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Effect of Soil Properties on Arbuscular Mycorrhizae Fungi (AMF) Activity and Assessment of Some Methods of Common bean (*Phaseolus vulgaris* L.) Inoculation in Lubumbashi Region (DR. Congo)

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Abstract

In the Lubumbashi plain, the dominant soils are acidic Ferralsol that contain small amount of available phosphorus and nitrogen. The organic matter content is also limited. This study evaluated the effect of Arbuscular mycorrhizal fungi (AMF) on root colonization in *Phaseolus* beans growth performance and yield. Three soils with a pH 5.8; 6.2 and 6.5 respectively were included. We also studied the effect of increasing doses of P_2O_5 in beans by providing 25kg of P_2O_5/ha, 50kg P_2O_5 ha^{-1} in combination with AMF inoculation. The results show a high frequency of colonization at a pH of 5.8 (69%); a plant height of 56 to 58 cm with 10 leaves and 6 pods per plant for a yield of 2095kg ha^{-1}. However, above pH 6.2, root colonization is low (31 to 61%) and plant height ranges from 27 to 39 cm with 4 to 6 pods for a yield between 631 and 1479 kg ha^{-1}. The effect on plant response of 25kg P_2O_5 ha^{-1} in combination with AMF inoculation was statistically significant for all measured parameters. Finally, this study compared the efficacy of inoculum produced on three different way on colonisation efficiency of beans. Inoculum produced by using *Plantago lanceolata* as host species contained more spores and resulted in better-colonized roots compared to inoculum coming from *Sorghum vulgare* or clay inoculum.

Keywords: Poor and acidic soils, mycorrhizae, phosphorus, beans.

INTRODUCTION

The soils in the Lubumbashi plain (Haut-Katanga, DR. Congo) have mainly a pH of 4.7 to pH 5.2, only few soils have approximately a pH of 6.0. Nitrogen levels range from 0.034 to 0.460% and free iron (Fe_2O_3) easily reaches 12.9% in some areas [1]. This acidity is unfavourable for the availability of phosphorus essential for the synthesis nucleic acids, proteins and increases seed formation [2-4]. Phosphorus uptake by the plant is optimal at soil pH ranging from 6 to 6.5 [5, 6], because below pH 6, aluminium ($Al3^{+}$), iron ($Fe2^{+}$; $Fe3^{+}$), manganese ($Mn2^{+}$), calcium ($Ca2^{+}$) and magnesium (Mg^{2+}) dominate cation exchange capacity and phosphorus blocking becomes important [7]. The uptake of P is in the form of primary (H_2PO^{4-}) or secondary (HPO_4^{2-}) orthophosphates, which unfortunately are only present in very low concentrations in most tropical soils [8]. Under this conditions the use of organic matter or chemical fertilizers can essential to enhance P uptake. However, arbuscular mycorrhizae are known to be able to extract phosphorus from the soil, make it available to crops and increase seed yield [9-13]. In this study, the soil pH effect was evaluated on root colonization of inoculated beans with arbuscular mycorrhizae fungi (AMF) and bean behaviour even in three soil types *in situ*. The efficiency of increasing doses of phosphorus (P_2O_5) combined with inoculum was also evaluated in ferralsols low in phosphorus and organic matter through a pot experiment. Finally, the efficacy of three types of inoculum was tested in combination with increasing doses of P_2O_5 on the behaviour of beans in ferralsols low in phosphorus and organic matter *in situ*.

MATERIALS AND METHODS

AMF Inoculums Production

Inoculum was produced with *Plantago* (*P. lanceolata* L)*; Sorghum* (*S. vulgare* L.) and leek (*Allium porrum* L.) according to the [14, 15] methods. *Sorghum* and *Plantago* were sown directly in the field 15cm x 10 cm either 80 plants/m² and *A. porrum* in pots at a density of 20 to 25 plants per pot (0.07m²). Three months later, roots were isolated and discoloured with 10% KOH at 90 ° C for 30 minutes and stained in trypan blue to see the mycorrhizae structures (hyphae, vesicles or arbuscles) that denote colonization by AMF. The number of colonized root were expressed in % [16]. The rhizospheric soil and colonised roots were collected as inoculum. The density of infectious propagules for the inoculum from *Sorghum* is 250 to 400 viable spores per 100g inoculum with roots colonized fort 70%. For Inoculum from *Plantago* roots has 200 to 300 spores per 100g with roots colonized 60 to 70%. Inoculum from *Allium* has 200-250 per 100g inoculum colonized roots 70%. AMF spores were described in each inoculum. The description was focus on spore's colour, the presence or absence of the pedicel and its mode of insertion on the spore, the ornamentation of the spore wall and layers well number [17, 18]. In all these inoculums, *Acaulospora*, *Ambispora* and *Gigaspora* genus are the most dominant.

