



Evaluating the Yield Advantage of Using *Rhizobium* Inoculant in Improved Varieties of Soybean and Beans

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ABSTRACT

The on-farm average yield of soybean (*Glycine max*) and beans (*Phaseolus vulgaris*) are 1t/ha and 0.8t/ha respectively. This is nearly a third of the potential yield, which is attributed to a number of factors including poor nodulation with the native rhizobia available in the soil. This study was done to assess the yield advantage for inoculation of selected improved soybean and bean varieties with *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* *bv phaseoli* respectively, on-station in lira and at five on-farm sites for two seasons. The study sites were located in the districts of Agago, Omoro, Kitgum, Amuru and Lamwo. The mother baby trial methodology was used to conduct participatory adaptive research. A randomized complete Block design was used for on-station trials. The trials included evaluating response of three improved inoculated and uninoculated soybean and bean varieties in each commodity against a local check. Results showed significant differences ($p < 0.05$) for both inoculated improved soybean and bean varieties. Inoculation of improved bean varieties significantly ($p < 0.05$) increased nodule numbers per plant, but was not significant for number of pods per plant. Also inoculation of improved soybean varieties significantly increased nodule numbers per plant, but was insignificant for number of pods per plant. Grain yield was also significantly ($p < 0.05$) higher in inoculated plots as compared to un-inoculated plots. Inoculation significantly increased yield in selected improved varieties in soybean and beans on average by 22.3% and 15%, respectively. In soybean MAKSOY 1N, MAKSOY 5N, MAKSOY 3N and LOCAL varieties were respectively improved by 14.13%, 23.33%, 64.40% and 42.8% compared to the non-inoculated controls. In beans NARO BEAN1, NARO BEAN2, NABE4 and LOCAL were respectively improved by 19.71%, 14.50%, 7.84% and 16.27% compared to the non-inoculated controls. It can be concluded that MAK-BIO-FIXER inoculum containing *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* *bv phaseoli* as an efficient approach for improving the nodulation process and increasing grain legume yield for selected improved varieties in soybean and beans respectively. Therefore, the use of these inoculum to enhance yield in these varieties can be recommended.

Keywords: *Bradyrhizobium japonicum*, *Glycine max*, *Phaseolus vulgaris*, *Rhizobium leguminosarum* *bv phaseoli*, inoculation, nodulation.

1. Introduction

Grain legumes are a very important source of dietary proteins and oil for humans (Joint, 1998). Agriculture today faces challenges such as soil fertility loss, climate change and increased attacks from pathogens and pests. Grain legume yield among farmers in Northern agro-ecology zone was less than a third of potential yield (PRELNOR, 2017). This is similar to findings by (Tittonell and Giller, 2013).

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There's need to increase grain legume yield to encourage its production by farmers as this will support sustainable agricultural production. Sustainable agricultural production is based on environmentally friendly approaches such as the use of biofertilizers, biopesticides and the return of crop residues (Mabrouk *et al.*, 2018).

About 78% of the atmospheric air is N, this gaseous substance cannot be used in this form by most living organisms until it has been fixed, that is, reduced (combined with hydrogen) to ammonia. Biological nitrogen fixation (BNF) is one of the most 'environmentally friendly' approach for obtaining nitrogen in agro-ecosystems since BNF uses energy derived from photosynthesis and does not accumulate excess nitrogen to cause pollution (Jensen and Hauggaard-Nielsen, 2003). BNF offers substantial source of N in cropping system since atmospheric N₂ is a renewable resource (Hungria and Bohrer, 2000) and accounts for 60% of nitrogen used in agriculture, lightening 10% and 25% from chemical fertilizers (Zahran, 1999). Grain legumes in addition to BNF contribute a significant input of N if vegetative legume residues are returned to the soil after grain harvest, thus offering potential benefit of subsequent cereal and other non-leguminous crops. Soybean has been reported to biologically fix nitrogen in the range of 85 - 154 kg N ha⁻¹ (Giller *et al.*, 2011). These grain legume attributes are important for cropping-system sustainability and climate change resilience. However, grain legumes face challenges in southern African countries such as low seed viability, poor nodulation with native rhizobia available in the soil, low availability and awareness of inoculants, lack of some essential nutrients in the soil and lack of market information (Vidigal *et al.*, 2019).

