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MYCORRHIZAL STATUS OF CULTIVATED AND WILD PEARL MILLET (*PENNISETUMGLAUCUM* (L.) R. BR) IN THREE AGRO-ECOLOGICAL ZONES OF SENEGAL

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ABSTRACT

Pearl millet (*Pennisetumglaucum* (L.) R. Br) is a cereal grown in the driest tropical and subtropical regions of Africa and Asia. Thanks to its strong capacity to adapt to the dry tropical climate and low fertility soils, millet plays an important role in the food security of local populations in these regions. It is able to establish a mycorrhizal symbiosis with arbuscular mycorrhizal fungi (AMF), which play a key role in the absorption of nutrients and the mitigation of abiotic and biotic stress in their host. We first evaluated the mycorrhizal status of cultivated and wild millet in three agro-ecological zones of Senegal by following a rainfall gradient and then the effect of inoculation with AMF on the growth of cultivated millet. We found that wild and cultivated millet establishedarbuscular mycorrhizal symbiosis but a low rate of mycorrhizationwas observed. The frequency of mycorhization was significantly higher in cultivated than in wild plants. We then evaluated the impact of AMF inoculation on plant growth and found very limited impact. These results suggest that millet is not very dependent on mycorrhizae although AMF may stimulate its growth.

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INTRODUCTION

Pearl millet (*Pennisetumglaucum* (L.) R. Br) is a major staple food and source of fodder and fuel in the arid and semi-arid regions of sub-Saharan Africa and India. The vegetative, reproductive and physiological characteristics of pearl millet make it a crop well suited for growth under difficult conditions, including low soil fertility, high pH, low soil moisture, high temperature, high salinity and limited rainfall where maize, rice, sorghum or durum wheat may fail (Vadez *et al*., 2012). Pearl millet grain is highly nutritious, with 8-19% protein, low starch, high fiber (1.2 g/100 g; Nambiar *et al*., 2011), and higher micronutrient concentrations (iron and zinc) than rice, wheat, maize and sorghum (Tako *et al*., 2015). It was domesticated in the central Sahel (Mali-Niger) about 4900 years ago as corroborated by archaeological and genomic studies (Burgarella *et al*., 2018). Despite the clear importance of pearl millet in agriculture, the production and productivity of this staple crop are very low, with an average grain yield of just 900 kg/ha. This low production is due, among other things, to climatic variability and low soil fertility, particularly in terms of nitrogen and assimilable phosphorus. It is well established that the association of cereal roots with AMF in the soil is likely to increase the hydromineral nutrition and productivity of these plants when grown under adverse soil conditions (Rodriguez *et al*., 2008; Ahanger *et al*., 2014; Salam *et al*., 2017). Cultivated pearl millet, like most cultivated plant species, can form a symbiotic association with these AMF. Arbuscular mycorrhizal fungi play a significant role in soil structure and aggregate stability (Rillig and

Mummey, 2006; Bitterlich *et al*., 2018) and plant water and nutrient uptake particularly in poor soils (Smith and Read, 2008). AMF can also form hyphal networks that can promote the colonization of neighboring plants of the same or different species (Van der Heijden and Horton, 2009). In Senegal, cultivated millet still coexists with wild millet around the plots or even inside some farmer's plots. Gene flows with wild relatives in the western and eastern Sahel have contributed to the increase in the diversity of cultivated millet in Africa (Burgarella *et al*., 2018). Although there are some studies on the response of cultivated millet to inoculation with AMF (Subba Rao *et al.*, 1985; Krishna *et al*., 1985), the mycorrhizal status of wild millet is not yet know. The aim of this study is to determine the colonization of cultivated and wild millet by AMF in field conditions in three agro-ecological zones of Senegal following a rainfall gradient. The response of cultivated millet to inoculation with AMF was than examined in green house conditions.

MATERIALS AND METHODS

Field experiments: The experimental site were located inDarou-Mousty (15°02'31"N, 16°02'53"W), Dya(14°13'60"N, 16°10'0"W) and Nioro (13°45'00"N, 15°48'00"W), Senegal, West Africa which are in the southern region of the Peanut Basin (13°45 N, 15°47 W) at 18m above sea level (Figure 1).

