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Improvement of arbuscular mycorrhizal fungi inoculum production by nutrient solution concentration and soil texture variation

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Key words: Inoculum production, arbuscular mycorrhizal fungi, nutrient solution, soil texture.

Abstract

Arbuscular Mycorrhizal Fungi (AMF) can increase the yield of plants from 50 to 200 %, but the large scale multiplication of AMF inocula is difficult because of their trophic nature, and this represents a big challenge in tropical areas. Within the framework of the study, two separate experiments were conducted in order to determine the most favorable conditions for AMF spore production. The effect of Rorison's nutrient solution (0, 4 and 8 ml/l) was tested on 6 types of AMF strains for spores production, while in the second experiment; it was the effect of soil-sand mixture variation (5-57 % clay content) that was evaluated in AMF spores production. A local variety of *Sorghum bicolor* was used as the host plant during the trials. Experiments were performed in completely randomized design with 4 replicates. Results showed that Rorison's nutrient solution and soil texture significantly ($P<0.05$) influenced plant growth, symbiotic and biochemical parameters. Nutrient solution induced significant increase in root colonization (5 to 36 %), and AMF spore production (12 to 23 spores/g of soil). The highest concentration of Rorison's nutrient solution promoted more spore formation, but that was not translated in plant yield. Soil texture variation had a significant impact on AMF root colonization and spores production, since mixture of sand and clay, with clay variation from 20-43% was found to favor both parameters. These data suggest that soil texture variation and nutrient solution concentration can significantly improve AMF spores production and *Sorghum* symbiotic performances.

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Introduction

Arbuscular mycorrhizal fungi **(**AMF) are associated with the roots of over 80 % of terrestrial plant species (Dalpé et Aiken, 1997). AMF are obligate symbionts that need a compatible plant host to complete their life cycle and produce spores, which are the main source of propagules used in crop production (Silva *et al.*, 2005). Among biofertilizers, mycorrhizal fungi represent the most significant group of soil microorganisms. Mycorrhizal colonization has been reported to induce many morphological, physiological and biochemical changes beneficial to host plants. Despite the importance of AMF in agriculture, their industrial production is fastidious, and many farmers could not easily access to AMF inoculums (Yeasmin *et al.* 2007).

Several methods to improve AMF inoculum production have been developed, such as the aeroponic (Hung *et al.*, 1988), monoxenic cultivation (St-Arnaud *et al.*, 1996) and the hydroponic (Hawkins and George, 1997). Although these methods have the advantage of producing spores suitable for studies on ontogeny (Wu *et al.*, 1995), ultrastructure (Maia and Kimbrough, 1998), and molecular biology (Sawaki and Saito, 2001); the high cost of their installation and maintenance prevent their use (Millner and Kitt, 1992). In addition, the reduction of the germination rate (Hung *et al.*, 1988) and production of spores with smaller diameter (Pawlowska *et al.*, 1999) have been observed in vitro culture compared with the traditional methods, in which the plant is cultivated in containers with different substrates. Substrate-based cultivation of AMF in pots, bags, or beds is the most widely adopted technique for AM fungal inoculum production because relatively low technical support is needed and consumables are cheap (Douds *et al.* 2006).

Environmental factors and soil conditions that favor the host plant growth tend to maximize mycorrhizal infection and sporulation (Monther and Kamaruzaman, 2012). Owing to the multiple factors affecting mycorrhization, this symbiosis is not well understood and it has not yet been efficiently exploited despite their enormous potentials (Covacevich, 2012). Gaur and Adholeya (2000) obtained maximum production of AMF inoculum in sand, which may have allowed better soil aeration, drainage, and oxygen supply, favoring root growth, in comparison to other tested pure substrates. Another advantage in AMF production in pot with sand is the low maintenance cost (Sylvia and Jarstfer, 1994). It is possible to enhance production of spores using Hoagland nutrient solution and mixtures of compost and vermiculite (Douds et Schenck, 1990; Douds *et al.*, 2006). One of the most important mechanisms to understand the effect of AMF on plant root system is the nutrition supply (Davamani *et al.*, 2010). However, a majority of studies have focused on the significance of phosphorus in AMF colonization of plant root system.

Few studies report the effects of nutrients and soil texture on AMF inoculum production in the world. In the present study, the influence of both nutrient concentration solution and soil texture variation in pot culture on AMF inoculum production was investigated.

Materials and methods

Study site and analysis

Protein analysis of plant, microbes and physico-chemical analysis of soils were conducted at the Biotechnology Centre of the University of Yaounde I (UYI) at Nkolbisson and in the Institute of Agricultural Research for Development (IRAD) of Cameroon respectively. The field experiment was conducted at the University of Yaoundé I Campus of Ngoa Ekelle, Cameroon.

Soil and sand collection

The different experiment were conducted with soils collected at the depth of 0-15 cm, in February 2010, from two different farms, one from Nkolbisson (3°51'N, 11° 30'E; grey oxisol), and the other from Soa (3°59'N, 11° 36'E; red oxisol), labeled SN and SS, respectively. Cassava (*Manihot esculentus*) and maize (*Zea mays*) were the crops grown on these farms. They physical and chemical characteristics are given

in Table 1. Coarse sand was collected from the Sanaga river, the larger river of Cameroon, about 918 km flowing in the Centre, Littoral and South regions in Cameroon. Soil and sand samples were air dried and sieved to pass 2 mm. SN was used as substrate to evaluate the effect of Rorison's nutrient solution and SS for the evaluation of soil texture on AMF inoculum production respectively.

