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Native arbuscular mycorrhizal fungi increased resistance of two plantain varieties (Fhia 21 and Orishele), under water deficit conditions in Cote d'Ivoire

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Abstract

A pot experiment was conducted to assess the potential of arbuscular mycorrhizal fungi to improve plantain resistance to water deficit conditions. For this purpose, three sources of inoculants were used: (1) a strain of *Glomus clavisporum* isolated from plantain rhizosphere in Azaguié region; (2) a fungal complex isolated from plantain rhizosphere in Azaguié (5° 38 'N, 4°05' W); (3) a fungal complex isolated from plantain rhizosphere in Bouaflé (6°59 'N, 5°45' W). These inoculants were tested, using the plantain varieties *Fhia 21* and *Orishele*, using three levels of water supply (100, 60 and 30%). Plant growth and others physiological parameters were measured and compared for all the treatments, including the control. Comprehensively, root colonization decreased with the reduction of water supply. The mycorrhizal plants had widest leaves, highest leaf water and chlorophyll contents for any level of water supply. As same, arbuscular mycorrhizal fungi significantly increased soluble sugars and proline contents with water deficit. These results demonstrated that arbuscular mycorrhizal fungi increase plantain photosynthetic activity and thus impact plantain growth in any water supply condition. The proline and soluble sugars are were sensitive molecules to water stress conditions and their productions in the plantain are were improved in presence of mycorrhizal fungi.

Keywords: Arbuscular mycorrhizal fungi, Cote d'Ivoire, plantain, resistance, water deficit

Résumé

Une expérience en pot a été réalisée pour évaluer le potentiel des champignons mycorhiziens à arbuscules (CMA) à améliorer la résistance du bananier plantain au déficit hydrique. Trois inocula ont été utilisées : (1) une souche de *Glomus clavisporum* provenant d'Azaguié, (2) un complexe de spores isolé provenant d'Azaguié (5 ° 38 'N - 4 ° 05' O) et (3) un complexe de spores provenant de Bouaflé (6 ° 59 'N - 5 ° 45' O). Ces inocula ont été testés sur les variétés *Fhia 21* et *Orishele* en considérant trois niveaux d'apport d'eau (100, 60 et 30%). La croissance des plants et d'autres paramètres physiologiques ont été mesurés et comparés pour tous les traitements. Il en ressort que la colonisation des racines a diminué avec la réduction de l'apport d'eau. Les plants mycorhizés avaient les feuilles les plus larges, la plus grande teneur en eau et en chlorophylle, quel que soit le niveau d'apport en eau. De même, les CMA ont augmenté de manière significative les sucres solubles et les teneurs en proline en condition de déficit hydrique. Ces résultats démontrent que les CMA augmentent l'activité photosynthétique du bananier plantain et ont un impact sur sa croissance dans toutes les conditions d'apport en eau. La proline et les sucres solubles sont des molécules sensibles aux conditions de stress hydrique et leur production dans le bananier plantain sont améliorées en présence de CMA.

Mots clés : champignons mycorhiziens à arbuscules, Côte d'Ivoire, bananier plantain, résistance, stress hydrique.

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Introduction

One of the most constraints for crop production is water deficit. It is considered as a major important factor limiting crop yields around the world (Guo et al., 2009). Water deficit reduces nutrient diffusion in soil and leads to a reduced root absorption capacity in crop plants. Banana and plantain are widely grown in the warm and humid tropics and are very sensitive to drought stress. They need between 1440 and 1920 mm of rain per year for growth and production. A strong reduction in yield is noticed when less than 30 mm of water is provided for four months (Nkendahand and Akyeampong, 2003). Generally, the symptoms are apparent by plant wilting, reduction in the net photosynthesis rate, stomatal conductance, water use efficiency, relative water content and gradually diminution in total chlorophyll content.

To overcome agricultural abiotic constraints, particularly drought stress, the use of symbionts and beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) could be a promising way. Arbuscular mycorrhizal fungi can colonize roots of the majority of terrestrial plant species to establish symbiotic association (Smith and Smith, 2012). They increase root surface area and enhance ability to explore nutrients beyond the nutrient depletion zone (Smith and Read, 1997). Indeed, different AMF species have been observed to colonize plantain roots (Jefwa et al., 2012). Inoculated plants with AMF fungi can improve crop production under water deficit conditions. Azcón and Barea (2010) showed that AMF associated with plant roots are able to develop a range of activities to increase plant growth and crop productivity under stressed conditions.

