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Molybdenum-Phosphorus Nutrition for Amelioration of Biological Nitrogen Fixation for Common Bean on a Ferralsol

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Abstract:
Common bean (Phaseolus vulgaris L.) is a principal food security crop in sub-Saharan Africa (SSA); a crop whose production is almost exclusively dependent on natural biological nitrogen fixation (BNF) as the source of nitrogen. The objective of this study was to determine the response of BNF in common beans, to different regimes of Mo-P application and rhizobia inoculants regimes in a Ferralsol. A screen house study was conducted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), in Uganda. A Ferralsol obtained from previously cropped fields with different types of legumes and cereals, and low in plant available P and Mo was used. Treatments included Mo applied at rates of 0, 0.6, 1.3 and 2.5 mg kg-1 of soil, equivalent to 0, 0.5, 1 and 2 kg Mo ha-1; P was applied at rates of 0, 38 and 76 mg kg-1 of soil, equivalent to 0, 15 and 30 kg P ha-1. Rhizobia inoculants, namely BioStacked (characterized as “stress tolerant inoculant” by the manufacturer, Becker Underwood in USA); and Mak Bio Fixer (strain CIAT 899). The study was laid out in a completely randomized design (CRD), in a split block arrangement, with 3 replicates and repeated three times. Common bean, variety NABE 4, a low land, fast cooking and tasty variety, commonly grown in Uganda, was the experimental crop. Results revealed that co-application of Mo and either of the two types of rhizobia inoculants had a significant ($P<0.005$) effect on the number of effective nodules and their dry weights; total shoot N and shoot dry weight. Pots treated with Mo at the rates of 0.5 to 1 kg ha-1, yielded the best overall. However, estimated values of biologically fixed nitrogen (total shoot N from rhizobia inoculated plots discounted for rhizobia un-inoculated plots), were not significantly ($P>0.005$) influenced by Mo application, rhizobia inoculation and/or both treatments. Additionally, intervening with P application in the Mo-rhizobia inoculant setup had no significant ($P>0.005$) effect on the status of estimated BNF generated by the common bean; implying that energy requirement for BNF was not a key limiting factor for the process. Overall, Biostacked rhizobia inoculant (exotic) tended to perform better at lower rates of Mo and P in terms of effective nodules and their dry weights, compared to Mak Bio Fixer (indigenous) that performed better at higher rates of Mo and P.

Keywords: BioStacked, Mak-Bio Fixer, Phaseolus vulgaris.

INTRODUCTION

Soil fertility decline constitutes a major challenge to the productivity of common bean (*Phaseolus vulgaris* L.) in sub-Saharan Africa (SSA), where beans are otherwise pivotal food crops; second after maize (*Zea mays* L.) (Snapp *et al.*, 2018). Most especially, soil nitrogen and phosphorus are heavily depleted, yet they play crucial roles in the metabolism of the crop, particularly in biological N fixation (BNF), which is the primary source of N in SSA's agricultural production systems (Giller, 2001; Dogbe *et al.*, 2002). Therefore, Rhizobia inoculation, involving the augmentation of Rhizobia bacterial cells, plays a key role in achieving robust nodulation, N fixation, plant growth and ultimately high grain yields in legumes, including common bean (Somasegaran & Hoben, 1994; Kandil & Ünlü, 2023). In the SSA region, where a substantial portion of arable soils is highly degraded (Snapp *et al.*, 2018), the efficacy of BNF in beans needs to be maximized. By and large, however, the initial micro-symbiont cell populations (titer) in the soil are often below the threshold (Singleton & Tavares, 1986; Thies *et al.*, 1991), thus hindering the rigorous take off of the BNF process in some legumes, including common beans.

More recently, however, significant progress has been made in the development of highly effective bean inoculants on a global scale (CIAT, 2008; Fahde *et al.*, 2023). Among those innovations is the "versatile" BioStacked also codenamed RHIZO-STICK® inoculant, crafted by the Becker Underwood Company in the USA (Westgate, 2011). This BioStacked inoculant reportedly contains viable cells of *Rhizobium leguminosarum bv. phaseoli*, which are resilience even in challenging and stressful environmental conditions (Westgate, 2011). It would be interesting to evaluate this material under SSA conditions, where different bio- and abio-stresses hinder the performance of the local inoculants of common bean (Westgate, 2011). The other factors speculated to partly explain the erratic responses of beans to Rhizobia inoculants in SSA, include inadequate soil molybdenum (Mo) (Kihara *et al.*, 2020) and/or available phosphorus (P). Molybdenum is an integral component of the nitrogenase enzyme, crucial for symbiotic nitrogen fixation (Hoffman *et al.*, 2014). While efforts have been made to understand the biochemistry of BNF, there is a gap in monitoring the sufficiency and functional environment of Mo for BNF in SSA soils, especially Ferralsols predominant in the SSA region.

The availability of soil phosphorus (P) is another key element influencing the functioning of the nitrogenase enzyme, providing the necessary energy for N fixation. Effective N fixation requires sufficient plant-available Mo and P in the soil (Hoffman *et al.,* 2014; Hubbell & Kidder, 2009). Unfortunately, over-weathered soils in SSA are characterized by low mineral reserves of Mo and P, with sesquioxides chemistry that is high in P fixation (Nziguheba et al., 2016; Peng et al., 2015). The deficiency, coupled with P fixation, could directly impact BNF, even when effective inoculants are administered. The objective of this study was to determine the response of BNF in common bean to different Mo-P levels under regimes of Rhizobia inoculants in a Ferralsol.

