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Response of Common Bean (*Phaseolus vulgaris L.*) to Endomycorrhizal Inoculation under Different Phosphorus Application Levels in South-Kivu, Eastern DRC

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have been reported to increase yield and phosphorus (P) uptake. However, it is still unclear how the common bean responds to mycorrhizal inoculation when there is a phosphate supply. This research focused on finding out how bean performance will be affected by mycorrhizal inoculation and increasing P dosages in order to reduce phosphate input. The study was conducted during the A 2021 cropping season in Kabare, while a split-plot design was used to compare two levels of inoculation and increasing phosphorus doses. *Rhizophagus irregularis* inoculation significantly improved mycorrhizal colonization, biomass, yield, and harvest index of beans at 0 and 30 kg P ha^{-1}. Bean plants inoculated with *R. irregularis* performed better in terms of biomass, yield, and harvest index at 30 kg P ha^{-1} than non-inoculated and inoculated plants at 60 and 120 kg P ha^{-1}, indicating the potential of AMF in lowering phosphate input. Phosphorus levels of 60 and 120 kg P ha^{-1} significantly decreased mycorrhizal infection, indicating the impact of inorganic P on the mycorrhizal symbiosis. In the ferralitic soils of Kashusha, mycorrhizal inoculation with *R. irregularis* may be a key tool for increasing bean production and ensuring phosphate fertilizer savings.

Keywords: Arbuscular mycorrhizal fungi; Phaseolus vulgaris L.; Phosphorus nutrition; South-Kivu

ABSTRAK

Jamur mikoriza arbuskula (AMF) dilaporkan dapat meningkatkan hasil dan serapan fosfor (P). Namun, masih belum jelas bagaimana respon kacang-kacangan terhadap inokulasi mikoriza ketika ada pasokan fosfat. Penelitian ini berfokus untuk mengetahui bagaimana kinerja biji kopi akan dipengaruhi oleh inokulasi mikoriza dan peningkatan dosis P untuk mengurangi masukan fosfat. Penelitian dilakukan pada musim tanam A tahun 2021 di Kabare, sedangkan rancangan petak terpisah digunakan untuk membandingkan dua tingkat inokulasi dan peningkatan dosis fosfor. Inokulasi Rhizophagus irreguleris secara signifikan meningkatkan kolonisasi mikoriza, biomassa, hasil, dan indeks panen kacang pada 0 dan 30 kg P ha-1. Tanaman kacang-kacangan yang diinokulasi R. irreguleris menunjukkan hasil yang lebih baik dalam hal biomassa, hasil, dan indeks panen pada 30 kg P ha-1 dibandingkan tanaman yang tidak diinokulasi dan diinokulasi pada 60 dan 120 kg P ha-1, yang menunjukkan potensi FMA dalam menurunkan penambahan fosfat. Kadar fosfor 60 dan 120 kg P ha-1 menurunkan infeksi mikoriza secara signifikan, hal ini menunjukkan adanya pengaruh P anorganik terhadap simbiosis mikoriza. Pada tanah ferralitik di Kashusha, inokulasi mikoriza dengan R. irreguleris merupakan kunci utama untuk meningkatkan produksi kacang-kacangan dan memastikan penghematan pupuk fosfat.

Kata kunci: *Jamur mikoriza arbuscular; Phaseolus vulgaris L.; Nutrisi fosfor; Kivu selatan*

INTRODUCTION

The main factors limiting agricultural yield, particularly for legumes, are land degradation and decreased soil fertility. The performance and yield of the common bean, in particular, are more dependent on the soil's supply of macronutrients, particularly phosphorus. Regarding nutrient absorption, beans absorb more phosphorus than any other nutrients (Chekanai et al., 2018). It was also demonstrated that a significant increase in yield was obtained with increasing doses of phosphate fertilizers in common beans. Thus, under phosphorus deficiency conditions, plants remain weak, and organ growth deformation occurs due to defective cell division (Liang et al., 2022).

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According to Chekanai et al. (2018), Legumes that have access to phosphorus may produce twice as much biomass, which raises bean yield. Additionally, soil microorganisms like arbuscular mycorrhizal fungi are crucial for optimizing soil nitrogen cycling and plant mineral nutrition. They therefore actively participate in phosphorus absorption and solubilization in many agro-systems (Veresoglou et al., 2019).