Experimental set up

During this study, three experiments were set up. In the first experiment, soil pH were studied on common bean root colonization by AMF. In a second experiment, the interaction between AMF and different doses of P_2O_5 was studied. In the last, three AMF inoculum efficacy were studied on common bean. Before each experiment the mycorrhizae spores were isolated from experimental soil after 100g soil wet sieving in 45µm soil sample, topped with another 1mm mesh sieve, which retained the large particles [16]. Isolated spores were identified according to procedures of Trap [17] and INVAM [18]. Spores identification focus on spores colour, presence or absence of the pedicel and its mode of insertion on the spore, ornamentation of the spore wall and wall layers on the spore. The most dominant morpho-species belonged to the genera *Gigaspora*, *Acaulospora* and *Archaeospora* and the maximum density was 120 spores per 100g of soil. The variety RWR 2154, which is bio-fortified with iron, was used in our experiments. Its height ranges from 30 to 50 cm with an erect growth habit and a vegetative cycle of up to 90 days. The number of pods varies between 15 and 20 per plant and the pod contains an average of 6 seeds. The seeds are cream-colored with black streaks and yield easily reaches 1.8 to 2.5 tons/ha [19].

In the first experiment, the pH effect was studied in 3 different sites. The first site has a deep clay Geric Ferralsol, with a pH of 6.5; nitrogen content % 0.096; carbon content % 0.970 and phosphorus ppm 10.3. The second and third sites are Plinthosols, which have lateritic on the surface. In the second site, there is a soil pH of 6.2; nitrogen content % 0.156; carbon content % 1.718 and phosphorus ppm 15.1. In the third site, there is a soil pH of 5.8; nitrogen content % 0.212; carbon content % 2.227 and phosphorus ppm 8.07. In the map (figure 1), sites experiment are showed:

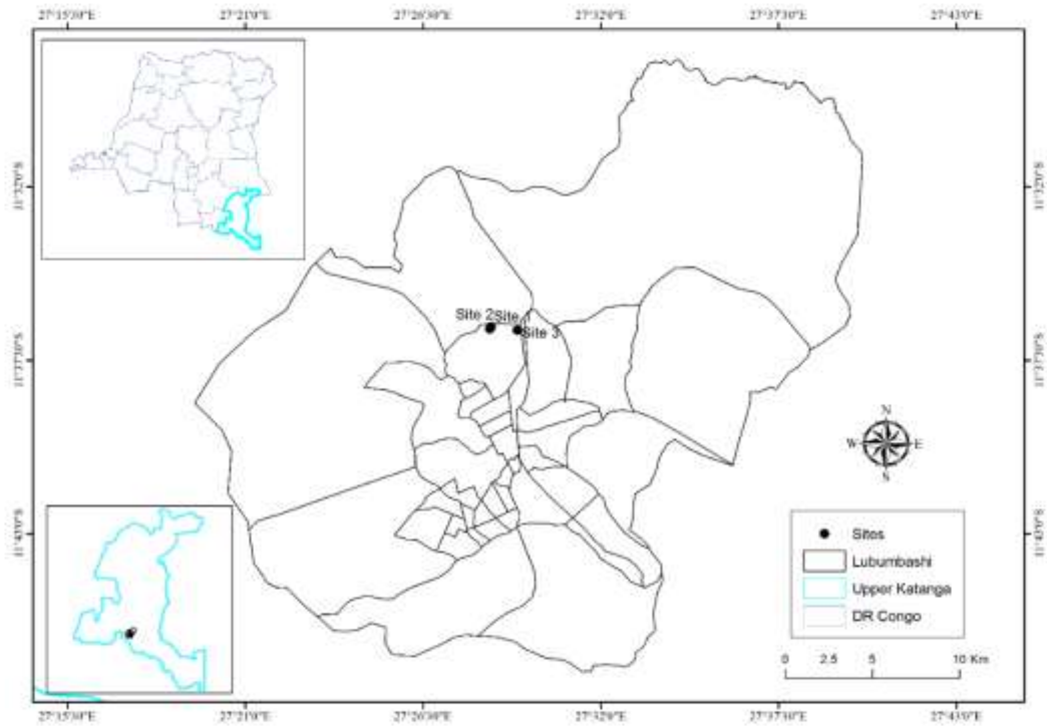

Fig-1: Sites experiment location on common beans inoculation by AMF in the Lubumbashi

Experiment was set-up on factorial 3 x 2 design (3 sites with AMF inoculum plots and without AMF inoculum plots in each site) with 4 replications. The inoculum used in this experiment come from *Plantago* using 100g of inoculum per seed hole. The plots were 1.5 m wide and NPK fertilizer supply was constant at 20kg nitrogen, 40kg P_2O_5 and 20kg K_2O ha^{-1}. Sowing was done at spacings of 20cm x 20 cm with two seeds per seed hole. The fields were weeded twice in the 3rd and 5th week after sowing and the experiment lasted 3 months (February to May 2019). In table 1 the characteristics of the used soil are given:

