Effect of Single Superphosphate and Arbuscular Mycorrhizal Fungi on Growth and Bambara groundnut *(Vigna subterranea* (L.) Verdc.) Yield

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Part 1- Scientific Research

Abstract

The aim of this study was to reduce food insecurity by improving the yield of Bambara groundnut with phosphorus fertilizer. The experiment was carried out in the field at the University of Yaounde I. The experimental design was a split plot with three factors; landraces (V1 and V2), single superphosphate doses (0, 50, 100, 150 and 200 kg P_2O_5.ha^{-1}) and AMF inoculum (M0: -AMF and M1: +AMF). P_2O_5 doses and AMF (composites *Gigaspora margarita*, *Acaulospora tuberculata* and *Glomus intraradices*) significantly boosted growth (number of branches, shoot height) and yield of Bambara groundnut. The dose 150 kg P_2O_5.ha^{-1} significantly increased the yield by 100% compared to the control (0 kg P_2O_5.ha^{-1}+M0) in V1. AMFs significantly increased the yield by 87.5% compared to the control in V2. P_2O_5 and AMF independently improved the grain yield. However, low doses of P_2O_5 (100 kg.ha^{-1}) may be associated with AMF to maximize grain yield of Bambara groundnut.

Key-words: Arbuscular mycorrhizal fungi, Bambara bean, fertilizers, phosphate starvation, phosphorus.

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a neglected legume native to northern Cameroon and northeastern Nigeria (Temegne et al., 2018a). It is used for food and feed because its seeds contain on average 63% of carbohydrates, 19% of proteins and 6.5% of fats (Bamishaiye et al., 2011). They are rich in calcium, potassium, iron (De Kock, 2013), essential amino acids (methionine, leucine, isoleucine, lysine, phenylalanine, threonine, valine, tryptophan) (De Kock, 2013; Yao et al., 2015) and vitamins (E: 3, 18 ± 0.15 mg / 100 g, C: 1.17 ± 0.20 mg / 100 g and A: 26.05 ± 0.14 mg / 100 g). It is used in the traditional pharmacopoeia to treat diarrhea, anemia, abscesses, internal injuries, ulcers, infected wounds, epilepsy, cataracts, menorrhagia during pregnancy, nausea in pregnant women, kwashiorkor and venereal diseases; and prevents heart disease, eye disease and colon cancer (Brink et al., 2006; Jideani & Diedericks, 2014). Bambara groundnut also contains kaempferol, an antioxidant polyphenol, which reduces the risk of many chronic diseases such as cancer (Jideani & Diedericks, 2014; Yao et al., 2015). Bambara groundnut has the ability to tolerate poor soils, drought and salt stress (Taffouo et al., 2010; Temegne, 2011; Jideani & Diederiks, 2014; Tsoata et al., 2015, 2016, 2017a, b).

Numerous studies have been carried out on optimizing the productivity of Bambara groundnut in Cameroon through biological and chemical fertilization (Ngakou et al., 2012; Temegne et al., 2015, 2017b, 2018b; Temegne, 2018). However, to the best of our knowledge, no work has yet been done on the combined effect of biological and chemical fertilization on the yield of Bambara groundnut. Soils in Cameroon, like most tropical soils, are poor in nutrients (Syers et al., 2008; Mbogne et al., 2015). Arbuscular mycorrhizal fungi (AMF) improve the water and mineral nutrition of plants (Onguene et al., 2001; Nwaga et al., 2010; Temegne et al., 2017a, 2018b). But the extreme poverty of some soils requires additional input (organic or chemical fertilizer) in addition to mycorrhizal fertilizer. Research has shown that high doses of chemical fertilizers inhibit mycorrhization (Bhadalung et al., 2005; Mbogne et al., 2015; Temegne et al., 2017a, b, 2018b). Since chemical fertilizers are the most accessible, it is therefore necessary to determine the minimum dose of chemical fertilizer that does not affect the effectiveness of mycorrhization.

