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Characterization Of Fungal Endophytes And Arbuscular Mycorrhizal Fungi From Different Soils Of Cameroon To Increase Onion (Allium Cepa L.) Productivity

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Abstract :
Onions are a highly prized spice due to their nutritional and medicinal properties. However, they face numerous diseases that reduce yields, with few studies conducted on the morphological diversity and effectivenes of fungal inoculation in stimulating growth and yield in Cameroon. Therefore, experiments were designed to characterise and study the plant response to inoculation with fungal endophytes and arbuscular mycorrhizal fungi (AMF). A factorial block design, comprising pots with sterilised soil from 3 agro-ecological zones (AEZ) in Cameroon and sand substrates, was employed to evaluate the effectiveness of these inoculations. A real-time polymerase chain reaction (PCR) was conducted on the fungal 28S gene. The present study demonstrated that fungal endophytes exhibit a high degree of morphological diversity, including hyphae, spores, and colonies. In contrast, AMFs display a diversity of morphotypes and spores in the three AEZs (I, II, and V). The AMF morphotype that appeared most frequently was the smooth black morphotype, with a frequency of 100%. The AEZ with the highest number of spores per gram of soil was AEZ V, with a count of 64.5 spores per gram of soil. The resutls of the real-time PCR demonstrated the presence of AMF curves that were identical to those observed in the positive control (Glomus hoï). The results also indicate that the most effective fungal endophyte isolate, PDA DB13, inoculated, significantly increased the number of leaves by +94%, plant height by +158% and onion bulb weight by +154%, while the AMFs increased the number of leaves by +49%, plant height by +141% and onion bulb weight by +148% compared with the control.

Key Word : *characterization ; AMF ; fungal endophytes ; PCR ; yield ; onion.*

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I. Introduction

The onion (Allium cepa) is a biennial plant of the Liliaceae family. It is characterised by concentric, fleshy scales that surround a stem, thus constituting a bulb. It is consumed in a raw state in salads or cooked in a variety of dishes, often combined with other vegetables. The plant has been cultivated since its adoption around the 1960s, due to its nutritional virtues. The plant contains a considerable quantity of vitamins, sulfur, silica, iodine, sodium, and numerous other elements, rendering it an efficacious tonic [1]. Onion has been demonstrated to possess a number of medicinal properties that may be beneficial in the prevention of cardiovascular diseases, including atherosclerosis [2]. Additionally, it offers economic advantages, generating substantial profits on an annual basis. The quantities produced provide the 15,000 producers with a total of over 47 billion FCFA annually, generated from an area of more than 6,000 ha. In the mid-term evaluation report of the Program to Improve the Competitiveness of Family Agropastoral Farms (ACEFA), the Far North of Cameroon observed a notable increase in onion production, with a 38.27% increase from a few producers over three consecutive years [3]. In 2012, the production of dry season onions was 95,000 tonnes, resulting in an income of approximately 12 billion [4]. It is estimated that 85% of onion production in Cameroon is supplied by the administrative regions of the North and Far North, where onion represents the second most important commercial crop after cotton.

In Cameroon, agriculture represents over 40% of the gross domestic product (GDP) and constitutes the primary source of employment and income-generating activities for the majority of the population (56%) [5]. Over the past decade, there has been a significant decline in the yield of onions due to a number of factors,

including the impact of various diseases and conditions on this crop. The thrips tabaci, which farmers referred to as the Yirli in English, is a local disease caused by an insect vector of a virus. This disease has precipitated a significant economic downturn in the onion sector in the far north of Cameroon. The social survey indicates that losses are approximately 70% at harvest. It is typical for national onion production to experience losses of 23% during storage and transport. Producers estimate that losses may reach up to 100% of the harvest. The most commonly encountered symptoms are consistent with those typically associated with fungal damage, according to [6].

A considerable body of research has demonstrated that biological control represents an environmentally sustainable approach to safeguarding plants against pathogens present on crops. Among the biological control methods that are recommended, the use of beneficial microorganisms, such as arbuscular mycorrhizal fungi and endophytes, remains an effective approach to combat plant diseases, increase their growth and yield, while combating global warming. However, soil degradation across the country represents a significant limitation for onion productivity. Experimental studies have shown that microbial communities, particularly AMF, play an essential role in the mineral and, in particular, water supply, through a symbiotic relationship with plants. This symbiosis is most prevalent in over 80% of land plants, as demonstrated by Nwaga et al. [7]. These microorganisms are capable of transporting water and mineral nutrients to the plant, and in return, benefit from the carbon compounds released by the host plant. It has been demonstrated that certain recently discovered mycorrhizae are capable of functioning as super biofertilizers (*Piriformospora indica*) and of protecting plants against toxic elements. Research conducted on onion mycorrhization by [8] has indicated that the species of mycorrhizal fungi in question enlarge the surface area of the root by 70 to 118% in comparison to the control. *Glomus clarum*, a species of arbuscular mycorrhizal fungus, demonstrated an improvement in the absorption of nitrogen and phosphate by 35 and 290 mg/plant, respectively. This resulted in a 83% increase in the dry weight of onion bulbs compared to the control treatment, 90 days after the plantation. [9] also demonstrated that arbuscular mycorrhizal fungi (AMF) exhibit antifungal properties and enhance onion biomass by approximately 50% in comparison to the control.