MATERIALS AND METHODS

A screen house experiment was conducted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) in Uganda, over two consecutive rounds (December 2015 to April 2016) using field soil obtained from farm fields.

Treatments and Design

Treatments included varying rates of molybdenum, P and types of rhizobia inoculants (local-MakBio, and exotic- Biostacked). Molybdenum, in the form of sodium molybdate (analytical grade) was applied at 0, 0.6, 1.3 and 2.5 mg kg^{-1} soil (equivalent to 0, 0.5, 1.0 and 2 kg ha^{-1}).

Phosphorus was applied at rates of 0, 38 and 76 mg P kg^{-1} soil, equivalent to 0, 15 and 30 kg P ha^{-1}. Two rhizobia inoculants were used, namely the local Mak Bio Fixer, obtained from the Department of Agricultural Production at Makerere University; containing a Rhizobium strain CIAT 899, packaged in unsterile peat with estimated cells per gramme (Table 2); and the exotic BioStacked produced by Becker Underwood company in the USA (Westgate, 2011)., imported and stored under refrigeration. BioStacked was rated by the manufacturer as "containing a high population of rhizobia cells per gramme" (Table 1), in sterilized peat with inbuilt sticker and antifungal agents (Westgate, 2011). The main plots consisted of the two inoculants (Mak Bio Fixer and Biostacked), while the sub-plots were the Mo and P application rates, as described below. The experiment was laid out in a completely randomized design in a split block, with three replicates and three repeats.

Administration of Rhizobia inoculants

Rhizobia inoculants were administered on the seeds carefully, to avoid Rhizobia cross contamination. This was done in sterilized plastic basins and using quantities prescribed by [4]. For this purpose, the inoculation process started with control pots, where no rhizobia inoculation was applied. This was followed by administration of respective pots with Mak Bio Fixer. The strain used in this inoculant is technically known as CIAT 899, was originally characterized by the Center for International Tropical Agriculture (CIAT) in the early 1990s (Ormeno-Orrillo *et al.*, 2012)). The strain is known to be stress tolerant, especially in terms of soil acidity and high temperatures (Ormeno-Orrillo *et al.*, 2012; Martinez-Romero *et al.*, 1991).]. However, its performance in response to levels of Mo and available P in a Ferralsol, is yet to be established. A day prior to planting of common bean, variety NABE 4, all bean seeds were soaked in distilled water for seeds to imbibe uniform moisture prior to planting. On the planting day, in order to dress the seeds with inoculants, a sticker material comprising of cane sugar (tea spoonful), dissolved in distilled water, was prepared. Specifically, one tea spoonful (approximately 20 g) was dissolved in 300 ml of distilled water using a coca cola soda bottle. Then, 1 kg of common bean seeds (NABE 4 varieties) was put in a clean plastic basin and fully dressed with the sticker, by manually sprinkling the bottle contents while hand-mixing the beans. The powdered inoculant was sprinkled on the sticky seeds, and gently hand mixed to uniformly dress rhizobia inoculant around the seed. The dressed bean seeds were planted at the uniform rate of 3 seeds per pot.

The entire process of inoculation followed with Mak Bio Fixer [4], was repeated in the case of BioStacked inoculant. The dressing in this case was also done prior to planting to enhance the bacteria-seed adherence and also increase survival of the bacterial cells [2]. However, because BioStacked inoculant already contained inbuilt/engrained sticker material, the process of sticker administration was omitted in this case. The administration of this inoculant involved sprinkling of distilled water, just sufficient to wet the seeds. Then the inoculant was sprinkled on the wet seeds and gently hand mixed to ensure uniform distribution of the material around the seed.

Pre-experimental Inoculum Cell Density (Titre)

Prior to administration of both inoculants, a test of cell counts on the rhizobia inoculants was initially performed to ensure an informed starting point of rhizobia cell density. The method used was the most probable number (MPN) described by [4]. The results of the cell count assessment are presented in the Table 1.

Table 1: Inoculant population estimated using Most Probable Number (MPN) prior to inoculation

Rhizobia inoculant	Population counts (Cells g^{-1})
Mak Bio fixer	6.7×10^6
BioStacked	4.6×10^7

The experiment was setup on benches in the screen house and watered with distilled water at 2–3-day intervals. One week after germination, the seedlings were thinned to one plant per pot. Weeding was done manually whenever weed plants appeared in the pots. The bean plants were routinely inspected for pests and diseases symptoms.

Bean Variety Used

The bean genotype used was NABE 4 variety, which is popularly grown in the country at altitudes 1000-1800 meters above sea level. It is reportedly tasty, swells on cooking and cooks fairly fast. It is also estimated to yield 1.5- 2 metric tons per hectare, and matures in 80-85 days after sowing (Sebuwufu *et al*. (2015).

Bulk Soil Collection and Routine Analysis

The bulk soil sample used for this study was collected from the Makerere University Agricultural Research Institute Kabanyolo (MUARIK) in Uganda. The source fields had undergone continuous cropping of various crops, including of cereals (maize) and legumes (beans and soybean), the latter occasionally applied with inoculants enhancers of BNF. The bulk soil samples (0 – 20 cm soil depth) were saved in gunny bags lined with polythene, in quantities of 50 kg and totaling to 500 kg. The bags were emptied on clean polythene sheets in shades, and the soil dried for seven days prior to further sub-sampling for further routine laboratory analysis. A composite soil sample obtained from the bulk sample was used to provide baseline soil properties prior to the experiment. The sample was pulverized using a porcelain mortar and pestle, before sieving using a 2-mm wire mesh. It was subjected to routine analysis for soil pH, available P, Kjeldahl N and exchangeable base (Page *et al.*, 1982). Plant available molybdenum content was analysed using the calorimetric thiocyanate method (Stewart *et al.*, 1974). Soil analytical results before the experiment are presented in Table 2.