It has been demonstrated that AMFs increase yield of common beans by promoting pod growth and productivity (Chekanai et al., 2018). Therefore, inoculation with effective AMF strains might enhance bean biological nitrogen fixation and phosphorus use efficiency, resulting in phosphate fertilizer reductions. However in South Kivu province, where the use of fungal biofertilizers has not yet been explored, the response of common bean (*Phaseolus vulgaris* L) to mycorrhizal inoculation under phosphorus supply situations is still poorly reported. Furthermore, it is yet unknown what phosphorus dose should be used in combination with an AMF inoculation to ensure effective bean performance. Based on the aforementioned context, this study was started with the objective of figuring out how common bean performance in South Kivu will be affected by AMF inoculation when increasing phosphorus doses. The objective was to minimize phosphate application in yhe field.

MATERIALS AND METHODS

The study area

The study was conducted in the Kabare territory in the South-Kivu Province during the cropping season from September to December, 2020–2022 at the Université Evangélique en Afrique's experimental field located in the Kashusha locality. One of 11 territories in South Kivu Province, Kabare is located at 28°45' and 28°55' E (longitude), 2°30' and 2°50' S (latitude), and 1460' to 3000m elevation. The monthly temperature (average) is 19.67°C, and the annual rainfall is 1601mm (Chuma et al., 2022a). The natural environment of the Kabare area is dominated by mountains, the highest peaks of which are Mount Kahuzi (3300m) and Mount Biega (2700m). The major soil type in Kabare is clay loam. It is a rich and productive soil, but it has been substantially depleted due to overexploitation and water erosion exposure, with a very poor nitrogen and phosphorus balance. The average annual precipitation is 1601±154 mm, and the monthly temperature is 19.672.3°C (Chuma et al., 2022b).

The Kabare area is bounded on one side by Kahuzi Biega National Park and on the other by Lake Kivu (Mugumaarhahama et al., 2021; Chuma et al., 2022b). This region's large number of lakes and woods results in bimodal rainfall and an equatorial climate with two seasons. The first is the growing season, which runs from September to June, and the other runs from July to August, specifying two agricultural seasons in the area (Pypers et al., 2011; Chuma et al., 2022a). Common beans are the most common leguminous crop grown in this zone's villages each year, with an estimated farmed area of 151,627.27 hectares. It is worth mentioning that the Kabare territory is the most densely inhabited of all the territories in South Kivu, putting significant strain on the soil and natural resources (CAID, 2019). According to Pypers et al. (2011), most farmers in this area do not have access to agricultural inputs and have limited opportunities to improve soil fertility.

Biological materials and experimental design

The experiment was conducted during the period from September to December 2021. The plant material consisted of the variety HM21-7, one of the most cultivated varieties in the study area, and is also preferred by the population. It is a biofortified variety released by the "Institut Nationale pour l'Etude et la Recherche Agronomique (INERA-Mulungu)" in collaboration with Harvest-Plus project. The seeds were produced by INERA-Mulungu within its leguminous crops platform and obtained through the Harvest-Plus organization. The fungal material consisted of an exotic strain of arbuscular mycorrhizal fungus, *R. irregularis*, whose inoculum was obtained from the collection of the Common Laboratory of Microbiology (LCM-IRD/ISRA/UCAD). This strain was selected based on its efficiency in improving the growth performances, nutrition capacity, and drought tolerance capacity of many crop species, including maize, cowpea, and beans (Le Pioufle et al., 2019; Begum et al., 2023). Inoculum production of this selected strain was carried out separately in the greenhouse on maize (*Zea mays* L.) as a host plant (Le Pioufle et al., 2019; Ndeko et al., 2020). Maize was grown on sterilized sandy soil from Sangalkam (121°C for one hour) for four months. Spore production was stimulated following an interruption in watering three weeks before harvest. The obtened inoculum after production was a mixture of infected root fragments with the rate of mycorrhization of ~80-95%, spores, and AM mycelia from the trap cultures and contained about of of 15 spores per gram of soil. After production, the *Rhizophagus irregularis* inoculum was stored at 2-4 °C in a cold room for two weeks before use.