N and P are major nutrients essential for plant growth (Morel et al., 2006). Bambara groundnut has the capacity to fix atmospheric N due to the rhizobial symbiosis (Ngakou et al., 2012; Musa et al., 2016). P is a limiting factor for crop yield on more than 30% of the world's arable land (Vance et al., 2003) and remains a limiting mineral for Bambara groundnut growth. The objective of this work was to evaluate the combined effect of chemical and biological fertilization on the growth and yield of Bambara groundnut.

Material and methods

Study Site- The trial was conducted at the Experimental Farm of the Faculty of Science, University of Yaounde I. The site is located in the Centre Region and is characterized by rainfall of 1,617 to 1,800 mm.year^{-1}. The average air temperature varies from 23 to 24 °C. It belongs to the humid forest agro-ecological zone with bimodal rainfall pattern, characterized by acidic ferralitic soils. It is governed by a Guinean equatorial climate with four seasons: a long rainy season from September to November, a long dry season from December to February, a short rainy season from March to June and a short dry season from July to August. The soil of the site is sandy clay (Table 1). The low C/N ratio reflects a rapid decomposition of organic matter. It leads to a dysfunction of the clay-humic complex.

Plant material-The plant material used consisted of two landraces of Bambara groundnut (V1: white seeds, V2: red seeds) bought from the local market.

Experimental design- The experimental design of the study was a split plot with three factors; doses of phosphate fertilizer (0, 50, 100, 150 and 200 kg P_2O_5.ha^{-1}), landraces (V1 and V2) and mycorrhizal treatment (M-: control, M: mycorrhizae). After clearing and plowing, a plot of 27 m x 18 m was divided into three parallel blocks of 27 m x 6 m. The spacing between the blocks was one meter and 0.5 m between the experimental units. Using a double decameter, a string and stakes, 20 experimental units of 2 m x 2 m were formed per block (60 units in total). Bambara groundnut seeds were sown (two seed.hole^{-1}), at the same time as the mycorrhizal inoculant (5 g.hole^{-1}), i.e. 49 holes for each unit. One plant per hole was maintained at emergence for a total of 2,940 plants. Plot maintenance was done by hand weeding and hoeing around the plants.

Ten plants per experimental unit were used for the evaluation of the number of branches and measuring the height of the plants. These data were taken weekly, from the second week after sowing until the tenth week. At harvest, yield parameters were determined and seed P content measured.

Data Analysis

The collected data were subjected to an analysis of variance (ANOVA) using the IBM SPSS software (Statistical Package for the Social Sciences) version 20. The Student-Newman-Keuls test at the 5% threshold allowed to rank the averages.

Table 1. Physico-chemical analysis of the soil of the study sites. pH: potential of hydronium ion, OC%: total organic carbon, N%: total nitrogen, C/N: ratio between proportion of total organic carbon and proportion in total nitrogen, P: phosphorus, Ca: calcium, Mg: magnesium, K: potassium, Na: sodium

Sand	Clay	Silt	OC	N	C/N	pH	Ca	K	CEC	Mg	Na	P Bray
%							cmol.kg^{-1}					µg.g^{-1}
43.68	43.4	12.92	2.1	0.167	12.6	6.21	6.84	0.23	6.53	0.62	0.04	2.37

Results

Mycorrhization significantly ($p <0.001$) favored Bambara groundnut growth in the field. Overall, at high doses, phosphate fertilization reduced the beneficial effect of AMF (Arbuscular mycorrhizal fungi) on the number of branches (Table 2) and the height (Table 3) of Bambara groundnut. Thus, the highest number of branches at V1 (77 ± 12) was observed at mycorrhizal treatment + 0 kg P_2O_5.ha^{-1}, eight (8) weeks after sowing (WAS) (Table 2). Two (2) to 6 WAS, the number of branches was significantly ($p <0.001$) higher at V1, but 8 WAS, it was more important ($p <0.001$) at V2 (Table 2).

Eight (8) WAS, the highest height (35.8 ± 2.1 cm) from V2 was obtained at mycorrhizal treatment + 50 kg P_2O_5.ha^{-1} (Table 3). This height decreased significantly with mycorrhizal treatment + 200 kg P_2O_5.ha^{-1} (34.6 ± 2.4 cm). Plant height of V2 was significantly ($p <0.001$) greater than that of V1 throughout the experiment (Table 3).