Table 2: Some physico-chemical properties of experimental study soil from Makerere University Agricultural Research Institute, Kabanyolo, Uganda

Soil properties	Test values	Critical values*
Soil pH (1:2.5 H2O solution)	5.65	5.50
Soil Organic Matter (%)	1.06	3. 0
Total N (%)	0.10	0.25
Available Mo (mg kg^{-1})	0.12	0.20
Available P (mg kg^{-1})	3.57	15.00
Potassium (cmol. kg^{-1})	0.26	0.38
Magnesium (cmol. kg^{-1})	1.83	0.20
Calcium (cmol. kg^{-1})	3.60	5.00
Soil textural class	Sandy clay	-

*Critical values (Page *et al.*, 1982)

Bulk Soil Processing

Before establishing the experiment, the remaining bulk soil samples were freed of visible plant materials and stones before it was sterilized in open metallic drums using firewood. The sterilization process was done >100 °C maintained for approximately 8 hours. After cooling for approximately 2 hours, 2 kg of soil were loaded per pot; following a layer of gravel-sand of 3 cm lined up at the bottom of the pot. The pots had been labelled with treatment identification, in readiness for treatment administration. The pots had earlier been perforated at the bottom (4 holes) and on the sides (6 holes) using hot 6-inch nail, to prevent water logging and to allow smooth air circulation in the soil.

Data Collection

Data were collected on number of nodules (effective) and plant growth parameters. Root nodules sample collection and enumeration was by uprooting of three plant replicates at 50% flowering stage. Nodules for each plant were cleaned using distilled water. Then, a surgical blade was used to dissect each nodule cross-sectionally, to assess for the presence of leghemoglobin, which is represented by a pink/reddish colour of the nodule interior [4]. This characteristic is depictive of effective nodules for a normally functioning BNF system.

Each nodule that displayed pink/reddish colour was subsequently oven-dried in aluminum foil, for oven dry weight determination. Drying was done at 60 °C for 48 hours. After cooling in a desiccator for about 12 hours, the materials were weighed using a Denver Instrument Company
The remaining plant parts after harvesting the root nodules, were also separately oven dried at 60 °C for 48 hours; cooled in a desiccator and weighed for shoot dry weight. Then, each plant sample was grounded into fine powder using porcelain mortar and pestle. The powder so produced was used for analysis for total shoot nitrogen content using the Kjeldahl technique (Page *et al*., 1982; Christensen, 1928)

Plant Sample Analysis

Dried plant samples were grounded using a porcelain mortar and pestle into fine powder. About 0.5 g of a plant sample was taken into digestion tube and the digestion mixture added (Christensen, 1928); the solution was digested at 360 °C following Kjeldahl N method described in the laboratory manual by (Page *et al*., 1982; Christensen, 1928).

Data Analysis

All data collected were entered in Excel Microsoft software and then subjected to statistical analysis in a three-way analysis of variance (ANOVA), using GenStat software (Version 13.0, VSN International, 2008). Fisher's Protected Least Significant Difference (LSD) was used to separate treatment means that were found significant at $P \leq 0.05$ level. Correlations were run on data for number of effective nodules and weight of effective nodules, with shoot nitrogen content and shoot dry weight.

RESULTS

Number of Effective Nodules

The three-factor interaction (P, Mo and rhizobia inoculants) was not significant ($P>0.05$) for number of effective nodules obtained; however, application of Mo together with rhizobia inoculants caused a significant effect ($p<0.05$) (Table 2). Generally, co-application of Mo with Mak Bio Fixer consistently steadily increased the number of effective nodules across the application rates. On the other hand, co-application of Mo with BioStacked inoculant generally resulted in

variably effective nodule numbers across the application rates. Besides, the number of effective nodules were generally less responsive to Mo with BioStacked inoculant than with Mak Bio Fixer, to the extent that even without Mo in the control plots, the number of effective nodules in the BioStacked plots increased by more than 16-folds compared to barely 3-folds for its Mak Bio inoculum counterpart.

Table 3: Performance of number of effective nodules in response to Mo application in common bean on a Ferralsol in Uganda

	Mo Applied (kg ha^{-1})			
Rhizobia Inoculants	0	0.5	1.0	2.0
No inoculation	0	0	0	0
Mak Bio Fixer	3	5	30	49
BioStacked	16	23	16	31
$LSD_{0.05}$	18.00			

Application of P together with rhizobia inoculants also had a significant effect ($p<0.05$) on the number of effective nodules (Fig. 1); whereby P application had an overall improvement on nodule numbers. Addition of 15 kg P ha^{-1} raised the number of effective nodules by 27% for both Mak Bio Fixer and BioStacked rhizobia inoculants. However, application of P beyond 15 kg ha^{-1} suppressed the effect of the BioStacked inoculant (54%) to a level displayed by the control, leaving Mak Bio Fixer inoculant insignificantly stable (Fig. 1).