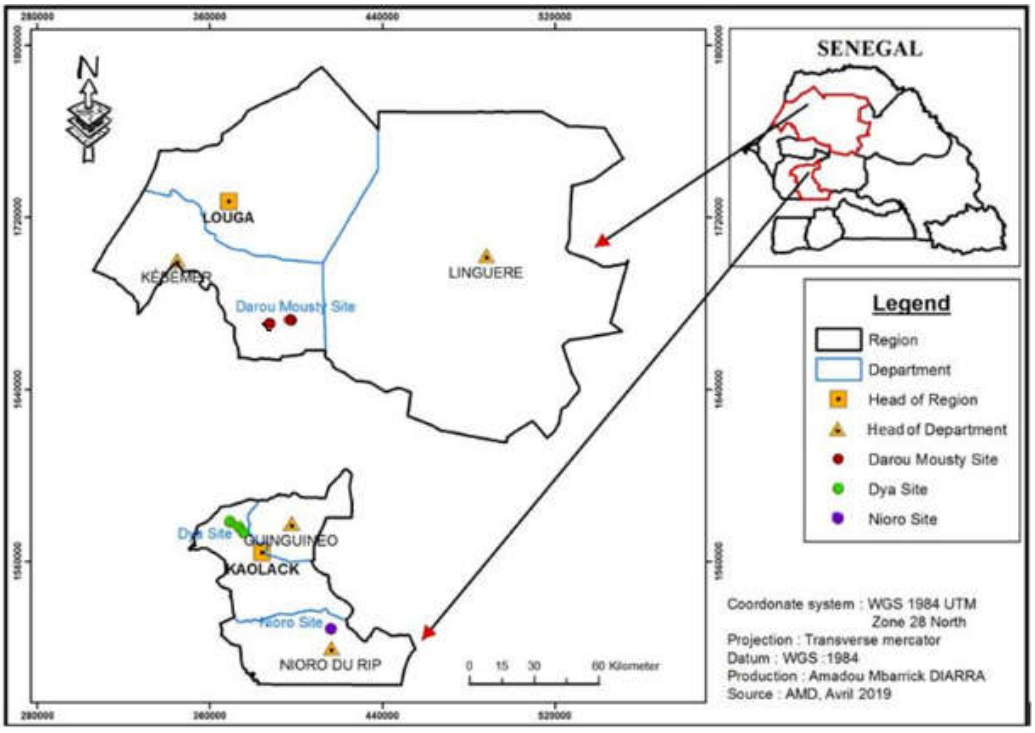

Figure 1. Locations of the experimental sites

The two major crops cultivated at the site are millet (*Pennissetumglaucum* (L.) R. Br.) and peanut (*Arachishypogaea* L.). The mean annual precipitation is 300-750mm and mainly comes between July and September. The mean air temperatures range from 20.0 to 35.7°C. The soil is a Deck-Dior (Badiane *et al*., 2000) loamy-sand (finesandy, mixed Haplic Ferric Lixisol), a leached ferrugineous tropical soil. Top soil (0-30 cm) has sand content of >90%, organic matter and total N contents of 0.52 and 0.03% respectively, total P content of 70 mg kg^{-1}, and mean pH (water) of 6.2. The experiment was conducted during the 2016 rainy season. Pearl millet Souna 3 variety was used on all sites in order to limit plant genotype effect. The farmers followed their traditional agricultural practices (fertilization or not, previous crops, etc.) that were recorded as well as the cropping history and the vegetation around (Table 1). At plant maturity, soil and root samples were taken. From each cultivated plot, a set of five replicates was collected. Each biological replicate came from the roots of five plants harvested from each cultivated plot in a 10 meters space from each other. Roots of wild relatives were collected within or around the cultivated plots. For each individual plant, approximately 500 g of rhizospheric soil was collected at a depth of 0 - 20 cm for analyses. Roots were placed in plastic bags in ice and transported to the laboratory for the detection and observation of AMF. A total of 60 root samples were collected: two plant types (cultivated and wild), six plots and five replicates per plot.

Greenhouse experiment

Soil and plant materials: Pot trials were carried out (July 4th to October 2nd, 2017) with the same variety of pearl millet (Souna 3). The treatments consisted of uninoculated millet plants and millet plants inoculated with a cocktail of three strains of AMF applied in a mixture of inoculants. The soil used was collected from the locality of Sangalkam (about 30 km east of Dakar, Senegal). The soil sampled consisted of 88.8% sand, 5.4% silt, 5.8% clay, 0.6% organic matter, 0.3% total C, 0.02% total N, 333.5 ppm total K and 41.4 ppm total P. It was sieved with 2 mm sieves, sterilized at 120°C for 48 h and placed in plastic bags (7 kg of soil per pot). Seeds of Souna 3 millet were rinsed thoroughly in sterile distilled water before being sown directly into PVC tubes containing plastic bags (4 grains per pot) with the culture substrate. Seedling were thinned to one plant per pot eight days after sowing.