Landraces, P_2O_5 doses, AMF and their interactions significantly affected ($p <0.05$) the yield components of Bambara groundnut (Table 4). Except for the diameter and P content of seeds, mycorrhization significantly ($p <0.001$) increased the yield components (100-grain weight, number of pods plant^{-1}, weight of pods plant^{-1}, weight of seeds plant^{-1}) of

Bambara groundnut (Table 4). Thus, in V1 landrace, the dose 150 kg P_2O_5.ha^{-1} increased the yield by 100% compared to the

control (0 kg P_2O_5.ha^{-1} + M- (without AMF)). In V2, the AMFs

increased the yield by 87.5% compared to the control (0 kg P_2O_5.ha^{-1} + M- (without AMF)).

Overall, high levels of P_2O_5 decreased the effectiveness of mycorrhization on some yield parameters (Table 4). Indeed, the highest 100-grains weight was observed at mycorrhizal treatment + 0 kg P_2O_5.ha^{-1} (V1: 111.6 ± 1.7 g). It decreased significantly ($p <0.001$) at mycorrhizal treatment + 150 kg P_2O_5.ha^{-1} (V1: 89.6 ± 2.5 g). The highest pod weight (V2: 24.8 ± 5.1 g) was obtained at mycorrhizal treatment + 100 kg P_2O_5.ha^{-1}. It decreased significantly ($p <0.001$) at mycorrhizal treatment + 200 kg P_2O_5.ha^{-1} (V2: 12.9 ± 2.1 g).

Overall, mycorrhization increased the percentage of double pods (Table 5). The number of double, triple and quadruple pods were greater in the V2 landrace compared to V1 where the triple and quadruple pods were non-existent.

Discussion

The biological fertilizer (AMF) tested does not significantly affect the diameter of Bambara groundnut seeds. Indeed,

plowing and/or weeding would have caused a disruption of the hyphae networks which allow the improvement of water and mineral supply, thus reducing the effectiveness of mycorrhization. This result is similar to those of Bourou et al. (2011) who found that *G. mosseae* had no effect on height and neck diameter of tamarind trees. But these results are contrary to those obtained by the same authors with the strain *G. aggregatum* for the height of the tamarind tree.

AMFs significantly improve the growth, yield and P content of Bambara groundnut seeds. Similar results were obtained by Megueni et al. (2011) on cowpea in the field and Temegne et al. (2017a, 2018a) on Bambara groundnut in pot experiments. In addition, Ngakou et al.

(2012) working on the effect of inoculation of Rhizobium and mycorrhizae on Bambara groundnut found that they improved grain yield compared to control. The grain yield obtained in our study varied from 0.6-1.9 t.ha^{-1}. This yield is higher than that obtained by Ngakou et al. (2012) who recorded a yield of 0.5-0.9 t.ha^{-1}. This result highlights the importance of using native AMF strains for sustainable Bambara groundnut production (Temegne et al., 2017b). The increase in P contents of cowpea leaves (*Vigna unguiculata* (L) Walp) inoculated with *G. clarum* was observed by Megueni et al. (2011). This result could be explained by the fact that mycorrhizae secrete enzymes that hydrolyze mineral substances which are indirectly accessible to the roots. The improvement of water and mineral nutrition as well as the better development of plants amended with the biological fertilizers are due to the development by these fungi of a network of hyphae. These hyphae take water and nutrients to allocate to plants. The work of Tsane et al. (2005) on the growth of banana vitroplants showed that *Glomus* sp improved the neck circumference, the height, the leaf area, the number of leaves emitted, as well as the plant dry matter as compared to the control.

Table 2. Effect of mycorrhization and P_2O_5 doses on the number of Bambara groundnut branches. M-: without AMF, M+: with AMF. For each column, the averages followed by the same letter are not significantly different at the 5% threshold.