Soil-sand mixtures.

A quantity of 240 kg of SS and 100 kg of coarse sand were used. Six soil-sand mixtures were prepared in different proportions (v:v): 0:1 (M0), 1:3 (M1), 1:1 (M2), 2:1 (M3), 3:1 (M4) et 1:0 (M5) and the granulometric characteristics of the mixtures were determined (Table 2).

The report of the different values of fractions on French textural diagram (17 classes) show that the mixtures had different soil textures according to Richer de Forges *et al.* (2008). The M0 mixture had a sand texture, M1 a sand clay texture, M2 a very clayey sandy texture, M3 a clayey sandy texture, M4 a clayey sandy texture and M5 a clayey texture.

Experimental designs

Two separate experiments were done.

Experiment 1: Rorison's nutrient solution: In order to evaluate the effect of Rorison's nutrient solution on AMF inocula production (Table 3), the experiment was performed in a completely randomized design. It was a 3 x 6 x 4 factorial design: 3 concentrations of Rorison's nutrient solution at 0, 2 and 4 times the stock solution (0, 4 and 8 ml/litre) and 6 types of AMF inocula (S1, S2 , S3, S4 and S5) + 1 control (without AMF), in 4 replicates, in a total of 72 experimental plastic pots. The substrate, 300 kg of SN (dry weight) was mixed with coarse sand in a 1:3 proportion (v:v) of soil and sand. The substrate was sterilized in an autoclave (120° C for 1h). A quantity of 4 kg of soil/sand mixture of each treatment was introduced in 72 plastic pots of 5 litre volume. The pots were irrigated once a week for three months with different Rorison's nutrient solutions (Table 4) and with distilled water the other day to avoid accumulation of salts.

Experiment 2: Soil-sand mixtures (soil textures): In order to evaluate the effect of soil textures or soil-sand mixtures on AMF inoculum production, the experiment was performed in a completely randomized design. It was in factorial of 6 x 2 x 4: 6 soil-sand mixtures in proportions of: 0:1 (M0), 1:3 (M1), 1:1(M2), 2:1(M3), 3:1(M4) et 1:0 (M5) x 1 type of AMF inoculum (S6) + 1 control (without AMF), in 4 replicates, in a total of 48 experimental plastic pots of 5 liter content. The substrates were sterilized in autoclave (120° C for 1h). A quantity of 4 kg of each mixture was introduced in 48 plastic pots. The pots were irrigated with deionized water at the same volume to avoid accumulation of salts.

Source of fungal inoculum and inoculation

The sources of selected AMF inocula (Table 3) were from the Soil Microbiology Laboratory resource collection, the Biotechnology Center of the University of Yaoundé I. They were propagated with *Sorghum* plants pots containing sterile substrate. These AMF are well defined and have been used for many experiments in nursery or in fields (Nwaga *et al.*, 2004). Pots were irrigated with Rorison's nutrient solution (twice a week) and tap water to bring the soil moisture to field capacity the other days. After four months, plant shoots were cut off and pot materials containing soil, mycorrhizal roots, hyphae and spores were thoroughly mixed and used as fungal inoculum or biofertilizer mycorrhiza. Root colonization percentage (Kormanik et Mc Graw, 1982) and number of spores (Schenck, 1982) were assessed to determine inoculum potential. Both inocula had an average of 33-53% root colonization and 3-6 spores/g spores density.

Based on the spore density, 50 spores/g approximately for each AMF treatment (S1= 17 spores/g, S2= 12 spores/g, S3= 10 spores/g, S4= 10 spores/g, S5= 9 spores/g and S6= 9 spores/g), were inoculated in each pot. The same quantity of sterilized AMF substrate was added to the control pots.

Host plant and nutrient solution

Seeds of sorghum (*Sorghum bicolor* L.), Damougari cultivars were obtained from IRAD Maroua Station, Cameroon. Seeds were surface sterilized with 1% (v/v) sodium hypochlorite solution for 3 min, and were rinsed three times with distilled water. Each pot received three seeds of sorghum. All the pots of the two experiments were allowed to grow under natural conditions. After two weeks of sowing, the treated pots were irrigated once a week for three months with the Rorison's nutrient solution, while control was irrigated with distilled deionized water.

Harvesting and measuring the parameters

Plants were harvested after 14 weeks and the pots dried for 2 weeks to stimulate sporulation of AMF. Growth parameters (Plant height (PH), Shoot dry weight (SDW), yield), symbiotic parameters (Mycorrhizal responsiveness (MR), Mycorrhizal dependency (MD), root colonization percentage (RC), number of spores (NS)) and protein content (PC)) of 12 plants per pot per treatment were determined.

The plants height was measured from the base to the apex of the stem. Dry weight of shoots was determined after oven-dried at 70◦C for 48 h to constant weight in order to obtain shoot dry weight (SDW). MD values were calculated according to Plenchette *et al.* (1983) and were determined by expressing the difference between dry weights of mycorrhizal with that of non-mycorrhizal plants. MD was calculated by % MD = (Dry weight of mycorrhizal plant – Dry weight of non mycorrhizal plant) x 100/ (Dry weight of mycorrhizal plant). MR was calculated by % MR = (Dry weight of mycorrhizal plant – Dry weight of non mycorrhizal plant) x 100/ (Dry weight of non mycorrhizal plant).