Table-1: Location of study sites and experimental soil classification

	Site 1	**Site 2**	**Site 3**
Coordinates	**S** 11°36.545'	**S** 11°36.439'	**S** 11° 36.502'
	E 027°29.410'	E 027°28.601'	E 027° 28.559'
Elevation (m)	1285	1268	1267
Soil colour	2,5YR 3/6	5YR 4/3	5YR 3/2
	Dark red	Reddish brown	Dark reddish brown
Sys and Schmitz [20]	Red Latosols	Lateritic soil	Lateritic soil
IUSS Working Group WRB [21]	Geric Ferralsols	Plinthosols	Plinthosols
pH w	6.5	6.2	5.8
N (%)	0.096	0.156	0.212
C (%)	0.970	1.718	2.327
P Bray2 (ppm)	10.3	15.1	8.07
Cation exchange capacity (CEC)	8.70	12.30	12.60
Exchanges cations content: cmol (+) per kg soil			
Ca	6.38	7.63	7.03
K	0.52	0.80	1.38
Mg	1.90	2.03	2.07
Na	0.06	0.07	0.07

The root colonization frequency was evaluated according to Walker *et al.*, [16] procedures. The plant height (cm), the number of leaves per plant and the leaves chlorophyll content were assessed 30 days after sowing. The chlorophyll content was measured on the third leaf using a KONICA MINOLTA chlorophyll meter. The number of pods per plant and yield (kg of beans) were also evaluated.

The second experiment was made in pots 18 cm diameter. The soil that used was Geric Ferralsol from site 1 (Table-1). Each pot had from bottom to top 2 cm thick coco-peat, soil, inoculum (1cm), a thin layer of soil experimental (1cm) and coco-peat fibers on the surface. The layered structure of the substrate assured a good rooting and the infiltration of water. The experiment was set up on 2 x 3 factorial design with 5 replications. The inoculum that used in this experiment came from leeck (*Allium porrum*) using 100g inoculum per pot (seed hole). Treatments were as follows: a control, 25 kg P_2O_5 ha^{-1}; 50 kg P_2O_5 ha^{-1}; inoculum; inoculum + 25 kg P_2O_5 ha^{-1} and the inoculum + 50 kg P_2O_5 ha^{-1}. The experiment was installed at the campus of the faculty of agricultural Sciences of the University of Lubumbashi (DR Congo) under a shaded net filtering sunlight up to half and limiting thus the evaporation of water. The experiment started in November 2017 and run to January 2018 under temperatures varying between 15 and 30 ° C with 11 to 12 hours of light. Root colonization frequency (%), plant height (cm), number of leaves per plant, leaves chlorophyll content (SPAD) were assessed 30 days after sowing. The number of pod per plant and number of grains per pod were also assessed.

The third experiment was carried out in a Geric Ferralsol in site 1 **(**Table-1). The experimental design was a 4 x 3 factorial with 4 replications; plot size was 1m². The efficacy of three inoculum types was studied and their combination to different doses of P_2O_5. The first AMF inoculum came from *Sorghum*, the second came from *Plantago* and the last was a burned clay (550°C), which contain 3000 AMF spores per 400g of clay. These spores isolated from soil were putted in clay to facilitate crop inoculation procedures, which consists in coating the seeds before sowing. A total of 12 treatments were designed as follows: a control, 25 kg P_2O_5 ha^{-1}; 50 kg P_2O_5 ha^{-1}; inoculum from *Sorghum*; inoculum from *Sorghum* + 25 kg P_2O_5 ha^{-1}; inoculum from *Sorghum* + 50 kg P_2O_5 ha^{-1}; inoculum from *Plantago*; inoculum from *Plantago* + 25 kg P_2O_5 ha^{-1}; inoculum from *Plantago* + 50 kg P_2O_5 ha^{-1}; clay inoculum; clay inoculum + 25 kg P_2O_5 ha^{-1} and clay inoculum + 50 kg P_2O_5 ha^{-1}. Sowing was done at spacing of 20 cm x 20 cm with two seeds per seed hole. The trial was started from February to May 2019. The fields were weeded at the 3rd and 5th week after sowing. At 30 days after sowing, the bean root was isolated and discoloured in 10% KOH at 90 ° C for 30 minutes and stained in trypan blue to assess root colonization frequency (%) [16]. The plant height (cm), the number of leaves per plant, the leaves chlorophyll content (SPAD), the number of pod per plant and beans yield were assessed.

STATISTICAL ANALYSIS

The Chapiro Wilk test was applied to verify data distribution. The variance analysis according to the general linear model was applied to compare the averages. This method was chosen to prevent data transformations, as data distribution was non-normal. The Duncan's test was applied to separate the averages in case of difference (α = 0.05). All statistical analysis were done using SAS 4.1 software.

RESULTS

Effect of Soil properties on root colonization and common bean performance

The frequency of root colonization is higher in soils with pH 5.8 and 6.2 (47±31 % to 53± 21%) compared to soil with pH 6.5 that has an average colonization of 25±13 %. The average colonization is statistically not different between inoculated and non-inoculated plants thus natural AMF colonized the root (35±24 % versus 48±25 % colonization). Inoculation of beans into soil with a pH of 5.8 leads to a higher frequency of colonization (69±24 %) compared to soil with a non-inoculated pH of 6.5, which provides 19±13% (Figure-2).