Landraces	Doses P_2O_5 (kg.ha^{-1})	AMF	2	4	6	8
V1	0	M-	4.6±0.6abc	12.3±3.6cde	33.3±7.4bcdef	47.6±8.5a
		M+	4.8±0.4cd	14.0±1.6ef	41.4±6.4gh	77.0±11.1ghij
	50	M-	4.4±0.5abc	12.6±1.2de	30.4±4.5abcd	49.0±6.5a
		M+	4.7±0.5bcd	16.6±2.9h	42.1±7.7gh	64.2±12.9cdef
	100	M-	4.6±0.6abc	13.8±1.9ef	35.2±9.5def	58.5±15.8abcd
		M+	5.1±0.6d	14.5±1.9fg	32.4±6.1bcde	58.3±8.3abcd
	150	M-	4.8±0.6bcd	16.1±2.9gh	46.2±10h	76.0±17.9fghij
		M+	5.1±0.6d	14.9±1.4fgh	37.6±4.5efg	65.0±8cdefg
	200	M-	4.6±0.5abc	15.1±2.4fgh	39.3±6.3fg	61.5±11.4bcde
		M+	5.1±0.3d	18.5±2.6i	46.6±11h	75.0±21.0fghij
V2	0	M-	4.3±0.5abc	8.8±1.9a	24.8±6a	52.2±10.5ab
		M+	4.6±0.5abc	11.3±2.6bcd	27.4±3.2ab	69.4±6.3defghi
	50	M-	4.3±0.4ab	9.70±1.8ab	25.4±5.8a	55.3±11.4abc
		M+	4.5±0.5abc	10.9±1.3bcd	28.3±3.8abc	70.9±7.3efghi
	100	M-	4.3±0.4ab	10.4±2 abc	29.2±3.9abcd	65.1±13.6cdefg
		M+	4.6±0.5abc	11.7±1.4bcd	34.1±3cdef	77.4±9.7hij
	150	M-	4.2±0.4a	11.2±1.5bcd	28.7±6.5abc	68.8±15.2defgh
		M+	4.4±0.5abc	11.3±2.2bcd	27.6±4.2ab	64.8±6.5cdefg
	200	M-	4.4±0.5abc	12.7±2.6 de	34.3±6.3cdef	80.9±17.5ij
		M+	4.5±0.5abc	12.3±1.7cde	35.1±5.7def	84.3±14.2j

The single superphosphate applied significantly improved the growth and the yield of Bambara groundnut. Similar results have been observed by Nweke & Emeh (2013) on *V. subterranea*, by Nkaa et al. (2014) on *V. unguiculata*, and by Temegne (2014) on *Brachypodium distachyon*. P is needed in large amount, and it is involved in the key functions of several plants, including energy transfer, photosynthesis, sugar and starch transformation, nutrient movement in the plant, and transfer of genetic traits (Jemo et al., 2010). This result indicates that phosphate amendment stimulates the vegetative growth of Bambara groundnut. It also emphasizes that soil nutrient depletion slows down and/or reduces plant growth since nutrients are not readily available for plant uptake in control plots (not fertilized with P_2O_5). This unavailability of nutrients therefore explains the reduction in plant height, leaves and branches development compared to plants fertilized with P. The significant increase observed in these parameters as a function of the increasing doses of P_2O_5 applied could also be explained by the nitrogen and phosphorus interaction in the soil rhizosphere (Benedyka et al., 1992; Kamchoum et al., 2018). Indeed, P availability increases nitrogen absorption by the plant. In addition, Shaheen et al. (2007) and Nuemsi et al. (2018) have shown that nitrogen is important for the improvement of plant growth because it provides the basic constituent of proteins and nucleic acids. Similarly, Silva et al. (2012) reported that inorganic fertilizer rapidly provides important elements at the early stage of plant growth and development stage. In all legume such as Bambara groundnut, the release of nitrogen from symbiotic nodules and dead legume roots increases the availability of nitrogen in the soil. So, the amount of nitrogen in the soil should be increased in the presence of legumes. The increase in Bambara groundnut growth under phosphate fertilization could also be explained by the fact that nutrient release rates are much higher in phosphate fertilizer. This release would therefore promote better growth of plants fertilized with P_2O_5 compared to control plants.