The multifunctional properties of AMF to improve banana growth, nutrition and tolerance to drought during the nursery phase have been proved and suggest that AMF could stimulate the production of planting material of good quality with a high nutrient content and more adapted to environmental factors such as fertility and drought (José et al., 2012). Studies showed that AMF are able to increase banana growth and nutrient uptake (Declerck et al., 1995; Kavoo-Mwangi et al., 2013) or provide tolerance to drought stress (Nwaga et al., 2011, Séry et al., 2016). Several studies reported that AMF could enhance the ability of plants to cope with drought stress by improving plant nutrition and increasing plant metabolism tolerated to water deficit (Boomsma and Vyn, 2008), improving absorption capacity and vegetative growth (Wu et al., 2004) increasing root volume and dry weight (Boureima et al., 2007). Accumulation of some metabolites in plant tissues is an important mechanism to overcome abiotic stress (Barzana et al., 2014). Moreover, free amino acid, such as proline, is a contributor to osmotic adjustment in water deficit plants. Proline plays an important role in drought resistance. Some studies indicated reduction in proline level in AMF-inoculated plants under water deficit (Asrar et al., 2012; Pavla et al., 2013) when others highlighted increase in proline accumulation in mycorrhizal plants subjected to water deficit (Goicoechea et al., 1998; Suravoot et al., 2013). Accumulation of soluble sugars induced by AMF symbiosis is a positive response to water deficit since it can protect cellular components such as cell membranes and proteins, and sustain plant physiological activity (Serraj and Sinclair, 2002). Proline and soluble sugars act as osmoprotectants, two plantain varieties such as *FHIA 21* and *Orishele*, two improved varieties of plantain in extension in Côte d'Ivoire.

thereby, facilitate water uptake and stabilize macromolecular structures and subcellular membranes under drought stress (Gomes et al., 2010). Water stress also had a negative effect on chlorophyll content, which indicates a decrease in photosynthesis. The rate of photosynthesis is higher in mycorrhizal plants compared to non-mycorrhizal plants (Auge, 2001). Plant tolerance to drought stress differed with AMF isolate associated to plant (Nwaga et al., 2011). Likewise, Pagano et al. (2013) have shown that AMF may be systematically and functionally diverse with abundant ecological differentiation and specialization to environment.

In this study, we selected two contrast plantain production regions in Côte d'Ivoire in order to identify local specific beneficial fungi as inoculants to improve plantain resistant to drought stress.
The aim of this study was to assess the potential of AMF to improve resistance to water deficit of two plantain varieties such as FHIA 21 and Orishele, two improved varieties of plantain in extension in Côte d'Ivoire.

Materiels and Methods

Arbuscular mycorrhizal fungi strains isolation, characterization and multiplication
AMF spores were isolated from plantain rhizosphere in Bouaflé (6°59 'N, 5°45' W) and Azaguié (5°38 'N, 4°05' W), two main plantain production areas in Côte d'Ivoire. AMF spores were separated from soil samples by wet sieving or decanting (Gerdemann and Nicolson, 1963). Morphological spore description was performed by mounting spores on glass slides and staining them with polyvinyl-lactic acid glycerol (PVLG) mixed with Melzer's reagent (1:1 vol/vol) (Brundrett et al., 1994). AMF identification was based on species description provided by International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and the classification of Glomeromycota proposed by Schüßler and Walker (2010) and revised by Redecker et al. (2013).
One isolate of *Glomus clavisporum* obtained from plantain soil in Azaguié region, a spore complex from Azaguié region and a spore complex obtained from Bouaflé region were used. AMF inoculants were multiplied in pot cultures with sterilized fine sand as a substrate. Maize (*Zea mays* L.) was used as a host and was cultured for three months in a greenhouse under natural conditions. Mycorrhizal inoculum consisted of spores extract in pot cultures after trapping.