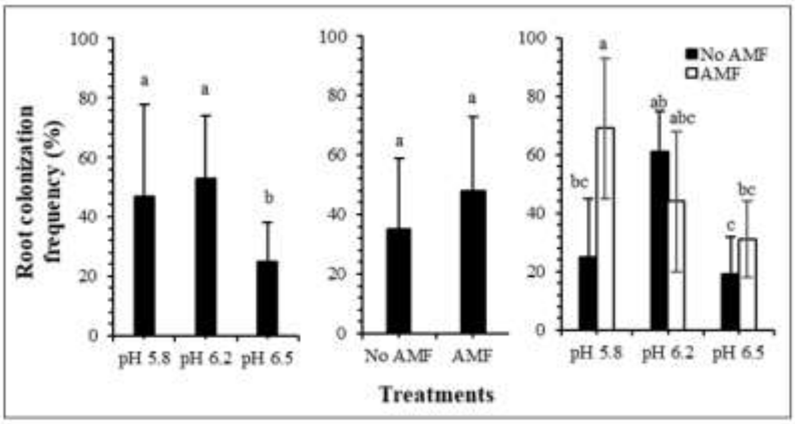

Fig-2Frequency of root colonization by AMF in common beans. Left: soil properties effect; middle: mycorrhizae effect: No AMF: non-inoculated treatments and AMF: inoculated treatments; right: soil properties x AMF interaction graph

Soil pH had a significant effect on plant height and number of leaves the 30th day after sowing (Table-2). The highest plant height is 57±8 cm with 10 leaves per plant founded in soil pH of 5.8. No differences in SPAD values could be detected. (38±3 to 40±6 SPAD units). Inoculation did not have a significant effect on plant height, number of leaves or chlorophyll level after analysis of variance. On the other hand, inoculation in combination with a pH of 5.8 gives the best result for plant height s (56±10 to 58±6 cm) and the number of leaves (10±2 leaves per plant) compared to other treatments. SPAD values are also higher which is 42±35 SPAD units in pH 5.8, followed by AMF inoculated treatments in soils with a pH of 6.2 or 6.5. In the latter, without inoculum, the chlorophyll level in the leaves is 36±3 SPAD units.

Table-2: Plant height, number of leaves per plant and leaves chlorophyll content 30 days after seedling

Treatments	Plants height (cm)	Number of leaves per plant	Leaves chlorophyll content (SPAD units)
Soil pH			
pH 5.8	57±8a	10±2a	39±5a
pH 6.2	33±12b	6±2b	40±6a
pH 6.5	34±7b	6±1b	38±3a
Mycorrhizal inoculation			
Non-Mycorrhizal inoculation	43±12A	7±2A	38±5A
Mycorrhizal inoculation	39±15A	7±3A	40±4A
Soil pH x Mycorrhizal inoculation interaction			
pH de 5.8 Non-mycorrhizal inoculation	58±6a	10±2a	36±3b
Mycorrhizal inoculation	56±10a	10±2a	42±5a
pH de 6.2 Non-mycorrhizal inoculation	39±9b	6±1b	42±6a
Mycorrhizal inoculation	27±12b	6±1b	37±4ab
pH de 6.5 Non-mycorrhizal inoculation	35±9b	6±1b	36±3b
Mycorrhizal inoculation	33±5b	6±1b	40±3ab

SPAD=*Soil Plant Analysis Development. The means that do not share any letters in a group in each column are significantly different after the analysis of variance (ANOVA) and the Duncan multiple comparison test (P<0.05)*

Table-3 shows that the highest number of pods is recorded for the soil with pH 5.8 (7 pods per plant) resulted in a high yield (1709 kg beans ha^{-1}). Inoculation also resulted in a significant difference in pod number and yield (7 pods per plant and 1477kg beans ha^{-1}) compared to non-inoculated treatments (5 pods per plant and 877kg beans ha^{-1}). The Highest yield was obtained when a pH 5.8 was combined with an AMF inoculation (2095 kg ha^{-1}). AMF Inoculation into a soil pH of 6.2 gives 1479 kg $ha^{-1}$of beans yield while the lowest yield was obtained on soil pH 6.5 non-inoculated plots (631±141 kg ha^{-1}).

Table-3: Number of pods per plant and beans seed yield

Treatments	Number of pods per plant	Yield (kg ha^{-1})
Soil pH		
pH 5.8	7±4a	1709±959a
pH 6.2	5±2b	1078±723ab
pH 6.5	5±1b	743±278b
Mycorrhizal inoculation		
Non-Mycorrhizal inoculation	5±1B	877±511B
Mycorrhizal inoculation	7±3A	1477±927A
Soil pH x Mycorrhizal inoculation interaction		
pH de 5.8 Non-mycorrhizal inoculation	9±5a	1323±711ab
Mycorrhizal inoculation	6±2bc	2095±1114a
pH de 6.2 Non-mycorrhizal inoculation	5±2cd	678±178b
Mycorrhizal inoculation	4±2d	1479±872ab
pH de 6.5 Non-mycorrhizal inoculation	6±2bc	631±148b
Mycorrhizal inoculation	5±1cd	855±148b

The means that do not share any letters in a group in each column are significantly different after the analysis of variance (ANOVA) and the Duncan multiple comparison test (P<0.05)

Increasing doses of P_2O_5 and AMF effect on common bean

The average frequency of root colonization by AMF is higher in treatments which received 25kg of P_2O_5 ha^{-1} (64±5%) compared to 50kg of P_2O_5 ha^{-1} and the control which had 46±14% to 47±14% of root colonization (figure 3 left). Root colonization frequency was 61±8 % on non-inoculated plots versus 49±16 % founded in inoculated plots (Figure-3 middle). The interaction factor shows that the dose of 25k g of P_2O_5 ha^{-1} non-inoculated provides 66±5% of root colonization while the 50kg of P_2O_5 ha^{-1} + AMF inoculum treatments provides 40±15% of root colonization (Figure-3 right).

