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# Mycorrhizal Inoculation Effect on Water Deficit Tolerance of Cashew Seedlings (*Anacardium occidentale* L.) and Soil Nutrients Availability

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***Authors' contributions***

*This work was carried out in collaboration among all authors. This work was carried out in collaboration among all authors. Authors AA and AYN designed, performed the study, statistical analysis and wrote the first draft of the manuscript. Author AB helped to set up trial and managed results discussion. All authors read and approved the final manuscript.*

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## ABSTRACT

This research aims to evaluate the effect of Arbuscular Mycorrhizal Fungi (AMF) in improving the resilience of cashew seedlings to water deficit and soil nutritional status. The split-plot experimental design was used. The treatments consist of two factors, the two-level water regime (30% useful water reserve and 70% useful water reserve) in main plots and the three-level inoculation (no inoculation, *Glomus mosseae* and *Glomus aggregatum*) in subplots. Each treatment is replicated nine times. The study was conducted at Agronomic Experimentation Station of the University of Lomé between June to November 2020. Induction of deficit hydric started three months after the setting up of the trial and lasted two months. At the end of the water shortage cycle, growth parameters were measured and leaf and soil samples were taken for laboratory analysis. Parameters assessed include mycorrhization rate, relative water content, leaf proline content, malondialdehyde content, mycorrhizal dependency, plant biomass and mineral content of the soil. The results show good mycorrhization rate, 70.86% for *Glomus aggregatum* and 54.92% for *Glomus mosseae* with mycorrhiza dependency of 12.87% and 11.74% respectively. Mycorrhizal

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inoculation reduced water stress symptoms in addition to the plant's intrinsic protective mechanisms. This was reflected in lower leaf proline and malondialdehyde content and improved relative water content of stressed but inoculated plants compared to uninoculated plants. The AMF also improved the availability of mineral nutrients in the soil, which resulted in better growth of inoculated plants under both water stress and normal watering conditions. The overall assessment of the research suggested that AMF can be used to improve cashew seedlings resistance against drought and to improve their growth through improvement of soil nutrient availability.

*Keywords: Cashew seedlings; water deficit; arbuscular mycorrhizal fungi; proline; malondialdehyde; soil quality.*

## 1. INTRODUCTION

In Togo, agricultural production is mainly rainfed [1]. This type of agriculture is increasingly faced with the problem of declining yields because of problems of climate change [2] thus limit the production of most crops including cashew nuts.

Cashew nut germination requires sufficient moisture; sowing must occur when the rains effectively resume in the case of a rainfed crop [3]. Irregular rainfall and the earlier end of the rainy season in production areas [4] increase the risk of drying out of germinated seedlings and slow down plant growth. Indeed, it has been reported that water deficit at the seedling stage significantly affects height, number of primary branches, number of secondary branches and canopy diameter [5]. To cope with this problem of seedlings loss in the first season, most of cashew nuts producers resort to direct seeding thus limiting the adoption of grafting improved planting material [6]. [7,8] have demonstrated the positive effect of the last rains before flowering on cashew productivity. These rains constitute water reserves, valuable for flowering and good fruiting that take place in the dry season, phases when the cashew tree's water demand is maximum [9]. Irrigation could be a solution to this problem [9]. However, its high cost, its negatives impacts on soil and water scarcity remain major challenges. This situation calls on the agricultural world to think of other more ecological alternatives, including Arbuscular Mycorrhizal Fungi (AMF). In fact, under natural conditions, the vast majority of plants, including forest trees, live in symbiotic association with AMF that supply them with water and mineral elements [10]. These exchanges not only allow for better growth of both symbiotic partners, but also better plant resistance to biotic and abiotic environmental stresses [11]. Thus, in an environmental preservation approach, AMF could play a major role by serving as an alternative to irrigation. It is in this perspective that this study was conducted. The objective of this study is to evaluate the effect of AMF in improving the resilience of cashew seedlings to water deficit and soil nutritional status.

## 2. MATERIALS AND METHODS

### 2.1 Biological Material

The plant material consisted of cashew seeds from the local clone Affem 17, provided by the Togolese Institute of Agronomic Research (ITRA). This clone was selected for its agronomic characteristics (good productivity and good kernel output ratio or KOR) which makes it a potentially cashew elite tree in Togo. The seeds (cashew nuts) that were used have an average weight of 6g. In addition, two strains of AMF were used: *Glomus mosseae* and *Glomus aggregatum*. These strains were provided by the Laboratoire Commun de Microbiologie (LCM), Senegal. The genus *Glomu*s was chosen for its predominance in cashew orchards and the rapid germination of its spores [12,13]. The inocula used contained an average of 155 spores for *G. aggregatum* and 114 spores for *G. mosseae* in one gram of sand.