It would be beneficial to conduct trapping studies on soils from various agro-ecological zones of Cameroon with the aim of promoting greater and more effective biodiversity. A number of studies have confirmed that Cameroon is home to a rich AMF potential and a highly diverse array of AMF species, which could be exploited for the sustainable utilisation of natural resources [10, 11]. This study was carried out to to examine the morphological diversity of fungal endophytes and AMF and assess their efficacy in enhancing the growth and bulb yield of onion (*Allium cepa* L.).

II. Materials And Methods

Experimental site of the study

The experimental field trial was carried out from November 2022 to Mars 2023 in Bokle, a locality situated 15 km east and south of Garoua, the capital of the North Cameroon region (9° 23' 02" N 13°30'08" E, altitude = 187 m). Garoua has a savannah climate with a dry winter (Aw) according to the Koppen-Geiger classification and an average annual temperature of 800 mm. The climate is tropical and belongs to the sodano-Sahelian category.

Preparation of the study substrate

Soil samples were collected from the top layer (0-15 cm) of different agro-ecological zones of our study site in Cameroon for fungal endophyte trapping and AMF extraction. The samples were sieved with a 2-3 mm diameter sieve and mixed with coarse sand in a soil/sand ratio of 3 :1 (V/V), respectively, according to the modified protocol of Adamou et al. [8]. The samples were then homogenised using a shovel. The resulting mixture was then placed in 5-litre polypropylene plastic bags (with small holes previously drilled to facilitate aeration of the soil and the evacuation of excess water). Subsequently, the bags were transported to the experimental site. Another portion of the mixture was autoclaved at 121°C for 50 minutes, after which it was used to fill the plastic pots at a rate of 5 kg per pot. This was done in order to test the effectiveness of the fungal endophytes and AMF on onion growth.

Plant and Biological Material

The onion bulbs used in this study (Galmi violet variety) came from FEPRODEX (Federation of Onion and Garlic Producers of the Far North Region) in Cameroon. Its growing cycle is 160 days, the shape of bulbs is round usually purple or white in colour. The fungal endophyte isolates were collected from a variety of localities across Cameroon, while the AMFs were similarly sourced from these same locations. Additionally, the inoculum for the reference strain of AMF, Glomus hoi, was supplied by the Department of Biological Sciences at the University of York in England.

Trapping and isolation of fungal endophytes

A completely randomised system was established for the purpose of trapping fungal endophytes. A total of eight treatments were employed, each representing a soil sample from one of three agro-ecological zones of Cameroon (AEZ). The locations included in the study were Pitoa, Bocklé, Meskine, Yagoua (Zone I), Foret and Yaounde (Zone V), and Marza and Dang (Zone II). Each treatment was replicated five times.

In order to isolate endophytic fungi, the roots of each treatment were harvested and surface disinfected using the modified method described by [12, 13]. Subsequently, the roots were rinsed on several occasions with running water, after which they were cut into small segments (0.5 to 1 cm). The samples were then immersed in 80% ethanol for a period of three minutes, followed by a two-minute rinse with 4% sodium hypochlorite. This was then followed by a further two minutes of immersion in sterile distilled water, after which the samples were treated with 75% ethanol for one minute. This was then followed by a further eight to ten rinses with sterile distilled water. They were then dried using sterile filter paper. The cut and dried roots were then cultured on PDA medium. The plates were then incubated at 28°C for 7 days until fungal growth was observed [14, 15].

AMF Spore Extraction and Enumeration

The number of AMF spores contained in the plastic pots of trapping soil was assessed 3 to 4 months after sowing. The pots were left without watering for a two-week period after trapping to stimulate AMF sporulation, and then 100g of soil from each treatment was taken to assess sporulation. Spores were extracted using the method of Schencke et al. [16]. To achieve this, 100 grams of soil were introduced into a one-litre Erlenmeyer flask. Approximately 300ml of tap water was then added to the mixture, which was homogenised. The mixture was then allowed to stand for 15 seconds before undergoing a series of sieving operations through a column of sieves with decreasing mesh sizes of 700µm, 200µm, 100µm and 45µm, respectively. The washing and decanting process was repeated three times, and the contents of the sieves were transferred separately into labelled boxes. The spores were observed at magnifications of 10 to 40 using a stereo microscope.
Calculate the number of spores/g according to the formula : $N = n \times 27.76/100$
With n the average of the number of spores of the three repetitions.

