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Mineral nutrition of mycorrhized tropical gum tree *A. senegal* (L.) under water deficiency conditions

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A pot experiment was set to examine the effects of arbuscular mycorrhizal fungi (AMF) on mineral nutrition of a tropical legume tree (*Acacia senegal)* under three different water status. *Acacia senegal* seedlings were inoculated with three species of AMF, *Glomus intraradices, Glomus fasciculatum* or *Glomus mosseae*. Three water levels (field capacity, moderate water deficiency and severe water deficiency) were applied to the pots after transplantation. *A. senegal* seedlings were colonized by the three AM fungi. Twelve weeks after water stress imposition, uptake nutrient of *A. Senegal* was enhanced by mycorrhizal inoculation under moderate and severe water status. Root colonization varied from 30.4 to 62.5%. The lowest intensity (30.4%) was observed on field capacity associated with *G. intraradices* and the highest root colonization (62.5%) was observed on severe water deficiency associated with *G. fasciculatum*. Relative improvement was noted in the foliar nutrient content nitrogen (N), phosphorus (P), potassium (K) and shoot water content of the inoculated plants, whatever the water regime. Mycorrhizal inoculation has no significant effect on shoot calcium (Ca), magnesium (Mg) and sodium (Na) compared to uninoculated plants. *G. fasciculatum* was the most efficiency fungus in nutrient foliar of *A. senegal* plants under water defiency conditions. Inoculating *A. senegal* plant with the arbuscular mycorrhizal fungus *G. fasciculatum* increased ability to acquire N, P, and K under water deficiency conditions.

Key words: Arbuscular mycorrhizal fungi, *Acacia senegal*, mineral nutrient, water deficiency.

INTRODUCTION

The major factors affecting plant growth in Sahelian zone agrosystem are water and nutrient (Floret et al., 1993). Water availability has been recognized as the most critical determinant of plant survival rate after transplantation in Sahelian zones. Thus, it is necessary to improve the level of efficiency in the plant capture and use of nutrients which is important in plant growth. In recent years, there has been increasing evidence that the microbial communities of soil and plants have an important role in the development of sustainable agriculture. Among the microorganism living in plant rhizosphere, arbuscular mycorrhizal fungi (AMF) have

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Table 1. Characteristics of the soil of study.

Component	Contents
Clay	3.6%
Silt	1.6%
Fine silt	2.9%
Fine sand	51%
Coarse sand	40.9%
Organic matter	1.06%
Total carbon	2.5
Total nitrogen	0.33
Conductivity (µS/cm)	658
Total phosphorus (ppm)	47
Available phosphorus (ppm)	3.1
pH (sol/water ratio 1:2)	6.7
pH (sol/KCl ratio 1:2)	4.5

been found to be essential components of sustainable soil-plant systems (Bucher, 2007). Therefore, mycorrhizal inoculation with suitable fungi has been proposed as a promising tool for improving restoration success in semi-arid degraded areas (Garbaye, 2000).

The symbiotic relationship between AMF and the roots of higher plants contributes significantly to plant nutrition and growth (Augé, 2001). These positive responses in productivity to AMF colonization have mainly been attributed to the enhanced uptake by AMF of relatively immobile soil ions such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and manganese (Mn) (Smith and Read, 2008), but it involve the enhanced uptake and transport of more mobile nitrogen (N) ions, particularly under drought conditions (Liu et al., 2007). Since nutrient mobility is limited under drought conditions, AMF may have a larger impact on overall plant growth and development in water deficiency conditions (Sánchez-Díaz and Honrubia, 1994).

The objective of the present study was to examine the effects of AMF on foliar nutrient of *A. senegal* seedlings under water deficiency conditions.