### 2.2 Trial Set-up and Conduct

The trial was conducted in a greenhouse, in 10 liters' pots at the at Agronomic Experimentation Station (SEAL) of the University of Lomé. The bottoms of the pots were pierced with four holes to let the water drain after watering. Each pot was filled with 10 kg of substrate made of soil taken from the 0 to 30 cm horizon at the SEAL. It is a ferralitic soil with a sandy-loam texture whose characteristics are summarized in Table 1. It was sieved to 2 mm and sterilized by heating before being potting.

**Table 1. Physico-chemical characteristics of the soil used as substrate for the trial before sterilization**

| Parameters | Content |
|---|---|
| Total organic matter (%) | 1.16 |
| Total organic carbon (%) | 0.67 |
| Total nitrogen (%) | 0.04 |
| C/N ratio | 16.8 |
| Total phosphorus (ppm) | 32.8 |
| Total potassium (ppm) | 42.9 |
| Calcium (ppm) | 228.8 |
| Sodium (ppm) | 20.47 |
| pH-$H_2O$ | 6.5 |
| Water electrical conductivity (µS.cm$^{-1}$) | 60 |
| Clay (%) | 9.9 |
| Slit (%) | 1.6 |
| Sand (%) | 87.6 |
| Field capacity (Ɵfc) pF2,5 (%) | 5.69 |
| Permanent wilting point (Ɵwp) pF4,2 (%) | 2.87 |

Cashew seeds were disinfected by soaking for 15 minutes in a 15% sodium hypochlorite solution and rinsed three times with distilled water [14]. To reduce germination gaps, nuts were pre-sprouted in sand before transferring to the pots [15] at a rate of one plant per pot. At transplanting, 20 g of fungal inoculum was incorporated into growing medium [16]. Irrigation was then reduced to 70% of the useful water reserve (UWR) until the application of the water deficit three months later. The water deficit induction consisted decreasing irrigation from 70% of the UWR (control treatment) to 30% of the UWR (stressed treatment). The split-plot experimental design was used. There were nine replicate with the two-level water regime (70% UWR and 30% UWR) in main plots and the three-level AMF inoculation (no inoculation, *Glomus mosseae* and *Glomus aggregatum*) in subplots. The lack of water cycle lasted 60 days. Useful water reserve was calculated using the following formula [17,18].

UWR=(Ɵfc 2.5 - Ɵwp 4.2) x Tfine x E x Da

Where, UWR: useful water reserve; Ɵfc 2.5: moisture at field capacity in %; Ɵwp 4.2: moisture at permanent wilting point in %; Tfine: % fine particles in soil; E: soil depth in dm; Da: soil bulk density.

The irrigation of the plants is done by successive weighing of the pots, at a periodicity of 3 days. During each weighing, the volumes of water corresponding to the different treatments were adjusted. The temperature and relative humidity of the ambient air in the greenhouse were measured daily at 8:00 a.m., 2:00 p.m. and 5:00 p.m, during the whole trial period. The average environmental conditions were as follows: a 12 H photoperiod, average temperatures of 24°C, 35°C, 20°C, on the one hand, and average relative air humidity of 65%, 41%, 55% respectively, on the other hand, 8:00 a.m., 2:00 p.m. and 5:00 p.m respectively. At the end of the water shortage cycle, growth parameters were measured and leaf and soil samples were collected for laboratory analysis.

## 2.3 Measurement of the Mycorrhization Rate

It was evaluated just before the application of the water deficit (three months after the setting up of the trial). The method adapted from [19] is used. Plant roots were harvested and rinsed thoroughly with tap water to remove soil. They are then placed in tubes containing 10% KOH and heated to 90°C for 1 hour 15 minutes in a water bath. Next, the root fragments were rinsed with water to remove the KOH and then reimmersed successively in hydrogen peroxide ($H_2O_2$ at 10 vol) for 40 minutes and in hydrochloric acid (HCl at 1%) for 30 minutes. Next, they are rinsed again with water and then stained with trypan blue (0.05%) and heated in a water bath for 30 minutes. The roots thus prepared are kept in the tubes in which a few drops of glycerol are added. The observations were made on 10 fragments per experimental unit and was done with an optical microscope at 400 times magnification. The mycorrhization rate is calculated according to the following formula:

$$MR = \frac{Number\ of\ mycorrhized\ root\ fragments}{Total\ number\ of\ root\ fragments\ observed} \text{x}100$$

Where MR: mycorrhization rate
Each root fragment showing at least one infection point (arbuscules or vesicles) is considered as mycorrhized.

