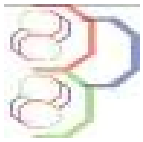

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Effect of native arbuscular mycorrhiza fungi inocula on the growth of Cowpea [*Vigna unguiculata* (L.) Walp.] in three differents agro ecological zones in Burkina Faso

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ABSTRACT

Objective: The use of symbiotic microorganisms as mycorrhizal fungi to improve the availability of nutrients to plants is of great importance in agriculture. In this study, we were interested in the response of cowpea, variety KVX 396-4–5-2D, to mycorrhizal inoculation in the context of selection of effective Arbuscular Mycorrhizal Fungi (AMF) to improve cowpea productivity.

Methodology and results: Six native AMF inocula (Talé Mossi, Pissila, Worou 1, Worou 2, Yakouta 1 and Yakouta 2), composed of spores contained in cowpea crop soils, were tested in cowpea plants under greenhouse conditions. Microscopic assessment of mycorrhizal colonization and biomass production were evaluated at the flowering/fruiting stage.

Results showed variability among inocula regarding their response to cowpea growth. The frequency of mycorrhizal colonization of inoculated plants was generally high (92%), but the intensities remained low (57.65%). However, Yakouta 2 inoculum has recorded the best frequency (92%) and intensity (57.65%) of mycorrhizal colonization. Inoculation of cowpea with Yakouta 2 inoculum resulted in a significant increase (4 folds) in shoot (3.33 g) and root (1.5 g) biomass compared to control plants (0.5 and 0.3 g respectively for shoot and root biomass).

Conclusion and application of findings: From these results, it appears that Yakouta 2 seems to be the most efficient for the growth of cowpea. This generally showed a beneficial effect of inoculation of cowpea suggesting that an endomycorrhizal strain selection could be carried on for cowpea inoculation *in situ*.

Key words: arbuscular mycorrhizal fungi, inoculation, cowpea, Burkina Faso.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is one of the most important grain legumes. In Burkina Faso, it occupies an important place in the diet of the population. The benefits associated with its production and strong demand both internal and export place cowpea in the strategic sector for food security in Burkina Faso. Unfortunately, its production is characterized by many constraints as low rainfall and the soil poverty in mineral elements such as nitrogen and phosphorus. This adverse conditions as not only a constraint on the vegetation, but also impede the development of the microbial

flora of the soil essential for plant nutrition and growth. However, it is well established that the association of legumes with arbuscular mycorrhizal fungi (AMF) can increase hydro mineral nutrition and significantly reducing parasitic infection and therefore allows to increase their productivity even when these plants grow on soils relatively poor (Hadiarto & Tran, 2011; Rooney *et al.*, 2011; Zhao *et al.*, 2015; Frosi *et al.*, 2016; Yooyongwech *et al.*, 2016). Moreover, the competitiveness with native strains of microorganisms makes inefficient inoculation with exotic AMF. The native strains seem more apt to this effect and it is therefore appropriate to find effective indigenous AMF to improve cowpea growth. The mycorrhizal symbiosis, a relationship involving an exchange for mutual benefits between plants and AMF, is one of the biological associations between plants and microorganisms widely studied. These microorganisms improve hydro mineral nutrition of the host plant and in return received energy and carbonaceous resources they need to complete their life cycle (germination, growth, reproduction) (Harrison *et al.*, 2002). However, the symbiosis functioning is not always optimal and depends on soil characteristics, AMF species, host plant mycorrhizal dependence and environmental conditions. Thus, native AMF strains may be more suitable and their effectiveness could be improved by the inoculation practice with selected strains.

For this purpose, in this study we were interested in the response of a cowpea variety commonly grown in Burkina Faso to native mycorrhizal inoculation in a perspective of selecting effective mycorrhizal fungi strains to improve legume productivity.

MATERIAL AND METHODS

Material

Sampling sites: The study sites are located in 3 climatic zones of Burkina Faso (table 1). Soils (0–40 cm) were collected in 6 fields spread over 4 sites of the 3 climatic zones. The physico-chemical characteristics of soils of the 6 selected fields are presented in table 3. Soil sampling was conducted in cowpea flowering/fruiting stage.