A total of 30 fragments of stained roots (about 1 cm long) for each treatment were cleared for 10 min in 10% KOH at 121°C in autoclave, rinsed with water, acidified in 5% HCl for 2 min, and stained for 30 min in 0.01% Fuchsine acid dissolved in destaining solution (14:1:1 (v/v/v) lactic acid:glycerol:water) (Kormanik et Mc Graw, 1982). These roots were examined with a compound microscope (40 and 100 x) to ascertain the presence of AM structures. Colonization percentage was calculated as colonization = (Number of colonized segments/number of total segments viewed) × 100. The average number of AMF spores /g of soil were determined according to Schenck (1982). The soluble protein content of the shoot plants was determined according to Bradford (1976).

Statistical analysis

Data presented as the means of 4 replicates ± standard deviation were analyzed by ANOVA test using the software SPSS 16.0 for windows. Differences bbetween the treatments were tested using the Student-Newman-Keuls test at 5% level.

Results

Experiment 1

Growth parameters

There were significant differences between fungi treatment in each level of nutrient solution. Significant differences were observed in the influence of nutrient solution on AMF plant colonization. Twice concentrated of Rorison solution combined with *Gigaspora margarita* giving the highest performances (Table 5).

The same level of nutrient solution combined with *Scutellospora gregaria* performed better in term of increasing SDW (Table 6). The application of 4 times Rorison's nutrient solution concentration significantly reduced the PH, SDW, and yield of the *Sorghum,* compared to other treatments (Fig. 1). AMF inoculation significantly enhanced the yield of the *Sorghum*. However, the best yield was observed when 2 times nutrient solution combined with *Scutellospora gregaria* was applied. Generally, growth parameters decreased significantly with an increase in nutrient concentration solution, compared to non-mycorrhizal treatment.

Symbiotic parameters

The results showed that AMF spore production and RC of the host plant varied significantly with regard to Rorison's nutrient concentration and the types of

inocula. Host plants treated with *Glomus hoï* (S1) and *Gigaspora margarita* (S4) that did not receive nutrient solution showed highly significant RC compared to control and other treatments (Fig. 2).

On the other hand, higher significant RC were obtained in *Sorghum* treated with Myco 2 (S3) and *Scutellospora gregaria* (S5), when 2 times Rorison solution were applied compared to other treatments. Significant RC (36%) of host plant treated with *Glomus intraradices* + *Scutellospora gregaria* (S2) was only found when 4 times nutrient solution was applied.

Table 1. Physical and chemical characteristics of soils used in this study.

Parameters	Soils	
	SN	SS
Moisture (%)	0.81	7.35
Total organic matter (%)	2.49	0.62
Organic carbon (%)	1.45	0.36
Total nitrogen (%)	0.15	0.12
C/N	9.67	3.00
Available P (mg/kg)	0.03	4.90
Al^{3+}+ H^{+} (cmol/kg)	0.01	0.02
Ca^{2+} (cmol/kg)	0.90	1.07
Mg^{2+} (cmol/kg)	0.20	0.57
K^{+} (cmol/kg)	0.07	0.24
Na^{+} (cmol/kg)	0.02	0.01
S (cmol/kg)	1.19	1.89
CEC ou T (cmol/kg)	1.82	4.41
V=S/Tx100	65.38	42.85
pH water	6.43	6.23
pH KCl	5.76	5.62
Clay (%)	8.00	53.25
Loam (%)	2.47	14.50
Sand (%)	89.53	32.25

Table 2. Granulometric characteristics of different soil-sand mixtures.

Fractions (%)	Soil-sand mixtures					
	M0	M1	M2	M3	M4	M5
Clay	5.11	20.40	32.75	38.75	42.69	57.25
Loam	1.14	2.35	4.10	7.68	11.80	12.25
Sand	93.75	77.25	63.05	53.57	45.50	30.50

Significant increase in NS of *Glomus hoï* (S1) and *Glomus intraradices* + *Scutellospora gregaria* (S2) in *Sorghum* was found when 2 times nutrient solution was applied compared to other treatments (Fig. 3). The application of 4 times nutrient solution induced significant higher NS of Myco 2 (S3), *Gigaspora margarita* (S4) and *Scutellospora gregaria* (S5) than other treatments.

Experiment 2

Growth parameters

The results showed that growth parameters of *Sorghum bicolor* varied with the different soil textures. Non mycorrhizal control plants produced the least height and SDW (Fig. 4, 5 and 6) compared to the mycorrhizal plants. Mycorrhizal inoculation significantly increased the PH and SDW of the host plant. These parameters increased with clay percentage from 5 to 43%. SDW and PH values varied with soil texture.

Table 3. Characteristics of arbuscular mycorrhizal fungi used. ud: undetermined, RC (Root Colonization), NS (Number of Spores), DRB (Dry Root Biomass), RD (Root Development index).

Types of mycorrhiza fungi	Characteristics			
	RC (%)	NS (Spores g^{-1})	DRB (g)	RD (1-5)
S1: *Glomus hoi*	33	3	57	2
S2: *Glomus intraradices+Scutellospora gregaria*	40	4	99	4
S3: *Glomus clarum+Gigaspora margarita* (Myco 2)	57	5	57	2
S4: *Gigaspora margarita*	53	5	99	3
S5: *Scutellospora gregaria*	ud	6	97	4
S6: *Glomus intraradices+Glomus clarum*	ud	5	ud	ud

Table 4. Rorison's nutrient solution composition.