High levels of P_2O_5 decrease the effectiveness of mycorrhization. This result is similar to those of Oliveira et al. (2015), who showed that mycorrhization increases plant development at low P supply. The work of Ingleby et al. (2007) and Temegne et al. (2018b) have shown that large intakes of soluble phosphates decrease the rate of mycorrhizal infection and may result in the elimination of the positive effects of this association on plant yield. However, different degrees of sensitivity can exist between species. This fact could justify the absence of inhibition of mycorrhization by phosphate fertilization on certain parameters. In addition, simple superphosphate is known as a fertilizer that mineralizes slowly.

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Authorship Contributions

Author TNC designed the study, developed the research protocol, performed the data collection and the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Author GWF contributed to the field experiment. Author NFD contributed to the data collection in the field. Authors WGA and NNG corrected the draft and proofread the final manuscript. Author APA translated the draft of the manuscript into English. Authors TVD and YE supervised the work

Table 3. Effect of mycorrhization and P_2O_5 doses on the height of Bambara groundnut. M-: without AMF, M+: with AMF. For each column, the averages followed by the same letter are not significantly different at the 5% threshold.

Landraces	Doses of P_2O_5 (kg.ha^{-1})	AMF	Time after sowing (Week) 2	4	6	8
V1	0	M-	11.8±2.8 abcde	19.4±2.6 a	22.1±2.8 a	24.0±3.2 a
		M+	11.9±1.8 abcdef	20.0±1.6 a	23.8±2.2 a	25.7±2.7 abc
	50	M-	10.4±2.0 a	20.9±2.4 ab	22.8±1.9 a	25.1±3.0 ab
		M+	10.9±0.8 ab	20.2±1.1 a	24.2±1.0 a	26.6±1.5 abc
	100	M-	11.1±2.1 abc	20.7±2.5 ab	23.2±2.3 a	24.7±2.3 ab
		M+	12.7±2.3 bcdef	20.8±1.1 ab	22.3±1.9 a	26.1±2.6 abc
	150	M-	11.5±2.1 abcd	20.1±2.0 a	23.8±2.2 a	25.8±3.3 abc
		M+	13.5±2.9 ef	22.7±1.1 bc	24.4±1.4 a	28.2±1.4 c
	200	M-	11.1±1.7 abc	21.1±1.8 ab	22.3±1.6 a	25.6±3.4 abc
		M+	13.4±2.5 def	21.7±1.7abc	24.4±2.7 a	27.0±3.6 bc
V2	0	M-	11.9±1.8 abcdef	22.9±3.7 bc	29.4±1.9 bc	32.2±3.1 de
		M+	12.9±1.4 cdef	26.5±1.9 de	31.1±2.9 cde	34.1±3.0 ef
	50	M-	12.1±1.3 abcdef	23.4±4.0 c	28.8±2.0 b	31.2±2.1 d
		M+	13.4±0.9 def	26.6±1.8 de	30.8±2.6 cd	35.8±2.1 f
	100	M-	12.6±1.9 bcdef	25.5±2.8 d	31.2±3.1 cde	31.5±2.3 d
		M+	13.3±1.0 def	27.2±3.9 de	33.2±2.6 f	35.7±4.9 f
	150	M-	12.3±1.5 bcdef	26.3±2.6 de	29.6±2.7 bc	32.8±3.6 de
		M+	13.9±1.3 f	28.2±2.3 e	32.4±1.9 de	36.1±2.3 f
	200	M-	13.3±1.9 def	25.8±1.6 d	31.9±3.1 de	32.9±2.9 de
		M+	13.8±2.1 f	27.3±1.2 de	32.7±2.4 de	34.6±2.4 ef

Table 4. Effect of mycorhization, P2O5 and landrace on Bambara groundnut yield. M-: without AMF, M+: with AMF (5 g.hole-1). For each column, the averages followed by the same letter are not significantly different at the 5% threshold.