Plant material and culture substrate
Two plantain varieties (*Fhia 21* and *Orishele*) two months old derived from PIF technique obtained from CNRA (*Centre National de la Recherche Agronomique*) were used. This planting material was disinfected and roots removed. Plants were acclimated in sterilized compost after two weeks and planted into 12 l pots containing 10 kg sterilized mixture soil and clean sand in the proportion of three volumes soil for one volume sand. Soil parameters were (pH = 7.1; organic matter = 2.81 %; total nitrogen = 0.15 %; available phosphorus = 55 ppm). Soil and sand were sieved (mesh diameter = 2 mm) and autoclaved (120°C, 2 Kg/cm^2, 2 h 30 min) two consecutive days.

Inoculation and water deficit application method
Plants were inoculated with 150 spores per pot for AMF treatments and watered up to field capacity for 60 days to allow successful establishment of AMF symbiosis; then three levels of water supplied: 100% (as control), 60% (moderate stress) and 30% (severe stress) were applied. In this study, 100% ó 3000 ml of water, 60% ó1800 ml of water and 30% ó 900 ml of water.

Experimental design and growth conditions
The experiment consisted in a randomized complete block design with four inoculation treatments applied both for the two plantain varieties: (1) plants inoculated with Glomus clavisporum; (2) plants inoculated with a complex of AMF spores from Bouaflé; (3) plants inoculated with a complex of AMF spores from Azaguié; (4) non-inoculated plants (control). Three replicates of each treatment were performed for a total of 72 pots. Experiments were conducted in a greenhouse at Institut National Polytechnique-Félix HOUPHOUET-BOIGNY de Yamoussoukro under natural photoperiod for four months.

Mycorrhizal colonization and morphological parameters
After 60 days under water stress, root fragments were sampled, washed and cleared in 10 % KOH solution and stained with 0.05 % trypan blue according to Phillips and Hayman (1970).
Root colonization was estimated according to Trouvelot et al. (1986). Twenty root segments of l cm length per treatment were examined for the presence of arbuscules, vesicles, or hyphae. The two parameters retained were: (1) colonization intensity and (2) colonization frequency corresponding to the ratio between root fragments colonized by AMF mycelium and the total number of root fragments analysed.

Plant length and leaf surface measurements were done every 14 days after water supply. Leaf surface was measured according to Champion (1963).

Physiological and biochemical parameters

Leaf water content (LWC) was determined and calculated according to the equation of Levitt (1980). Leaf chlorophyll content was determined according to Lichtenthaler and Buschmann (2001). Chlorophyll was extracted in 80 % (v/v) acetone from 1 g of fresh leaf sample in the dark at room temperature. Absorbance was measured at 664 and 649 nm in a UV/VIS spectrophotometer. Chlorophyll a, b and total concentrations were calculated using the Porra (2002) equation:

Chl a = 13.36 x DO (664) – 5.19 x DO (649)

Chl b = 27.43 x DO (649) – 8.12 x DO (664)

Total chlorophyll = Chl a + Chl b

Fresh leaves (FL) were collected for the determination of free proline and total soluble sugars contents. Leaf proline content was determined according to Trolls and Lindsley (1955) by measuring the quantity of colored reaction product of proline with ninhydric acid. Proline was determined by spectrophotometric analysis at 528 nm. The concentration of proline was calculated using L-proline for t he standard curve. Soluble sugars were extracted from 100 g leaf tissues in hot 80% (v/v) ethanol by Dubois et al. (1956) method; the absorbance was measured at 490 nm.

Data analyses

Data were subjected to ANOVA with STATISTICA 7.1. Fisher's least significant difference (LSD) was used for post-hoc comparisons to determine differences between means within and among treatments. Differences were considered significant at $p < 0.05$.

Results

Identification of AMF spores

A total of 12 AMF species were identified at both areas with different population relative abundances (Table 1). *Gomus clavisporum, Rhizophogus intraradices, Acaulospora scrobiculata* and *Funneliformis mosseae* appeared as the most dominant species at both sites. The species *Glomus clavisvorum* was observed with high proportion in most study sites of these two agroecological regions.

Influence of water supply on mycorrhizal colonization

The observation of roots after 60 days of water stress showed effective AMF root colonization for all the observed plants. No mycorrhizal colonization was observed in non-inoculated plants. Mycorrhizal colonization of both plantain varieties varied with water stress and was higher in plants grown under good water supply than plants grown under water stress conditions. Root colonization decreased with water reduction (Figure 1). The percentage of root colonization was highest with the complex AMF spores of Azaguié for the three levels of water supply than the other AMF treatments.