Rhizobium-legume symbiosis system is considered the most efficient and important process in crop production. It plays a role in sustaining long-term soil fertility in different agricultural systems (Amarger, 2001). Leguminous plants in partnership with *Rhizobium* have the ability to convert the atmospheric nitrogen into usable forms. The use of *Rhizobium* inoculation meets 50-70% of the crop nitrogen requirement and increases legumes productivity (Ndakidemi *et al.*, 2006).

In Uganda farmers still find fertilizer costly, hence low usage at only 1.9kg/ha against a world average of 141kg/ha (FAO, 2016). The requirements for Fertilizer-N are predicted to increase in the future, however the use of high doses of fertilizers has a negative and an unpredictable impact on the environment and contaminates the soil, water and other natural resources (Otieno *et al.*, 2009 and Kanianska, 2016). It's well noted that yield limitation is a function of many factors, however, most farmers in Uganda usually grow soybean without applying inoculants. In Malawi grain yield in soybean inoculated with *Bradyrhizobium japonicum* was significantly higher in inoculated plots as compared to un-inoculated plots with ranges of 524 to 1868 kg ha⁻¹ for inoculated soybean and 246 to 905 kg ha⁻¹ for un-inoculated soybean (Njira *et al.*, 2013). Currently, new varieties are being released for soybean and beans, but there's very little information available on their response to MAK-BIO-FIXER.

2. Materials and Methods

2.1. Site and Research design / Methodology

The mother baby trial methodology was used to conduct participatory adaptive research. The Mother-Baby methodology analyzes the average treatment effects of technologies when there is self-selection among host farmers and plots and substantial heterogeneity (Snapp *et al.*, 2002). This methodology is essential in order to develop, asses and test various agronomic practices in a manner that incorporates diverse farmer priorities and can evaluate performance across a range of management practices and edaphic conditions in a quantifiable and repeatable manner.

The central mother trial was replicated within the site at Ngetta Zonal Agricultural Research and Development Institute (NgeZARDI) in season 2019A and 2019B. NgeZARDI is located in northern Uganda at 02°.29573'N; 032°.92092'E; 1, and at 101 meters above sea level (masl). Average daily temperature is 25 °C; while maximum temperature is 29 °C. The climate is moist, sub-humid, with a mean annual rainfall of 1,639.1 mm that is bi-modally distributed from March- June and August-December (Kumakech *et al.*, 2014). The design used for the on-station trial was a randomized complete Block design in split plot arrangement with two main plot treatments (inoculated and uninoculated), each with four (4) sub plot treatments replicated three times i.e. NARO BEAN 1, 2, NABE 4 & local check for beans and MAKSOY 1N, 3N, 5N& Local check for Soy bean. This therefore presented two different studies for beans and soybean genotypes respectively. The baby trials are replicates of the mother trial. Five on farm trials were established in 2019A and 2019B each farmer's field representing

a replicate. The replicates contained the different varieties for each of soybean and beans as subplot treatments, inoculated and uninoculated as the main plot treatments.

Soybean trials were established in the districts of Agago, Omoro, Kitgum and Lamwo and beans in the districts of Lamwo, Amuru and Omoro. Table 1 shows sites soils characteristics. The on station main plots located in Lira district measured 19m x 31m and subplots 5mx5m with interplot spacing of 1.5m and interblock spacing of 2m. The beans were spaced at 50cm x 15cm and soybean at 60cmx 10cm. On-farm plots measured 5x5m with similar crop spacing, inter plot and inter block spacing as on-station. Land preparation was done using hand hoe, weeding was done twice at 3weeks and 7weeks respectively. Harvesting was done at crop physiological maturity.

Table 1: Characteristic of soils

District	pH	OM %	P -----ppm-----	Ca	Mg	K	Sand -----%-----	Clay	Silt	Textural Class
Omoro	6.0	4.9	28.6	1722.6	568.2	103.9	55	22	23	Sandy clay loam
Lamwo	6.5	3.6	29.3	1758.2	721.2	369.1	53	23	24	Sandy clay loam
Amuru	5.8	2.7	24.9	939.8	640.4	313.5	53	22	25	Sandy clay loam
Lira	5.8	2.9	12.6	828.8	551.8	300.8	65	17	18	Sandy loam
Agago	6.3	2.3	0.2	1951.2	217.4	335.2	59	20	21	Sandy clay loam
Kitgum	6.5	3.6	29.3	1758.2	721.2	369.1	53	23	24	Sandy clay loam

2.2. Mineral fertilization

All plots received triple superphosphate (46% P₂O₅) during soil preparation at the rate of 100 kg ha⁻¹, while muriate of potash (60% K₂O) was applied at the rate of 40 kg ha⁻¹. All treatments received ammonium nitrate (34% N) at a rate of 50 kg N ha⁻¹ at planting time as an activator dose.

