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Response to Inoculation with Arbuscular Mycorrhizal Fungi of Two Tomato (*Solanum lycopersicum* L.) Varieties Subjected to Water Stress under Semi-Controlled Conditions

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Abstract

In arid and semi-arid regions, the growth and development of cultivated plants, especially tomato (*Solanum lycopersicum* L.), are severely limited by water deficit. Thus, to cope with this constraint, the plant establishes symbiotic relationships with arbuscular mycorrhizal fungi (AMF) in the soil whose extension of the hyphae allows a better and deeper exploration; this notably improves the hydromineral nutrition of the plant. Therefore, the choice of fungal partner becomes crucial for the establishment of a crop in water-deficient soil. In this context, the contribution of AMF to the water stress tolerance of two varieties of tomato plants was assessed under semi-controlled conditions. Parameters, such as the mycorrhizal frequency, intensity of mycorrhization, relative mycorrhizal dependency, growth, and biochemical parameters (carbon, nitrogen, phosphorus, and proline contents) of plants subjected to three levels of water stress (T100, T70, and T30), were evaluated. The highest frequencies and intensities of mycorrhization and relative mycorrhizal dependencies were obtained with plants of the *Xewel* variety inoculated with *Rhizophagus fasciculatus* (F: 95.24%, 88.35%, and 13.64%; M: 40.52%, 37.52%, and 11.22%; D: 23.7%, 54.4%, and 78.82%) and in those of the *Lady Nema* variety inoculated with *Claroideoglomus etunicatum* (F: 95.12%, 87.01%, and 15.25%; M: 40.66%, 37.99%, and 11.42%; D: 19.27%, 57.01%, and 70.98%), respectively at water regimes of T100, T70 and T30. These same symbiotic couples recorded, at T30, the best survival rates (+ 40%) and the higher aerial (77% and 74%) and root dry weights (80% and 59%). Plants of the *Xewel* variety inoculated with *R. fasciculatus* recorded the highest contents of carbon (T70: 30.59% and T30: 21.55%) and phosphorus (T70: 0.18% and T30: 0.17%). Plants of the *Lady Nema* variety recorded the highest nitrogen contents with 3.51% and 3.20%, respectively at T70 and T30. Plants of the *Lady Nema* variety, inoculated with *C. etunicatum*, also recorded the highest proline contents (572.25, 739.44, and 1165 nmoles·g^{-1} of fresh material), followed by those of the *Xewel* variety inoculated with *R. fasciculatus* (580.36, 763.65, and 1112.11 nmoles·g^{-1} of fresh matter), respectively at T100, T70, and T30. For the *Lady Nema* variety, the best fungal partner is *C. etunicatum*, followed by *R. fasciculatus* and, finally, *Funneliformis mosseae*. However, for the plants of the *Xewel* variety, *R. fasciculatus* is the most efficient, followed by *F. mosseae* and *C. etunicatum*. This suggests that, in tomatoes, the efficiency of mycorrhizal symbiosis under water stress conditions is not only dependent on the host plant but on both associated symbiotic partners. Hence, it is a need for screening to identify the best symbiotic couples in a stressful environment.

Keywords

Solanum lycopersicum, Water Stress, Arbuscular Mycorrhizal Fungi, Growth, Carbon, Nitrogen, Phosphorus, Proline, Tolerance