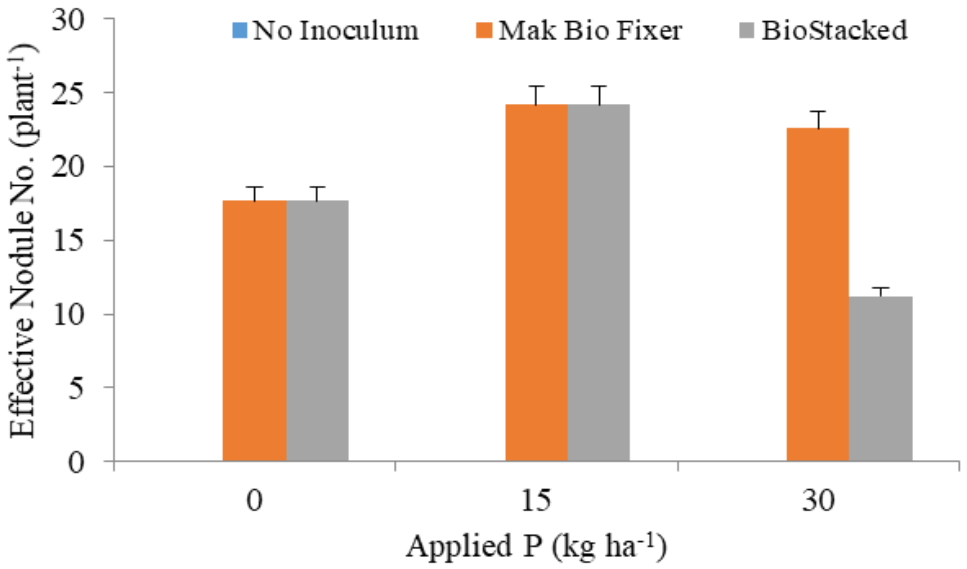

Figure 1: Phosphorus by inoculant type interaction on common bean number of effective nodules per plant.

Effective Nodule Dry Weight

The three-factor interaction (P, Mo and rhizobia inoculants) was again not significant ($P>0.05$) on the effective nodule dry weight. However, application of P with each of the rhizobia inoculants had a significant effect ($p<0.05$) on effective nodule dry weight (Fig. 2); with the nodules under Mak Bio Fixer responding the most. The control plots (without rhizobia application) presented no evidence of effective nodules.

Table 4: Performance of rhizobia inoculants on effective nodule dry weight in common bean on Ferralsol in Uganda

Rhizobia Inoculants	Effective nodule dry weight (g nodules plant^{-1})
No inoculation	0.00
Mak Bio Fixer	0.19
BioStacked	0.17
$LSD_{0.05}$	0.09

Application of P also increased the dry weight of effective nodule with both types of inoculants (Fig. 2); in the order of 65 and 67%, for Mak Bio Fixer and Biostack rhizobia inoculants, respectively. Although the BioStacked inoculant effect was suppressed (45%) to a level below the control that of the Mak Bio Fixer inoculant remained fairly stable (Fig. 2).

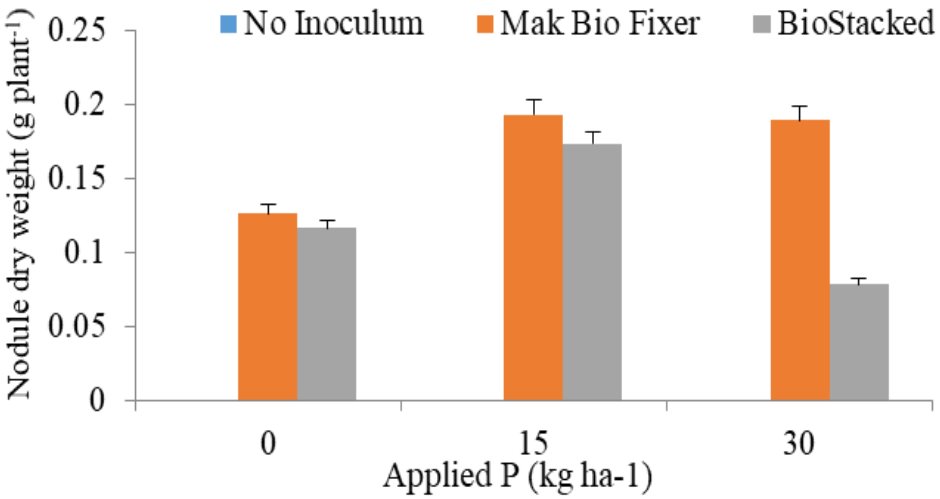

Figure 2: Phosphorus by rhizobia inoculant type interaction on common bean effective nodule dry weight.

Plant Shoot N Content

The three-way factor interaction (P, Mo and rhizobia inoculants) was not significant (P>0.05) on the shoot N content. However, application of co-application of Mo with either of the two rhizobia inoculants significantly increased the content of total N percent contained in the plants (Table 5). It is clear that the slightest presence of Mo at 0.5 kg ha^{-1} caused a spike in the content of total N in the bean plants whose seeds were dressed with Mak Bio Fixer. The level of total plant N was maintained until after the application rate of 1 kg Mo ha^{-1} when the total plant N content dropped sharply to less that unity percent.

Table 5: Molybdenum x Rhizobia inoculants performance on shoot N content in common bean

	Mo applied (kg ha^{-1})			
Rhizobia Inoculants	0	0.5	1.0	2.0
No inoculation	1.15	1.25 (0.10)	5.45 (4.30)	3.98 (2.83)
Mak Bio Fixer	3.02	5.45 (2.43)	5.26 (2.24)	3.46 (0.44)
BioStacked	1.19	0.79 (-0.40)	4.85 (3.66)	4.56 (3.37)
$LSD_{0.05}$	2.17			

*Values in parentheses represent response values after subtracting the control values.