## 2.4 Evaluation of the Hydric Status of the Leaves

This status was evaluated through the relative water content (RWC). It was measured according to the method of [20] and carried out on four leaves of each experimental unit.

$$RWC\ (\%) = \frac{Wi - DW}{WT - DW} \text{x}100$$

Where, RWC: Relative water content; Wi: weight of leaves immediately after sampling, WT: weight of leaves after 24 hours of soaking in distilled water in the dark and WD: dry weight of leaves after oven drying at 70 °C for 48 hours.

## 2.5 Determination of Leaf Proline

The determination of proline was done according to the Bogdanov method adapted to leaves [21]. It consisted in measuring the absorbance at 510 nm of an aqueous leaf extract (25 mg/ml water) and a standard proline solution (32 µg/ml). To 0.5 ml of leaf extract or proline standard solution, or distilled water (for the blank) contained in 5 mm test tubes, are added 1 ml of formic acid (100%) and 1 ml of ninhydrin (3%). After vigorous shaking for 15 minutes at room temperature, the mixture in the test tube is boiled for 15 minutes. Then, 2.5 ml of 50% 2-propanol is added to the mixture and incubated in a water bath at 70 °C for 10 minutes. After cooling the mixture to room temperature for 45 minutes, the absorbance was read at 510 nm with a spectrophotometer (type Uviline Connect series 940).

In order to calculate the proline content, the proteins were extracted and determined. Fresh leaves (0.5g) were ground in 4 ml of 0.1 M sodium phosphate buffer (pH 7) containing 1 mM Ethylene Diamine Tetra acetic Acid (EDTA), 1 mM ascorbic acid and 1% polyvinylpyrrolidone (PVP). The crushed material was then centrifuged at 4°C at 14000 rpm for 10 minutes. The resulting supernatant was used for protein determination according to [22] method. The proline content of the leaves was then determined using the following formula

$$\text{Proline content (mg/g mf)} = \frac{\frac{Ae}{As} \text{x} \frac{Ms}{Mf}}{Qp}$$

Where, Ae: Absorbance extract; As: Absorbance standanrd solution; Ms: Standard proline mass; Mf: fresh matter and Qp: Protein quantity.

## 2.6 Extraction and Determination of Malondialdehyde

Malondialdehyde (MDA) was determined by the method of [23] on 250 mg of fresh plant material collected and ground. The crushed material was then suspended in 5 ml of trichloroacetic acid (5% w/v) containing 1.25% glycerol. The homogenate was centrifuged at 12000 rpm for 10 min and filtered through Wathman paper No. 1. The supernatant was collected in test tubes. To 2 ml of supernatant, 2 ml of 0.67% thiobarbituric acid (prepared in distilled water) is added. The whole mixture is vortexed, heated for 30 min in a water bath at 100 °C, cooled in ice and then centrifuged for one minute. The absorbance is measured at 532 nm and then at 600 nm. The optical density is then corrected by subtracting the non-specific absorbance at 600 nm. The amount of MDA is calculated using a molar extinction coefficient of 155 $mM^{-1}.cm^{-1}$, according to the Beer-Lambert law:

Absorbance=$\in$ x L x [C]

Where $\in$: Molar extinction coefficient; L: width of the cell (1cm); [C]: MDA concentration ($mg.g^{-1}$ fresh matter).

## 2.7 Measurement of Plant Biomass and Mycorrhizal Dependency

Five months after inoculation, whole plants (leaves, stem and roots) are harvested. They are weighed immediately to determine the total fresh biomass, then oven dried at 70°C for 48 hours to obtain the total dry biomass.