1. Introduction

Water stress is considered to be one of the major factors limiting crop production under natural conditions [1]. It greatly affects the growth and development of plants. The tomato (*Solanum lycopersicum* L.) needs about 40 to 60 cm of water during its growth [2]. One of the crucial stages that require a lot of water is flowering. However, in most areas where it is cultivated, water is unavailable in quantity and quality; the plants, then, find themselves in a situation of water stress. The consequences of this stress in the tomato are a decrease in the number of flowers and, therefore, the number of fruits, and a decrease in transpiration and photosynthesis, resulting in a slowdown in growth [3]. In reaction to stress, the plant can develop control strategies such as avoidance or tolerance, to restart its growth, for example, by promoting the development of new roots, most often in an area close to the surface, in order to absorb more water [4]. The plant also responds by protecting and reducing the transpiring surface area and, therefore, the exposure to water loss, to keep its water potential as high as possible [5]. However, these intrinsic plant strategies may not be sufficient. Thus, several authors have shown that the exploitation of the microbiological potential of soils, in particular that of arbuscular mycorrhizal fungi (AMF), could make it possible to promote the adaptation of plants to water stress [6]. Among soil microbial communities, mycorrhizal fungi constitute a key component in plant-microbiome-soil interactions. These fungi, present in the rhizospheres of most ecosystems, form symbiotic associations with the roots of about 80% of terrestrial plant species [7]. In exchange for the carbon resources received from the host plant, these fungi improve the uptake and transport to the plant of very poorly mobile nutrients, such as phosphorus [8], increase drought tolerance [7], and reduce the effect of pathogen infections [9]. Mycorrhizal symbiosis also gives plants tolerance to heavy metals [10] and organic pollutants [11]. In addition, positive interactions have been demonstrated between mycorrhizal fungi and soil bacterial communities [12].

In fact, plant root infection by mycorrhizal fungi leads to the development of mycelial hyphae, which indirectly increases the volume of soil accessible to the host plant resulting in better mobilization of soil nutrients [13]. AMF improve the nutritional capacity of plants to uptake mineral elements such as N, P, K, trace elements, etc. Indeed, AMF constitute a type of association characterized by the formation of intracellular structures called arbuscules. These are the sites of nutrient and carbon exchanges between the symbiotic partners [14]. Through specific transporters and enzymes, the mycorrhization of plant root systems by AMF also improves water uptake through the development of a telluric mycelial network that allows plants to explore a large volume of soil [15] and to transfer forms of organic nitrogen (amino acids) which are difficult to assimilate by the roots alone [16]. However, the major nutrient, the supply of which is ensured exclusively by the mycorrhizal symbiosis in association with bacteria, is phosphorus. AMF also have the ability to increase the products of photosynthesis [17] as well as the accumulation of osmolytes such as proline [18]. Indeed, a mycorrhizal root explores a volume of soil much larger than a single root (for 1 cm of the root, there is 10 m of mycelium) and the fungal filaments have a much smaller diameter than the roots, *i.e.* 1/100 mm *versus* 1/10 to 1 mm, allowing them to penetrate into the microporosity of the soil and find the water that persists there during dry periods [19].

It, therefore, seems judicious to study and favor the inoculation of plants with symbiotic fungal strains that are effective in promoting their tolerance to water stress. This study aimed to estimate, in semi-controlled conditions, the positive effect of mycorrhization by AMF on the growth of plants of two tomato varieties (*Solanum lycopersicum* L.) subjected to the increasing intensity of water stress *i.e.* 100%, 70% and 30% watering at field capacity (T100, T70, and T30) to determine the best symbiotic couples and to promote the association of mycorrhizae with tomato cultivation in dry Sahelian zones.

2. Materials and Methods

2.1. Plant Material

The plant material consists of seeds of the two best performing hybrid tomato (*Solanum lycopersicum* L.) varieties identified during *in vitro* condition work, namely the *Lady Nema* and *Xewel* varieties. The seeds were supplied by the company Tropica Sem-Senegal (Technisem Novalliance Group), located in Dakar city. Their characteristics and the storage conditions (**Table 1**) were identical to those used in the study of osmotic stress applied *in vitro* [20].

2.2. Fungal Material

To assess the impact of inoculation on the growth and development of tomato plants under water stress, three strains of arbuscular mycorrhizal fungi (AMF) were used. These were *Claroideoglomus etunicatum*, *Rhizophagus fasciculatus* and *Funneliformis mosseae* [21], whose former names were, respectively *Glomus etunicatum*, *Glomus fasciculatum* and *Glomus mosseae*. They belong to the collection of the Joint Laboratory of Microbiology (LCM**, IRD/ISRA/UCAD**) of the ISRA-IRD research center in Dakar, Bel-Air (Senegal). The origin and references of these strains are specified in **Table 2**.