Morphological Characterisation of Fungal and AMF Endophytes

Macroscopic analysis consisted of the colour, appearance of the mycelium, partitioning, spore shape, odour, margin and pigmentation of the colonies in accordance with the microbiological standard set forth by Sarangthem and Momota [17]. Additionally, methylene blue and fresh state analysis was conducted on these fungal endophytes. The colonised roots were analysed for AMFs according to their vegetative and reproductive systems.

Experimental trial

A pot experiment was conducted in a factorial block design with 2 factors : the variety (V1 : Galmi purple onion) as the main factor and the treatments : Control (T0), CMA, CMA+UREE, PDA F, PDA CB6, HK B41, HK B7, PDA DB13, HK, HKCB7 and PDA 19) as a secondary factor was adopted. Each treatment had 6 replicates, i.e. 6 plants per treatment for a total of 72 plants evaluated.

To evaluate the efficacy of fungal endophytes and AMF on the growth of onions in pots, CMA were inoculated into each pot (10 cm deep) with 10 g of inoculum containing 50 spores of the CMA species per pot. Fungal endophyte isolates were inoculated by introducing 45 ml of inoculum containing 1×10^8 cells/ml of each fungal endophyte isolate per pot into each seed hole. After inoculation, an onion bulb was placed in each pot, close to the inoculum. All pots were watered daily.

Real-time PCR

A real-time PCR assay was employed to ascertain the presence of the target sequence of the 28S gene in the AMF sample.

DNA extraction was carried out as follows : The DNA sample to be extracted was incubated on a heating block for 15 minutes at a temperature of 56°C. Then 250 µL of absolute ethanol was added, the mixture was vortexed for 15 seconds and centrifuged briefly. Incubation took place at room temperature for 5 minutes. The columns were identified and the mixture transferred to the corresponding columns, centrifuged at 6800g for 1 min. The columns were then transferred to new collection tubes. 500µL of wash buffer was then added and the mixture centrifuged at 14000g for 1 min. The columns were transferred to new collection tubes and centrifuged at 14000g for 3 min and onto new labelled sterile 1.5mL microtubes. 50µL of elution solution was introduced into each column and incubated for 1 min at room temperature and centrifuged at 14000 g for 1 min. The columns were discarded, checking for the presence of eluate in the 1.5 mL microtube [18].

PCR was carried out to determine whether the AMFs extracted from the different soils possessed the 28S gene as the AMF target DNA. The master mix was prepared as follows : MMix Eppendorf 20 µL (12.4 µL

molecular water, 4 µL 5X EvaGreen, 0.8 µL forward primer and 0.8 µL reverse primer, 2 µL DNA template). Fragments of the 28S CMA gene were amplified using primers 28G1 (5'-CATGGAGGGTGAGAATCCCG-3') and 28G2 (5'-CCATTACGTCAACATCCTTAACG-3') as described by Bruno et al. [19]. The following thermal cycler parameters were employed : One cycle of denaturation was performed at 95°C for 15 minutes, followed by denaturation at 95°C for 15 seconds, primer annealing at 58°C for 30 seconds, and a final extension step at 72°C for 30 seconds. The amplification products were analysed on a BIO-RAD thermal cycler and the results were visualised on a computer.

Statistical Analysis

The data collected were subjected to an analysis of variance (ANOVA) using SPSS version 25.0 software. A Duncan test at the 5% threshold was used to compare the means.

III. Results

Morphological characterisation of fungal endophyte isolate

Table I presents the morphological characterization of fungal endophyte isolates isolated from soil samples from different AEZs of Cameroon. This characterization is based on four criteria, namely : the type of hyphae ; spore types ; color and appearance of colonies cultured on agar medium. We note that 30% of the isolates are non-partitioned and powdery. The type of spores and the color of the colonies are more diverse.

Table I. Morphological characterization of fungal endophyte.