2.3. Inoculation method

The MAK-BIO-N-FIXER inoculant containing *Bradyrhizobium japonicum* bacteria (10⁶ viable cell/ g inoculum) for soybean and *Rhizobium leguminosarum bv phaseoli* (10⁶ viable cell/ g inoculum) for beans were used as a biological nitrogen fixer. The source of inoculant is Makerere University, Kampala. Inoculation process in both cases involved mixing 1 spoonful of sugar in 300mls of clean water (1 soda bottle) to form a 5% sugar solution inoculum sticker. 15kg of Soybean / bean seeds was weighed and poured in a clean basin and then the inoculum sticker added to it. The inoculum sticker (slurry) of 1:1 ratio in 5% sugar solution was mixed thoroughly with 15kg seeds of soybean/beans until each seed was evenly coated. The coated seed was covered with paper/cloth and left to dry in the shed for 30 minutes and planting done within 24 hours of inoculation.

2.4. Plant sampling and analysis

Plant sampling was done using systematic random sampling selecting after every 2 plants from within the net plot of eight ridges. 10 soybean/bean plants per subplot were sampled at podding stage, after data was collected from each of these plants on the following three parameters; number of pods, total number of nodules. Grain yield (kg/ha) was collected at physiological maturity. Total number of nodules per plant was collected through destructive sampling for nodule counts and nodule effectiveness at flowering according to standard procedures. 10 plants, two from each row was dug up, and uprooted from a net plot of 5m x 5m.

2.5. Data Analysis

Data collected were subjected to two-way Analysis of Variance (ANOVA) according to the procedures outlined by Snedecor and Cochran (1980) using Xlstat edition 2020. Separation of treatment means was done using least significant differences (LSD) at 0.05 level of probability.

3. Results

3.1. Effect of Bradyrhizobia inoculum on the performance of soybean

Data presented in (Tables 2 and 3) (Fig.1, 2 and 3) show the response of number of pods per plant, total number of nodules per plant and grain yield (kg/ha) of soybean to *Bradyrhizobium* inoculation at harvest.

The number of pods is significantly higher in uninoculated soybean plants than in inoculated. There is no significant difference in the total number of nodules in inoculated soybean plants compared to uninoculated plants. On the other hand, the grain yield of the inoculated plants is significantly higher than those of the un-inoculated plants ($p = 0.0001 < 0.05$) (Table 2). Rhizobial inoculation has a significant influence on grain yield. In non-inoculated plants the yield is $1360.00 \pm 30.70 \text{ kg ha}^{-1}$. It is $1663.33 \pm 32.90 \text{ kg ha}^{-1}$ in inoculated plants, an increase of 22.30% compared to uninoculated plants.

Furthermore, inoculation has no influence on the number of pods and the total number of nodules in the different varieties of soybean. However, the grain yield of the different varieties was significantly improved by inoculation ($p = 0.0001 < 0.05$) (Table 3). The MAKSOY 1N, MAKSOY 5N, MAKSOY 3N and LOCAL varieties were respectively improved by 14.13%, 23.33%, 64.40% and 42.8% compared to the non-inoculated controls.

Table 2: Number of pods per plant, total number of nodules per plant and grain yield (kg/ha) as influenced by *Bradyrhizobium* inoculation

Treatments	Number of pods (per plant)	Total number of nodules (per plant)	Grain yield (kg/ha)
Uninoculated	$63.54 \pm 0.28 \text{ a}$	$55.8 \pm 0.39 \text{ a}$	$1360.00 \pm 30.70 \text{ b}$
Inoculated	$43.83 \pm 0.22 \text{ b}$	$65.04 \pm 0.48 \text{ a}$	$1663.33 \pm 32.90 \text{ a}$

In each column, values are expressed as mean \pm SE. Different letters within column indicate significant difference between treatments according to the Turkey test at $P < 0.05$

Table 3: Effects of *Bradyrhizobium* inoculation on number of pods per plant, total number of nodules per plant and grain yield (kg/ha) of four varieties of soybean.