Mycorrhizal fungal inoculation improves leaf water, area and chlorophyll content

All AMF inoculants significantly increased leaf area and water content for both plantain varieties under all levels of water supply when compared with the non-inoculated plants. Leaf area and water content were reduced by water deficit conditions (Table 2 and 3). At 60 % of water supply, Glomus clavisporum, complex AMF spores of Bouaflé and complex AMF spores of Azaguié respectively increased leaf area by 28.24 %, 16.99 % and 30.89 % compared to non-mycorrhizal plants for Fhia 21 variety. For Orishele variety, leaf area increased respectively by 12.24 %, 11.46 % and 13.91 % compared to non-mycorrhizal plants. Under severe water supply condition, Glomus clavisporum, complex AMF spores of Bouaflé and complex AMF spores of Azaguié respectively increased leaf area by 44.99 %, 30.11 % and 38.78 % compared to non-mycorrhizal plants for Fhia 21 variety and increased respectively by 34.74 %, 35.32 % and 40.70 % for Orishele variety.

The three AMF treatments stimulated chlorophyll a, b and total content in leaves of both plantain varieties (Table 2 and 3). The content was reduced by water stress but remained high in mycorrhizal plants compared to non-mycorrhizal plants.

Table 1. AMF population relative abundances at both regions

AMF species	Azaguié	Bouaflé
Sclerocystis sinuosum	1.4 %	10 %
Rhizophogus intraradices	16 %	17 %
Glomus sp.	11 %	8 %
Entrophospora sp.	0.5 %	11 %
Claroideoglomus sp.	1.1 %	9 %
Funneliformis mosseae	9 %	13 %
Acaulospora scrobiculata	19 %	11 %
Glomus clavisporum	22 %	17 %
Scutellospora sp.	2 %	0.5 %
Ambispora sp.	5 %	0.7 %
Acaulospora sp.	9 %	1.9 %
Gigaspora sp.	4 %	0.9 %

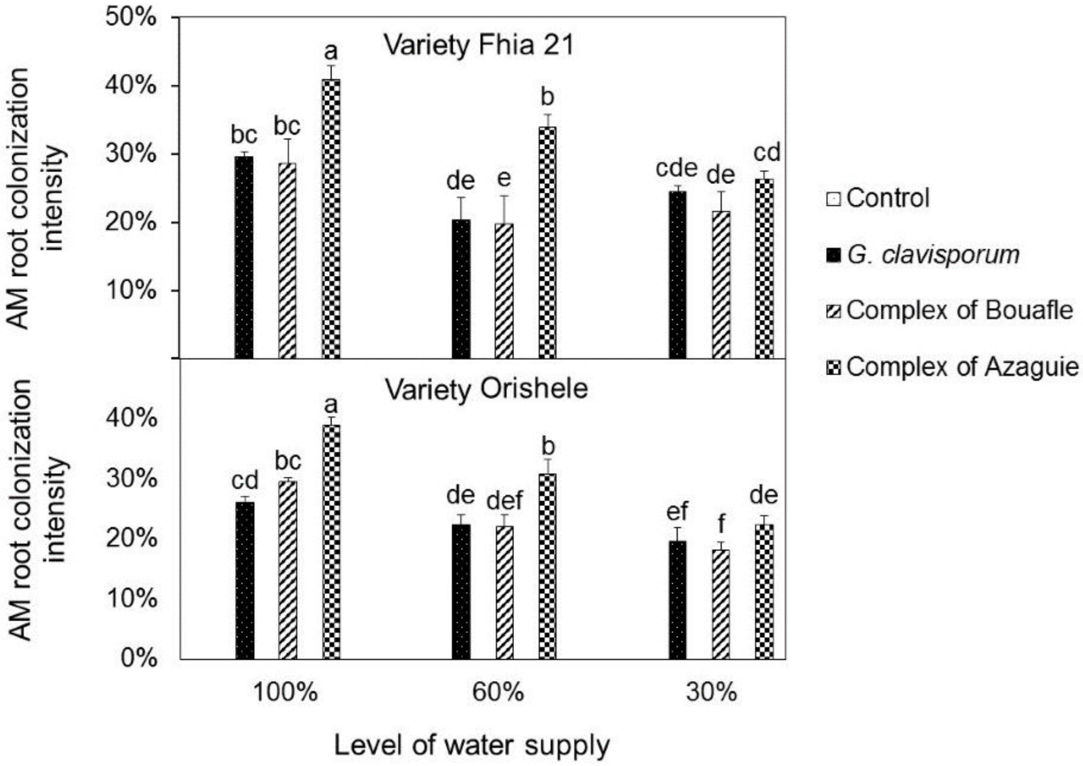

Figure 1. AMF root colonization of both plantain varieties under different levels of water supply as mean ± standard deviation. Means with different letters are significantly different (LSD, p = 5%)