Fungal materials and AMF inoculum production: The AM fungi used in this study were *Glomus aggregatum*, *G.fasciculatum* and *Rhizophagus irregularis.* They were propagated as pure cultures in a greenhouse using a mycotrophic plant (Zea mays) and sterilized (2 x 2 hours at

180°C) soil from Sangalkam (Senegal). After 3 months, maize roots and culture substrate were collected to assess spore density (Gerdemann and Nicolson, 1963) and the level of roots colonization by AMF (Trouvelot *et al*., 1986). The Colonized maize roots were cut into about 1 cm fragments and carefully homogenized in the culture substrate to form the AMF inoculum. For each AMF strain, the inoculum consisted of a mixture of sandy soil, spores and mycorrhizal root fragments.

Seedling inoculations and experimental design: The inoculation experiment was carried out as a completely randomized single-factor block device: mixed inoculations a cocktail of the three strains and a control represented by the uninoculated plants. This device comprises three successive blocks corresponding to three measurement time points (30, 60 and 90 day after sowing (DAS)). Twelve repetitions were carried out for each of the two treatments (Figure 2, photo 1). At the time of planting, 20 g of AMF inoculum was placed at a depth of about 4 cm in the center of pots and mixed with sterilized soil. Treatments without AMF received nothing as inoculum. Millet plants were watered regularly with tap water to field capacity.

water. Fine roots which are more likely to form mycorrhizae were collected, cleaned with KOH (10% (w/v)) at 80°C for 30 min, and stained with trypan blue (0.05% (w/v) in a 0.8% acid acetic solution) at 90°C for 35 min (Phillips and Hayman, 1970). Frequency and the intensity of colonization by AMF were evaluated for each treatment according toTrouvelot*etal*. (1986).

Statistical analysis

Statistical analyses were performed with the R software (version 3.5.1). For the field experiment, mycorrhizal infection percentages were subjected to a three-way analysis of variance (ANOVA) (sites xplots x plants). Mean values were compared using Tukey test (Honestly significant differences, HSD) at the significance levels $p<0.05$. For the greenhouse experimental, Shapiro normality test ($p<0.05$) was applied to all data. Two approaches were used: a parametric approach (single-factor analysis of variance (ANOVA) for variables tested for normality, followed by a comparison of means using the Tukey's test (HSD) at the significance level $p<0.05$.

Figure 2. A complete randomized block experimental design for the study of the responses of millet to inoculation with AMF

Table 1. Crop precedents and inputs used in the last year before the experiment for each experimental plot

Sites	Plots	Previous culture	Fertilization	Cooccurring plants
Darou Mousty	P1	Cowpea	Unfertilized	Striga Grasses
	P2	Fallow land over 10 years old	Fertilized (NPK 15-10-10)	Weed-free field
Dya	P3	Groundnut	Unfertilized	Trees Grasses *Sesbania rostrata*
	P4	Groundnut	Fertilized (NPK 15-10-10)	-
	P5	Orchard	Unfertilized	Mango trees Peppers Cassava Weed-free field Millet plants already uprooted
Nioro	P6	Groundnut	Fertilized (NPK 15-10-10 and Urea)	Grasses

Measurement of plant morphological traits and chlorophyll content: Chlorophyll content, plant height, collar diameter and number of tillers were measuredat each time point. For the chlorophyll content, we chose the third leaf from top to bottom, and made three measurements at three different locations on the same leaf with a SPAD meter. The height of the plant was determined from the base of the main shoot to the tip of the longest leaf. The diameter at the collar was determined by measuring the diameters of the axles at the base of the stem. For each measurement time point, individual plants were harvested, the above-ground and root biomass were weighed after drying in an oven for 72 hours at 70°C.

Estimation of root colonization by arbuscular mycorrhizal fungi: Roots were harvested and carefully washed with tap

For variables whose distribution does not follow the normal distribution, a non-parametric approach was used (median comparison).