Table 1: Characteristics of soil sampling sites

Sample site	Climatic zone	Localization	Rainfall
Yakouta 1	Sahelian	14°04' 57.0" N 0°08' 35.0" W	< 600 mm
Yakouta 2	Sahelian	14°04' 02.8" N 0°06' 41.9" W	< 600 mm
Pissila	Sudan-Sahelian	13°10' 23.0" N 0°49' 41.0" W	600 mm – 900 mm
Talé Mossi	Sudan-Sahelian	13°09' 58.0" N 0°53' 32.0" W	600 mm – 900 mm
Worou 1	Sudanian	11°08' 48.0" N 2°14' 42.0" W	> 900 mm
Worou 2	Sudanian	11°09' 16.0" N 2°15' 23.0" W	> 900 mm

Plant and Fungal material: The cowpea variety KVX 396-4-5 - 2D (KVX) commonly cultivated in Burkina Faso was used. The fungal material consists of AMF spores extracted from the soils of the study sites taken from the cowpea rhizosphere and used to produce inocula.

Culture substrate : The culture substrate was a soil collected around Ouagadougou and sterilized (121 °C, 20 min), whose characteristics are as follows: clay, 3.92%; total silt, 5.88%; total sands, 90.2%; total organic matter, 0.331%; total carbon, 0.192%; total nitrogen, 0.016%; C/N, 12; total phosphorus, 172.52 ppm; available phosphorus, 1.74 ppm; pH H_2O (p/v: 1/2, 5), 6.44.

Methods

Inocula production: Inocula production consisted to multiply spores of the native fungi strains contained in each soil of study sites. A mycotrophe plant, maize (*Zea mays*) was grown in 2 l pots containing, 250 g of soil (soil of fields containing native fungi) and 1750 g of sterile culture substrate under shelter used as a greenhouse. The six (6) field soils (Talé Mossi, Pissila, Worou 1, Worou 2, Yakouta 1 and Yakouta 2) have been used. Each treatment is repeated 3 times. Control received 2000 g of sterile culture substrate. These pots are regularly sprayed with tap water at field capacity. The inoculum was obtained after 6 months of culture and consisted of a mixture of spores, mycorrhizal roots fragments and soil (Spores: 1020–2230 spores/100 g sol); roots: 100% colonization).

Implementation of the test

Treatment of seeds and germination: The cowpea and maize seeds were superficially disinfected by soaking in ethanol 96% for 3 min, rinsed thoroughly with sterile

distilled water and then disinfected in calcium hypochlorite solution ($CaCl_2O_2$ at 3.3%, w/v) during 3 min and finally rinsed thoroughly with sterile distilled water before sowing. Concerning cowpea, each pot received three seeds and a wedge is carried one week after the plants emergence to leave only one plant per pot. For maize, ten seeds were sown per pot. **Inoculation and experiment lay out:** Before pots filling, culture substrate was homogenized, sieved with a 2 mm sieve and sterilized at 121 °C for 1 h. Pots of 1.5 litres have been used. Each pot received 1000 g of sterilized culture substrate. The inoculation was carried out at sowing time with 10 g of inocula for each inoculated treatment. Control pots have not inoculated. A total of 7 treatments (including 1 control) with 5 replicates per treatment has been retained. The device is a simple randomization complete block design. **Evaluation of density and identification of fungi spores of mixed inocula:** After inocula production, spores density was evaluated in each mixed inoculum. They were extracted by wet sieving according to the method of Gerdemann & Nicolson (1963) slightly modified. In a beaker (1 l), 100 g of inoculum is suspended in 500 ml of tap water and agitated vigorously to separate the fungal propagules and soil particles. The suspension is poured onto on sieves bunk decreasing mesh (250-50 µm). The same sol is again submerged, stirred, and the wet sieving repeated several times until clear water is obtained after agitation. The content of each sieve is retrieved, suspended in tap water in centrifuge tubes (25 ml corex tubes). A sucrose solution (60%) was injected using a syringe at the bottom of the tubes. The tubes were centrifuged at 3000 rpm for 10 minutes. Spores contained in each centrifuge tubes are collected at the water/ sucrose solution interface and rinsed with distilled water in a sieve of 50 µm. Three replicates of 100 g for each inoculum was used. After water extraction, the spores were observed under a stereomicroscope (magnification = 0,7x, 2x and 4x) and manually grouped according to morphological character as colour, shape, size, and then enumerated. Each type of spores was then mounted in Polyvinyl-lacto-glycerol (PVLG) (Koske & Tessier, 1983) supplemented with Melzer's reagent (v/v) (Morton, 1988) and observed under the microscope (magnification= 4x, 10x and 40x) for colour, thickness of the wall, number of wall layers, sporogenous cells, spores diameter, spores hypha suspensory attachment mode, and sometimes, spores ornamentation. Each type of spores was compared to AMF specimens of INVAM collection (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi, http://www.invam.caf.wvu.edu) and a genus was assigned to each homogeneous spores group.