Varieties	Number of pods (per plant)		Total number of nodules (per plant)		Grain yield (kg/ha)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
MAKSOY 1N	$58.53 \pm 0.91 \text{ a}$	$34.43 \pm 0.65 \text{ b}$	$31.70 \pm 0.75 \text{ a}$	$62.10 \pm 1.79 \text{ a}$	$1226.67 \pm 12.57 \text{ b}$	$1400.00 \pm 21.77 \text{ a}$
MAKSOY 5N	$75.70 \pm 0.96 \text{ a}$	$32.37 \pm 0.58 \text{ b}$	$95.57 \pm 2.05 \text{ a}$	$79.6 \pm 2.51 \text{ a}$	$1600.00 \pm 32.66 \text{ b}$	$1973.33 \pm 35.00 \text{ a}$
MAKSOY 3N	$65.90 \pm 1.49 \text{ a}$	$65.40 \pm 1.16 \text{ b}$	$49.63 \pm 1.21 \text{ a}$	$71.63 \pm 1.61 \text{ a}$	$1773.33 \pm 12.57 \text{ b}$	$2880.00 \pm 37.71 \text{ a}$
LOCAL	$55.03 \pm 0.85 \text{ a}$	$43.13 \pm 0.57 \text{ b}$	$46.60 \pm 1.07 \text{ a}$	$46.83 \pm 1.5 \text{ a}$	$840.00 \pm 39.25 \text{ b}$	$1200.00 \pm 67.99 \text{ a}$

In each row, values are expressed as mean \pm SE. Different letters within row indicate significant difference between treatments according to the Turkey test at $P < 0.05$

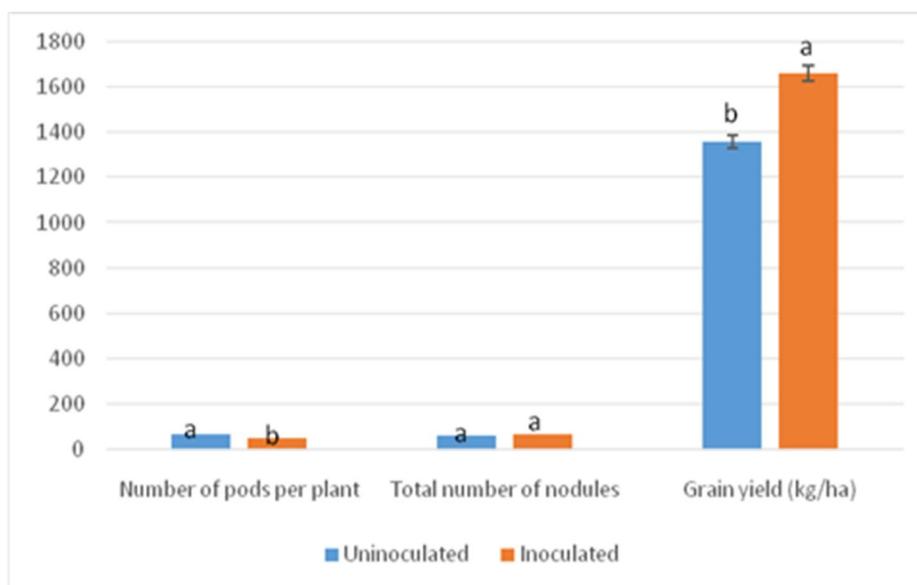


Fig. 1: Number of pods per plant, total number of nodules per plant and grain yield (kg/ha) as influenced by *Bradyrhizobium* inoculation