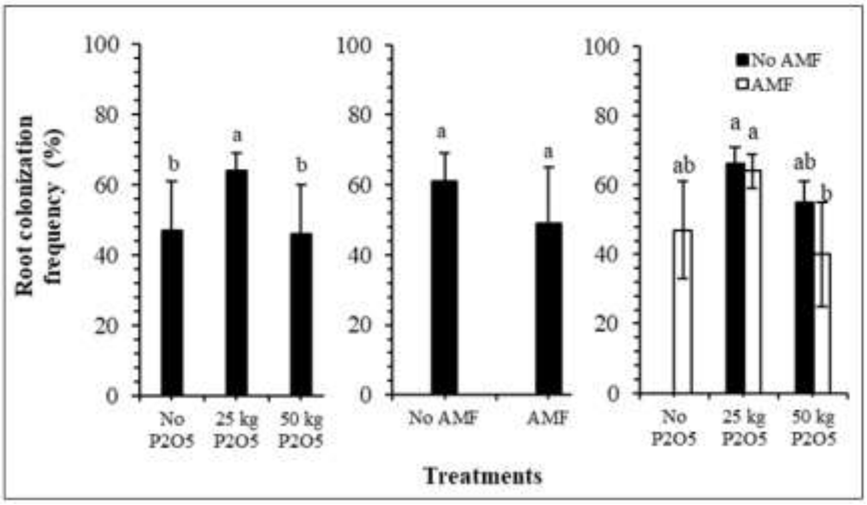

Fig-3: Frequency of root colonization by AMF in common beans. Left: phosphorus effect (kg P2O5 ha-1); middle: inoculum effect: No AMF: non-inoculated treatments, AMF: inoculated treatments with mycorrhizae; right: phosphorus (P_2O_5) x AMF inoculum interaction

At 30 days after sowing, plant height were not different between the phosphorus (P_2O_5) levels with average values between 9 and 11 cm per plant. The inoculated plants were mostly 11 cm high while the non-inoculated plants measured 9 cm. AMF inoculum combined to 25 or 50 kg P_2O_5 ha^{-1} scored better that the other treatments. The number of leaves was the same for the P_2O_5 effect, i. e. 4±0 to 5±1 leaves per plant. AMF inoculum treatments gives 5±1 leaves versus 3±1 leaves obtained in non-inoculated treatments. The results show that AMF inoculum combined to 25 or 50 kg of P_2O_5 provides highest plant height (11 cm) with

5±1 leaves per plant. Plants fertilized with 50 kg P_2O_5 had a leaves chlorophyll content of 37 SPAD units while those fertilized with 25 kg of P_2O_5 contained 32 to 34 SPAD units. Inoculated plants have a chlorophyll level of 36 SPAD units while non-inoculated plants contain 32 SPAD units. The interaction factor provides 38 SPAD on treatments that received 50kg P_2O_5 ha^{-1} combined to AMF inoculum as without AMF inoculum. The control has 33±3 SPAD units of leaves chlorophyll content (Table-4):

Table-4: Plant height, number of leaves and leaves chlorophyll content at 30 days after seedling

Treatments	Plant height (cm)	Number of leaves per plant	Chlorophyll leaves content (SPAD units)
Phosphorus (P_2O_5 kg ha^{-1})			
Unfertilizer	9±71a	4±0a	34±3ab
25kg P_2O_5 ha^{-1}	10±1a	4±1a	32±7b
50kg P_2O_5 ha^{-1}	10±1a	5±1a	37±5a
Mycorrhizal inoculation			
Non-mycorrhizal inoculation	9±1B	3±1B	32±6B
Mycorrhizal inoculum	11±1A	5±1A	36±4A
Phosphorus x mycorrhizal inoculation interaction			
Non-mycorrhizal Unfertilizer	8±0c	4±0b	33±3b
25kg P_2O_5 ha^{-1}	9±0bc	4±1b	26±4c
50kg $P_2O_5$$ha^{-1}$	9±1bc	4±1b	38±4a
Mycorrhizal inoculation Unfertilizer	10±0ab	4±0b	35±4ab
25kg P_2O_5 ha^{-1}	11±1a	5±1a	38±2a
50kg P_2O_5 ha^{-1}	11±2a	5±1a	35±6ab

SPAD*: Soil Plant Analysis Development. The means that do not share any letters in a group in each column are significantly different after the analysis of variance (ANOVA) and the Duncan multiple comparison test ($P<0.05$)*

For the number of pods as well as the number of seeds per pod not differences could be observed in relation to P_2O_5 fertilisation with an average of 2 pods containing 3 to 5 seeds (Table-5). Inoculation doubled the number of pods per plant and the number of seeds per pod (2 pods harvested versus 1 pod). Inoculum alone, as well as its interaction with 25 or 50 kg P_2O_5 ha^{-1}, significantly increases the number of pods per plant and the number of seeds per pod. The averages are 2 to 3 pods each containing 3 to 5 grains.