The experiment was set up following the local practices applied to bean planting in the study area. These included manual tillage and weeding. Farmers were prohibited from cutting bean leaves, a locally known practice. The biofortified variety used was already popularized in the area, and mycorrhizal inoculation and phosphate fertilization were the only innovative practices in the study area. The trial evaluated the bean's response to inoculation with *R. irregularis* under the application of increasing phosphorus doses. The set-up included four P levels in tems of P doses (0, 30, 60, and 120 kg P ha^{-1}, respectively D0, D1, D2, and D3) and two mycorrhizal treatments (inoculation with *R. irregularis* and without inoculation). Eight treatments (dose x mycorrhizal inoculations) were considered for this experiment. The treatments under study were randomized and repeated three times each.

Sowing was carried out in rows on the elementary plots according to the treatments under study, with a spacing of 40cm × 20 cm at a rate of 2 seeds per planting hole. The soil application method carried out the mycorrhizal inoculation before the sowing. For this purpose, 15g of mycorrhizal inoculum was placed at a depth of 5 cm just below and near the bean seeds to initiate the mycorrhization process upon emergence. Triple superphosphate (TSP) was used as a phosphorus source. A reference rate of 60 kg P2O5 ha^{-1} was considered and from which other rates were calculated. (Chuma et al., 2022a; Géant et al., 2020). Each plot received a targeted fertilizer application in accordance with the various treatments investigated. The application of mycorrhizal inoculum and fertilization to the plots occurred simultaneously as well. For treatments that were both fertilized and inoculated, the inoculum was positioned directly above the fertilizer in close proximity to the seed and separated from it by a thin layer of soil. The plants were collected after three months, and many characteristics were assessed.

Parameter measurements and methods

Measurement of growth and yield parameters

The different growth parameters were measured three weeks before harvest, except for plant biomass, which was determined one week before harvest. These parameters included collar diameter and height of the bean plants. After 90 days of cultivation, the bean plants were harvested. The different measurements were made on 20 bean plants collected randomly from each elementary plot. Indeed, the plants were carefully deseeded, and the aerial parts were separated from the root system. The roots were carefully washed with water, and a part was used to determine the root biomass, while another part was used for the evaluation of the mycorrhizal colonization rateof the common bean in the differents treatments. .

On the other hand, the aboveground parts were used to measure the shoot biomass. The shoot and root biomass of bean plants were determined after drying samples in an oven at 65°C for 72h. In addition, the dry weight of the shoot and root parts was measured using a balance. Yield parameters, however, were determined at harvest. These include the pods pods number pods number pods number per plant, the seeds number per pod, the 100-seed weight (g), and the yield(kg.ha^{-1}). The bean plants were harvested at full maturity when the pods and seeds had dried in the field.

Quantification of AMF root colonization, mycorrhizal growth response, and nodulation

To quantify mycorrhizal colonization, living miniature roots of bean plants (2g) were taken from the various plots as previously reported. Before cleaning and staining, the obtained root material was divided into small 1 cm fragments and kept in a 70% ethanol solution. Cleaning was then performed to remove the contents of the cells while leaving the root and fungal structures intact. Root fragments were submerged with ten percent KOH solution and heated in a water bath at 90°C for 1 hour. The roots were soaked in a 0.05% Trypan blue solution and then heated in a water bath at 80°C for 30 minutes. After the root coloring stage, roots fragments were transferred to a petri dish, and 20 fragments were fixed on the slide between the slide and the coverslip (Phillips & Heyman, 1970). One hundred root fragments (1 cm long) were examined for each sample. Finally, a light microscope inspection was performed at 10x and 40x magnification to determine whether AM fungal structures (Arbuscules, Vesicles, and Hyphae) were present. Based on the proportion of mycorrhizal roots, mycorrhizal colonization was evaluated by the methodology employed by Trouvelot et al. (1986) and reported by Ndeko et al. (2022). The scoring method was used to determine the frequency of root colonization and the percentage of root length colonized by AMF for each treatment. Mycorrhizal structures present included arbuscles, hyphae, and vesicles.