Mycorrhizal dependency (MD) was calculated for the dried matter using the following formula [24]:

$$MD\ (\%) = \frac{DBM - DB\ No\_M}{DBM} \text{x}100$$

Where DBM: Dry biomass of innoculed plants, DBNo_M: Dry biomass of control plants

## 2.8 Chemical Analysis of the Soil at the End of the Trial

In order to assess the variation of nitrogen, phosphorus, soil acidity and soil salinity at the end of the trial, soil samples were taken following the different treatments and analyzed at the Soil Water Plant Fertilizer (SEVE) laboratory of the Togolese Institute of Agronomic Research (ITRA). Electrical conductivity (EC) and pH (water) were determined on a 1/5 and 1/2.5 aqueous extract of the sample, respectively, after 15 minutes of magnetic stirring. Total Kjeldahl nitrogen, assimilable phosphorus (Olsen) were determined.

## 2.9 Statistical Analyses

Statistical analyses were performed using R software. The Shipiro Wilk and Levene tests were used to test the normality of the data and the homogeneity of the variances. When the conditions are satisfied, an analysis of variance (ANOVA) is done followed by a discrimination of the means according to the Student Newman-Keuls test (SNK) for the sources of variation that were found to be significant.

## 3. RESULTS AND DISCUSSION

## 3.1 Results

### 3.1.1 Mycorrhization rate

Microscopic observation of prepared roots identified mycorrhizal vesicles in the root cortex of AMF-inoculated plants (Fig. 1). The mycorrhization rate showed a significant difference ($p<0.001$) between treatments (Table 2). The *Glomus aggregatum* strain had a better mycorrhization rate of 70.86% compared to 54.92% for the *Glomus mosseae* strain. There was no mycorrhization for the control plants.

**Table 2. Mycorrhization rates tress months after trial establishment**

| Arbuscular mycorrhizal fungi | Mycorrhization rate (%) |
|---|---|
| *Glomus aggregatum* | 70.86 ± 15.80 a |
| *Glomus mosseae* | 54.92 ± 8.04 b |
| No inoculation | 00.00 ± 0.00 c |
| P-value | p<0.001 |

*Mean in each column followed by the same letter(s) are not significantly different according to the Student Newman-Keuls test*

### 3.1.2 Variation in relative leaf water content (WRC), foliar proline and malondialdehyde in leaves

The different treatments had a significant effect ($p=0.013$) on relative water content (Table 3). The water deficit induced a relatively low water loss, i.e. a decrease of 2.5% in plants inoculated with *Glomus aggregatum*, 3.2% in those inoculated with *Glomus mosseae* and 5.6% in uninoculated plants.

The different treatments had statistically significant effects for proline ($p<0.001$) and MDA ($p=0.015$) content (Table 3). The results show an accumulation of these two biochemical markers in leaves under water deficit, with high levels in stressed non-mycorrhized plants. Under water-deficient conditions, proline content increased by 114.63% in uninoculated plants, compared to 92.73% and 89.67% in *Glomus mosseae* and *Glomus aggregatum* inoculated plants, respectively. The increase in MDA content under water-deficient condition was relatively small compared to that of proline, with rates of 62.93% in non-inoculated, 52.27% and 35.23% in *Glomus mosseae* and *Glomus aggregatum* inoculated plants, respectively.

**Table 3. Relative water content, leaf proline and malondialdehyde levels by treatment**

| Irrigation | Inoculation | WRC (%) | Proline ($mg.g^{-1}$ mf) | Malondialdehyde ($mg.g^{-1}$ mf) |
|---|---|---|---|---|
| 70 % UWR | No inoculation | 90.75 ± 0.82 a | 2.94 ± 0.21 c | 3.48 ± 0.21 bc |
| | *G. mosseae* | 91.25 ± 0.72 a | 2.34 ± 0.32 d | 2.64 ± 0.31 d |
| | *G. aggregatum* | 91.56 ± 1.28 a | 2.42 ± 0.15 d | 2.98 ± 0.37 cd |
| 30 % UWR | No inoculation | 85.61 ± 0.68 c | 6.31 ± 0.38 a | 5.67 ± 0.22 a |
| | *G. mosseae* | 88.33 ± 1.43 b | 4.51 ± 0.27 b | 4.02 ± 0.25 b |
| | *G. aggregatum* | 89.27 ± 0.99 b | 4.59 ± 0.40 b | 4.03 ± 0.56 b |
| P-value | | p=0.013 | p<0.001 | p=0.015 |