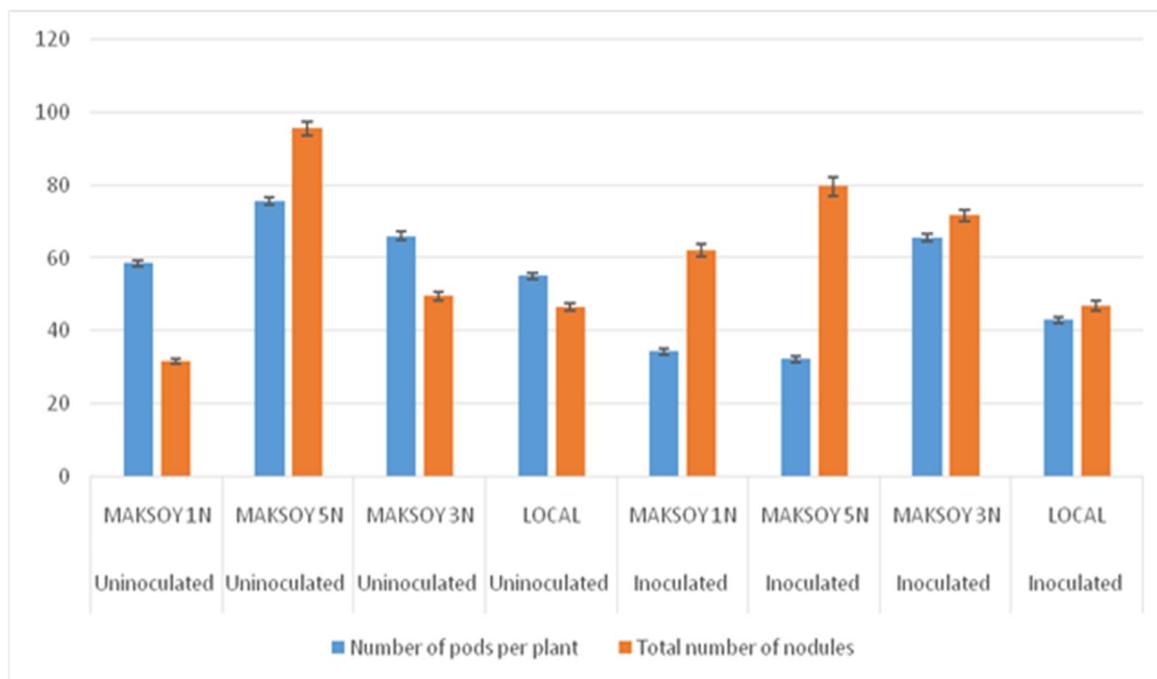


Fig. 2: Number of pods per plant and total number of nodules per plant as influenced by *Bradyrhizobium* inoculation

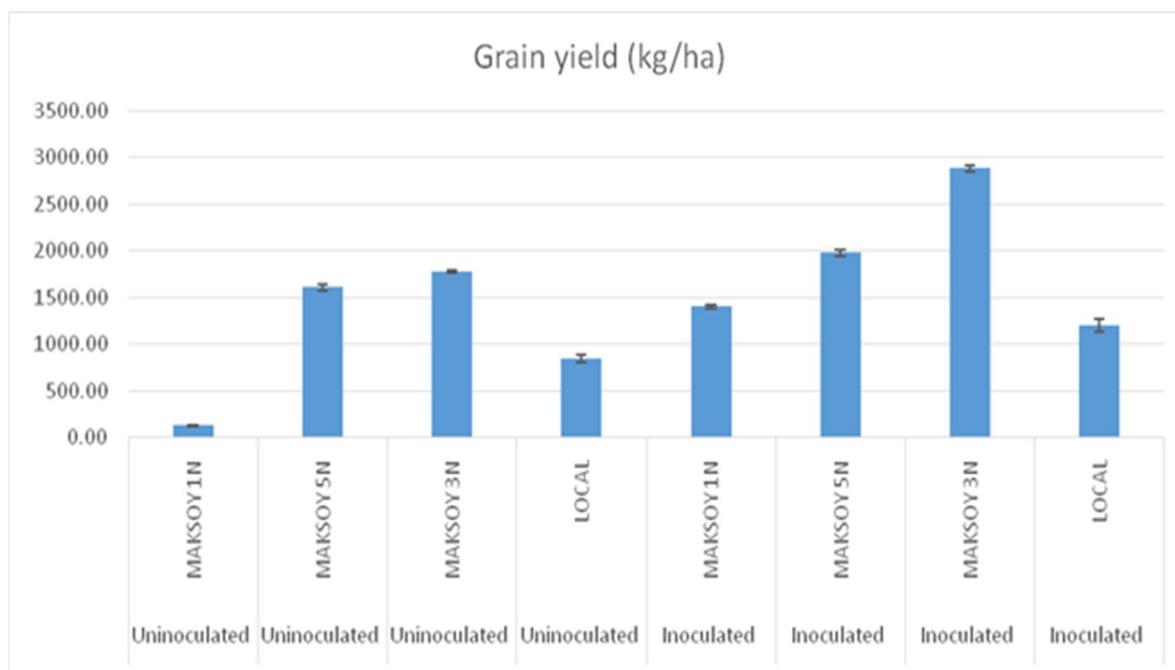


Fig. 3: Grain yield (kg/ha) as influenced by *Bradyrhizobium* inoculation

Effect of *Rhizobium leguminosarum bv phaseoli* inoculum on the performance of beans