Table-5: Number of pods per plant and number of grains per pod

Treatments	Number of pods per plant	Number of grains per pod
Phosphorus (P_2O_5 kg ha^{-1})		
Unfertilizer	2±1a	3±2a
25kg P_2O_5 ha^{-1}	2±1a	3±2a
50kg P_2O_5 ha^{-1}	2±1a	5±2a
Mycorrhizal inoculation		
Non-mycorrhizal inoculation	1±0B	3±1B
Mycorrhizal inoculum	2±1A	4±2A
Phosphorus x mycorrhizal inoculation interaction		
Non-mycorrhizal Unfertilizer	-	-
25kg P_2O_5 ha^{-1}	1±0b	3±2b
50kg $P_2O_5$$ha^{-1}$	1±1b	3±2b
Mycorrhizal inoculation Unfertilizer	2±0ab	3±2b
25kg P_2O_5 ha^{-1}	3±1a	5±1a
50kg P_2O_5 ha^{-1}	3±1a	5±1a

The voids (-) indicate the absence of plants due to mortalities recorded during the experiment. The means that do not share any letters in a group in each column are significantly different after the analysis of variance (ANOVA) and the Duncan multiple comparison test ($P<0.05$)

Efficacy of three types of AMF inoculum combined to P_2O_5

The control plants provides the lowest root colonization frequency by AMF (51±9 %) but inoculated plants gives 63±7 % to 69±5 % (Figure-4 left). The P_2O_5 fertilization did not significantly influence the root colonization; the average was 56±11 % to 60± 10% (Figure-4 middle). The interaction effect shows a better frequency of colonization on *Sorghum* inoculum (IFS) combined to P_2O_5 or without P_2O_5. The control plants and 50 kg P_2O_5 ha^{-1} gives the lowest root colonization 48±10 % or 51±10 % (Figure-4 right).

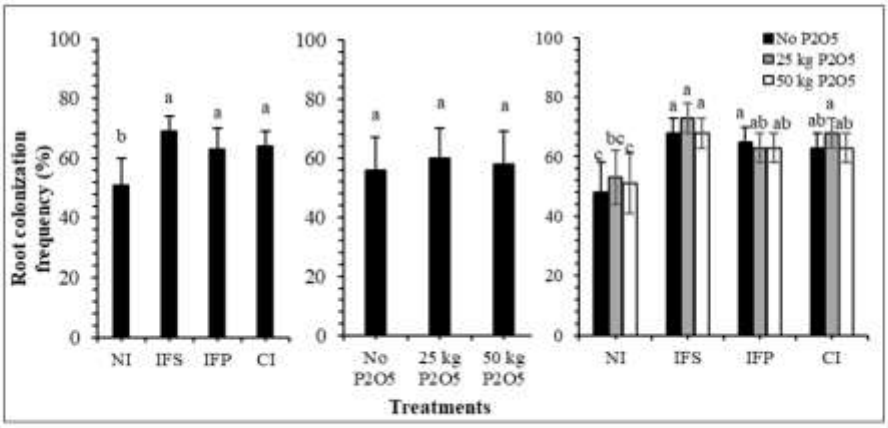

Fig-4: Frequency of root colonization by AMF in common beans. The left graph: inoculum effect; the middle: the phosphorus effect (P_2O_5); right: inoculum x phosphorus interaction. NI: non-inoculated treatments; IFS: inoculum from *Sorghum*; IFP: inoculum from *Plantago*; CI: clay inoculum

Results in Table-6 shows that the best height of the plants is obtained on treatments with the inoculum from *Plantago* (35 ± 12 cm) but the lowest height on the clay inoculum (18 ± 8 cm). P_2O_5 fertilization did not significantly influence plant height but interaction shows a best height of plants (41 ± 12 cm) when the *Plantago* inoculum is combined to 50kg of ha^{-1}. The clay inoculum or the treatment of 25 kg of P_2O_5 and the control, offer 18 ± 8 to 24 ± 10 cm plant height. *Plantago* inoculum is better for the number of leaves that is 25 ± 5 versus 5 ± 1 leaves per plant obtained on *Sorghum* inoculum treatments. The number of leaves was similar on P_2O_5 fertilized plots compared to the control (11 ± 8 to 12 ± 9 leaves per plant). *Plantago* inoculum give 26 ± 9 leaves when it has applied alone or combined to 25kg P_2O_5. *Sorghum* inoculum alone or combined to P_2O_5 offers the lowest number of leaves per plant (5 ± 1 leaves). *Sorghum* and *Plantago* inoculum offers the same leaves chlorophyll content as the control (34 ± 5 to 37 ± 3 SPAD units) while the clay inoculum gives 30 ± 5 SPAD units. An equal leaves chlorophyll content is observed whatever the fertilization. However, the interaction shows 39 ± 3 SPAD units chlorophyll content on *Plantago* inoculum alone and 28 ± 7 SPAD units when the clay inoculum is combined to 25 kg of P_2O_5 ha^{-1}.