*UWR: useful water reserve, WRC: water relative content, mf: fresh matter. Mean in each column followed by the same letter(s) are not significantly different according to the Student Newman-Keuls test*

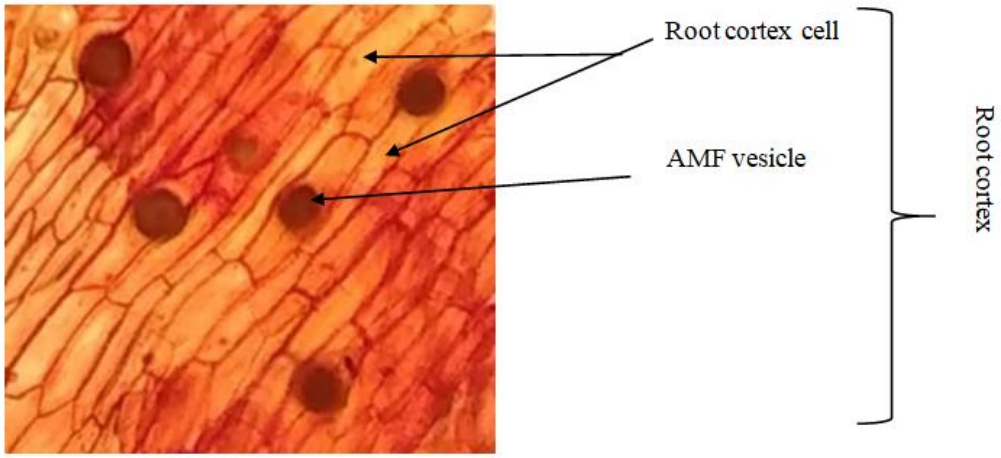


**Fig. 1. Root cortex of 3-month-old cashew tree observed under optical microscope (G x400) showing vesicles of arbuscular mycorrhizal fungi**

**Table 4. Correlation matrix (Pearson) between biochemical markers and relative water content**

| Variables | 70% UWR | | | 30% UWR | | |
|---|---|---|---|---|---|---|
| | **MAD** | **Proline** | **WRC** | **MDA** | **Proline** | **WRC** |
| MDA | **1** | | | **1** | | |
| Proline | **0.7089** | **1** | | **0.8381** | **1** | |
| WRC | **-0.5895** | -0.5207 | **1** | **-0.7740** | **-0.7154** | **1** |

*Values in bold are different from 0 with significance level alpha=0.5. MDA: malondialdehyde, UWR: useful water reserve, WRC: water relative content*

Correlation analysis shows a negative correlation between relative leaf water content and biochemical markers regardless of the water regime considered (Table 4). This correlation is not significant (r=-0.5207, p=0.0826) for proline in normal water regime while in deficit condition this correlation is significant (r=-0.7154, p=0.0089).

### 3.1.3 Variation in plant biomass

No interaction was found between water regime and inoculation on biomass. Tables 5 and 6 present the main effects of the different factors on biomass and mycorrhizal dependence. Water regime significantly affects both fresh and dry biomass. Induction of water deficit (30% UWR) resulted in a loss of 28.4% of fresh biomass and 31.5% of dry biomass. Inoculation of AMF had an interesting effect on biomass (p<0.001). However, both strains of AMF acted identically on biomass. Inoculation with *Glomus aggregatum* resulted in a gain in fresh biomass of 17.08% versus 14.82% for *Glomus mosseae*. For dry biomass the gain was 14.76% for *Glomus aggregatum* and 13.29% for *Glomus mosseae*. The mycorrhizal dependence seems to be more important for *Glomus aggregatum* (12.87%) than for *Glomus mosseae* (11.74%).

### 3.1.4 Variation in mineral nutrient levels in the growing medium

Soil analyzes five months after the establishment of the trial showed an overall improvement in nitrogen (N) and phosphorus (P) content and a decrease in soil acidity (Tables 7 and 8). Inoculation and water regime and their interaction were not significant (p>0.05) on the levels of the different soil elements measured.