Data presented in (Tables 4 and 5) (Fig. 4, 5 and 6) show the response of number of pods per plant, total number of nodules per plant and grain yield (kg/ha) of bean to *Rhizobium leguminosarum bv phaseoli* inoculation at harvest. The number of pods is significantly higher in uninoculated bean plants than in inoculated ones. There was also significant difference in the total number of nodules in inoculated bean plants compared to uninoculated ones. In addition, the grain yield of inoculated plants was significantly higher than those of uninoculated plants ($p = 0.0001 < 0.05$) (Table 4). Rhizobial inoculation has a significant influence on grain yield. In the uninoculated plants the yield was $1536.67 \pm 28.23 \text{ kg ha}^{-1}$ while it was $1766.67 \pm 35.25 \text{ kg ha}^{-1}$ in the inoculated plants, an increase of 14.96% compared to the uninoculated plants. Inoculation has no influence on the number of pods. However,

inoculation had a significant influence on the number of nodules also grain yield of the different varieties was significantly improved by inoculation ($p = 0.0001 < 0.05$) (Table 5). The beans varieties NARO BEAN1, NARO BEAN2, NABE4 and LOCAL were respectively improved by 19.71%, 14.50%, 7.84% and 16.27% compared to the non-inoculated controls.

Table 4: Number of pods per plant, total number of nodules per plant and grain yield (kg/ha) as influenced by *Rhizobium leguminosarum bv phaseoli* inoculation

Treatments	Number of pods (per plant)	Total number of nodules (per plant)	Grain yield (kg/ha)
Uninoculated	27.48 ± 0.14 a	55.47 ± 0.53 b	1536.67 ± 28.23b
Inoculated	17.20 ± 0.06 b	106.16 ± 0.44 a	1766 ± 35.25a

In each column, values are expressed as mean ± SE. Different letters within column indicate significant difference between treatments according to the Turkey test at $P < 0.05$

Table 5: Effects of *Rhizobium leguminosarum bv phaseoli* inoculation on number of pods per plant, total number of nodules per plant and grain yield (kg/ha) of four varieties of bean

Variety	Number of pods (per plant)		Total number of nodules (per plant)		Grain yield (kg/ha)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
NAROBAN1	20.37 ± 0.32 a	19.20 ± 0.19 b	50.33 ± 2.30 b	116.33 ± 1.65 a	1893.33 ± 90.65 b	2266.67 ± 83.15 a
NAROBAN2	23.30 ± 0.40 a	16.43 ± 1.70 b	30.03 ± 1.43 b	111.73 ± 1.70 a	1746.67 ± 35.00 b	2000 ± 54.43 a
NABE4	22.83 ± 0.37 a	15.53 ± 1.52 b	78.73 ± 2.38 b	91.90 ± 1.52 a	1360.00 ± 39.25 b	1466.67 ± 62.85 a
LOCAL	43.40 ± 0.69 a	17.66 ± 2.00 b	62.77 ± 1.90 b	104.62 ± 2.00 a	1146.67 ± 25.14 b	1333.33 ± 31.43 a

In each row, values are expressed as mean ± SE. Different letters within row indicate significant difference between treatments according to the Turkey test at $P < 0.05$

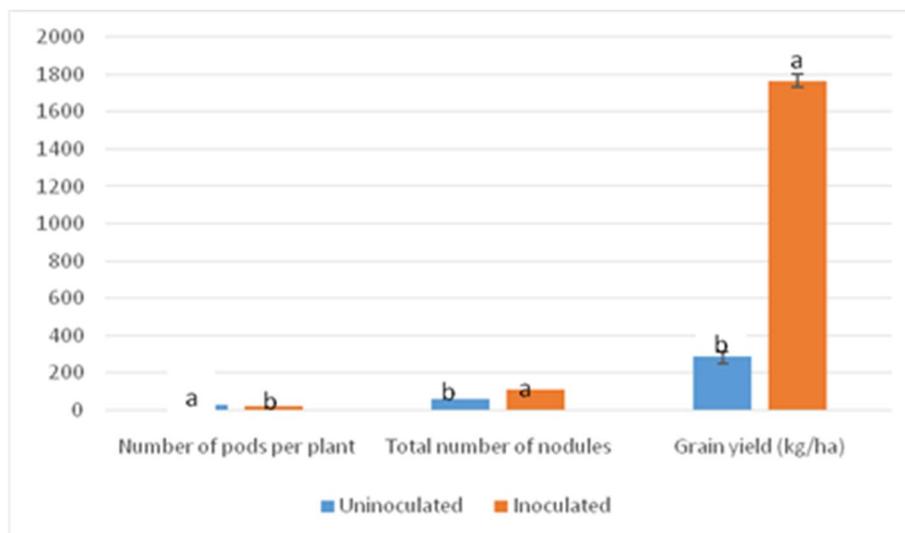


Fig. 4: Number of pods per plant, total number of nodules per plant and grain yield (kg/ha) as influenced by *Rhizobium leguminosarum bv phaseoli* inoculation