The behavior of total N under the influence of the BioStacked rhizobia inoculant contrasted somewhat with that of the Mak Bio Fixer described above (Table 5). Although application of Mo at the lowest rate (0.5 kg Mo ha^{-1}) tended to suppress the content of total plant N, application of Mo at higher Mo rates contrastingly sharply increased plant N to content nearly twice that of the control (no Mo application). Generally, with the exception of the lowest level of application of Mo, the rest of the rates increased total plant N content by levels higher than those of Mak Bio Fixer.

Application of P together with rhizobia inoculants also had a significant effect on shoot nitrogen content (Fig. 3). Application of 15 kg P ha^{-1}, without rhizobia inoculation, increased shoot N content by 54%. However, with Mak Bio Fixer, application of 15 kg P rate increased shoot N by 29; while with BioStacked inoculant, the increase was 41%. Application of P beyond 15 kg P ha^{-1} significantly suppressed shoot N content (15 to 19%), irrespective of the inoculant type including the control (Fig. 3).

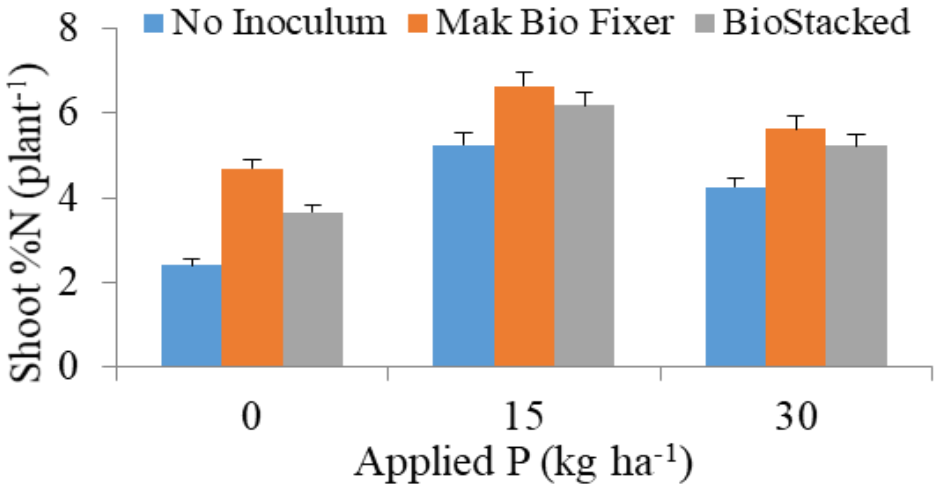

Figure 3: Influence of applied phosphorus and rhizobia inoculants on shoot N for common bean variety NABE 4.

With regard to the estimated BNF related shoot N content (total shoot N from inoculated pots minus total shoot N from uninoculated pots), it is clear that application of P elevated shoot N content slightly for all inoculated pots (Fig. 4). Overall, the BioStacked inoculant was more responsive to P application than its Mak Bio Fixer counterpart. On the other hand, application of P at the level beyond 15 kg ha^{-1}, again tended to suppress the estimated balance of shoot N by greater than two-folds in the case of Mak Bio Fixer; and by approximately 1.5 folds for BioStacked inoculant, and uninoculated control.

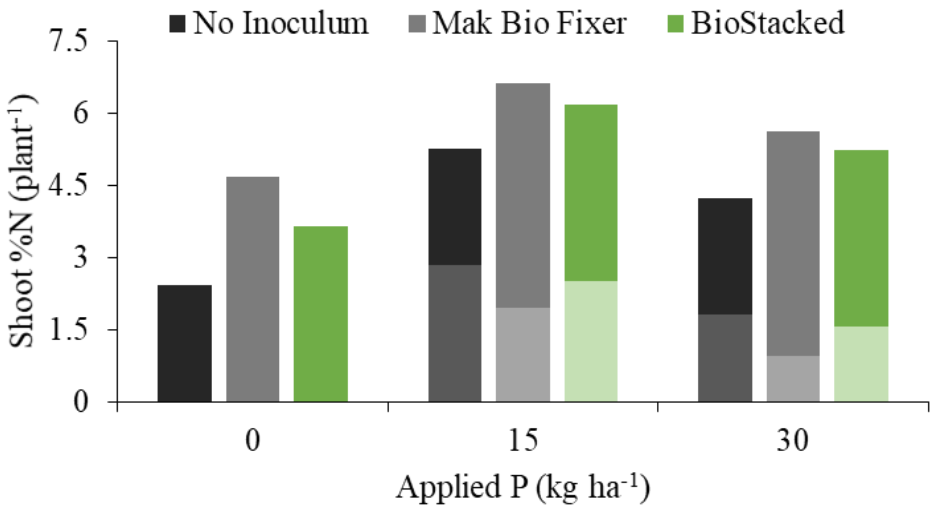

Figure 4: Influence of applied phosphorus and rhizobia inoculants on shoot N for common bean variety NABE 4. Stacked graph represents estimated N due to BNF, obtained by subtracting total shoot N in uninoculated plots from inoculated pots.

Shoot Dry Matter

The three-factor interaction (P, Mo and rhizobia inoculants) was not significant (P>0.05) on the shoot dry matter per plant.