To obtain sufficient inoculum, each AMF strain was propagated under a potted shade-house using a mycotrophic plant, maize (*Zea mays* L.), in sterilized (120˚C, 2 h) Sangalkam soil. After 3 months of cultivation, roots and culture

Table 1. Origins and characteristics of the tomato varieties.

Varieties	Origin	Characteristics
Lady Nema	Tropica Sem	Adapted to rainy and hot season, determined growth, good leaf cover, good yield, earliness of 75 - 80 days, good tolerance to nematodes, CMV (Cucumber Mosaic Virus), TYLCV (Tomato Yellow Leaf Curl Virus), resistant to TMV (Tobacco Mosaic Virus) and *Fusarium*
Xewel	Tropica Sem	Adapted to rainy season, determined growth, very good productivity, early (60 to 65 days), tolerant to TYLCV (Tomato Yellow Leaf Curl Virus) and *Fusarium*, Resistant to TMV (Tobacco Mosaic Virus)

Table 2. References of the strains of arbuscular mycorrhizal fungi from the LCM** collection (IRD/ISRA/UCAD**).

AMF	References	Abbreviations
Claroideoglomus etunicatum	[21] NCBI: txid937382 (Becker and Gerdemann BEG 176)	*C. etunicatum*
Rhizophagus fasciculatus	[21] NCBI: txid47032 (Thaxter sensu Gerdemann DAOM 227130)	*R. fasciculatus*
Funneliformis mosseae	[21] NCBI: txid27381 (Nicolson and Gerd.; Gerd. and Trappe DAOM 227131)	*F. mosseae*

**IRD: Institut de Recherché pour le Développement; ISRA: Institut Sénégalais de Recherches Agricoles; LCM: Laboratoire Commun de Microbiologie; UCAD: Université Cheikh Anta Diop.

medium were collected to assess spore density [22] and root colonization rate for each AMF strain [23] [24]. The corn roots colonized by each AMF strain were then cut into fragments of about 1 cm and mixed with the culture medium containing spores and hyphae, to constitute the inoculum.

2.3. Culture Substrates

The soil sampled at Sangalkam (Rough GPS position: Latitude. 14.7811˚, Longitude. −17.2278˚), located 50 km from Dakar, served as a substrate for the experiments, under semi-axenic conditions, of the AMF effect on the tolerance to water stress of tomato plants. The soil was taken from a horizon between10 to 20 cm deep. It was then sterilized by autoclaving at 120˚C for 96 hours to inactivate all native microflora. The physico-chemical characteristics of this soil are recorded in **Table 3**.

Table 3. Physico-chemical characteristics of Sangalkam soil [25].

Component elements	Content for 100 g of soil
Sand	88.8%
Silt	5.8%
Clay	5.4%
Organic material	0.6%
Total carbon	0.3%
pH	5.33
pH KCl	4.4
CE ($\mu s \cdot cm^{-1}$)	121.6
Nitrogen (%)	0.051
Pass (ppm)	62.244
Na^+ (meq/100g)	0.465
K^+ (meq/100g)	0.749
Fe^{2+} (ppm)	0.251
Mn^{2+} (ppm)	0.002
Cu^{2+} (ppm)	0.00001
Zn^{2+} (ppm)	0.01
C/N Ratio	14%
Calcium total	1.03 ppm
Magnesium total	0.30 ppm

Na^+: Sodium; K^+: potassium; Fe^{2+}: iron; Mn^{2+}: manganese; Cu^{2+}: copper; Zn^{2+}: zinc; Pass: Available phosphorus.

2.4. Methods

2.4.1. Experimental Set-Up and Culture Conditions

1) Experimental Set-up

The experiments were carried out under semi-controlled conditions in a shelter, at the Plant Biology Department (FST/UCAD). The effects of inoculation with AMF strains were studied with plants of *Lady Nema* and *Xewel* varieties which appeared to be the most tolerant to water stress after testing under *in vitro* conditions [20].

The adopted experimental set-up is a randomized block with 3 factors: inoculum, variety, and water stress (**Figure 1**).