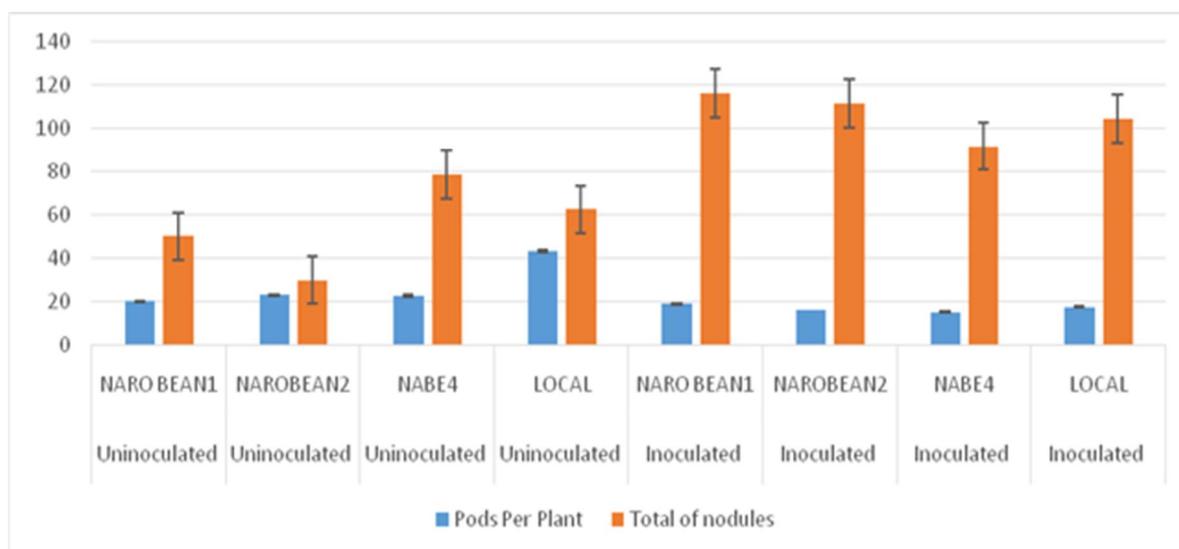


Fig. 5: Number of pods per plant and total number of nodules per plant as influenced by *Rhizobium leguminosarum bv phaseoli* inoculation

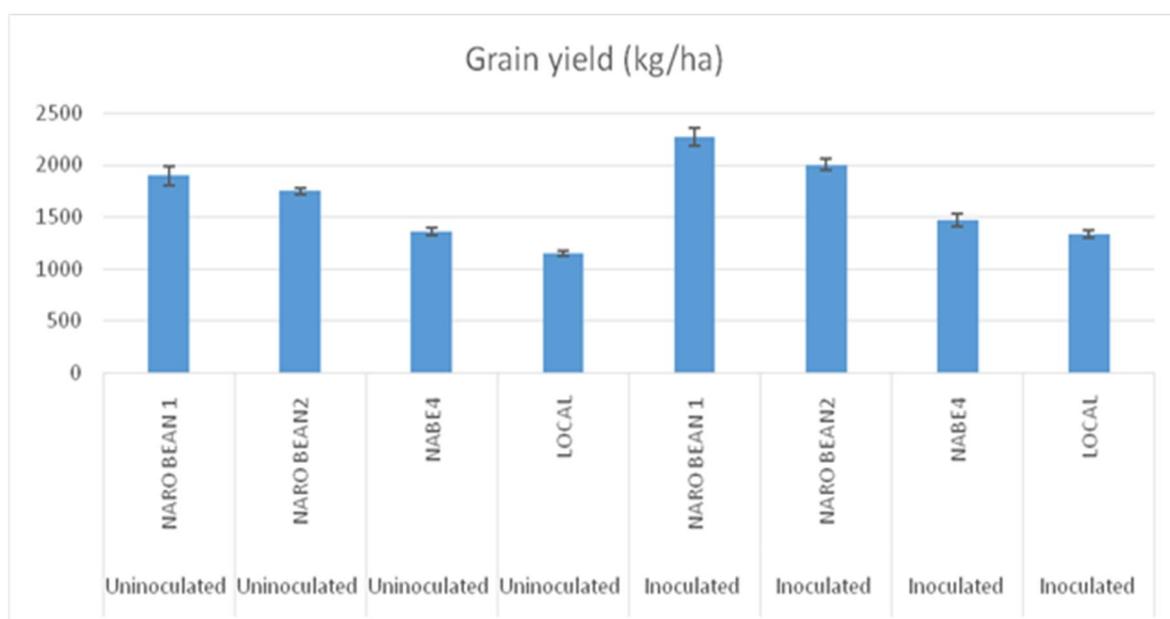


Fig. 6: Grain yield (kg/ha) as influenced by *Rhizobium leguminosarum bv phaseoli* inoculation