Under severe water stress condition, chlorophyll contents were not influenced by mycorrhization regardless of strain for Orishele variety. However, for the Fhia 21 variety only plants inoculated with Glomus clavisporum had significantly higher chlorophyll contents than non-mycorrhizal plants.

AMF improves soluble sugars and proline contents

The content of soluble sugars increased remarkably by colonization of AMF whatever the imposed water supply (Figure 2). In the absence of water stress, for Fhia 21 variety, soluble sugars pass increased significantly from 158.25 µmol / mg FM (control) to 231.57; 273.92 and 274.80 µmol/mg FM respectively in leaves of plants inoculated with G. clavisporum, Bouaflé spore complex and Azaguié spore complex. For Orishele variety, they soluble sugars increased significantly from 108.35 µmol/mg FM in leaves of control plants to 189.72; 177.22 and 252.93 µmol / mg FM respectively in leaves of plants inoculated with G. clavisporum, Bouaflé spore complex and Azaguié spore complex. Soluble sugars contents increased with the severity of stress, and remained significantly higher in mycorrhizal plants compared to non-mycorrhizal plants.

Whatever water supply, proline remained significantly higher in mycorrhizal plants compared to non-mycorrhizal plants (Figure 3). Nevertheless, under severe stress conditions (30 % of water supply), plants of Fhia 21 variety inoculated with Glomus clavisporum showed a significantly higher proline content compared with other AMF treatments. Under moderate water stress condition, for Fhia 21 variety, proline increased significantly from 1.40 µmol/mg FM (control) to 2.16; 6.48 and 9.45 µmol/mg FM respectively in leaves of plants inoculated with G. clavisporum, Bouaflé spore complex and Azaguié spore complex. For Orishele variety, proline increased significantly from 0.70 µmol/mg FM in leaves of control plants to 2.60; 2.85 and 1.73 µmol/mg FM respectively in leaves of plants inoculated with G. clavisporum, Bouaflé spore complex and Azaguié spore complex. In general, the proline content increased with the severity of the stress, and remained higher in mycorrhizal plants compared to non-mycorrhizal plants.

Discussion

This study investigated the potential of arbuscular mycorrhizal fungi to improve plantain resistance to water deficit using three sources of inoculants on two plantain varieties. All AMF inoculants significantly increased all the measured parameters for both plantain varieties under all water supply conditions when compared to the non-inoculated plants. This study also showed the decrease in plantain growth and development under water stress conditions. This decrease is in line with mycorrhizal colonisation intensity that also decreased with the reduction of water quantity (Abbaspour et al., 2012). This reduction of the intensity of AMF infectivity parameters under water stress conditions can be explained by the physiological changes of the host plant (Juniper, 1993). Decrease in plant photosynthesis products affect the status in root carbohydrates, and consequently the rate of mycorrhizal fungi colonization (Juniper, 1993; Thomason, 1990). Some authors (Rambal et al., 2003; Uhlmann et al, 2006) showed that water stress reduces mycorrhization rates, as also evidenced by our results. However, in presence of AMF, whatever the source of inoculation, the measured parameters were better than in absence of AMF inoculants. It was shown that through expanding root system, mycorrhizal plants improve hydraulic conductivity and water uptake and therefore increase drought tolerance (Boomsma and Vyn, 2008). Nevertheless, the potential of AMF to alleviate drought stress in host plants is limited to a certain water supply threshold as the establishment of AMF depends also on plant development (Pavla et al., 2013).