Table-6: Plant height, number of leaves per plant and leaves chlorophyll content at 30 days after seedling

Treatments	**Plant height (cm)**	**Number of leaves/plant**	**Leaves chlorophyll content (SPAD units)**
Mycorrhizal inoculum			
Non-mycorrhizal inoculation	24±8b	11±6b	34±5a
Inoculum from *Sorghum*	21±6bc	5±1c	35±4a
Inoculum from *Plantago*	35±12a	25±5a	37±3a
Clay inoculum	18±8c	7±1bc	30±5b
Phosphorus (P_2O_5 kg ha^{-1})			
Unfertilized	23±10A	11±8A	35±2A
25kg P_2O_5 ha^{-1}	24±9A	12±9A	33±5A
50kg P_2O_5 ha^{-1}	27±10A	12±8A	34±5A
Mycorrizal Inoculum x phosphorus (P_2O_5 kg ha^{-1})			
Non-mycorrhizal Unfertilized	24±10bcde	9±5bc	35±5abc
25kg P_2O_5 ha^{-1}	23±6cde	11±6b	34±4abc
50kg P_2O_5 ha^{-1}	27±6bcd	12±6b	34±6abc
Inoculum from *Sorghum* Unfertilized	21±6bcde	5±1c	36±5abc
25kg P_2O_5 ha^{-1}	22±7bcde	5±1c	35±1abc
50kg P_2O_5 ha^{-1}	21±5bcde	5±1c	35±4abc
Inoculum from *Plantago* Unfertilized	31±6abc	26±5a	39±3a
25kg P_2O_5 ha^{-1}	33±15ab	26±9a	35±1abc
50kg P_2O_5 ha^{-1}	41±12a	25±3a	37±2ab
Clay inoculum Unfertilized	15±10e	7±1bc	31±5acd
25kg P_2O_5 ha^{-1}	20±8cde	7±1bc	28±7d
50kg P_2O_5 ha^{-1}	18±8de	8±2bc	30±3cd

SPAD=*Soil Plant Analysis Development. The means that do not share any letters in a group in each column are significantly different after the analysis of variance (ANOVA) and the Duncan multiple comparison test (P<0.05)*

Inoculum from *Plantago* gives 12 pods per plant, followed by clay inoculum, which gives 9 pods per plant. Inoculum from *Sorghum* and the control gives 4 to 6 pods per plant. The best dose of P_2O_5 is 50 kg ha^{-1} (9 pods per plant) compared to 50 P_2O_5 kg ha^{-1} and control that offer 6 or 7 pods. The best combination that provide the highest number of pods per plant is the inoculum from *Plantago* with 50kg of P_2O_5 ha^{-1} (15 pods per plant). The yields obtained range from 275kg to 614kg ha^{-1} for the inoculum effect, which is highly significant in case of inoculum from *Plantago* or clay inoculum. The contribution of 50kg of P_2O_5 ha^{-1} allows having on average of 501 kg of beans compared to 25kg of P_2O_5 while the control gives only 300 to 370kg of yield ha^{-1}. The highest yield is 911 kg ha^{-1} when applying 50kg of P_2O_5 + inoculum from *Plantago* (Table-7).

Table-7: Number of pods per plant and yield (kg h^{-1})

Treatments	Number of pods/plant	Yield (kg/ha)
Mycorrhizal inoculum		
Non-mycorrhizal inoculation	6±2c	275±128b
Inoculum from *Sorghum*	4±2bc	308±132b
Inoculum from *Plantago*	12±5a	602±250a
Clay inoculum	9±4b	614±115a
Phosphorus (P_2O_5 kg ha^{-1})		
Unfertilized	6±3B	302±145B
25kg P_2O_5 ha^{-1}	7±4AB	373±183B
50kg P_2O_5 ha^{-1}	9±4A	501±260A
Mycorrizal Inoculum x phosphorus (P_2O_5 kg ha^{-1})		
Non-mycorrhizal Unfertilized	5±3de	229±109e
25kg P_2O_5 ha^{-1}	6±2de	263±121de
50kg P_2O_5 ha^{-1}	7±2de	335±140d
Inoculum from *Sorghum* Unfertilized	4±2e	238±88de
25kg P_2O_5 ha^{-1}	5±2de	312±169de
50kg P_2O_5 ha^{-1}	5±1de	374±121d
Inoculum from *Plantago* Unfertilized	7±3de	345±20de
25kg P_2O_5 ha^{-1}	14±1ab	550±33c
50kg P_2O_5 ha^{-1}	15±4a	911±92a
Clay inoculum Unfertilized	8±4cd	541±56c
25kg P_2O_5 ha^{-1}	8±4cde	586±136bc
50kg P_2O_5 ha^{-1}	11±4bc	714±77b

The means that do not share any letters in a group in each column are significantly different after the analysis of variance (ANOVA) and the Duncan multiple comparison test ($P<0.05$)

DISCUSSION

Soil properties and mycorrhizae effect on common bean

Results shows that number of AMF spores and hyphae or arbuscles and vesicles in inoculum are the key to facilitate root colonization. The soil pH also affect the frequency of root colonization by AMF. Soils with a low pH showed a higher colonization rate. At 5.8 pH, the average colonization rate is 53 ± 21% but at 6.2 pH, the root colonization rate is 47±31 %. In 6.5 soil pH, root are lowly colonized by AMF (25±13 %). Interaction between soil pH and AMF inoculum shows that AMF inoculation of beans growing in soils with a pH of 5.8 leads to a higher frequency of colonization (69%) whereas in soil with a pH of 6.5 without inoculum the colonization is only 19%. The plant height, the number of leaves per plant and the leaves chlorophyll content were also higher on plants which growing in the soil with pH 5.8 than with pH 6.2 or 6.5.