Code	Hyphae	Spore type	Colony color	Appearance
PDA F	Partitioned	Big and small	H and S beige	Powdery
PDAC B6	Partitioned	Small	H and S beige	Powdery
HK B41	Not partitioned	Small	H and S beige	Filamentous
HKB7	Not partitioned	Big and small	H white pink and S beiges	Filamentous
PDA DB13	Partitioned	Big rounds	H white et S green	Filamentous
DB13	Partitioned	Big rounds	H white et S green	Filamentous
HK	Partitioned	Small and rounds	H et S beige	Powdery
HKC B7	Not partitioned	Small	H white pink et S white	Filamentous
PDA 13	Partitioned	Small	H white et S pink	filamentous
PDA 19	Partitioned with internal spore	Small	H white et S white	filamentous

H : hyphae ; S : spores

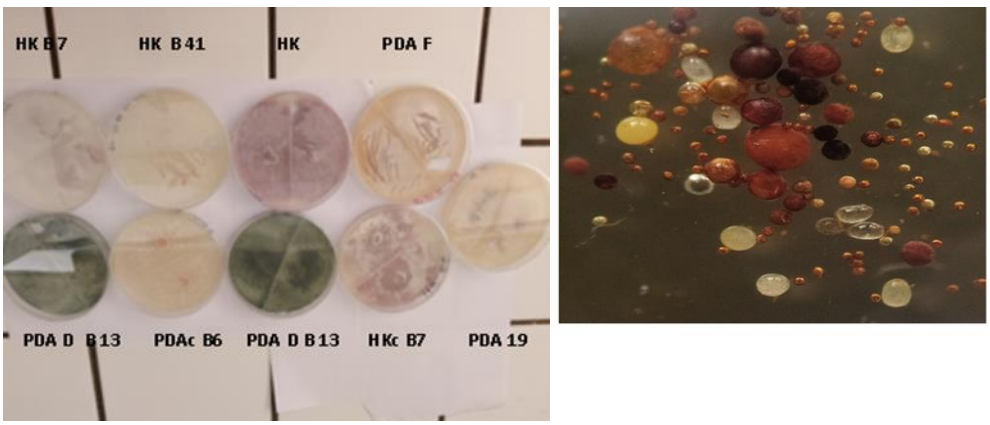


Figure 1.
a) Mocroscopic appearance of fungal endophyte isolates selected to test their effectiveness on onion growth and yield,
b) Fungal spores extracted from the 3 agro-ecologiques zones soil.
PDA F, PDA CB6, HK B41, HK B7, PDA DB13, HK, HK CB7 et PDA 19 are fungal endophytes isolates.

Morphological characterization of AMF spores

Table II presents the frequency of appearance of AMF morphotypes in the three AEZ of Cameroon. From this table, it can be seen that the most frequently occurring AMF morphotype is smooth black, accounting for 100% of all occurrences, followed by brown (92%), and then light (73%). The zone with the greatest number of morphotypes is ZAEV (24), followed by ZAEI (14). In contrast, the zone with the smallest number of morphotypes is ZAEII (10).

Table II. Frequency and number of AMF spore morphotypes extracted from different soils.

AEZ	I	I	I	I	II	II	V	V	V	V	V	T	Frequency (%)
Morphotypes/ sol	1	2	3	4	5	6	7	8	9	10	11		
RB					+							1	9
RW					+							1	9
Br		+	+	+	+	+		+	+	+	+	9	92
LD	+				+	+	+	+				5	45
SB	+	+	+	+	+	+	+	+	+	+	+	11	100
R					+							1	9
LBr				+			+	+	+			4	36
DBr				+			+		+			3	27
L			+	+		+	+	+	+	+	+	8	73
LW							+					1	9
W	+											1	9
G	+							+				2	18
LG										+		1	9
Morphotypes number	4	2	3	5	6	4	6	6	5	4	3	48	

The different colors of spores are represented by the letters : RW = rough white, LW = light white, LG = light golden, B = black, W = white, Br = brown, G = golden, R = rough, L = light, LD = light dirty, DBr = dark brown, LBr = light brown, RB = rough black, SB = smooth blac ; T= total

Diversity of AMF morphotypes and number of spores

Table III presents the diversity of morphotypes and the number of spores/g of soil according to different ZAE of soil samples. It appears from this table that the ZAE with the greatest number of spores/g of soil is ZAE V with an average value of 64.5 spores/g of soil (Campus and Foret), followed by ZAE II with a value average of 50 spores/g of soil (Dang and Marza) and AEZ I is the one which has the lowest average value in number of spores/g of soil of 42.75 spores/g of soil. Concerning the diversity of AMF morphotypes, the ZAE with the greatest diversity is ZAE V (6 and 7 morphotypes) respectively. Followed by ZAEII (4 and 6 morphotypes) and there less diverse is ZAE I (2, 3, 4 and 5 morphotypes). These observations allow us to conclude that the diversity of AMF morphotypes in an AEZ has a highly positive correlation with the average number of spores/g of soil in this AEZ.