Measurement of various parameters

Growth parameters: Plants height of all treatments were measured at the flowering/fruiting stage (45 days after sowing). To evaluate shoot and root biomass, all plants were harvested at the flowering/fruiting stage. All roots was harvested. These roots were cut at 1 cm long; and a subsample of 10 g was taken for the mycorrhizal colonization assessment. Remaining roots and plants aerial parts were dried at 70 °C for 72 hours to evaluate biomass.

Staining for Mycorrhizal Colonization: About 10 g of roots from each treatment was thoroughly washed and placed in falcon tubes and then cleared using 10% KOH. They were heated in 90 °C water bath for one hour. The roots were washed with tap water. Staining was then done by adding 0.05% trypan blue in lactic acid and heating in 90 °C water bath for 30 minutes (Phillips & Hayman, 1970) and observation under the microscope (magnification=10x). The mycorrhizal frequency and intensity were estimated by Trouvelot *et al.* (1986) method.

Statistical analysis: All data were tested for homogeneity of variance by Bartlett test before analyses. The % data were arcsine (√x) transformed, while other data was log (x+1) transformed wherever necessary to fulfill the assumptions of ANOVA. Data were statistically analyzed using the one-way analysis of variance (ANOVA) with XLSTAT 2015 statistical software and the means were compared using the Newman-Keuls test ($p < 5\%$).

RESULTS

Density and diversity of spores of AMF mixed inocula: Table 2 shows density and diversity of spores contained in the mixed inocula. Overall, the spores' density of all sites remains high. For AMF identification, in comparison to the specimens of INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi, www.invam.caf.wvu.edu) collection, the spores of inocula were grouped into four genera including *Scutellospora, Glomus*, *Entrophospora* and *Gigaspora*. *Entrophospora* genus is close to *Entrophospora infrequens* (Hall) Ames and Schneider species. A high density and diversity of AMF spores were observed in all inocula. Statistical analyses showed significant differences in the number of spores between different inocula ($P < 0.0001$). Pissila inoculum recorded the higher values (2230 spores/100 g of soil and 4 genera) and the

lowest density is observed on the Yakouta 2 inoculum (1020 spores/100 g of soil).

Table 2: Density and diversity of native spores per 100 g of soil from sites (Yakouta1, Yakouta 2, Talé Mossi, Pissila, Worou 1 and Worou 2).