RESULTS AND DISCUSSIONS

Mycorrhizal colonization rate of bean plants as affected by phosphorus levels

Our results indicateded that bean plants showed a low mycorrhization colonization rate regardless of the applied phosphorus dose. In contrast, mycorrhizal inoculation significantly boosted root colonization in plants infected with *R. irregularis* (Figure 1), and a significant interaction between the components was seen, as evidenced by a two-way ANOVA (Table 1). The findings showed that at 60 and 120 kg P, mycorrhizal colonization frequency and intensity of the bean plants were significantly

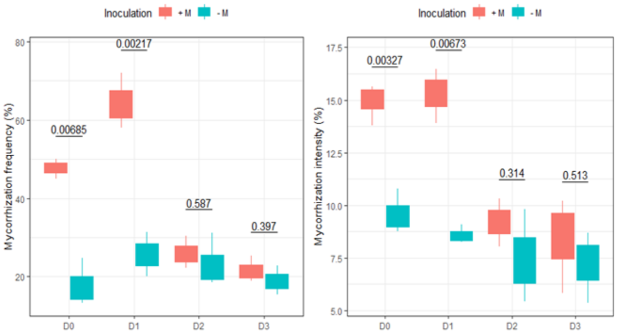

Figure 1. Arbuscular mycorrhizal fungal colonized root length (mycorrhizal frequency and mycorrhizal intensity) in percent of the total root length TSP fertiliser was used as a source of phosphorus at different doses levels: 0, 30, 60 and 120 kg P ha^{-1}

Table 1. Two-way analysis of variance for plant growth parameters and yield-related parameters for mycorrhizal and non-mycorrhizal bean plants grown under different phosphorus fertilization levels (0, 30, 60 and 120 kg P ha^{-1})

Indicators	P doses (df=3)		AMF (df=1)		P doses*AMF (df=3)	
	F	*P*-value	F	*P*-value	F	*P*-value
Mycorrhizal colonization						
Mycorrhizal frequency (%)	8.03	0.002	133	< 0,0001	7.39	0.003
Mycorrhizal intensity (%)	5.59	0.008	7.4	0.015	1.48	0.26
Growth parameters						
Aboveground biomass	13.45	0.000	49.91	< 0,0001	8.44	0.001
Belowground biomass	0.69	0.57	68.7	< 0,0001	1.03	0.4
Total Biomass	20.38	< 0,0001	309.24	< 0,0001	18.04	< 0,0001
Plant heigh	5.75	0.007	12.19	0.003	2.18	0.12
Collar diameter	3.93	0.028	14.91	0.001	5.44	0.009
Leaf area	12.9	0.000	0.13	0.71	0.15	0.92
Yield and yield components						
NPP	5.8	0.007	30.57	< 0,0001	0.59	0.62
NGP	7.57	0.002	11.57	0.004	3.59	0.037
100-grains weight	16.17	< 0,0001	21.56	< 0,0001	1.8	0.18
Yield (kg/ha)	12.07	0.000	166.77	< 0,0001	2.27	0.12
HI	0.64	0.59	4.68	0.046	0.95	0.43