Stock solution: macro-nutrients (g/l)	
Solution A: $Mg\ SO_4.\ 7H_2O$	120.02
Solution B: $Ca\ (NO_3)_2,\ 4H_2O$	238.04
Solution C: $KH_2PO_4,\ 3H_2O$	115.38
Solution D: micro-nutrients (g/l)	
Fe DTA	12.500
$MnSO_4,\ 4H_2O$	1.121
H_3BO_3	1.142
$(NH_4)_6\ Mo_{24}.4H_2O$	0.930
$ZnSO_4.7H_2O$	0.220
$CuSO_4.5H_2O$	0.198

The highest PH and SDW were obtained with soil-sand mixture M4: 3:1(v:v). This mixture had a clay sand texture (43% of clay). M4 mixture treated with AMF promoted better PH and SDW compared to other textures (Fig. 4, 5 and 6). We can conclude that plant height and dry biomass significantly varied with the soil texture.

Biochemical and symbiotic parameters

Soil-sand mixtures significantly influenced shoot protein concentrations (Fig. 7). Mycorrhizal inoculation significantly increased PC of the host plant. The highest PC was observed in M0 and M4, compared to other treatments. The minimal PC was observed in M1, the best when soil-sand mixture M4: 3:1(v:v) (43% of clay) was used as substrate compared to other treatments, at the exception of M0. PC of non mycorrhizal and mycorrhizal plants decreased at (M4) texture. M0 and M4 mixtures combined with AMF were the best textures which promoted PC. So, PC increased when percentage of clay increase.

The highest MR and MD were observed in M2 and M5 soil-sand mixtures compared to other treatments (Table 7). The minimal MR and MD were observed in M4.

MR and MD of the host plant varied with soil textures. But, these parameters decrease in M3 and M4, and continued to increase after M4. In general MR and MD increase when percentage of clay increases.

Root colonization and spore density were significantly influenced by the soil textures. The RC decrease when the soil-sand mixtures varied or the percentage of clay increased (Fig. 8). The highest RC percentage was observed in M1 (sand-clay texture) and the minimal in M5 (clay texture). These results showed that RC varied with the soil texture and sand-clay texture promoted the RC. On the order hand the clay texture significantly reduced the RC.

Table 5. Plant height (cm) of *Sorghum* 4 months after sowing (4 MAS) in variable level of nutrient solution.

	Rorison's nutrient solution concentration (times)		
AMF	0	2	4
S0	74.07±1.75 bcdef	77.25±3.00 bcde	77.75±2.84 bcde
S1	72.26±3.14 ef	72.74±1.23 def	79.00±4.34 b
S2	78.06±1.53 bcd	73.10±2.99 cdef	69.64±3.12 f
S3	77.08±1.42 bcde	78.71±3.51 bc	47.95±1.48 i
S4	79.11±1.96 b	90.87±1.05 a	64.28±1.72 g
S5	78.22±2.53 bcd	77.26±3.27 bcde	56.42±0.39 h

Means followed by the same small letter (line) does not differ (P< 0.05).

Table 6. Shoot dry weight (g/plant) of *Sorghum* 4 MAS.

	Rorison's nutrient solution concentration (times)		
AMF	0	2	4
S0	2.42±0.09 cd	2.47±0.12 c	2.79 ± 0.05 ab
S1	2.40±0.21 cd	2.05±0.13 e	2.55 ± 0.12 bc
S2	2.54±0.08 bc	2.38±0.14 cd	2.67 ± 0.15 bc
S3	2.39±0.06 cd	2.55±0.09 bc	1.98 ± 0.09 e
S4	2.82±0.15 ab	2.82±0.23 ab	2.03 ± 0.05 e
S5	2.79±0.16 ab	3.01±0.20 a	2.14 ± 0.12 de

Means followed by the same small letter (line) does not differ (P< 0.05).

Table 7. Mycorrhizal responsiveness (MR) and dependency (MD) of sorghum in different soil textures.

Soil textures	MR (%)	MD (%)
M0 (sand texture)	+550	+85
M1 (sand-clay texture)	+694	+87
M2 (clay too sand texture)	+2444	+96
M3 (clay-sand texture)	+353	+78
M4 (clay-sand texture)	+97	+49
M5 (clay texture)	+2419	

The NS increased from 3 to 12 g of soil when the soil-sand mixtures varied or when the percentage of clay increased, except in M4 where the number of spore decreased (Fig. 9). The highest NS were observed in M3 (clay-sand texture) and M5 (clay texture). The minimal were observed in M0 and M1 textures. On the order hand the sand texture significantly reduced the NS.

Discussion

Experiment 1

The results of this experiment showed that the three levels (0, 2 and 4 times) of Rorison's nutrient solution had a significant effect on growth and symbiotic parameters. The use of water as control, as well as the application of 2 times Rorison's nutrient solution concentration increased plant growth and grain yield of *Sorghum* colonized by AMF. These results are in accordance with those of Douds and Schenck (1990), who reported that addition of nutrient solution with low P resulted in an increase in shoot biomass of *Paspalum notatum* colonized by AMF.