Table III. Diversity of morphotypes and number of AMF spore in the different AEZ.

No	Samples	AEZ	Spore colors	Diversity of morphotypes	Number of spores / g
1	Marza	II	RB, RW, Br, LD, B, R	6	37
2	Campus	V	B, LBr, DBr, LD, B, l, LW	7	50
3	Meskine	I	W, B, LD, G	4	58
4	Dang	II	B, Br, L, LD	4	63
5	Foret	V	B, Br, LBr, LD, L, G	6	79
6	Pitoa	I	B, LBr, DBr, Br, L	5	28
7	Bocklé	I	B, L, Br	3	56
8	Yagoua	I	Br, B	2	29

The different spore colors are represented by the letters : B = black, W = white, Br = brown, G = golden, L = light, R = rough, LD = light dirty, DBr = dark brown, LBr = light brown. RB = rough black, RW = rough white, LW = light white

Demonstration of the presence of CMA in the roots of onion plants

Microscopic observations of onion roots after root staining revealed the presence of endomycorrhizal fungi which live in association with these roots. The presence of these endomycorrhizal fungi is illustrated by the colonization of roots by fungal structures of transport (hyphae), transfer (arbuscules), resistance (spores) and storage (vesicles). These mycorrhizal structures were observed under an optical microscope (Figure 2).

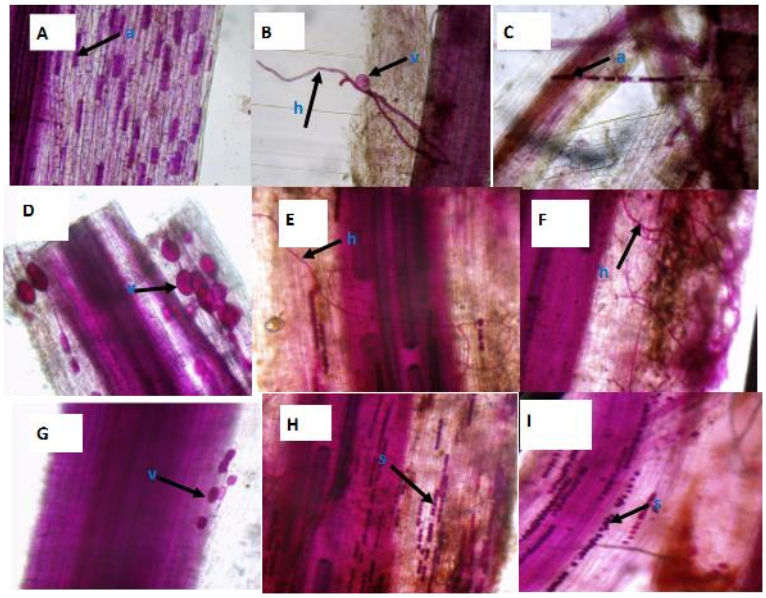
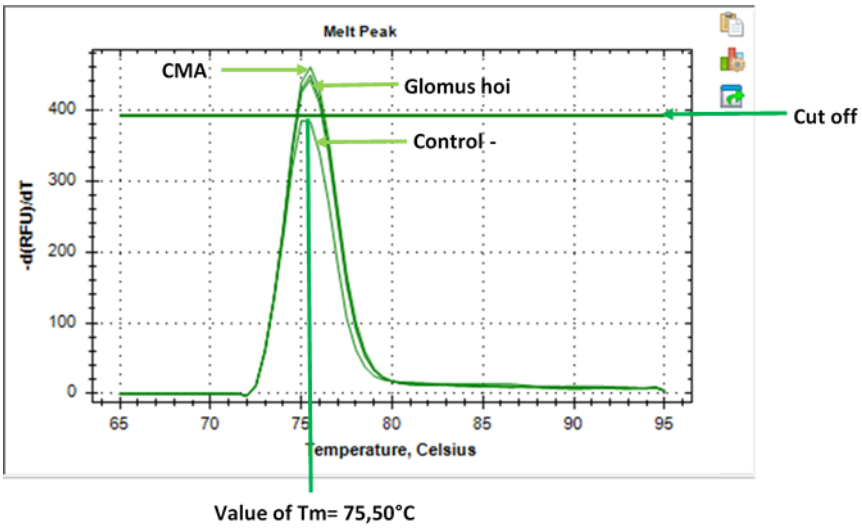


Figure 2. Colonization of onion plant roots by AMF fungal structures.
The letters A, B, C, D, E, F, G, H and I in upper case represent different images obtained after microscopic observations and the letters in lower case represent : v) CMA vesicles ; h) AMF hyphae ; s) spores ; a) Root cells containing AMF arbuscules after staining with acid fuchsin.