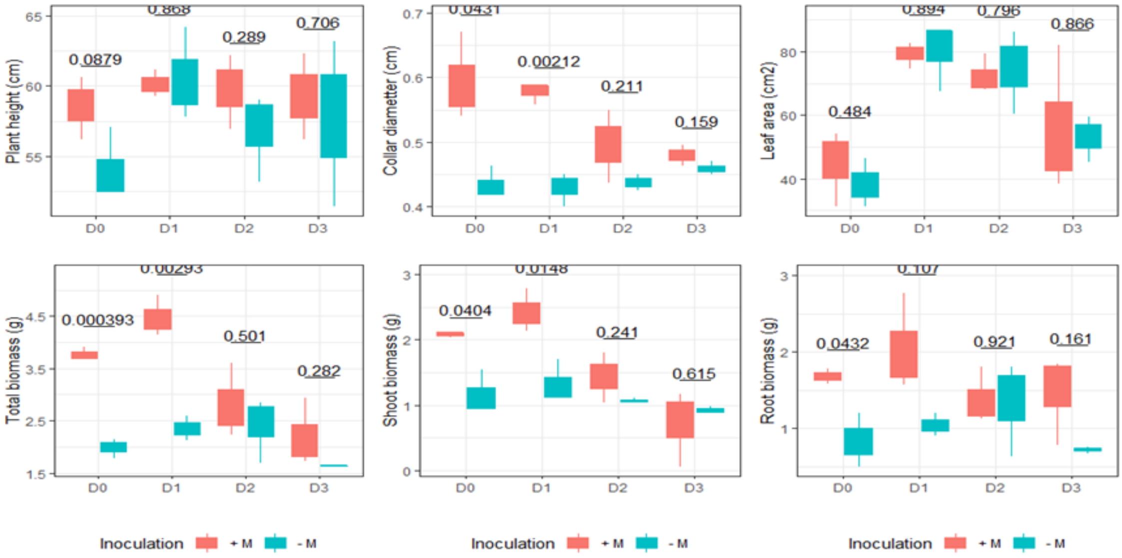

Figure 2. Growth parametres of bean plants (Plant height, Collar diameter, leaf area, total biomass, shoot biomass and root biomass) under mycorrhizal inoculation (+M and –M) and phosphorus doses application (D_0=0, D_1=30, D_2=60 and D_3=120 kg P ha^{-1}) in Kashusha

reduced. Additionally, fertilizer application rate had a negative impact on mycorrhizal colonization, arbuscle and vesicle formation in agro-systems (El-Sherbeny et al., 2022; Liu et al., 2020).

Bean growth parameters as affected by mycorrhizal inoculation and applied phosphorus levels.

Figure 2 shows the association between growth parameters and treatments. The findings demonstrated that, at 0 and 30 kg P ha-1, mycorrhizal inoculation greatly enhanced plant growth (Table 1). In comparison to non-inoculated plants and inoculated plants at levels of 60 and 120 kg P ha-1, the performance of inoculated plants was greater

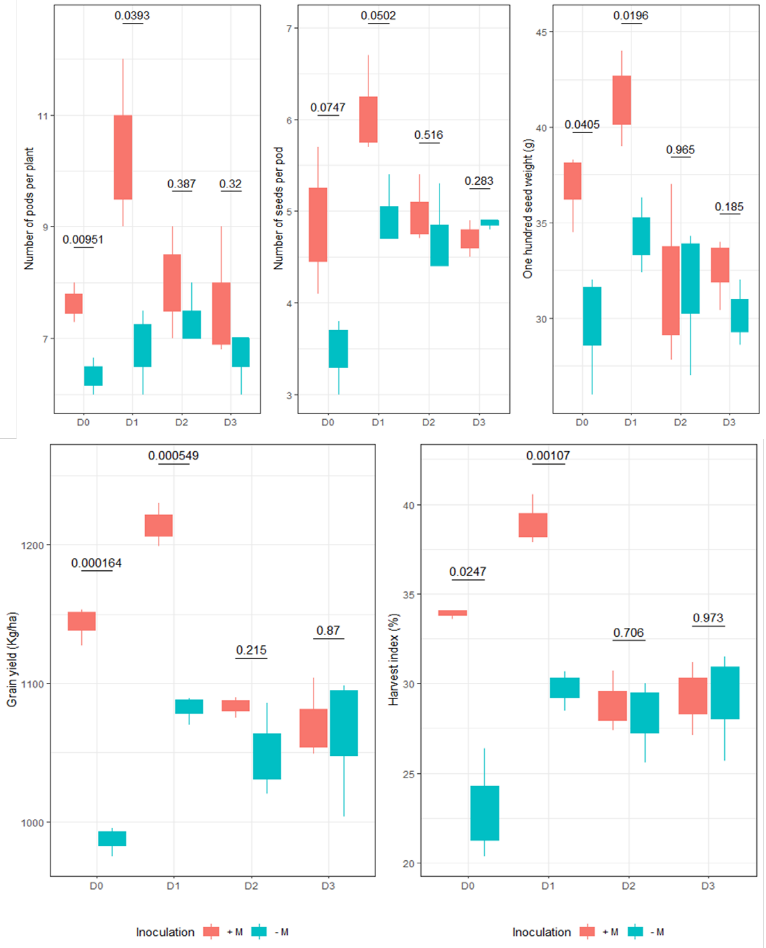

Figure 3. Bean yield-related parameters (pods number pods number pods number per plant, number of seeds per pod, one hundred weight, average grain yield and harvest index) as affected by *R. irregularis*   mycorrhizal inoculation and phosphorus levels application