Table 6: Performance of Mo application on shoot dry weight and plant response differentials without control values

Mo rates (kg ha^{-1})	Shoot dry weight (g $plant^{-1}$)
0	3.18
0.5	2.76 (-0.42)
1	5.86 (2.68)
2	4.04 (0.86)
$LSD_{0.05}$	1.84

*Values in parentheses represent response values after subtracting the control values

However, application of P together with rhizobia inoculants had a significant effect on shoot dry matter per plant (Fig. 5). Application of phosphorus at 15 kg P ha^{-1} together with Mak Bio Fixer rhizobia inoculant, increased shoot dry matter by 32%, but not with the BioStacked inoculants. Further increase in P rate suppressed shoot dry matter especially with Mak Bio-Fixer.

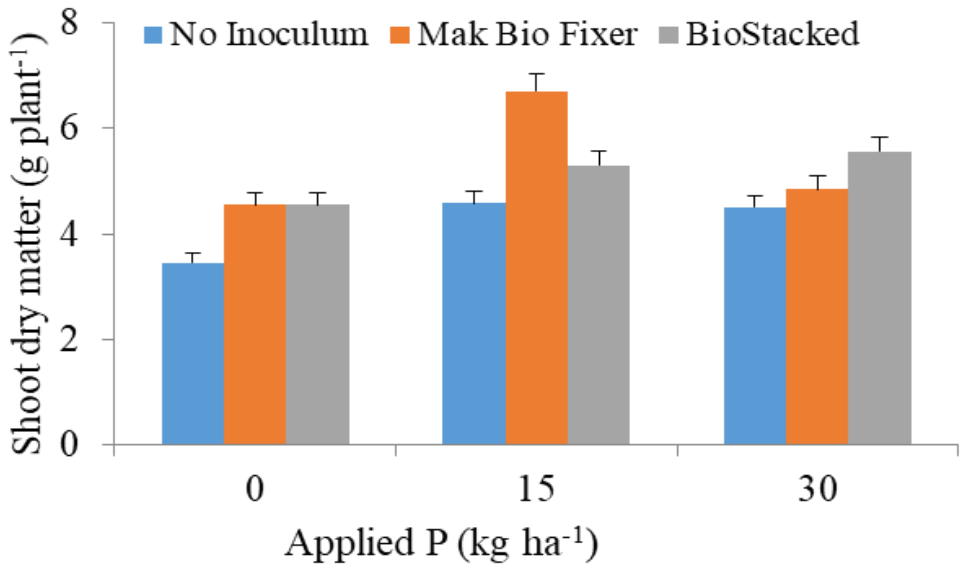

Figure 5: Application of phosphorus and rhizobia inoculants on shoot dry matter weight for commmon bean vairety NABE 4.

With regard to the estimated biologically fixed nitrogen related shoot dry matter (shoot dry matter from inoculated pots minus shoot dry matter from uninoculated pots), it is clear that application of P elevated shoot dry matter slightly for all inoculated pots (Fig. 6). However, Mak Bio Fixer inoculant was more responsive to P application at lower rate than its BioStacked counterpart. Then again, application of P at the level beyond 15 kg ha^{-1}, tended to suppress estimated balance of shoot dry matter by less than 1.8 folds in the case of Mak Bio Fixer; by approximately 0.25 folds for BioStacked inoculant, and by 0.08 folds uninoculated control.

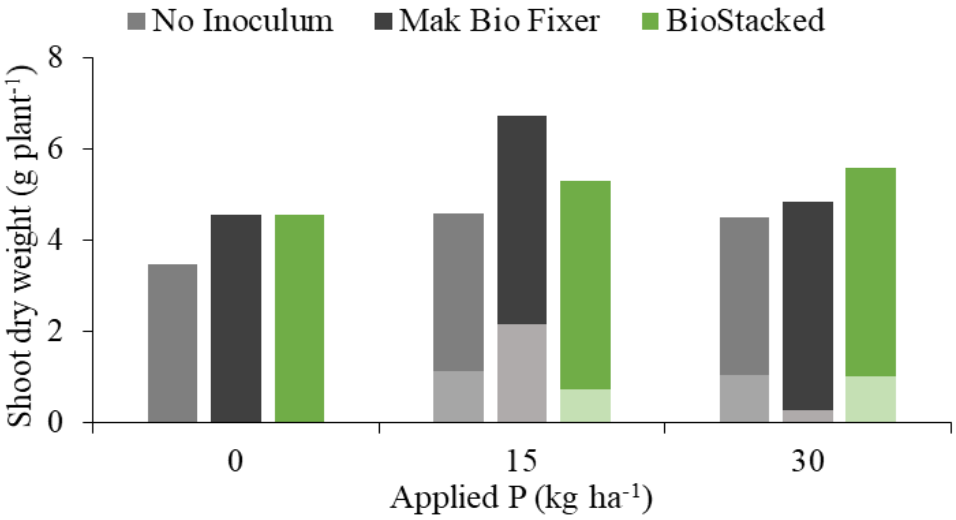

Figure 6: Influence of applied phosphorus and rhizobia inoculants on shoot dry matter for common bean variety NABE 4. Stacked graph represents estimated shoot dry weight due to BNF, obtained by subtracting total shoot dry matter in uninoculated plots from inoculated pots.

DISCUSSION

Number of Effective Nodules

The general increase in number of effective nodules per bean plant due to Mo application, irrespective of the type of rhizobia applied (Table 3), is proxy evidence that this nutrient is insufficient for biological nitrogen fixation in the study Ferralsol. This is the first time that soil Mo assessment has been done in Uganda in relation to BNF, in grain legumes production. This also explains the inconsistent feedback from farmers who use rhizobia inoculants from the BNF Laboratory at Makerere University. Therefore, in order to promote BNF for common bean production at MARIK farms, there is need for inclusion of micro-quantities of Mo in the inoculant packages supplied to end-users.