IV. Real-Time PCR Of 28S Gene With Melting Curve Analysis

The 5X EvaGreen (figure 3) gives the melting temperature (Tm) of 75.50°C for the CMA sample analyzed and the positive control (Glomus hoi). Real-time PCR analysis made it possible to determine that our CMA sample contains the target sequence of the 28S gene. The curves are above the threshold value. That means that our CMA sample contains a high microbial load with d(RFU)/dT greater than 400. The results obtained show the presence of CMA curves identical to the positive control (Glomus hoi). We note the presence of the 28S CMA genes with Tm equal to 75.50°C.



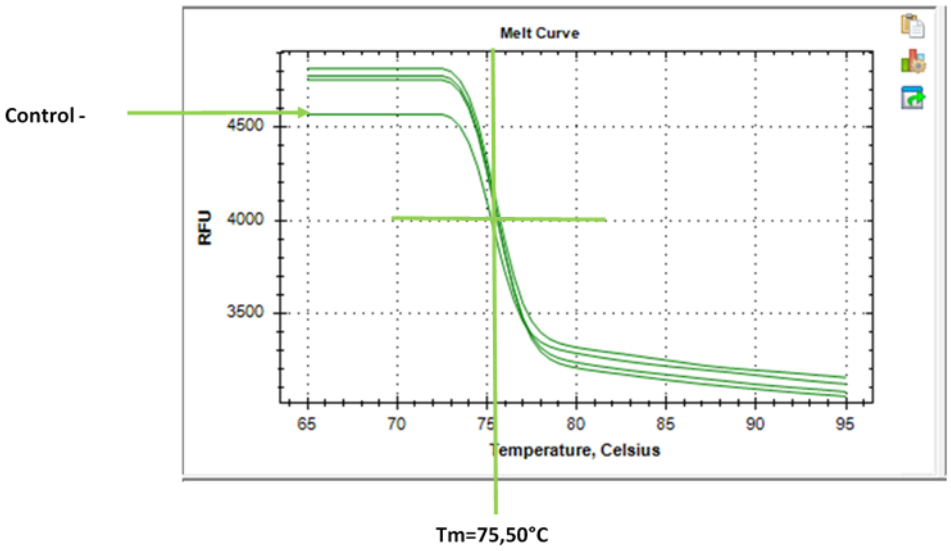
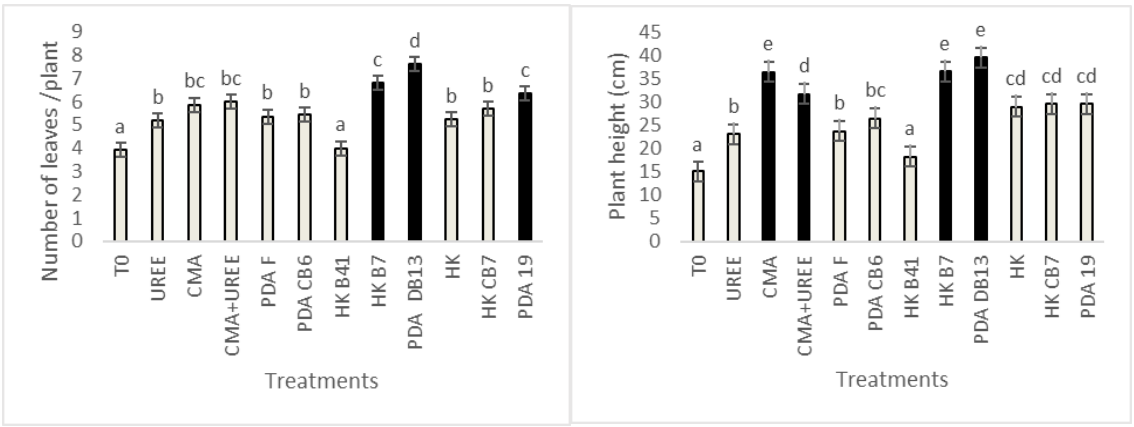


Figure 3. Melting curves of the 28S CMA gene in reel-time PCR.