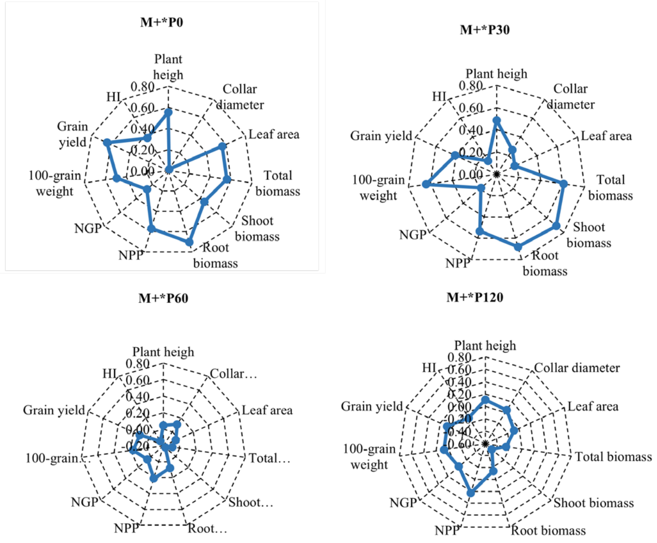

Figure 4. Mycorrhizal Inoculation Effect (MIE) for the growth and yield-related parameters of bean according to the mycorrhizal treatments M+*P0, M+*P30, M+*P60, M+*P120

in terms of collar diameter, aboveground biomass, and total biomass at 30 kg P ha-1. Regardless of the P dose, the mycorrhizal inoculation had no impact on the leaf area. These findings imply that mycorrhizal inoculation may contribute to increase bean growth and phosphate fertilizer input.

Bean yield and yield-related parameters as affected by mycorrhizal inoculation and applied phosphorus levels

Mycorrhizal inoculation increased bean yield and harvest index compared to non-inoculated plants, at 0 and 30 kg P ha^{-1} (Figure 3). Mycorrhizal efficiency was reduced with P-level treatment at 60 and 120 kg P ha$^{-1 \text{ rates}}$. Mycorrhizal inoculation with *R. irregularis* enhanced common bean yield, even at a high-level application (60 kg P. ha^{-1}), grain yield was greater but not statistically different from non-inoculated treatments. Mycorrhizal inoculation improves nitrogen absorption, biofortification, and crop production in most legumes (Liang et al., 2022). The reduction of mycorrhizal growth at a height P supply may be a result of the suppression of mycorrhizal inoculation's effects in this treatments.

The MIE index analysis revealed the response pattern of common bean to inoculation with *R. irregularis* under the different treatments. MIE values were calculated based on the growth and yield parameters of the bean to show the variation of growth and yield parameters following mycorrhizal inoculation. The response to mycorrhizal inoculation varied significantly across the treatments under study (Figure 4). Positive and significant MIE values (>0.5) E were observed in most of the growth and yield parameters in the inoculated treatment without phosphorus supply (M+*P0), which attests to the positive response of the bean to the mycorrhizal inoculation. These are plant height (0.55), leaf area (0.56), total, shoot, and root biomass (0.56, 0.45, and 0.71, respectively), pods pods number per plant (0.57), 100-seed weight