RESULTS

Mycorrhizal status of cultivated and wild pearl millet

We tested if wild and cultivated pearl millet plants established arbuscular mycorrhizal symbioses under field conditions in three agro-ecological zones of Senegal following a rainfall gradient. We observed arbuscular mycorrhizal symbioses in all samples from our three sites indicating that both cultivated and

Table 2. Summary of ANOVA for the effects of site, plot and plant type on the percentage of root length colonization (intensity) and frequency of root colonization

Factors tested	Intensity	Frequency
Sites	NS (P=0.79)	NS (P=0.75)
Plots	NS (P=0.06)	* (P=0.01)
Plants	NS (P=0.40)	** (P=0.008)
Sites x Plants	NS (P=0.73)	NS (P=0.65)
Plots x Plants	NS (P=0.15)	NS (P=0.19)

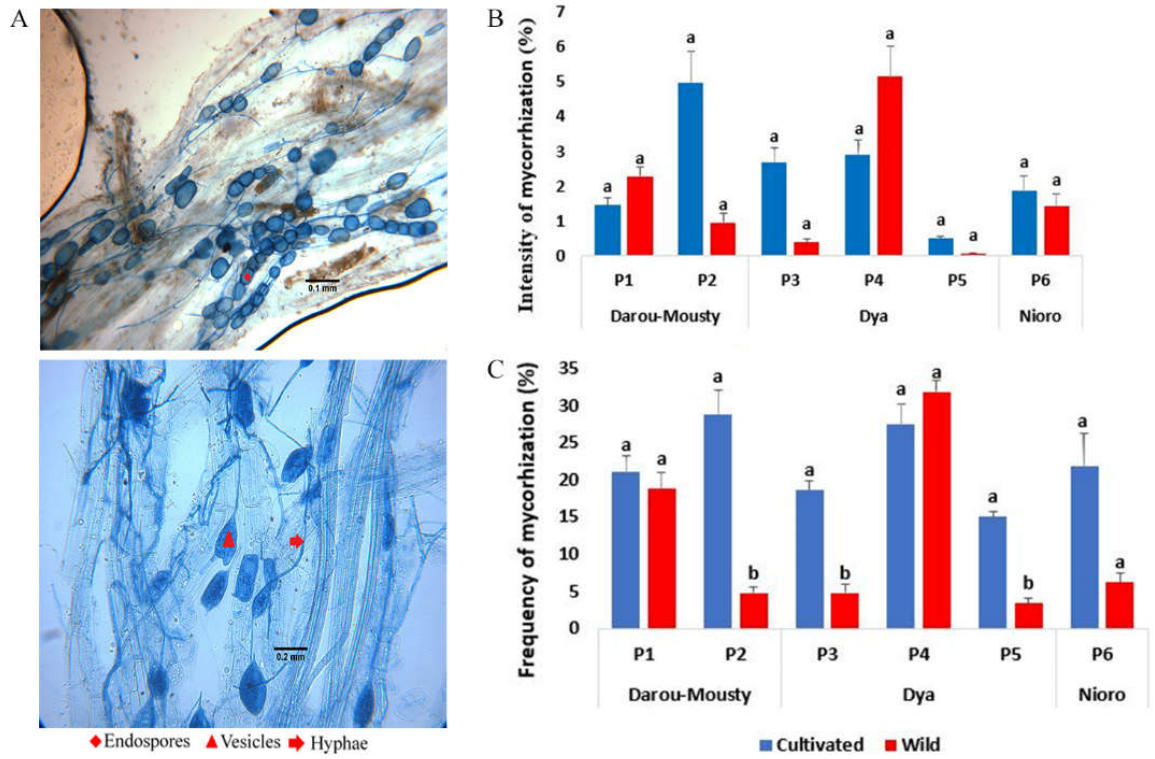

Figure 3. Mycorrhizal status of cultivated and wild pearl millet. (A) root fragments with typical arbuscularmycorrhizal (AM) structures (hyphae, vesicles and endospores), (B) percentage of root length colonized by AM fungi (intensity of mycorrhization), (C) frequency of root colonization