V. Effect Of Fungal Endophytes Isolates And CMA On Onion Growth Parameters

Plant growth (number of leaves and plant height) of onion was significantly ($p<0.05$) influenced by the inoculation of fungal endophyte isolates and AMF, as shown in (Figure 4). Treatment with fungal endophyte isolate (PDA DB13) is the most effective, as it induces the greatest increase in the number of onion leaves by +94% and plant size by +158% compared to the control (T0) and other treatments this is due to the fact that this isolate has the capacity to synthesize growth hormones. The CMA also increased the number of leaves by + 49% and the size of the plants by more than 141% compared to the control. The ability of the CMA to colonize the root of the onion early, allowed them to induce a positive effect on growth parameters. None of the treatments had a negative effect on the growth of onion plants. The fungal endophyte isolate HK B41 had the lowest performance compared to the control (T0). It still appears that the average values of HK B41 are higher than that of the control. We also note that the application of chemical fertilizer (Urea) in combination with CMA inhibited the action of these beneficial microorganisms, which explains the fact that plants height inoculated with CMA+UREA is lower than that of CMA.



VI. Effect Of Fungal Endophytes Isolates And CMA On Onion Bulb Weight

Almost all treatments had a favorable response to inoculation of fungal endophyte isolates and AMF. For bulb weight except : HK treatment (figure 5). Among all the treatments, the most effective isolate is PDA DB13 with the weight of bulbs (0.514g/plant) compared to the control (0.202g/plant), i.e. a significant increase in onion bulb yield of +154 %. We also note that the treatment with CMA had a positive response to inoculation of +148% compared to the control, because CMA improves the ability of onion plants to take up nutrients such as P, Zn and Cu, AMF is also known to enhance the acquisition of other plant nutrients such as water and nitrogen. The PDA 19 isolate also had a good yield in onion bulbs (0.501g/plant).

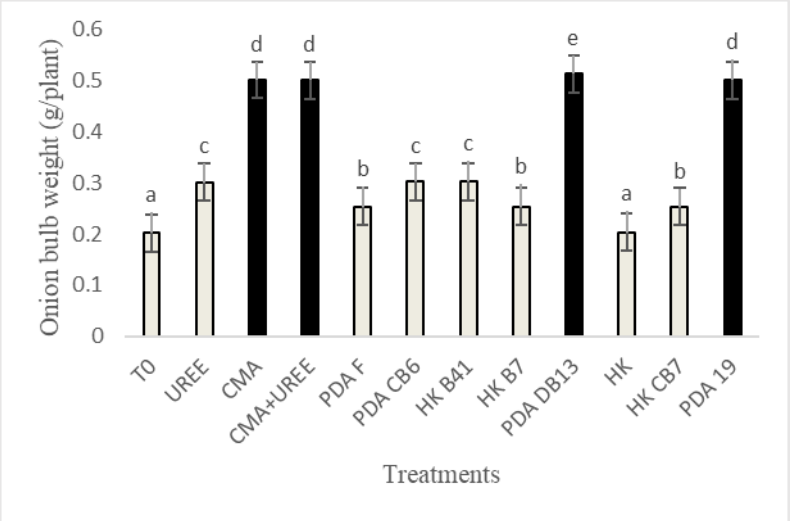


Figure 5. Effect of inoculation of fungal endophytes isolates and CMA on onion bulb weight. PDA F, PDA CB6, HK B41, HK B7, PDA DB13, HK, HK CB7 et PDA 19 are potential fungal endophytes isolates for increasing the number of leaves and plant height of the onion in plastic pots ; T0 (Control without fungal inoculation) ; UREE (Control with chemical fertilizer) ; CMA (Treated with arbuscular mycorrhizal fungi) ; CMA+ UREE (Treated with arbuscular mycorrhizal fungi and chemical fertilizer). The bars with the same alphabetical letter are not significantly different from each other at the threshold of 5% according to the Duncan test, means ± standard Error.