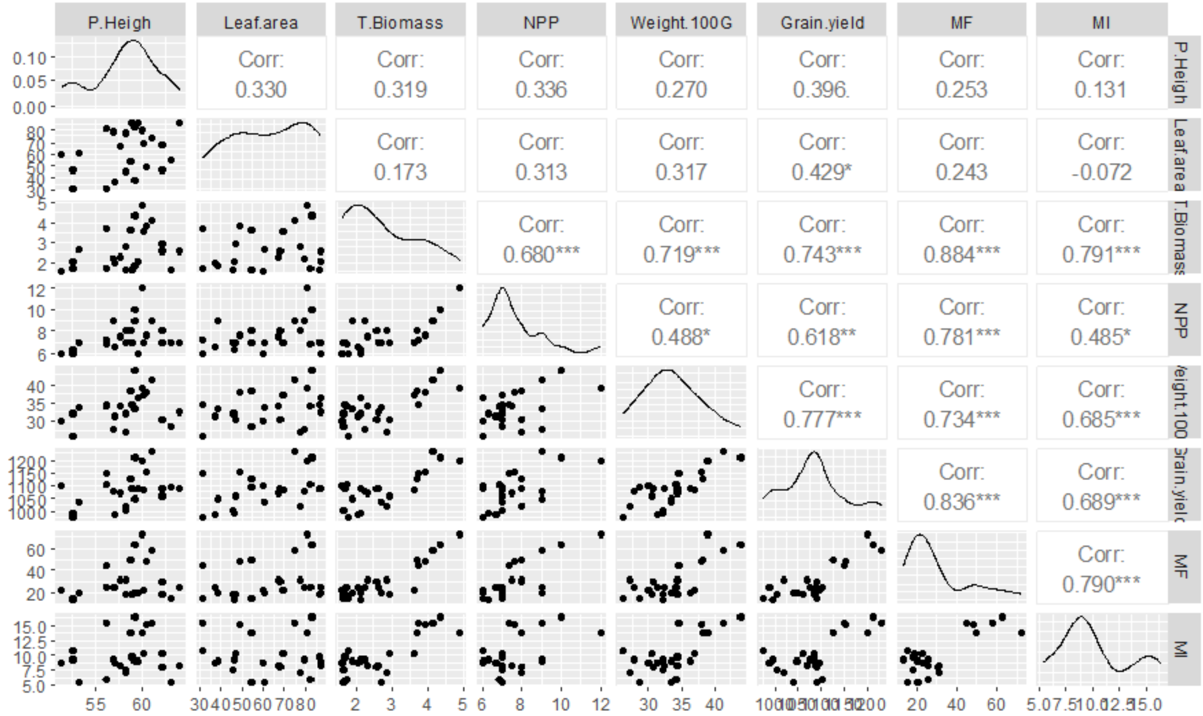

Figure 5. Pearson's correlation matrix and regression analysis between growth and yield-related parameters, considering AM inoculation and non-inoculated treatments

(0.49) and grain yield (0.64). The same trend was observed for the inoculated treatment with 30 kg P. ha^{-1} application. However, a strong response to inoculation was attested to the increase in total, shoot, and root biomass (MIE values were 0.61, 0.71, and 0.68, respectively). These results attest to the positive effects of mycorrhizal inoculation and a strong mycorrhizal dependency of the bean under low P application (Njaramanana et al., 2022). The mycorrhizal dependency is strongly linked to the genotype used, and it is also influenced and reduced by the contribution of mineral fertilizer inputs, especially phosphate fertilizers (Ortas & Bilgili., 2022). A drastic or total reduction of the mycorrhizal colonization and the mycorrhizal inoculation effect was also observed under the same conditions (Begum et al., 2023). However, for the inoculated treatments with an application of 60 and 120 kg of P. ha^{-1}, positive MIE values were observed but not significant (<0.5) for plant height, collar diameter, root biomass, pods number per plant, 100-seed weight, and grain yield, attesting the absence of response to mycorrhizal inoculation or the low mycorrhizal dependency of the bean under these treatments. Other variables, such as shoot and total biomass and pods number per plant, showed negative MIE values, indicating a depressive effect of mycorrhizal inoculation under these treatments.

The Pearson correlation analysis provided a relationship between mycorrhization variables (mycorrhization frequency and intensity) and growth, yield-related parameters, and grain yield to understand better the influence of inoculation with *R. irregularis* on the performance of common bean under Kashusha conditions (Figure 5). The results revealed a positive and substantial relationship between total biomass and the frequency and intensity of mycorrhization (r=0.884 and r=0.79, respectively). As a result, the plants' photosynthetic capability and nitrogen intake are boosted (Bağdatli and Erdoğan, 2019; Ndeko et al., 2020). The positive relationships between mycorrhizal parameters and the total biomass would be explained by a strong mycorrhizal dependency of the bean genotype used, which was enhanced under the conditions of low phosphorus application