4. Discussion

The major constraint limiting crop productivity is low fertility of soil (Diatta *et al.*, 2020). The success of leguminous crops depends on their ability to form efficient Nitrogen-fixing symbiosis with root nodule rhizobia. Rhizobia associated with legumes can be used to improve crop productivity at a very low cost (Zahran, 2009). Inoculation is essential where nitrogen fixing bacteria is low to enhance the effective population of rhizobium in the rhizosphere of the crop (Bhattacharjee *et al.*, 2008). Many workers have indicated the necessity of legume inoculation with effective and efficient rhizobial strains (Mekhemar *et al.*, 2007 Badawi *et al.*, 2011). It is clear that, plants inoculated with *Rhizobiaceae* yield more than uninoculated plants.

Our results show a positive effect of inoculating soybean and common beans with *Bradyrhizobium japonicum* and *Rhizobium leguminosarum bv phaseoli* respectively. The increased soybean and bean yield in all inoculated treatments might be attributed to the promoted vegetative growth owing to the availability of nitrogen via diazotrophy. The substantial increase in both grain

legume yield in response to inoculation compared with the control is in line with previous reports (Otieno *et al.*, 2009; Denton and Peoples 2010; Njira *et al.*, 2013 and Mabrouk *et al.*, 2018).

There was no significant difference in number of nodules following inoculation with *Bradyrhizobium japonicum* compared to the un-inoculated plants. This could be attributed to the presence of a high population of native rhizobia in the soil enabling nodulation in the uninoculated plots. However, significant number of nodules was observed following inoculation with *Rhizobium leguminosarum phaseoli* compared with the un-inoculated plots, implying that there was low population of native *Rhizobium leguminosarum phaseoli* compared to *Bradyrhizobium japonicum* in the soil (Pulver *et al.*, 1982)

There was significant difference observed in number of pods for both soybeans and beans in un-inoculated plots compared to inoculated plots. The grain yield was significantly higher in inoculated plots of both soybeans and beans compared to the un-inoculated plots. This could be attributed to the presence of nodules that were effective at fixing nitrogen. The results also indicate that both the improved soybean and beans genotypes respond differently to inoculation. Inoculation significantly increased yield in selected improved varieties in soybean and beans on average by 22.3% and 15%, respectively. In soybean MAKSOY 1N, MAKSOY 5N, MAKSOY 3N and LOCAL varieties were respectively improved by 14.13%, 23.33%, 64.40% and 42.8% compared to the non-inoculated controls. In beans NARO BEAN1, NARO BEAN2, NABE4 and LOCAL were respectively improved by 19.71%, 14.50%, 7.84% and 16.27% compared to the non-inoculated controls.

5. Conclusion

It can be concluded that exploitation of efficient MAK-BIO-FIXER inoculum containing *Bradyrhizobium japonicum* and *Rhizobium leguminosarum bv phaseoli* improved the nodulation process and grain legume yield for selected improved genotypes in soybean and beans by an average of 22% and 15%, respectively.

6. Recommendation

Among the soybean varieties maksoy 3N had the best response in terms of yield increase due to *Bradyrhizobium japonicum* inoculation followed by the local check and in beans NARO BEAN 1, had the best response followed by the local check. Hence, *Bradyrhizobium japonicum* and *Rhizobium leguminosarum bv phaseoli* inoculum are efficient for improving yields among improved varieties for soybean and beans respectively. The farmers saved seeds (local check) also responded positively to use of inoculant. The low cost of this inoculant makes it affordable to the local farmers, this can be a great opportunity to improve on farm yield.

Limitations

In this study the amount of nitrogen fixed by MAK-BIO-FIXER inoculum was not assessed. The estimation of nitrogenase activity, as well as the dry weights of the root nodules and the nitrogen content of the plant were not evaluated to test the efficiency of the tested bacterial inocula.

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