**Table 5. Effect of water regime on biomass**

| Water regime | Total fresh biomass (g) | Total dry biomass (g) |
|---|---|---|
| 70% UWR | 21.06 ± 1.63 a | 0.2382 ± 0.020 a |
| 30% UWR | 15.07 ± 1.45 b | 0.1631 ± 0.017 b |
| P-value | p=0.003 | p=0.005 |

*UWR: useful water reserve. Mean in each column followed by the same letter(s) are not significantly different according to the Student Newman-Keuls test*

**Table 6. Effect of inoculation on biomass and mycorrhizal dependency**

| Inoculation | Total fresh biomass (g) | Total dry biomass (g) | Mycorrhizal dependency (%) |
|---|---|---|---|
| No inoculation | 16.33 ± 3.08 b | 0.1835 ± 0.033 b | - |
| *G. mosseae* | 18.75 ± 3.47 a | 0.2079 ± 0.045 a | 11.74 |
| *G. aggregatum* | 19.12 ± 3.38 a | 0.2106 ± 0.047 a | 12.87 |
| P-value | p<0.001 | p<0.001 | |

*Mean in each column followed by the same letter(s) are not significantly different according to the Student Newman-Keuls test*

**Table 7. Variation of the substrate characteristics according to the water regime**

| Water regime | pH | ECw (µs.cm$^{-1}$) | N (%) | P (ppm) |
|---|---|---|---|---|
| 70% UWR | 7.16±0.18 | 290.67±43.25 | 0.052±0.006 | 42.71±1.99 |
| 30% UWR | 7.05±0.17 | 292.55±18.77 | 0.055±0.006 | 42.34±1.85 |
| Significance | p>0.05 | p>0.05 | p>0.05 | p>0.05 |
| Initial substrate | 6.5 | 60 | 0.04 | 32.8 |

*ECw: water electrical conductivity*

**Table 8. Variation of the substrate characteristics according to the inoculation**

| Inoculation | pH | ECw (µs.cm$^{-1}$) | N (%) | P (ppm) |
|---|---|---|---|---|
| No inoculation | 7.03±0.12 | 305.83±29.89 | 0.054±0.004 | 43.81±1.36 |
| *G. mosseae* | 7.15±0.23 | 278.33±20.72 | 0.052±0.006 | 41.45±2.23 |
| *G. aggregatum* | 7.12±0.17 | 290.67±42.21 | 0.055±0.006 | 42.32±1.32 |
| Significance | p>0.05 | p>0.05 | p>0.05 | p>0.05 |
| Initial substrate | 6.5 | 60 | 0.04 | 32.8 |

*ECw: water electrical conductivity*

Induction of water deficit resulted in a 0.86% decrease in soil phosphorus compared to the soil under normal watering (70% UWR). Nitrogen content increased by 5.77% in soils under water deficit (30% UWR). Inoculation led to a decrease in N and P content in the soil. This decrease was 3.70% for N and 5.38% for P for *Glomus mosseae*. For *Glomus aggregatum*, there was a 3.40% decrease in phosphorus and a 1.86% increase in nitrogen in inoculated soil compared to uninoculated soil. A slight decrease in acidity was observed in the inoculated soil. The same trend was observed for this parameter in the normally watered soil. The water electrical conductivity (ECw) of the soil at the end of the trial increased drastically by at least 384.45% compared to the initial substrate for both the watering regime and the inoculation.

## 4. DISCUSSION

The high mycorrhization rate (more than 50%) found for the two inoculated strains of the genus *Glomus* testifies to the high mycorrhization potential of this genus [13]. In addition, *Glomus aggregatum* and *Glomus mosseae* have been identified as some of the most efficient strains of AMF for inoculation of cashew trees [12]. The absence of mycorrhizal symbiosis in the uninoculated plants is explained by sterilization, which would have destroyed all the spores of the AMF attached to the soil used as substrate.

The cashew tree would retain a significant amount of water under the effect of dehydration, as shown by the low water loss measured (2.5-5.6%). This would imply an avoidance strategy [25] that could be linked, on the one hand, to an optimization of water absorption by the roots and, on the other hand, to a complex set of root morphological characteristics (depth, mass and volume, ramifications). This strategy is also favored by mycorrhizal inoculation [26]. The suction capacity developed by the roots conditions the maintenance of a good water potential at the level of the leaves in plants subjected to hydric or saline stress [27,28]. The low water loss associated with stressed but inoculated plants may be explained by the role of AMF in storing water molecules in extra-matricial and root parts for gradual diffusion to the plant [29]. These results are similar to those of [30] who proved that under water stress condition, the relative water content of leaves of orange plants

inoculated with AMF had higher relative water content compared to leaves of uninoculated plants.