VII. Discussion

Table 2 presents the frequency of appearance of CMA morphotypes in the 3 AEZs of Cameroon. It appears from this table that the morphotype of CMA which appears most frequently is smooth black with a frequency of appearance of 100%, followed by brown 92% and then light 73%. The zone which has the greatest number of morphotypes is AEZV (24), followed by AEZI (14) and the zone which has the smallest number of morphotypes is AEZII (10). Alongside these results, the data in Table 3 show us the diversity of morphotypes and the number of spores/g of soil according to different AEZ soil sampling. It appears from this table that the AEZ with the greatest number of spores/g of soil is AEZ V with an average value of 64.5 spores/g of soil (Campus and Foret), followed by AEZ II with a value average of 50 spores/g of soil (Dang and Marza) and AEZ I is the one which has the lowest average value in number of spores/g of soil of 42.75 spores/g of soil. Concerning the diversity of AMF morphotypes, the AEZ with the greatest diversity is AEZ V (6 and 7 morphotypes) respectively. Followed by AEZII (4 and 6 morphotypes) and there less diversified is ZAE I (2, 3, 4 and 5 morphotypes). These observations allow us to conclude that the diversity of AMF morphotypes in an AEZ has a highly positive correlation with the average number of spores/g of soil in this AEZ. These results are in agreement with the work of Nowo et al. [20]. Who showed that inoculation of AMF species (Glomus hoi, Scutellospora gregaria, Rhizophagus intraradices and Gigaspora margarita) to Allium porrum plants on soil samples from AEZ V increases extra-root sporulation of (153 spores/g). This diversity and high concentration of spores could be very beneficial for increasing crop yields after inoculation. In addition to AMF spores, several other fungal endophytes (9) were isolated after trapping and are highlighted by morphological characterization of the spores. Different isolates obtained. This characterization is based on four criteria, namely : the type of hyphae ; spore types ; color and appearance of colonies cultured on agar medium. Among these isolates we were able to identify a few which turned out to be interesting as beneficial endophytes for plants. Sontsa et al. [21] also isolated a significant diversity of fungal and bacterial endophytes in the soils of the 5 agro-ecological zones of Cameroon. They obtained twenty-six isolates, i.e. 20 bacterial endophyte isolates and 6 fungal endophyte isolates pre-selected and morphologically characterized. Likewise [22] isolated endophytes tolerant to abiotic stress and used as biological fertilizer to alleviate stress and produce crops in tropical areas. Concerning the presence of endomycorrhizal fungi in the roots. Root colonization is illustrated by an abundance of fungal structures : transport (hyphae), transfer (arbuscules) ; resistance (spores) and storage (vesicles). These mycorrhizal structures were observed under an optical microscope (Figure 2). Such an abundance of fungal structures shows that the onion (Allium cepa L) is a very mycotrophic plant. The work of [23] corroborate the presence of these characteristic structures of endomycorrhizal fungi in the roots of the Plantago lanceolata plant after inoculation with AMF and root staining. To be certain of the presence and adequate concentration of CMA in our inoculum, a PCR analysis was carried out. The 5X EvaGreen (Figure 3) gives the melting temperature (Tm) of 75.50°C for the sample ofCMA analyzed and the positive control (Glomus hoi). Real-time PCR analysis made it possible to determine that our CMA sample

contains the target sequence of the 28S gene of mushrooms. The curves are above the threshold value. Which means that our CMA sample contains a high microbial load with d(RFU)/dT greater than 400. The results obtained show the presence of CMA curves identical to the positive control (Glomus hoi). We note the presence of the 28S CMA genes with Tm equal to 75.50°C. Similar work was carried out by [19] which revealed the existence of two new AMF species isolated from the rhizosphere of Brazilian soils. The results obtained on the growth of onion plants show that the application of different treatments based on beneficial microorganisms significantly improves ($p<0.05$) the growth of this plant (Figure 4). The work of Plenchette [24]. (1991) also revealed that inoculation of onions with CMA (Glomus caledonium and Glomus epigaeus) improves growth by up to 500% compared to the control, compared to 235% to 176% on the same plant inoculated with Glomus clarum and Glomus intraradices by [11] against 600% obtained by [25]. In general, the application of microbial biofertilizers (CMA and fungal endophytes) increases the yield of onion bulbs. Almost all treatments had a favorable response to inoculation of fungal endophyte isolates and AMF. Among all the treatments, the most effective isolate is PDA DB13 with the weight of bulbs (0.514g/plant) compared to the control (0.202g/plant), i.e. a significant increase in onion bulb yield of +154 %. This ability of the isolates to increase the productivity of the onion can be explained by the fact that they allowed this plant to absorb more nutrients compared to the non-inoculated control. These results are in agreement with the work of Adamou et al [8] who demonstrated that inoculation of onion plants with AMF significantly increased the dry weight of onion bulbs from +66% to +83% compared to the control 90 days after sowing. Several other authors have also shown that inoculation of onion plants with microbial biofertilizers helps improve their production [9, 26, 27].

VIII. Conclusion

This study shows that fungal endophyte isolates have a great morphological diversity and CMA a diversity of morphotypes and spores in the 3 AEZ (I, II and V) of Cameroon. The results obtained suggest that the symbiosis between CMA-onion and fungal-onion endophytes is of great importance for improving onion productivity. The majority of the isolates tested stimulated an increase in leaf number, plant size and bulb yield. From this work it appears that the most effective isolate is PDA DB13. CMA can also be recommended to improve onion growth parameters and yield. A better knowledge of the interaction between these beneficial microorganisms could provide information on their mode of action and their ability to provide different services during symbiosis with plants. The best selected isolates need to be tested in the field to better evaluate their performance.