Samples	Number of spores	Genus
Pissila	2230.33(38.41)[1]a[2]	*Scutellospora; Gigaspora; Glomus; Entrophospora.*
Talé Mossi	1439.67(43.88) b	*Scutellospora; Gigaspora; Glomus.*
Worou 1	1553.33(40.55) b	*Scutellospora ; Gigaspora ; Glomus.*
Worou 2	1206.67(17.64)c	*Scutellospora ; Gigaspora ; Glomus.*
Yakouta 1	1246.33(66.93)c	*Scutellospora ; Gigaspora ; Glomus.*
Yakouta 2	1020(34.64)d	*Scutellospora ; Gigaspora ; Glomus.*
Significance level	< 0.0001	

[1] Standard error of the mean
[2] Values in the same column (spore numbers) with the same letter are not significantly different according to the Newman-Keuls test (p<0.05).

Spore abundance, when compared to the chemical characteristics of the soils from the study sites (table 3) was negatively correlated with the soil's available phosphorus (R^2=0.3, P = 0.2) (Fig. 1).

Table 3: Physico-chemical characteristics of soil sampling sites

Samples	Clay (%)	Total silt (%)	Total sand (%)	Total organic matter (%)	Total carbon (%)	Total nitrogen (%)	C/N	Total phosphorus ($mg.kg^{-1}$)	Available phosphorus ($mg.kg^{-1}$)	pH H_2O
Yakouta 1	3.92	5.88	90.2	0.557	0.323	0.029	11	258.78	23.68	6.86
Yakouta 2	3.92	5.88	90.2	0.529	0.307	0.027	11	258.78	26.03	6.91
Pissila	7.84	15.69	76.47	0.809	0.469	0.038	12	123.23	2.75	6.03
Talé Mossi	5.88	15.69	78.43	0.941	0.546	0.038	14	160.2	2.82	6.07
Worou 1	5.88	17.65	76.47	1,324	0.768	0.058	13	166.36	3.15	5.82
Worou 2	5.88	17.65	76.47	1,605	0.931	0.077	12	135.55	2.82	6.12

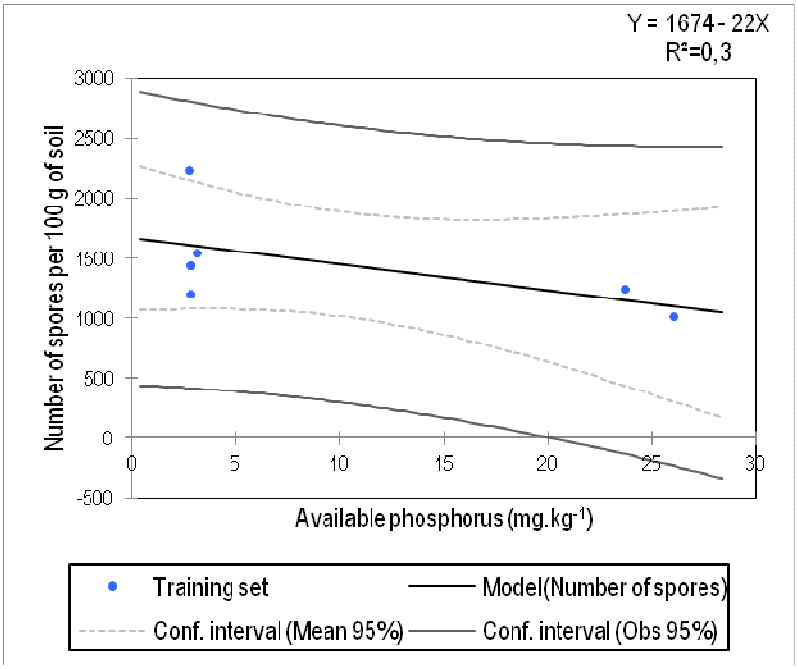


Figure 1: Regression of number of spores by Available phosphorus (mg.kg^{-1})

Growth parameters: Growth parameters (table 4) show variability depending inocula type. For plants height, inoculation with Yakouta 2 and Worou 1 improved significantly ($P < 0.0001$) cowpea growth compared to control treatment. This enhancement is 81.5% and 53.3% higher than control for inoculation with Yakouta 2 and Worou 1 respectively. The highest value is obtained with Yakouta 2 (19.6 cm). Concerning KVX plants biomass, statistical analyses show also that inoculation with Yakouta 2 and Worou 1 improved significantly ($P < 0.0001$) shoot and root biomass. However, total biomass is significantly improved ($P < 0.0001$) by inoculation with Yakouta 2, Worou 1 and Pissila. The highest biomass is obtained with Yakouta 2 mixed inoculum.