Landraces	P_2O_5 Doses ($kg.ha^{-1}$)	AMF	100-grain weight (g)	Seed diameter (mm)	N° of pods $plant^{-1}$	Pod weight $plant^{-1}$ (g)	Seed weight $plant^{-1}$ (g)	Grain yield ($t.ha^{-1}$)	Seed P content (%)
V1	0	M-	83.2±1.6c	11.9±0.7ab	10.8±2.0a	9.0±2.7ab	6.4±2.0ab	0.8±0.2ab	0.28±0.1d
		M+	111.6±1.7h	12.4±1.4abcd	15.2±5.5ab	14.3±2.4bcde	10.2±1.6bcdef	1.2±0.2bcdef	0.21±0.1ab
	50	M-	84.2±2.0c	12.2±0.6abc	12.5±4.2ab	10.1±3.8abc	7.2±2.7abc	0.9±0.3abc	0.21±0.1ab
		M+	100.4±2.9f	12.4±0.8abcd	17.0±1.5abc	15.4±0.9bcdef	10.7±0.6bcdef	1.3±0.1bcdef	0.22±0.1ab
	100	M-	74.2±1.8a	11.1±0.3a	10.5±3.7a	6.9±2.4a	4.5±1.5a	0.6±0.2a	0.22±0.1ab
		M+	98.8±1.9f	12.5±1.1abcde	13.6±3.8ab	12.0±2.9abcd	8.6±2.3abcde	1.1±0.3abcde	0.21±0.1ab
	150	M-	92.6±2.6e	12.3±1.2abc	18.7±7.5abc	18.7±7.2def	13.2±5.2fg	1.6±0.6fg	0.23±0.1b
		M+	89.6±2.5de	12.1±0.7abc	20.4±3.9bc	16.7±2.2cdef	11.6±1.4	1.4±0.2cdef	0.20±0.1a
	200	M-	108.8±1.4g	12.9±0.9bcdef	14.6±5.9ab	16.5±8.2cdef	11.5±6.0cdef	1.4±0.7cdef	0.21±0.1ab
		M+	97.6±2.6f	12.8±0.8bcde	27.0±9.0d	22.1±3.8fg	15.7±2.7g	1.9±0.3g	0.23±0.1b
V2	0	M-	77.4±4.7b	13.4±1.5bcdef	10.9±2.2a	10.8±2.7abc	6.4±2.0ab	0.8±0.2ab	0.32±0.1ef
		M+	83.6±4.3c	12.5±0.9abcde	18.6±2.8abc	20.2±4.0efg	12.1±2.3def	1.5±0.3def	0.32±0.1ef
	50	M-	90.8±2.4de	14.0±1.4ef	16.7±5.2abc	14.3±5.3bcde	8.5±2.3	1.0±0.3abcde	0.29±0.1d
		M+	88.4±2.6d	13.0±0.8bcdef	14.2±6.2ab	17.8±5.4def	10.9±3.4cdef	1.3±0.4cdef	0.26±0.1c
	100	M-	84.0±2.1c	13.2±1.0bcdef	15.4±7.2ab	18.5±8.2def	11.1±5.1cdef	1.4±0.6cdef	0.30±0.1de
		M+	91.0±3.5de	13.6±0.8cdef	23.0±6.6cd	24.8±5.1g	12.5±2.6efg	1.5±0.3efg	0.32±0.1ef
	150	M-	92.6±4.0e	14.3±1.6f	18.0±9.4abc	16.7±8.2cdef	9.2±4.6bcdef	1.1±0.6bcdef	0.32±0.0ef
		M+	91.2±3.4de	13.6±0.8cdef	14.0±4.0ab	18.4±1.8def	11±1.2cdef	1.3±0.1cdef	0.34±0.1fg
	200	M-	76.6±2.2ab	13.9±1.1def	11.1±4.5a	12.4±5.5abcd	7.6±3.6abcd	0.9±0.4abcd	0.39±0.1h
		M+	79.0±4.6b	12.6±1.4bcde	12.4±3.6ab	12.9±2.1abcd	7.9±1.2abcde	1.0±0.1abcde	0.35±0.1g

Conclusion

Mycorrhization significantly improves growth (plant height, number of branches) and the yield of Bambara groundnut. The effectiveness of these fungal species in increasing plant yield and nutrient uptake varies with single superphosphate doses. High phosphate levels (200 $kg.ha^{-1}$) reduce the effectiveness

of mycorrhization. We plan to test the effect of various combinations of local AMF strains found in the Bambara groundnut rhizosphere on yield and build a production unit for this biological fertilizer.