The difference of plant height, number of leaves per plant and leaves chlorophyll content was 23 to 40% in favour of soil at pH 5.8. A significant effect was also observed on bean yield, which was 2095 kg/ha for a pH of 5,8, while for soils with pH 6.2 or pH 6.5 the yield varied from 631 to 885 kg ha^{-1} with 20% difference between yield values averages. This reflects the contributions of AMF in mineral assimilation in plants, with a positive effect on bean yield as demonstrated by Pushpa *et al.*, [13] and Ouzounidou *et al.*, [22]. In the African tropics, the pH range most suitable for bean cultivation is 6 to 7.5 [23]; however, our results show the possibility of optimizing mycorrhizae inoculation of beans at soil pHs between 5.8 and 6.2 because beyond that, there is a significant decrease in root colonization of AMF.

AMF and increasing doses of P_2O_5 effect on common bean

Colonization of bean roots was stimulated by the low dose of P_2O_5 (25kg ha^{-1}). While, the dose of 50kg P_2O_5 ha^{-1} gave a low root colonization frequency.

The high phosphorus use reduce AMF activities on crop root, Mukerji *et al.*, [14] also proved this. The excessive supply of P_2O_5 significantly reduced the activity of AMF for the treatment image of 50kg P_2O_5 ha^{-1}, which leads to a 40% colonization while in soil not inoculated but simply fertilized with 25kg P_2O_5 ha^{-1}, colonization reaches 66%. The decrease in colonization is explained by the inactivity of fungi when the soil solution contains a high available phosphorus content [24]. On average, the frequency of colonization was the same between inoculated and non-inoculated plants without considering the phosphorus added. However, crop inoculation remains important because it provides new sources of infectious propagules with a high root colonization [25, 26]. The plant height, as well as the number of leaves and the chlorophyll level in the leaves, were higher for inoculated treatments than for non-inoculated treatments and the differences ranged from 5 to 25%. Mortalities were recorded during second experiment and led to the total loss of control plants (not inoculated not fertilized) compared to inoculated or fertilized treatment. AMF are really bio fertilizers and bio-protectors in common bean production; Abdel-Fattah *et al.*, [27]; Abdel-Fattah *et al.*, [28]; Sabry *et al.*, [29] confirm also that. In pot experiment, the results are similar to those found by Neeraj and Singh [30]; Bhattarai *et al.*, [31] showing that beans inoculated with mycorrhizae assimilate enough phosphorus and nitrogen that are beneficial for good vegetative growth and high seed production.

Efficacy of three types of AMF inoculum combined to P_2O_5

Inoculated plants showed a statistically similar frequency of colonization (63±7 to 69±5 %) compared to the controls (51±9 %). Root intensity colonization (root portion which contain hyphae or arbuscles or vesicles expressed in %) could be different between AMF inoculum types but it was not assessed in this study. Fertilization did not induce any difference in colonization but its interaction with inoculum shows that 25 kg P_2O_5 ha^{-1} is sufficient to stimulate root colonization. Inoculum from Plantago provides high plant height and a higher number of leaves per plant, especially when combined with 50kg P_2O_5 ha^{-1}. This inoculum contains enough colonized roots (up to 15g per 100g of inoculum); the number of propagules (spores and hyphae) in AMF inoculum influence significantly root colonization by AMF, Gaur and Adholeya [25] and Zakaria *et al.*, [26] confirm that. The number of pods per plant was also higher on inoculum treatments from Plantago especially when combined with 50kg P_2O_5 ha^{-1}; the average was 15 pods/plant while 50kg P_2O_5 ha^{-1} provided only 7 pods/plant. The same was true for the yield, which was 911kg/ha for inoculum treatment from Plantago+50kg P_2O_5 ha^{-1}, followed by clay-based treatment+50kg P_2O_5 ha^{-1} (714kg beans ha^{-1}).

CONCLUSIONS

Arbuscular mycorrhizal fungi play an important role in sustainable production. Results shows a high root colonization of the bean when inoculated into soil with a pH between 5.8 and 6.2. The bean inoculated into a soil of pH 5.8 produces twice the number of pods and therefore a higher yield. In soil low in phosphorus and organic matter, a low dose of 25kg P_2O_5 ha^{-1} combined with mycorrhizae inoculum is sufficient to stimulate root colonization of the crop. The best type of inoculum should contain not only mycorrhizae spores but also colonized roots to facilitate the colonization of the crop.

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