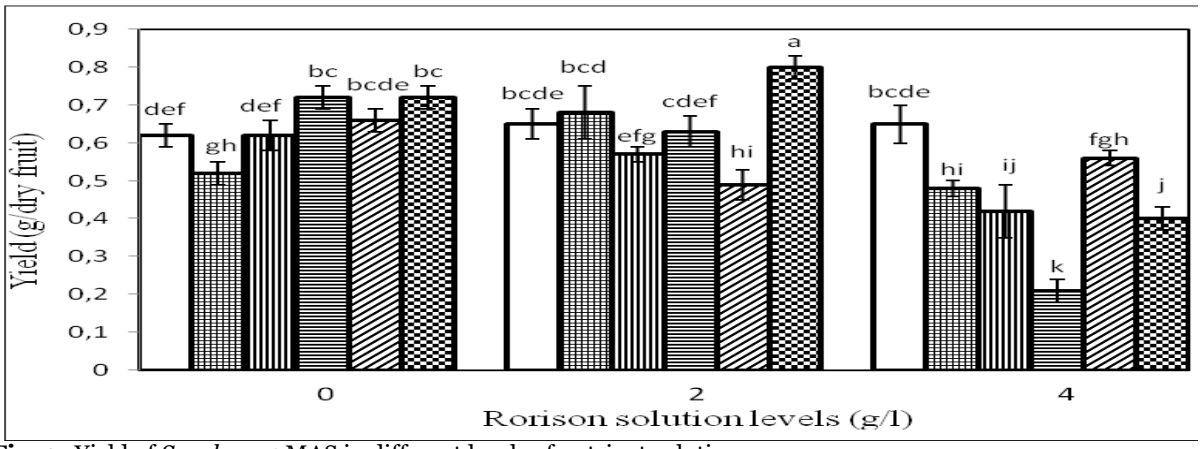

Fig. 1. Yield of *Sorghum* 4 MAS in different levels of nutrient solution.

Control without AMF (S0) *Glomus hoï* (S1), *Glomus intraradices* + *Scutellospora gregaria* (S2), Myco 2 (S3), *Gigaspora margarita* (S4), *Scutellospora gregaria* (S5). Histograms with the same letters are not significantly different at 5% (Newman Keuls test).

The decrease in growth and grain yield at 4 times Rorison nutrient solution concentration could be due to the modification of soil characteristics and the high levels of salts (N and P nutrients) in this nutrient solution. This result is in accordance with those of Ebrahim and Nasser (2013). Therefore, it was reported that AMF colonization and extra radical hyphae growth were suppressed when plants were grown with high levels of nutrients; but this level of elements was not toxic to the plant and do not suppressed growth totally (Liu *et al.,* 2000). Ngonkeu (2003) reported that, the rate of growth and germination of AMF strains varied with solution acidity of the substrate; this has been shown to have a mark effect on some AMF species and these could consequently affect the growth and grain yield of *Sorghum*. In this experiment the soil (SN) used was acidic.

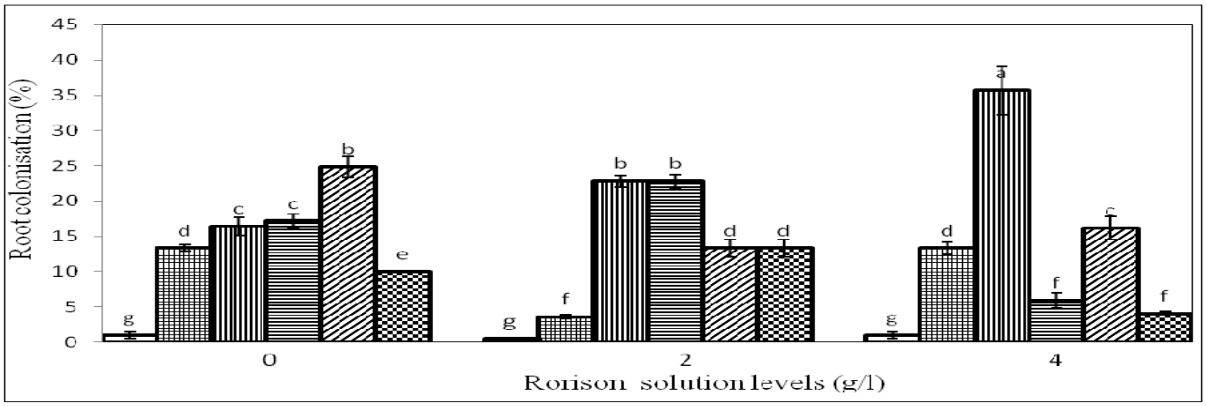

Fig. 2. Root colonization of *Sorghum* 4 MAS.

Control without AMF (S0), *Glomus hoï* (S1), *Glomus intraradices* + *Scutellospora gregaria* (S2), Myco 2 (S3), *Gigaspora margarita* (S4), *Scutellospora gregaria* (S5). Histograms with the same letters are not significantly different at 5% (Newman Keuls test).

The addition of Rorison's nutrient solution did not have a significant effect on *Sorghum* and tomato root colonization decrease (Ebrahim and Nasser, 2013). In contrast, RC of *Sorghum* in this study varied significantly with the level of nutrient solution and also with the type of AMF inocula used. This variation could be due to the high nutrient concentration of P and N in Rorison's nutrient solution that might have significantly induced or reduced root colonization. Douds and Schenck (1990) demonstrated that, the

addition of a nutrient solution void of P significantly increased the percentage of root length and colonization, compared to addition of water. However, high level of P has been shown to reduce RC, as confirmed by the results of Liu *et al.* (2000). In contrast, the root colonization of *Glomus intraradices* + *Scutellospora gregaria* was high in comparison to other treatments and control. This implies that these AMF could be adapted to support high P levels or high nutrients in the soil.