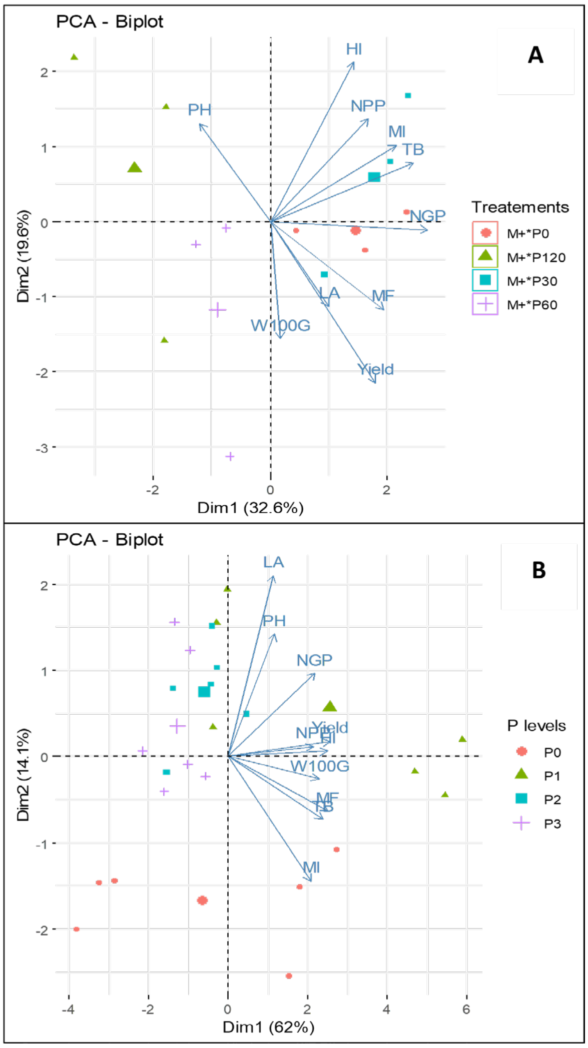

Figure 6. Principal component analysis (PCA) with the 11 growth and yield-related parameters of bean plants according to the different treatments, (A) and the phosphorus levels application (B, in blue arrows), and the two AMF parameters, MF (% of AM frequency of the root system) and MI (% of AM intensity of the root system); The 16 different colored individuals at the 95% level represent all P treatments (M+*P0, M+*P30, M+*P60 and M+*P120) in A and P levels of P0 (no phosphorus applied), P30 (application of 30 kg P ha^{-1}), P60 (application of 60 kg P ha^{-1}) and P120 (application of 120 kg P ha^{-1}) in B.

(Njaramanana et al., 2022). Similarly, a positive correlation was observed between bean plant height and mycorrhization frequency and intensity, but the correlation was not significant (r=0.253 and r=0.131). As for yield, the bean mycorrhization rate was highly and positively correlated with all yield-related parameters except for the number of grains per pod, which would be more related to the genotype used. An increase in mycorrhization frequency and intensity significantly improved the pods number per plant (r=0.781 and r=0.485), 100-seed weight (r=0.734 and r=685), and bean yield per ha (r=0.836 and r=0.689). It was indicated that inoculation with the efficient strain of AMF could improve the performance of most crops (Ndeko et al., 2022; Njaramanana et al., 2022). The results in Figure 6 indicate that bean yield can be explained by several factors depending on the treatment. It was noted that yield was positively linked to mycorrhization frequency in the M+*P0 and M+*P30 treatments, while no relationship was observed in the other treatments.

For beans, a positive effect on yield was observed by other authors under low phosphorus application. Moreover, under these conditions, mycorrhizal inoculation also improves nutrient uptake, nutrient translocation to the fruit-bearing parts of the plant, and pod formation and filling (Mott et al., 2022). This result would explain the positive influence of mycorrhization rate on common bean grain yield. On the other hand, a more or less complete suppression of the effect of mycorrhizal inoculation was observed under intensive phosphorus applications (Lang et al., 2022).

CONCLUSIONS

Our findings revealed that bean growth and yield have been improved by mycorrhizal inoculation, particularly when less phosphorus was used. (0 and 30 kg P ha^{-1}) and in the control treatments. Applying very high doses of phosphorus (60 and 120 kg P ha^{-1}) drastically reduced mycorrhizal colonization of bean plants and the efficiency of the *R. irregularis* strain and, consequently, bean performance and yield. Field-scale mycorrhizal inoculation with the R. irregularis strain could potentially increase bean yields at low phosphorus levels and save phosphate fertiliser.

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