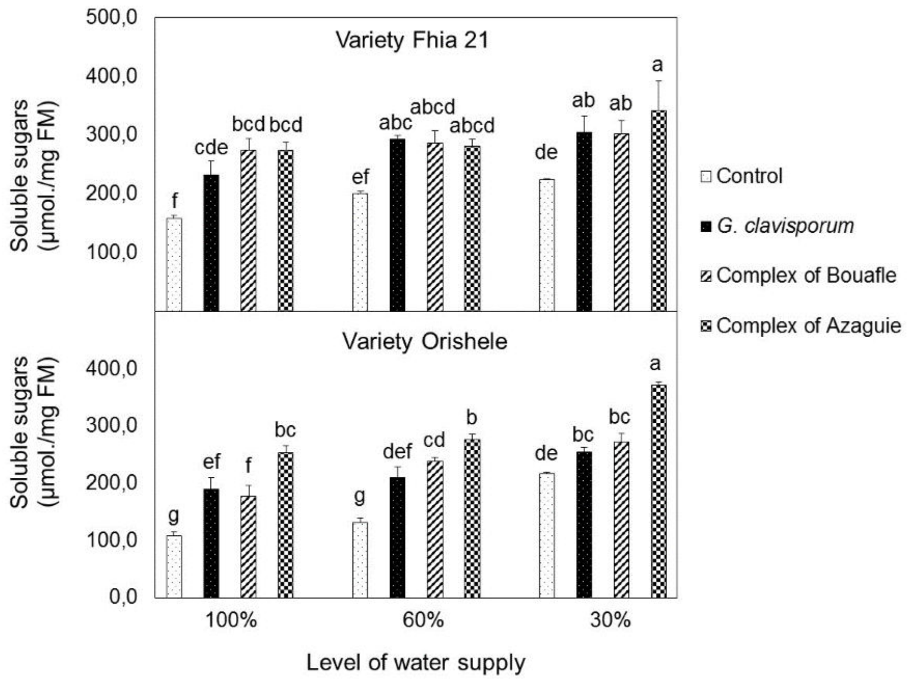

Figure 2. Soluble sugars of both plantain varieties under different levels of water supply presented as mean ± standard deviation OR standard error. Means with different letters are significantly different (LSD, p = 5%).

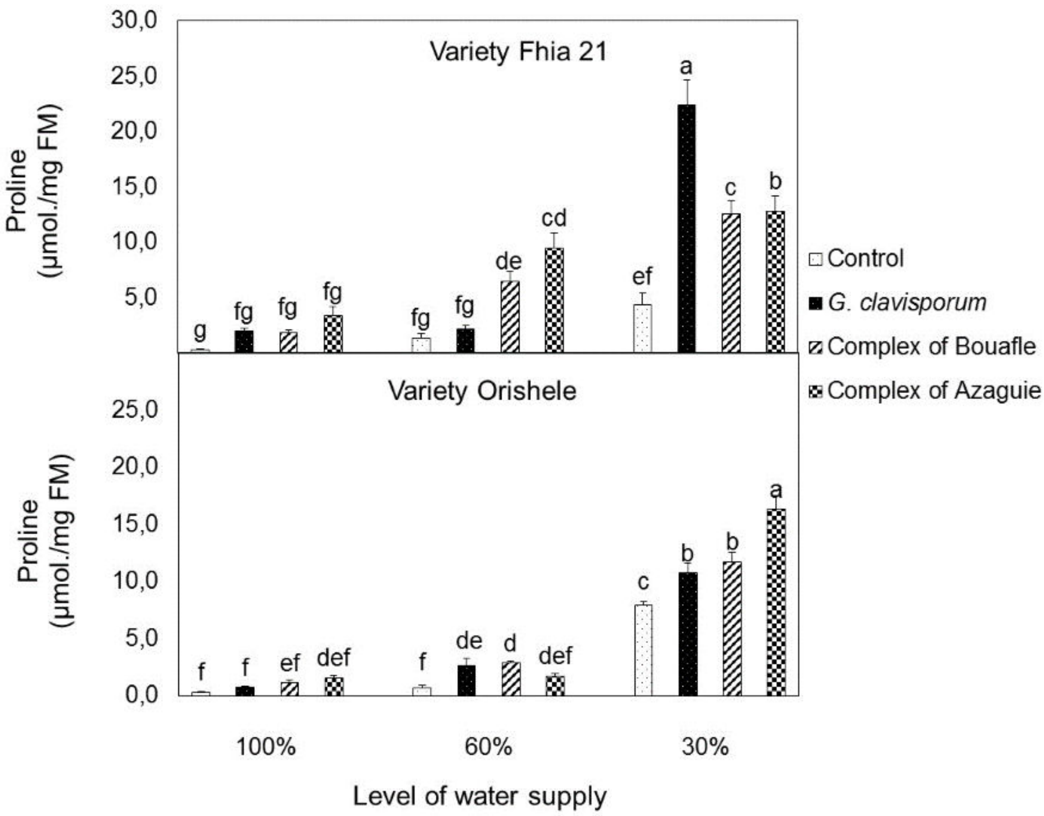

Figure 3. Proline of both plantain varieties under different levels of water supply presented as mean ± standard deviation OR standard error. Means with different letters are significantly different (LSD, p = 5%).