- The inoculum factor has four levels: the un-inoculated control plants and the plants inoculated, respectively with the strains of *Claroideoglomus etunicatum*, *Rhizophagus fasciculatus*, and *Funneliformis mosseae*.
- The variety factor has two levels: *Lady Nema* and *Xewel*.
- The water stress factor has three levels: control T100 (plants watered at field capacity), medium stress T70 (plants watered with 70% of field capacity), and severe stress T30 (plants watered with 30% of field capacity). The field capacity is the maximum water holding capacity of the soil. It corresponds to the quantity of water retained, after 48 hours of free water dripping, by a soil previously saturated with water [26].

For each water stress condition, a total of 10 plants/variety/inoculation condition was used, *i.e.* 240 plants in total. Plants were watered to field capacity every day and maintained under these conditions for 2 weeks before the application of water stress.

2) Culture Conditions

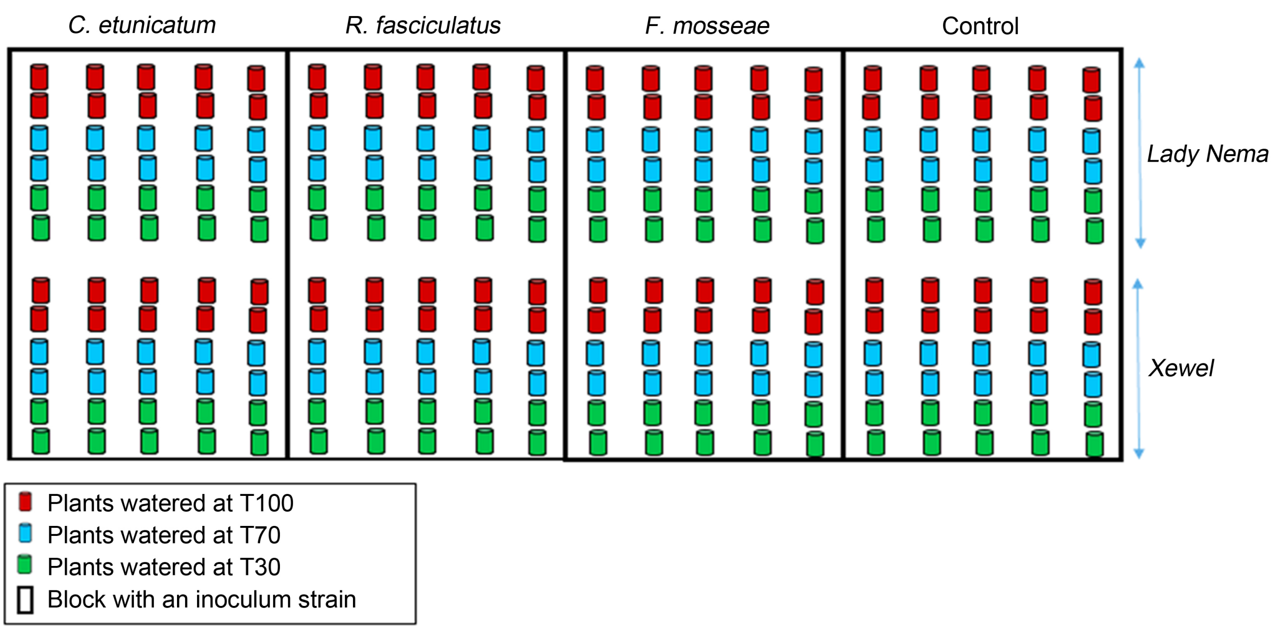

Figure 1. Experimental device for the test under shade in semi-controlled conditions.

The containers consisted of black polyethylene bags (30 cm × 13 cm) filled with 2 kg of substrate. The sowing of the seeds, initially soaked for 2h, was done by sowing 2 seeds per bag. Thinning, consisting of leaving one plant per bag, was carried out after emergence, *i.e.* two weeks after sowing. The duration of the experiment, carried out entirely under semi-controlled conditions under shelter, was 3 months.