MATERIALS AND METHODS

Planting of materials

The experiment was conducted at the Department of Plant Biology (University Cheikh Anta Diop/Senegal). The soil used in this study was collected from Sangalkam (50 km from Dakar, Senegal). Soil was sterilized by autoclaving at 12°C for 1h. Characteristics of the soil were given in Table 1. Seeds were scarified and surface-sterilized with concentrated sulfuric acid for 10 min then washed in sterile distilled water before germinated on sterile water agar at 0.8 at 30°C in the dark for 48 h. The germinated seeds were transferred to plastic bags used in nursery. Four germinated seeds were sown into each bag containing approximately 500 g sandy soil of Sangalkam and thinned to one seedling per pot, 1 week after emergence. All seedlings were watered daily for 1 week to allow proper establishment.

AMF Inoculation

The three species of AMF were used: *Glomus mosseae*, *Glomus fasciculatum* and *Glomus intraradices* obtained from the Laboratory of Fungal Biotechnology (LBC) of the Plant Biology Department (University Cheikh Anta Diop, Sénégal). Mycorrhizal inoculum for each endophyte consisted of mixed soil, spores, mycelium and infected root fragments obtained using maize as host plant. Inoculum from each AM fungus possessed similar infected characteristics (an average of 40 spores per gram and 85% of infected roots).

Experimental design and growth conditions

Plants were inoculated with one of three AMF species by placing 20 g of inoculum directly in the substrate at the position of the roots. The seedlings were subdivided into three blocks. Each block had a plant control and AM inoculation treatment with *G. mosseae*, *G. fasciculatum* or *G. intraradices* each in five replicates. The first block of control plants was kept to 100% of field capacity (FC). The second block of moderate water-deficiency (MWD) plants was maintained at 50% FC and the last block of severe water-deficiency (SWD) plants was maintained at 25% FC. Plants were raised from January to March in a greenhouse with the following conditions: average day/night temperature 30/25 ± 2°C, and relative humidity maintained between 55 and 65%. Plants were harvested after 3 months.

Measurements and chemical analysis

The percentage of mycorrhizal root infection was estimated by microscope observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v), according to Phillips and Hayman (1970). The relative arbuscular richness *(*that is, percentage of arbuscules observations among the number of AMF in roots) were calculated using the grid-line intersect method (Giovannetti and Mosse, 1980).
Chemical analysis of plants and soil were conducted at the Analysis and Characterization Service (SERAC) of the University of Franche-Comte in France. Plant mineral element compositions [Ca, Mg, sodium (Na), and K] were assessed after digestion with HNO_3 + H_2O_2. Elements were determined using flame atomic emission spectrometry (VARIAN spectra A 220FS) following the French AFNOR standards (FD T90-019, 1984). Kjeldahl Nitrogen was determined by volumetric determination using the French AFNOR standards (NF EN 25663, 1994). The content of the four mineral elements was expressed in mg. kg^{-1} of dry weight. The phosphorus was determined by atomic absorption (GANIMEDE P) using a molecular adaptation following the French AFNOR standards (NF EN 6878, 2005).

Statistical analysis

Statistical procedures were carried out with the software package R version 2.5. Two factors analysis of variance (ANOVA) was performed to partition the variance into the main effects and the interaction between inoculation and water status.

RESULTS

Symbiotic development

As shown in Table 2, roots of control seedlings were

Table 2. Effects of water status on mycorrhizal colonization (%) of *A. Senegal* seedlings.

Treatment	**AM colonization (%)**		
	FC	**MWD**	**SWD**
Control	0.00^{d}	0.00^{d}	0.00^{d}
G. intrardices	30.40^{c}	$37.2\ 0^{bc}$	43.60^{c}
G. fasciculatum	50.10^{a}	55.60^{a}	62.50^{a}
G. mosseae	40.95^{b}	50.35^{a}	55.40^{b}

Values in columns followed by different letters are significantly different ($p \leq 0.05$). FC: Field capacity; MWD: Moderate water deficiency; SWD: Severe water deficiency.

Table 3. Foliar nutrient (mg/kg dry matter) of *A. Senegal* shoot under water status subjected to four different treatments.