As demonstrated in this study, similar results were obtained in the case of inoculated corn with Glomus mosseae (Abdelmoneim et al., 2014) and the inoculated rice with Glomus intraradices (Michel et al., 2011) under stress conditions where inoculated plants had significant increase of growth parameters. Mycorrhizal symbiosis produces physical, biochemical and physiological changes on colonized roots that lead to a better general status of the plant and its different organs (Barea et al., 1997, please add new ref).

Mycorrhizal plants contained more chlorophyll a and chlorophyll b concentration in leaves than non-mycorrhizal plants under water stress conditions. These results corroborated previous results (José and Marta, 2008; Asrar et al., 2012). Shinde and Khanna (2014) reported higher amount of chlorophyll pigments in mycorrhizal potato plants and Shinde and Jaya (2015) reported the same trend with pea under water stress condition. However, the content of chlorophyll in leaves of mycorrhizal and non-mycorrhizal plants was reduced with increasing water stress.

In order to support drought stress, plants accumulate some specific molecules including organic solutes like soluble sugars, proline or other amino acids to regulate the osmotic potential of cells (ref). This allows water absorption improvement under water stress conditions (Zhang et al., 2010). In this study, under water deficit stress, mycorrhizal plants synthesized more proline and soluble sugars contents in leaf tissues of both plantain varieties than well-watered conditions. These results are in line with observations made in leaf tissues of mycorrhizal *Poncirus trifoliate, Macadamia tetraphylla, Oryza sativa* and pea plants (Fan and Liu, 2011, Suravoot et al., 2013, Ruíz-Sánchez et al., 2011, Shinde and Jaya, 2015). The greater content of proline in leaves of mycorrhizal plants probably plays a key role as osmolyte participating in osmotic adjustment (Hassine et al., 2008) to control water uptake and maintainance in leaves. This suggests that mycorrhizal plants might deploy a better capacity for osmotic adjustment relative to the non-mycorrhizal plants under water stress, which was supported by better leaf turgor in the mycorrhizal plants. On the other hand, AMF inoculation helps soluble sugars accumulation under adverse conditions, potentially resulting in a decrease of osmotic potentials in host cells. Evelin et al. (2009) suggested that sugar accumulation in AM inoculated plants is due to AMF colonization but not to P improvement, because AM-mediated C pools and AM-induced hydrolysis of starch to sugars may involve in the process, irrespective of P status of AM and non-AM plants. Some studies have shown a reduction in proline levels in AMF plants under water deficit (Nwaga et al., 2011; Pavla et al., 2013). The response of plantain mycorrhizal symbiosis may therefore depend on fungi strains and plantain varieties. These results corroborated those obtained with the varieties ELAT and PITA 21 in Cameroon where *Glomus* sp. and *Scutellospora* sp. strains allowed better development and tolerance to water stress of inoculated plants (Nwaga et al., 2011).

Conclusion

This study demonstrated that mycorrhizal colonization can mitigate the adverse effects of water stress on treated plants. *Glomus clavisporum*, complex AMF spores of Bouaflé, and complex AMF spores of Azaguié increased all growth parameters for both plantain varieties under well-watered and drought stress. Mycorrhizal plants had more chlorophyll contents than non-mycorrhizal plants under all water supply conditions. The greater contents of proline and soluble sugars in leaves of mycorrhizal plants may provide high energy to promote Fhia 21 and Orishele plantain varieties growth under water deficit stress. This study suggests that the use of indigenous AMF isolated in tropical soil is a promising approach to alleviate water stress damage in plants. It also suggests that mycorrhizal inoculation is able to stimulate tolerance of plantain under water deficit condition by increasing accumulation of organic solutes.

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