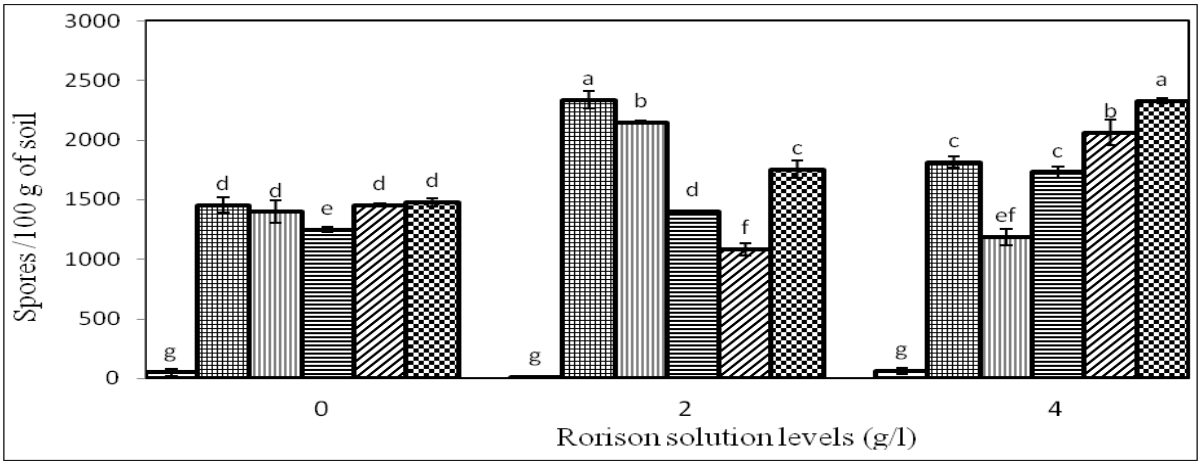

Fig. 3. Spore density by AMF 4 MAS.

Control without AMF (S0), *Glomus hoï* (S1), *Glomus intraradices* + *Scutellospora gregaria*) (S2), Myco 2 (S3), *Gigaspora margarita* (S4), *Scutellospora gregaria* (S5). Histograms with the same letters are not significantly different at 5% (Newman Keuls).

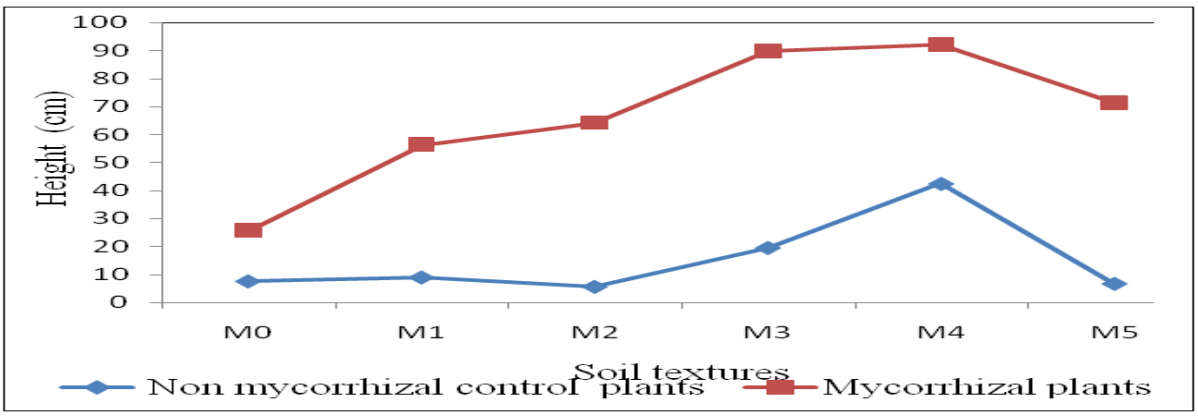

Fig. 4. Plant height of *Sorghum* 4 MAS in different soil textures.

M0 (sand texture), M1 (sand-clay texture), M2 (clay too sand texture), M3 (clay-sand texture), M4 (clay-sand texture), M5 (clay texture).

The sporulation in the present study varied with AMF inoculum type and the level of application of nutrient solution. Certain AMF must adapt to produce spores at a given level of nutrients. According to Douds and Schenck (1990), sporulation of *Acaulospora Iongula* exhibited a patterned response to N:P indices of the nutrient solutions. The decrease of NS by *Glomus hoi* (S1) and *Glomus intraradices* + *Scutellospora gregaria* (S2), when treated with 4 times nutrient solution concentration could be due to the addition of P and N, or to the ability of certain AMF like *Glomus intraradices* to produce spores inside the root. Only spores in the soil were counted in this study. According to Silva *et al.,* (2005), the production of spores by *Glomus margarita* and *Scutellospora heterogama* increased significantly after addition of Tris-HCl buffer in Hoagland with 3 µM P and Long Ashton II with 15.9 µM P. On the other hand, the

same author notice that spores production by *Glomus etunicatum* was improved when the substrate was irrigated with Hoagland with 3 µM P + Tris-HCl buffer and Hoagland with 20 µM P solutions. Sporulation decreased as P increased, at a given P level, sporulation was greater at a higher N level (Douds and Schenck, 1990). In addition, the effect of nitrogen on the sporulation of AM fungi differs among grass species and in some species nitrogen may suppress sporulation (Saito *et al.*, 2011).