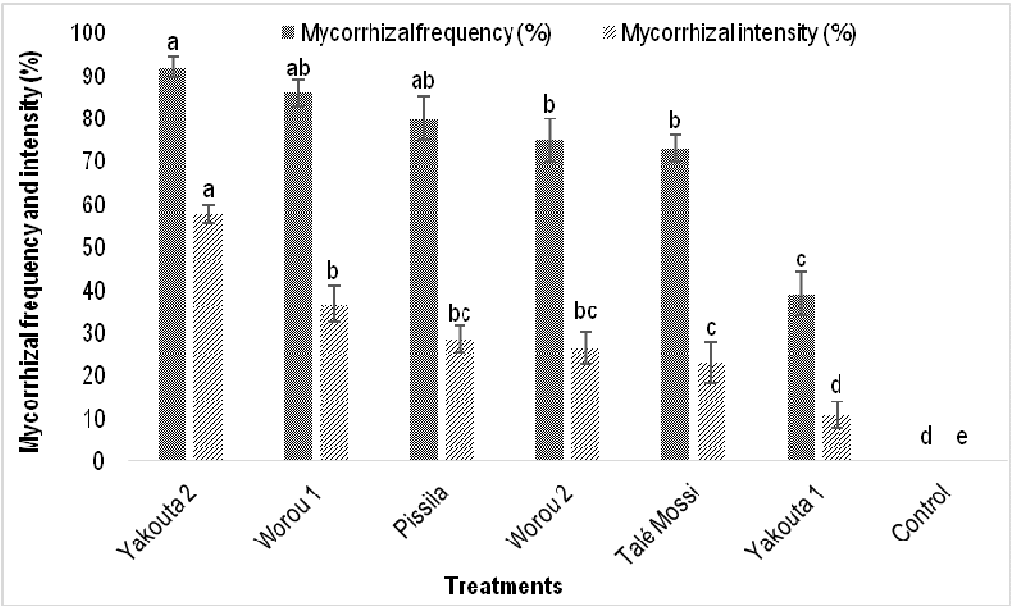
Table 4: Plant height, aerial biomass, root and total biomass 45 days after sowing of cowpea variety of KVX 396-4–5-2D (KVX) inoculated with 6 mixed inocula (Yakouta1, Yakouta 2, Talé Mossi, Pissila, Worou 1 and Worou 2).

Treatments	Height (cm)	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
Yakouta 2	19.6(0.62)[1]a[2]	3.33(0.25)a	1.5(0.11)a	4.83(0.27)a
Worou 1	17.1(0.33)b	2.72(0.17)a	1.25(0.05)b	3.97(0.21)a
Worou 2	11.1(0.43)c	0.87(0.2)b	0.29(0.09)c	1.17(0.21)bc
Talé Mossi	10.7(0.54)c	0.65(0.09)b	0.29(0.02)c	0.94(0.11)c
Pissila	10.2(1.37)c	1.68(0.66)b	0.49(0.13)c	2.17(0.67)b
Yakouta 1	9.4(0.99)c	0.69(0.07)b	0.47(0.04)c	1.16(0.04)bc
Control	10.8(0.49)c	0.5(0.05)b	0.3(0.04)c	0.8(0.07)c
Significance level	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[1] *Standard error of the mean*

[2] *Values in the same column (plant height, shoot, root and total biomass) with the same letter are not significantly different according to the Newman-Keuls test (p<0.05).*

Mycorrhizal frequency and intensity: The results on mycorrhizal parameters are assigned in figure 1. Overall, mycorrhizal frequency is high, but the intensity remains low for these mixed inocula. Statistical analyses showed significant differences between inoculated treatments and control. However, there is variability between inoculum. Yakouta 2 gives the highest mycorrhizal frequency (92%) and intensities (57.62%).