Treatment	**N**	**P**	**K**	**Na**	**Ca**	**Mg**
FC						
Control	34.30^{a}	1.58^{a}	11.73^{b}	2.75^{a}	34.18^{a}	6.15^{a}
G. intraradices	32.60^{a}	1.40^{a}	13.22^{b}	1.88^{b}	32.22^{a}	5.16^{a}
G. fasciculatum	39.80^{a}	1.64^{a}	15.71^{a}	2.44^{a}	30.01^{a}	6.10^{a}
G. mosseae	37.40^{a}	1.51^{a}	12.97^{b}	2.23^{a}	29.99^{a}	5.00^{a}
MWD						
Control	36.30^{c}	1.48^{b}	9.63^{c}	2.10^{a}	31.74^{a}	4.41^{a}
G. intraradices	35.80^{c}	1.65^{b}	14.96^{b}	1.70^{b}	25.60^{b}	4.88^{a}
G. fasciculatum	57.00^{a}	2.27^{a}	20.54^{a}	1.93^{a}	30.02^{b}	4.46^{a}
G. mosseae	43.00^{b}	1.70a	17.18^{a}	1.87^{b}	25.46^{b}	4.47^{a}
SWD						
Control	32.80^{d}	1.18^{b}	7.00^{c}	0.89^{a}	25.30^{a}	3.99^{b}
G. intraradices	60.00^{c}	1.55^{b}	10.33^{b}	0.79^{b}	23.50^{a}	3.36^{b}
G. fasciculatum	74.10^{a}	2.02^{a}	17.10^{a}	0.90^{a}	25.28^{a}	5.07^{a}
G. mosseae	67.00^{b}	1.46^{b}	10.38^{b}	0.92^{a}	24.19^{a}	4.05^{a}

Values in columns followed by different letters are significantly different ($p \leq 0.05$). FC: Field capacity; MWD: Moderate water deficiency; SWD: Severe water deficiency.

observed after water treatments, indicating the absence of mycorrhiza. *A. senegal* seedlings were infected by any of AM fungi and the average root colonization varies from 30.4 to 62.5% (Table 2). Results showed that *G. fasciculatum* and *G. intraradices* had the highest and the lowest AM fungus colonization among the three AMF, regardless of water status. Water deficiency increased root colonization.

Foliar nutrient

Nutrient concentrations in the shoots of the seedlings after harvesting are shown in Table 3. *A. senegal* shoot N, P, and K contents showed that the nutrient uptake was a function of the applied treatment. Colonization by *G. fasciculatum* improved the content of these nutrients more than the other two fungi. The greatest differences among inoculated plants were found for foliar K. This fungal effect was more obvious under water deficiency conditions where nutrient uptake by *A. senegal* plants is more limited. Moreover, the damaging effects of water deficiency conditions on nutrient acquisition of control plant were reduced in inoculated plant (Table 3). As observed for mycorrhizal colonization, the highest foliar concentrations of N, P, and K were seen in the plants inoculated with *G. fasciculatum* under water deficiency condition. Likewise, the field capacity did not have an effect on foliar nutrient concentrations in shoots.

The presence of water deficiency conditions reduced the contents of N, P and K by 13, 25.3 and 36.4%, respectively, in the control plants. However, the most

Table 4. Significance level (F-values) of effects of differents factors and factors interaction on foliar nutrient based on analysis of variance (ANOVA).

Variable	Inoculation	Water status	Inoculation x Water status
Df	3	2	6
N	1779.07***	9671.15***	181.10***
P	1636.975***	18.437***	32.294***
K	365.666***	23.892***	47.761***
Na	4.4865**	92.4347***	1.1571^{ns}
Ca	5.4989**	30.8064***	1.8789^{ns}
Mg	8.6854***	25.7115***	2.0110^{ns}

*, **, *** and ns indicate the level of significance at $P \leq 0.05$, 0.0, 0.001 and the absence of significance respectively.

active fungus in increasing water deficiency tolerance was *G. fasciculatum*. It increased N, P, and K concentrations by 125, 86.4 and 144%, respectively over those values recorded in control plants under severe water stress (Table 3). Shoot N, P, and K concentrations for water deficiency conditions were significantly higher than field capacity. Analysis of variance was seen to have significantly increased all foliar nutrient concentration by inoculation and water status. Statistical results also show that combined factors were significant for the N, P, and K contents (Table 4).