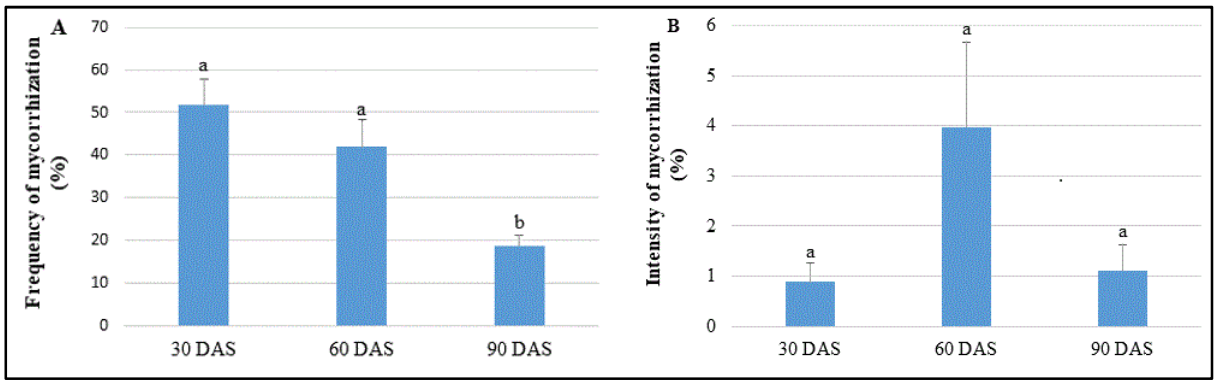

Figure 4. Effect of inoculation with AMF on frequency (A) and intensity (B) of root colonization

wild pearl millet always established mycorrhizal symbioses under field conditions (Fig. 3A). However, the percentage of root length colonized by AMF (≤ 5%) and frequency of root colonization (≤ 30%) were low under fields conditions (Fig. 3B). An ANOVA (Table 2) revealed significant plant type (cultivated vs wild pearl millet; $p = 0.008$) and plot ($p = 0.015$) effects on frequency of root colonization (Fig. 3C). Cultivated plants showed a higher frequency of root colonization than wild pearl millet, indicating that cultivated plants were more colonized than their wild relatives. On the other hand, no significant differences in percentage of root length colonized by AMF were detected.

Effect of inoculation with AMF on pearl millet growth and physiology: We next evaluated the impact of AM symbiosis on cultivated pearl millet by analyzing the growth and physiology of plants grown in sterile soil with or without AMF inoculum. A significant difference in mycorrhizal frequencies was noted between the three measurement times ($p = 0.000$) in inoculated plants. Frequency of millet root system infection was found to be higher at 30 DAS (51.83%). This frequency decreases progressively to 41.91% at 60 days and 18.75% at the end of the experiment (90 DAS; Figure 4A). Mycorrhizal intensitysalso varied with time, but these differences were not statistically significant ($p = 0.127$, ANOVA).

Table 3. Effect of AMF inoculation on chlorophyll content and millet growth

Number of DAS / treatments	Chlorophyll content	Height (cm)	Collar diameter (cm)	Number of tillers	Above-ground biomass (g)	Root biomass (g)
30 DAS						
NM	30.48 ± 4.89 a	87.45 ± 7.53 b	12.71 ± 1.57 b	4.16 ± 0.81 a	11.25 ± 2.96 b	4.04 ± 2.33 a
M	28.60 ± 3.88 a	92.46 ± 5.22 a	13.55 ± 1.49 a	3.88 ± 1.14 a	13.92 ± 2.59 a	4.41 ± 1.71 a
60 DAS						
NM	35.91 ± 5.33 a	186 ± 40.94 a	16.85 ± 2.86 a	5.71 ± 1.45 a	64.86 ± 24.61 a	17.07 ± 9.76 a
M	35.73 ± 6.53 a	186.16 ± 33.57 a	16.47 ± 2.83 a	5.50 ± 1.47 a	60.10 ± 24.89 a	11.13 ± 5.48 a
90 DAS						
NM	51.78 ± 8.16 a	243.25 ± 53.99 a	16.62 ± 2.41 a	5.66 ± 1.07 a	105.63 ± 40.72 a	56.65 ± 37.51 a
M	49.71 ± 11.2 a	260.50 ± 28.89 a	16.5 ± 2.43 a	6.16 ± 1.33 a	112.38 ± 24.96 a	50.47 ± 25.19 a
Factorstested						
Age : 30	NS	**	*	NS	*	NS
60	NS	NS	NS	NS	NS	NS
90	NS	NS	NS	NS	NS	NS

Photo 1. Block with millet plants used for the measurement of the different parameters

By the fourth week (30 DAS), the intensity of mycorrhization was very low (0.88%). This intensity increased to 3.97% at 60 DAS and decreased to 1.11% at the end of the experiment (90 DAS; Figure 4B). Inoculation with AMF had no significant effect on the chlorophyll content, the number of tillers and root biomass of millet plants throughout the duration of the experiment. However, a significant positive effect was observed at 30 DAS on height (p = 0.006), collar diameter and above-ground biomass (p = 0.029). At 60 and 90 DAS, the results showed that the growth parameters of the control and inoculated plants were not significantly different (Table 3).