Values of the same parameter (mycorrhizal frequency or intensity) with the same letter are not significantly different according to the Newman-Keuls test (p<0.05) Significance level for mycorrhizal frequency and intensity (P < 0.0001).
Figure 1: The mycorrhizal Frequency and intensity of KVX 396-4–5-2D plants inoculated or not with mixed inocula (Yakouta1, Yakouta 2, Talé Mossi, Pissila, Worou 1 and Worou 2).

DISCUSSION

The results of spores' number show a variability depending study sites. When comparing the spores density and study soils sites physicochemical characteristics, it appears that spores abundance is inversely correlated to soil mineral richness, especially phosphorus. This could be explained by the fact that plant prefers to directly use nutrients when they are available in the soil and directly accessible by these roots. Which does not promote the mycorrhizal symbiosis establishment reducing the hyphae abundance in soil so the proliferation AMF spores (Haro, 2016). These results are in agreement with those of Olsson *et al.* (1997), Saidou *et al.* (2009) and Alguacil *et al.* (2010), which showed that the soil phosphorus richness decreases the spores abundance of this inoculum. Also, Shi *et al.* (2014) showed that fertilization reduced AMF hyphae abundance in the soil. The arbuscular mycorrhizal fungi genus found in our study sites do not seem to be specific either to the study site or to the climatic zone. However, Pissila sites have recorded the greatest diversity (four different genera). This could be explained by low mineral content of the soils in this study site, including phosphorus. The high content of phosphorus in the soils of the other study sites resulted in a reduction of the hyphae, so abundance of AMF and the mycorrhizal species. These results are in agreement with the conclusions of Olsson *et al.* (1997) and van Diepen *et al.* (2010). Alguacil *et al.* (2010) and Liu *et al.* (2012) showed that inorganic fertilizers often reduce the AMF diversity. The result of mycorrhizal frequency and intensity shows that no control treatments were colonized by mycorrhiza suggesting that the differences observed for the different parameters are mainly due to the effect of inoculated AMF strains. The mycorrhizal inoculation with mixed native inocula improves cowpea variety KVX growth. However, this improvement depends on the parameters (biomass and height) and the inoculum used. This could be explained by the host preference of the AMF that would determine their effectiveness. Similar results were found by Mensah *et al.* (2015) who showed that the response of plants to mycorrhizal inoculation with AMF ranges from very beneficial to harmful. In addition, mixed inocula contain at least 3 different genera whose effects may be added. These results are in accordance with those of Haro *et al.* (2012). Indeed, these authors have shown that the use of mixed native strains is more suitable than single AMF exotic strains, as mixed inocula contain at least two different genera whose effects can be synergetic. In

addition, each genus could contain several AMF species. The improvement of KVX growth by inoculation could be the effect of the improvement of host plant hydro mineral nutrition. Our results are in accordance with those of Haro *et al.* (2015), Diop *et al.* (2013) and Zhu *et al.* (2015) which showed that biomass production is enhanced by mycorrhizal inoculation. However, these mycorrhizal parameters are not proportional to the number of spores contained in each inoculum. Plants inoculated with Yakouta 2 inoculum, which has recorded the lowest number of spores, presented the highest mycorrhizal frequency and intensity. These results, indicates that AMF contained in Yakouta 2 inoculum are performants to improve KVX growth.

CONCLUSION

This study, conducted with the aim to select native mycorrhizal strains able to improving cowpea growth showed that the use of native strains could be well appropriate. It also showed that the mixed inoculum contains effective strains. It appears from this study that this mixed inoculum can be used as AMF biofertilizers. These AMF biofertilizers could be used to improve the cowpea growth. However, it appears that the effectiveness of the AMF symbiotic activity depends on mycorrhizal strains. The mixed inoculum Yakouta 2 was more beneficial to KVX, but it would be interesting to carry out in situ inoculations in order to confirm their efficiency.

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