Na, Ca, Mg concentration declined with increasing water deficiency conditions (Table 3). Na concentrations of inoculated plant with *G. intraradices* decreased significantly with the water regime. Ca concentrations of inoculated plant decreased significantly under moderate deficiency condition, but not under FC and SWD conditions. Magnesium concentrations in different treatments did not change significantly in comparison with control except for Mg shoot content in severe water deficiency condition.

DISCUSSION

Human activity is one of the main factors reducing soil fertility in the vast Sahelian area. As it was observed in tropical gum trees, water deficiency conditions reduce plant mineral nutrition in areas distress from water limitation. In this study, *G. fasciculatum* was the most efficient fungus in terms of *A. Senegal* performance, and mostly in improving foliar nutrient concentration. Differences among AMF presenting foliar nutrient improvement under water deficiency conditions have already been reported (Qu et al., 2004; Miransari et al., 2007). When soil water is limited, K plays an important role in the control of water relations, helping to maintain a high tissue water level, even under osmotically impaired conditions. It is well documented that K, as the most prominent inorganic solute, plays a key role in osmoregulation processes in photosynthesis (Smith et al., 2004; Li et al., 2006). It should also be noted that AM plants are able to absorb higher rates of P, which, among other important roles in the plant, can markedly enhance root growth (Miransari et al., 2008; Smith and Read, 2008). The increase of the uptake may help explain the important growth effects obtained in inoculated plants under water deficient conditions (Audet and Charest, 2006).

In our study N, P, K concentrations in inoculated plants were particularly increased under drought conditions. This is in agreement with the studies of other researchers comparing AM species under different stresses (Ruiz-Lozano et al., 2003). While water status significantly affected the concentration of N, P, and K, AMF species exerted a significant effect on all nutrient concentrations. Table 3 shows that the high efficiency of all AMF on the use of these macronutrients by tropical gum tree. Accordingly, previous researchers have found that the efficiency of AM increases with increased level of stress (Audet and Charest, 2006; Subramanian et al., 2006).

Significant interaction of water status and AMF on the concentrations of N, P, and K indicate differential nutrient uptake efficiencies for different AM-host plant combinations (Table 4). In agreement with these ideas, Subramanian and Charest (1997) contend that AM symbiosis improved drought tolerance of a tropical tree, primarily through the enhanced uptake of slowly diffusing nutrients. In fact, foliar nutrient have been found to increase in inoculated plants (Mortimer et al., 2008), but not under water deficiency condition. The opposite effect was observed by Huett et al. (1997). Na, Ca, Mg concentrations in inoculated plants were lower than those of the controls, however, improved Mg concentration under severe water condition was observed. The same results were found by Monzon and Azcon (1996) for inoculated plants.

The support of symbiotic associations between these fungi and tropical gum tree may be suggested in application of inoculation for successfully improve mineral nutrient uptake of seedlings in Sahelian zone agrosystem.

Conclusion

We may conclude that especially under water deficiency conditions, AMF enhances foliar essential nutrient uptake. This role is very much dependent on different species of arbuscular mycorrhiza. Accordingly, the symbiosis of *A. Senegal* with more efficient AM specie *G. fasciculatum* under such conditions can be very beneficial in water deficiency condition. The next step should be transposing this greenhouse experiment to the field.

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Abbreviations: AMF, Arbuscular mycorrhizal fungi; **FC**, field capacity; **MWD**, moderate water deficiency; **SWD**, severe water deficiency.

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