DISCUSSION

Pearl millet is a cereal crop that adapts very well to hot and dry environmental conditions. It is cultivated in the driest tropical and subtropical regions of Africa and Asia. Thanks to its strong capacity to adapt to the dry tropical climate and low fertility soils, pearl millet plays an important role in the food security of local populations in these regions. Some studies predict a decrease in millet productivity of about -6% in the most arid areas of Africa by the year 2100 (Berg *et al*., 2013). It is generally known that adaptation to arid conditions can be associated with root traits such as well-developed architecture (Lynch, 2011), or even general interactions with certain soil microorganisms (Grover *et al*., 2011), in particular the association with mycorrhizal fungi (Rodriguez *et al*., 2004). Our study focuses on the impact of mycorrhization on millet growth. We firtst evaluated if pearl millet enter AM symbiosis in field conditions in Senegal. Analysis of the mycorrhizal status of cultivated and wild millet in three contrasting regions of Senegal indicated that under natural conditions, both cultivated and wild millet established mycorrhizal symbiosis but mycorrhizal intensities are very low (5%) suggesting that the interaction was limited. Cultivated millet showed higher frequencies of root colonization than wild millet. One possible explanation for this observation is that wild genotypes may have developed adaptations to nutrient-poor environments and are less dependent on mycorrhizal infection than cultivated genotypes (Koide *et al*., 1988). This may also be related to the modification of root characteristics during millet domestication (Eissenstat *et al*., 2015; Chen *et al*., 2016). This low level of *mycorrhizal colonization* could also be explained by the low density of endomycorrhizal plants and the frequent soil disturbances in the plots studied, which could reduce the density of AMF propagules needed to colonize millet roots in sandy soils where C is minimal (Vestberg *et al*., 2011; Peay *et al*., 2013; Porazinska *et al*., 2018). We next evaluated the impact of AM symbiosis on pearl millet growth and physiology in pot by inoculation with a cocktail of three AMF strains (*G. aggregatum*, *G. fasciculatum* and *R. irregularis*) in

sterile soil. Our results show that inoculation with AMF does not improve millet growth. Indeed, the chorophyll content of inoculatedplants is lower than that of controls, however the difference is not significant. Therefore we cannot think of a depressive effect of mycorrhization in this case because the depressive effects of mycorrhization reported in the literature are generally attributed to deficient conditions of photosynthesis, which was not the case in this experiment. It has been shown that the mycorrhizal fungi strains used have been able to stimulate growth in several fruit trees (Guissou *et al*., 1998; Bâ *et al*., 2000; Thioye *et al*., 2017). However, the response of plants to mycorrhization depends not only on the fungal species (Plenchette *et al*., 1982) but also on the species of the host plant whose mycorrhizal dependency (Plenchette *et al*., 1983) is mainly related to soil fertility and root system morphology (Baylis, 1975). It is important to note that pearl millet is, among all grasses, considered to have a low mycorrhizal dependency, although very important growth stimuli have been reported (Subba Rao *et al*., 1985). This mycorrhizal dependency also fluctuates according to the millet genotype (Krishna *et al*., 1985) and the mycorrhizal strains used (Krishna & Dart, 1984). It would be interesting to carry out further experiments in which plants would be inoculated with the 3 strains of AMF separately (*G. aggregatum*, *G. fasciculatum* and *R. irregularis*) and the impact of stressful environmental conditions could be evaluated to assess the behavior of pearl millet associated with each of these strains of AMF.

CONCLUSION

We thus conclude that the mycorrhization of millet remained relatively weak and did not induce the classical growth stimulation reaction which is mainly a function of the capacity of AMF to develop an important network of hyphae in the rhizosphere (Tinker, 1978). From an agronomic point of view, these results suggest that AMF are not a potential target for millet productivity improvement. Therefore, it would be important to explore other avenues concerning other fungi associated with millet roots.

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