➢ **The problem solved**

This study shows that it is possible to reduce the amount of chemical fertilizer used in agriculture by combination with organic fertilizers. We showed that mycorrhization significantly increases the yield of Bambara groundnut. In landrace V2 for example, mycorrhizae increased the yield by 87.5% compared to the control. A maximum dose of 100 kg P2O5.ha-1 combined with mycorrhizae helped to improve Bambara groundnut yield without inhibiting the effectiveness of mycorrhization.

➢ Full description of the methodology used

At first, a field trial was set up. The soil was removed from the Bambara groundnut rhizosphere. This soil was sandwiched between two layers of sterile sand in 500 g bags perforated in the lower third section. Two sorghum seeds were sown in bags and placed in bins to retain moisture. The plants grew under these conditions in a greenhouse until maturity. At this stage, the plants were transferred to the shelter and stressed until the shoot and the substrate were dry. The substrate was poured on dry paper and stirred to homogenize the contents. For each bag, a 100 g sample of substrate taken from each trapping bag was used to extract the spores by wet sieving through a series of sieves ranging from 400 to 38 µm (Gerdemann & Nicholson, 1963). The spore suspension contained in the sieves was centrifuged on a 50% sucrose gradient (Mbogne et al., 2015). After rinsing, it was poured onto a gridded surface filter paper to facilitate counting of the spores. The latter were counted using a binocular loupe according to their size, their colors, their shapes and the presence or absence of a hypha suspensor. Mycorrhizal spores were mounted between slides and lamellae in PVLG (Polyvinyllactoglycerol) with Melzer's reagent (Josserant, 1983) and identified on the basis of morphological descriptions (sizes, colors, shapes, ornamentations, wall characteristics, hypha suspensor) published by INVAM (International Culture Collection of Arbuscular Mycorrhizal Fungi). The retained strains were multiplied by trapping to produce an inoculum needed for the field trial. The average number of spores is expressed per 100 g of dry substrate. A good inoculum must contain at least 30 spores per gram of substrate. The field trial was performed as described above.

➢ **Description of the technology and know-how required for the successful transfer**

For an effective transfer of technology, a production unit will be established in which the inoculum will be produced. Depending on the locality, different types of inoculum may be produced if it is realized that the dominant species are different after spore extraction and identification of native strains. Then, we will organize seminars with the producers not only to make them know the bio fertilizer, but especially to train them on its use. Subsequently, several production units or a distribution network will be set up to ensure the supply of producers in the main Bambara groundnut production areas.

➢ Description of all steps required to achieve marketability- analysis of the target population;- study of competitors;- product analysis (strengths and weaknesses);- study of opportunities by referring to a similar product (other fertilizers);- define the price of the product (mycorrhizae);- to advertise.

Table 5. Effect of mycorrhization on the distribution of Bambara groundnut pods. M-: without AMF, M+: with AMF (5 g.hole^{-1}).

Landraces	P_2O_5 (kg.ha^{-1})	AMF	% solitary pods	% Double pods	% Triple and quadruple pods
		M-	91	9	0
	0	M+	84	16	0
		M-	97	3	0
	50	M+	90	10	0
		M-	96	4	0
	100	M+	74	26	0
		M-	81	19	0
	150	M+	95	5	0
		M-	85	15	0
V1	200	M+	81	19	0
		M-	59	35	6
	0	M+	38	43	19
		M-	53	45	2
	50	M+	35	60	5
		M-	47	46	7
	100	M+	67	31	2
		M-	61	34	5
	150	M+	29	67	4
		M-	37	46	17
V2	200	M+	38	43	19

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