The superiority of number of effective nodules with BioStacked inoculant at the lower rates of Mo application (≤0.5 kg Mo ha^{-1}), to Mak Bio Fixer inoculant, is difficult to explain from these study results. Unfortunately, the exact components of BioStacked inoculant package were not disclosed at the time of inoculant specimen acquisition from the mother company (Becker Underwood). It may be that Mo could have been deliberately included or otherwise as a contaminant during inoculant processing stages. On the other hand, it is possible that the rhizobia strain in the BioStacked inoculant is tolerant to low concentrations of Mo in the soil, to meet its BNF requirements (Christensen, 1928).

The contrasting superior performance of Mak Bio Fixer at the higher rates of the Mo application, compared to its BioStacked counterpart (Table 3), further demonstrates the sensitivity of CIAT 899 (*Tropici strain*) as a native rhizobia strain, to the sufficiency of Mo in the soil, resulting from its (Mo) application (Ormeno-Orrillo *et al.*, 2012). The bacterial strain code named CIAT899 (*tropici strain*) in Mak Bio Fixer inoculant has been previously characterized to be tolerant to high temperatures, high levels of acidity and is reportedly symbiotically more stable than other non-indigenous rhizobia strains (Kihara *et al*., 2020; Ormeno-Orrillo *et al.*, 2012; Martinez-Romero *et al.*, 1991). Overall, this study has demonstrated that BioStacked inoculant promotes production of more effective nodules than does its Mak Bio Fixer counterpart, especially at lower levels of Mo application in the study soil. However, in the absence of the BioStacked inoculant, Mak Bio Fixer will benefit greatly from application of molybdenum at ≥0.5 kg ha^{-1}.

The lack of 3-factor interaction (P, Mo and rhizobia inoculants), and yet the presence of a significant effect of Mo by inoculant type on number of effective nodules per plant (Table 3), raises interesting results, in light of the earlier hypothesis that P was a possible constraint to the performance of nitrogenase enzyme to result in BNF. This suggests that the limitation to the performance of nitrogenase enzyme may have been due to something else rather than inadequate soil P *per se*. It can also be speculated that the positive interaction effect between P and rhizobia inoculants, with or without Mo (Fig. 1), was due to increased efficiency of the performance of nitrogenase, despite the limited quantities caused by low Mo supply under natural soil ecosystem (Mfilinge, *et al.*, 2014; Samago *et al.*, 2018). Nevertheless, further investigations are recommended to verify these inferences.

Effective Nodule Dry Weight

The significant effect of applied rhizobia inoculants on effective nodule dry weight (Table 4) is difficult to explain from this study. However, it can be theorized that the inoculant enhancement of BNF in the effective nodules, resulted in better growth of the host plant and greater photosynthetic activity, which in return supplied more photosynthates to the N source nodules,

thus becoming denser and possibly larger in size. It can also be speculated that the expanded nodule size became a sink for further uptake of nutrients from the soil, which possibly also added to the weight of the recipient nodule. Literature related to this phenomenon is hard to find, although a few reports (Njira *et al*., 2013; Njira *et al*., 2013; Gedamu *et al*., 2021) Asserted that BNF inoculants in soybean increased nodule dry weight with rhizobia inoculation. However, they did not reveal whether the effect was linked with effective nodules as such; neither did they articulately explain their relationships.

The superiority of Mak Bio Fixer inoculant over the BioStacked in promoting effective nodule dry weight (Table 4) could be attributed to the former being more adapted to the study tropical soil conditions than the later. Several authors (Ormeno-Orrillo *et al.*, 2012; Sohlenkamp *et al*., 2018) have observed that CIAT 899, represented as Mak Bio Fixer in the present study, is versatile at stressful pH values (pH $\leq$4.5) with genes encoding response regulators and membrane transporters, plus enzymes involved in amino acids and carbohydrates metabolism and proton extrusion. It also implies that BioStacked inoculant is not as stress tolerant under study soil conditions as has been prescribed by its manufacturers (Westgate, 2011).

The significant effect of joint application of P and rhizobia inoculants on effective nodule dry weight (Fig. 2) could be attributed to enhanced growth of the plants, including the nodule dry weight. Gedamu *et al*., (2021) also observed that most nodule components of soybean were positively influenced by application of co-application of P and rhizobia types in Ethiopia. On the other hand, the effect of rhizobia strains on nodule dry weight likely manifests through nitrogenase enzyme, which is also a function of Mo (Hoffman *et al*., 2014). The observation could be explained by the possibility that rhizobia inoculants contained Mo as an unintended ingredient, which in part catered for Mo supply in the study soil (Table 3). Various inoculant carriers have been reported to contain various levels of Mo, naturally inborn or deliberately impregnated by human activity (Zhou *et al.*, 2017). Because Mo is required in minute quantities by plants, including for BNF, the quantities delivered by the inoculants sometimes over shadow the deficiency of Mo in the soil. There is need for assessment of the level of content of Mo in both Mak Bio Fixer and Biostacked inoculants.