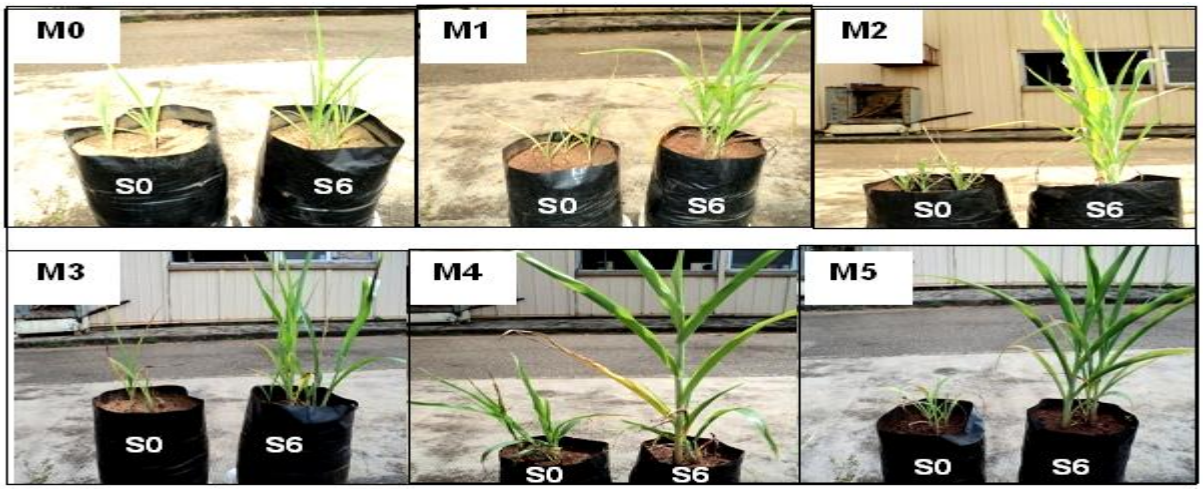

Fig. 5. Plant height of *Sorghum* 3 MAS. S0 (control without AMF), S6 (AMF: *Glomus intraradices* + *Glomus clarum),* M0 (sand texture), M1 (sand-clay texture), M2 (clay too sand texture), M3 (clay-sand texture), M4 (clay-sand texture), M5 (clay texture).

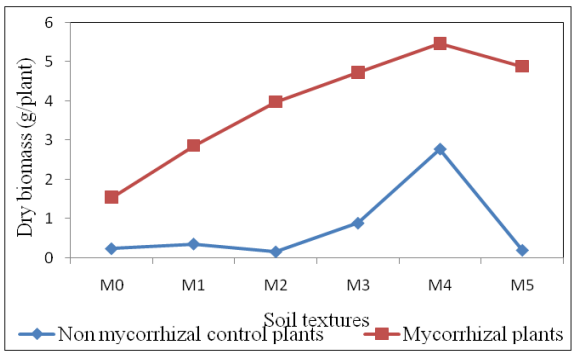

Fig. 6. Dry shoot biomass of *Sorghum* 4 MAS. M0 (sand texture), M1 (sand-clay texture), M2 (clay too sand texture), M3 (clay-sand texture), M4 (clay-sand texture), M5 (clay texture).

Experiment 2

The inoculation by AMF (*Glomus intraradices* + *Glomus clarum*) on *Sorghum bicolor* grown in soil with different textures increased PH from 25 to 90 cm, SWD from 1.5 to 5.5 g/plant and PC from 400 to 600 µg/g, compared to the control. The yield increased by mycorrhiza inoculation was between 33-66% in maize grown in an Oxisol/Ultisol under field conditions and on the other hand, mycorrhiza inoculation increased P biomass content from 40 to 280% for cowpea and 40 to 390% for pearl millet, compared to the control (Nwaga *et al.*, 2004, 2010). Different mycorrhiza inoculation methods may increase sorghum biomass under farm conditions in Cameroon from 0.43 t to 1.17 t/ha (Nwaga *et al.*, 2004). Inoculation with mycorrhizal fungi increased the yield of green peppers and potatoes in a high P soil (Douds *et al.*, 2003, 2007).

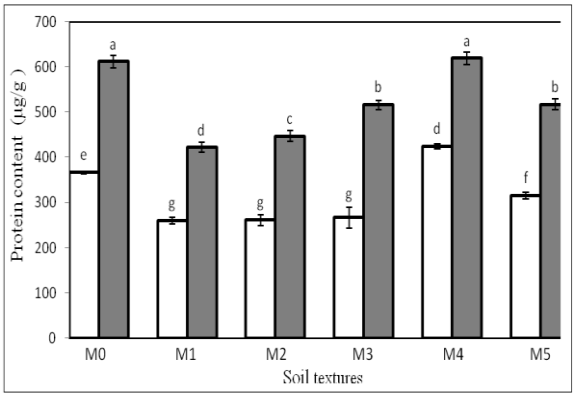

Fig. 7. Shoot protein content of *Sorghum* 4 MAS. ☐ Control plant (without AMF) ■ AMF inoculated plant (*Glomus intraradices* + *Glomus clarum*) M0 (sand texture), M1 (sand-clay texture), M2 (clay too sand texture), M3 (clay-sand texture), M4 (clay-sand texture), M5 (clay texture). Histograms with the same

letters are not significantly different at 5% Newman Keuls test.

