAL 379 (66.83) (Table 3). However, effective nodules were observed in the plots inoculated by the strain alone (without N fertilizer) and the strain with P fertilizer alone (Fig. 2). The nodules in plots with N fertilizer were light green to light pink in color. The size of the nodules from the plots inoculated with the strain alone was as large as the well matured field pea seed and pinkish in color when dissected whereas the size of most nodules of the other plots with N fertilizer are small and green to light pink in color. The less effectiveness of the nodules in inoculated plots with N fertilizer may be due to the preference of the crop to the applied N fertilizer than soothing the strain for symbiotic association to fix atmospheric N.

Figure 2. Effective nodules

Table3. Effect of *Bradyrhizobium japonicum* on the nodule number of soybean

Treatment	*Mean nodule number per plant*		
	2006	2007	Combined
1	0	0	0
2	0	0	0
3	106	32	69
4	67	58	63
5	0	0	0
6	0	0	0
7	91	42	67
8	98	37	68
9	0	0	0
10	0	0	0
11	62	46	54
12	99	44	72
13	0	0	0
14	0	0	0
15	70	33	52
16	87	43	65
LSD (0.05)	39.96	18.33	30.45
C.V (%)	13.98	16.05	14.93

Similarly, Table 4 showed that there was significant difference in nodule volume among the treatments. The maximum nodule volume was recorded by 46 kg N + 46 kg P_2O_5 + TAL 379 (3.592 ml) and TAL 379 alone (3.412 ml). Both nodule number and volume were high during 2006 than during 2007 (Table 3 and 4).

Table4. Effect of *Bradyrhizobium japonicum* on the nodule volume of soybean

Treatment	*Mean nodule volume in ml*		
	2006	2007	Combined
1	0.00	0.00	0.00
2	0.00	0.00	0.00
3	4.56	2.27	3.41
4	2.73	3.60	3.16
5	0.00	0.00	0.00
6	0.00	0.00	0.00
7	3.45	2.56	3.00
8	4.00	2.11	3.06
9	0.00	0.00	0.00
10	0.00	0.00	0.00
11	2.67	2.86	2.76
12	4.55	2.63	3.59
13	0.00	0.00	0.00
14	0.00	0.00	0.00
15	3.22	2.26	2.74
16	3.33	2.77	3.05
LSD (0.05)	1.46	0.8632	1.172
C.V (%)	40.75	39.33	40.05

Conclusion

In general, the over all result indicates that the strain (*B. japonicum* TAL 379) has good effect on nodulation and grain yield of soybean. Soybean needs *Bradyrhizobium japonicum* for nodulation and fixing atmospheric nitrogen and thereby increase yield and yield components. No *B. japonicum* was observed in the soil of the trial site in the non inoculated plots whereas good number of nodule was counted in all inoculated plots implying that the strain is exotic and should be introduced to the system with the crop to increase its yield. In addition, the strain was performed well and adapted to Finoteselam and similar agroecological zone and resulted in a significant crop yield increment. The grain yield of soybean was increased by the strain alone over the control by 19.3% (347 kg) ha^{-1}.

Therefore, for soybean to nodulate and fix atmospheric nitrogen and produce high grain yield, the seed should be inoculated with the *Bradyrhizobium japonicum* (TAL 379) and facilities to maintain and multiply the strain should be in place to help soybean growing farmers and to initiate other farmer to produce the crop for commercial use with reasonable cost.

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