Total Shoot Nitrogen

The significant positive response of total shoot N to Mo application (Table 5), in the absence of evidence of BNF, suggests the effectiveness of Mo on total N was *via* a path different from that of BNF. Molybdenum, apart from being a direct plant nutrient, is known to be part of two enzymes related to nitrogen utilization in plants; namely nitrogenase and nitrate reductase (Hoffman *et al*., 2014). Nitrogenase is a complex enzyme responsible for BNF, either symbiotically or asymbiotically. It comprises of component I (FeMo protein) and component II (Fe protein) within which molybdenum and iron are central in electron transport chain (Hoffman *et al*., 2014). On the other hand, nitrate reductase enzyme, which is largely resident in the roots of higher plants, is responsible for reducing nitrate ions at uptake, to amino forms which are readily and safely transported within the plants for ultimate utilization sites (Srivastava, 1980).

Although nitrate ions account for the bulk of mineral form absorbed by plants, their tissue accumulation is toxic to plant cells. Hence, with or without BNF occurrence in leguminous plants, nitrate reductase remains operational and accounts for most of the nitrogen found in plant tissues (Beevers & Hageman, 2003). It is, therefore, possible that the observation in the present study was primarily caused by the nitrate reductase path rather than its nitrogenase counterpart.

Although both nitrogen metabolism paths can be simultaneously operational, the nitrate reductase path usually suppresses its counterpart for reasons postulated to be heavy demand for energy of the latter, which the plant often avoids in a bid to save energy for other metabolic essential functions (Cannell & Thornley, 2000 ; Liu *et al.*, 2011).What is difficult to explain though is the sustained source of nitrate ions in a soil that tested low for total N, before we established the experiment (Table 3). Other researchers have alleged that increased availability of Mo in the soil boosts the plant's ability to mobilize nitrates from distant soil resources (Mendel & Haensch, 2002; Williams & Frausto da Silva, 2002; Sauer & Frebort, 2003; Kaiser *et al.*, 2005). The occurrence of this phenomenon is difficult to conceptualize based on existing knowledge. On the other hand, the absence of effect of rhizobia inoculants on total shoot N values may be attributed to unfavourable soil conditions for BNF such as inadequate supply of key nutrients like available P, which was critically low in the soil before the experiment (Table 3). It is estimated that up to 16 ATP molecules are utilized in BNF process to produce two molecules of ammonia Kaiser *et al.*, 2005). Hence, low supply of P as well as other nutrients greatly affected the performance of BNF in legume plants (Abdulameer, 2011; Weldu & Habtegebrial, 2013; Habtegebrial *et al.*, 2015).

With regard to the presumed BNF fraction (balance after discounting for control values in total shoot N, values in parentheses in Table 5), the limited effect of Mo application with or without application of rhizobia inoculants, also echoes the reasons presented above; that is soil P inadequacy (Table 2) which impacted the performance of nitrogenase enzyme, and cause a dysfunction in BNF. This observation implies that although Mo was able to increase the number and dry weight of effective nodules, with application of rhizobia inoculants (Tables 4 and 5), and the functionality of nitrogenase enzyme whose capacity was augmented by Mo application (Habtegebrial *et al.*, 2015).

The slight detectable increase in estimated BNF values based on shoot N, could be attributed to the explanation provided earlier those increased levels of P application, enhanced the performance of nitrogenase enzyme, despite the limited quantities caused by low Mo supply under natural soil (Kihara *et al.*, 2020). The lower response due to P application displayed by the BioStacked inoculant treated plants, suggests that this strain could not cope with abiotic stresses to the extent prescribed by the supplier company. On the other hand, the more stable performance of Mak Bio Fixer inoculant on the basis of the subject at hand, seems to underscore the fact that the inoculant was more resilient under the study conditions than its BioStacked counterpart (Martinez-Romero *et al.*, 1991)

Shoot Dry Weight

The lack of significant interaction effect of P and rhizobia inoculant types on bean shoot dry weight (Table 5), is further evidence that P application effect had no influence on BNF. In light of this, therefore, the significant effect of P *per se* on shoot dry weight (Figure 5) must have been through the nitrate reductase pathway, rather than the nitrogenase-BNF pathway. Other studies suggested that phosphorus application consistently showed a positive response to shoot dry matter on common bean (Turuko & Mohamme d, 2014; Fageria & Baligar, 2016; , Chikowoa *et al.,* 2018. However, more investigations are necessary to confirm these observations because the authors did not explain the mechanism through which P affected the plant.

The insignificant response of estimated shoot dry weight could be due to the effect of the applied phosphorus. Soil phosphorus is known to influence plant nutrient uptake and hence, increase the vigor of plant growth (Mfilinge, *et al.*, 2014). The positive response to P application with Mak Bio

Fixer rhizobia inoculant treated plants, supports the impression that this strain is adapted to the study soil conditions (Ormeno-Orrillo *et al.*, 2012) On the other hand, the slight response to P application of BioStacked rhizobia inoculant suggests that this strain is not as tolerant to abiotic stresses as originally prescribed by the supplier company.

CONCLUSIONS

This study has revealed that common beans are significantly responsive to Rhizobia inoculants when co-applied with either Mo or P on a Ferralsol in Uganda. The performance of the bean plants was superior when Mak Bio Fixer was co-applied with higher rates of Mo or P. In contrast, BioStacked, the exotic Rhizobia inoculant was less sensitive to application of Mo or P at higher rates. Thus, in the absence of soil fertility enhancers, namely, Mo or P, bean growers can benefit more from use of BioStacked Rhizobia inoculants for production of NABE 4 beans. In contrast, where bean growers are able to access sufficient supplies of Mo or P, they should better use the indigenous Mak Bio Fixer Rhizobia inoculant for production of common beans. By and large, it is clear that Mo is becoming a limiting factor for BNF in common beans on this type of Ferralsol.

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