The PC was high in mycorrhized *Sorghum* plant grown in sand owing to the fact that AMF has the ability to improve nutrient uptake and drought tolerance of plants. In Oxisol of Cameroon, Nwaga *et al.* (2011) found that AMF inoculation provided 30% more water to drought stressed banana under nursery conditions after 40 days and, under farm conditions may provide bigger banana bunches than the non mycorrhizal control. Generally, growth and soluble PC as a biochemical parameter increased with increase in the clay content (5 to 43%) soil texture, because of the fact that soil cation exchange capacity and fertility were improved. Clayed soils are more fertile than sandy ones because of their higher nutrient storage capacity from soil solution (Carrenho, 2007).

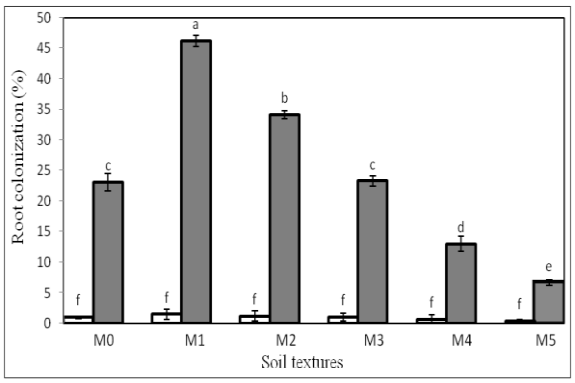

Fig. 8. Root colonization of *Sorghum* by AMF 4 MAS.

☐Control plant (without AMF) ■ AMF plant (*Glomus intraradices* + *Glomus clarum*) M0 (sand texture), M1 (sand-clay texture), M2 (clay too sand texture), M3 (clay-sand texture), M4 (clay-sand texture), M5 (clay texture). Histograms with the same letters are not significantly different at 5% (Newman Keuls test).

Plants and AMF aerobic organisms need both nutrient stored by clay and also aeration provided by sand, for that reason, soil-sand mixture M4: 3:1(v:v), having 43% clay was the best in terms of promoting growth and soluble PC as an indicator for an improvement of the efficiency of plant functioning. Generally, MR and MD increased because of the fact that clay percentage and nutrient richness in soil fertility of some mixtures were higher. Low soil fertility limits plant development and increases the dependency of plants on mycorrhizal association (Carrenho *et al.*, 2007). In this study, these parameters were improved in the mixtures with high clay and sand content; hence the physical characteristic of soil as substrate could significantly influence the MR and MD.

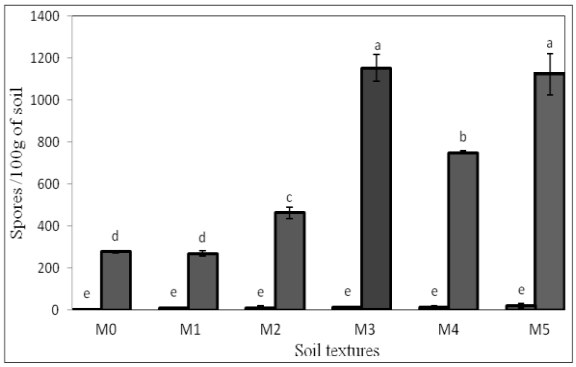

Fig. 9. Spore density of AMF 4 MAS.

☐ Control plant (without AMF) ■ AMF plant (*Glomus intraradices* + *Glomus clarum*) M0 (sand texture), M1 (sand-clay texture), M2 (clay too sand texture), M3 (clay-sand texture), M4 (clay-sand texture), M5 (clay texture). Histograms with the same letters are not significantly different at 5% (Newman Keuls test).

A significant decrease in RC was noticed with an increase in clay content, in soil-sand mixtures, probably owing to the fact that macroporosity of the soil decreased when clay content increased. The present results confirm observations of Carrenho *et al.* (2007); This author found that soil aeration is a key factor driving infection, colonisation and metabolic activity of the AMF and root colonization, they reported a significant reduction in root colonisation in *Sorghum* grown on clayed soil. A clay soil texture favors the deposition of suberin on the epidermis of the plants which increases resistance to infection by AMF (Koske and Gemma, 1995 cit. Carrrenho *et al.*, 2007). Plants in sand with big particle sizes of 0.50-0.78 mm had higher root fresh weights, spore production and higher percentage of mycorrhizal colonization than those with smaller particle sizes such as clay (Gaur and Adholeya, 2000).

Increased in spore number was observed when soil-sand mixtures varied with an increase in clay content, which could be correlated to the decrease of macro porosity of the soil, which increased micro porosity and water stress. The results indicating that an increase in soil micro porosity induced an increase in AMF spores production, which was in accordance with the studies conducted by Olfat and Khara (2012), who reported that sporulation by AMF is positively correlated with an increase in clay content.

Conclusion

Results showed that Rorison's nutrient solution addition could significantly increase the production of AMF spores. We also noticed that, clay and clay sand textures significantly enhanced the growth of plant, spore production and plant activity through an increase in soluble protein content. Further researches are needed in order to determine the best combination of soil-sand mixture, and different nutrient solution concentrations which could better improve AMF production.

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