Induction of water deficit resulted in accumulation of proline in the leaves of the plants, but inoculation led to a decrease in proline content in the leaves. The accumulation of proline under stress conditions is a common response of plants [31]. Proline is thought to act as an osmotic regulator and to protect membrane structures from dehydration [32]. It also provides a means of reduced carbon and nitrogen storage during stress. In this study, there was a negative correlation between proline content and relative leaf water content in water-starved plants. Thus, proline accumulation appears to be a symptom of plant suffering. Indeed, proline accumulation under water deficit may be related to its neosynthesis, increased protein hydrolysis, inhibition of its oxidation to hydroxyproline, or decreased incorporation into proteins [31]. Our results could be explained by the fact that AMF would exert a temporizing effect on water deficit by making water resources more available to the plant. Indeed, according to [33], AMF increase the soil/root exchange interface by 100 to 1000 times via their mycelial networks synonymous with better water nutrition for the plant. Moreover, thanks to their very fine hyphae, AMF have the capacity to mobilize water that is a priori inaccessible to plants. These results corroborate those of [30,34,35] who associate low proline contents with high relative water contents in mycorrhized plants under water stress conditions in aerial and root organs. In contrast to our results, some authors claim that there is a positive correlation between AMF inoculation and proline accumulation in plant organs under water stress conditions [36,37].

The accumulation of MDA in the leaves of stressed cashew plants reflects their sensitivity to water deficit [5]. Malondialdehyde is considered a good indicator of plant tolerance to different abiotic stresses [38]. Its determination provides information on the state of degradation of cell membranes [39]. It is a product of membrane lipid peroxidation [40]. Indeed, the water deficit induces an oxidative stress with formation of free radicals at the origin of this peroxidation. The peroxidation of membrane lipids would be associated with an insufficient functioning of the detoxification system, which could lead to damage of the main cellular components [41]. Thus, the low levels of MDA in stressed but inoculated plants compared to uninoculated stressed plants could be explained by the contribution of AMF in limiting the expression of water stress.

AMF inoculation had a significant effect on vegetative growth. These results can be explained by the fact that AMF while contributing to better water supply also act as biofertilizers [42]. Indeed, with their strong capacity to explore a larger nutritional surface than roots, AMF mobilize and make available to the plants nutrients that are inaccessible to them a priori. In addition, mycorrhizae by associating with plant roots, facilitate better root development thus allowing plants to better feed themselves [43]. This results in improved growth of mycorrhized plants [11,15]. Similar results have been reported on Jujube tree (*Ziziphus mauritiana* Lam) [44]. In contrast to our results, [16] found a non-significant effect of AMF inoculation on cashew seedling growth. It should be noted that their results do not mention the mycorrhization rate of inoculated seedlings. The low gain in dry biomass under water deficit conditions could be explained by the fact that under water deficit conditions the mycorrhizal symbiosis is negatively affected [45].

Nitrogen, available phosphorus and soil acidity at the end of the trial improved overall. However, electrical conductivity increased drastically. The increase in soil salinity can be explained by the fact that irrigation is always associated with a deposit of small quantities of salts that over time can become considerable [46]. The improvement in nitrogen and assimilable phosphorus content compared to the initial substrate is explained by the progressive mineralization process of the soil organic matter. The decrease in phosphorus and nitrogen content of inoculated soils compared to uninoculated soils is evidence of the role of AMF in decomposing and mineralizing plant organic matter and mobilizing nutrients for the benefit of the host plant [47,48]. This is especially the case for phosphorus, which has very low mobility in the soil [49]. By improving their transfer from the soil to the mycorrhized plants, AMF indirectly contribute to the decrease of these mineral elements in the soil, as is the case in our study. Inoculation also had a depressive effect on soil acidity. [16] also reported greater uptake of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) by cashew plants inoculated with *Glomus clarum* compared to control plants.

## 5. CONCLUSION

The objective of this study was to improve the resistance of cashew seedlings to water deficit through their inoculation with AMF. The inoculation of cashew seedlings reduced the pressure of water deficit through improved water and mineral supply to the seedlings. This was reflected in low leaf proline concentration and high relative water content in stressed inoculated plants in contrast to stressed uninoculated plants. Inoculation also had a depressive effect on oxidative stress characteristic of low MDA content in inoculated plants. The mycorrhizal symbiosis improved the availability of mineral elements in the soil, which resulted in better plant growth.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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