The inoculation was carried out at the time of sowing by providing 20 g of inoculum of the appropriate strain. The inoculum was characterized by a mycorrhization frequency of at least 85% and a spore density of approximately 40 for each fungal isolate. It was brought all around the seeds to a depth of 1 - 3 cm.

2.4.2. Parameters Measured

1) Mycorrhization Parameters

At the end of the experiments, the plants were harvested. Their roots were thoroughly rinsed with tap water to remove adhering sand particles. The observation of root colonization was made after staining according to the technique developed by [23].

Histological examination was performed under an optical microscope at 100x magnification, by mounting between slide and coverslip, 20 fragments of fine roots of approximately 1 cm long for each plant, selected at random. The root fragments were crushed with a few drops of glycerol. The presence of AMF structures (hyphae, vesicles, and arbuscules in the roots), allows an estimate of the colonization level of the root samples.

The intensity and frequency of mycorrhization were then evaluated by the method of [24].

- The frequency of mycorrhization (F) was evaluated without considering the extra- and intra-root development stage of the symbionts. Only the presence or absence of mycorrhizal propagules was counted. It was calculated by the following formula:

$$F(\%) = \frac{\text{number of mycorrhizal fragments}}{\text{Total number of fragments}} * 100$$

- Depending on the extent of colonization, a class is assigned to the root fragment and the intensity of mycorrhization is assessed as follows:

$$M(\%) = 95n5 + 70n4 + 30n3 + 5n2 + n1/N$$

n5, n4, n3, n2 and n1 respectively denoted the number of fragments of class 5, 4, 3, 2 and 1 and N was the number of fragments observed.

- Mycorrhizal dependency (MD) is defined by [27] as the degree to which a plant is dependent on the mycorrhizal condition in order to produce its maximum growth or yield at a given level of soil fertility. The relative mycorrhizal dependency (RMD) expresses the degree to which a plant responds to mycorrhizal inoculation under soil fertility conditions.

The relative mycorrhizal dependency was calculated according to the method of [28] using the following formula:

$$\mathrm{RMD}(\%) = \frac{\text{Total biomass of mycorrhizal plants} - \text{Total biomass of control plants}}{\text{Total biomass of mycorrhizal plants}} * 100$$

2) Agro-morphological Parameters

After 3 months of cultivation, plants were carefully removed from each polyethylene bag. Growth parameters such as fresh and dry weights of aerial and root parts were determined. After separation of the aerial and root parts, their biomasses were determined with a Sartorius precision balance (precision: 0.0001). The parts were dried in an oven (Binder brand) for 120 h at a temperature of 80˚C ± 0.5˚C, before weighing the dry biomass of the aerial and root parts, respectively.

For each treatment and each variety, plant survival rate (PSR) was calculated as follows:

$$\mathrm{PSR}(\%) = \frac{\text{Number of plants having survived}}{\text{Total number of plants tested}} * 100$$

This criterion was used to classify varieties according to their ability to tolerate water stress.

3) Dosage and Evaluation of Plant Mineral Contents

- The determination of Total Carbon was done by the method of [29] modified. Organic Carbon is oxidized with a mixture of 1N Potassium Dichromate ($K_2Cr_2O_7$) and concentrated H_2SO_4 (d = 1.84). The Carbon assay was carried out by Spectrophotometry at ƛ= 600 nm (Thermo Scientific Genesys 20).
- Total Nitrogen was determined as recommended by [30]. Mineralization was carried out in the presence of concentrated H_2SO_4 at 18 N, Salicylic Acid ($C_7H_6O_3$), Hydrogen Peroxide (H_2O_2) and Selenium powder as a catalyst. Through this mineralization, Nitrogen is transformed into Ammonium ions (NH_4^+) whose presence was determined by Spectrophotometry at ƛ = 660 nm (Thermo Scientific Genesys 20).
- Total phosphorus was determined according to the method of [31]. 500 mg of dry biomass from the aerial part of the plants were reduced to finely pulverized ashes and aliquots were resuspended with HCl, the vanadomolybdic reagent and permutted water. Thus, the solutions are colored yellow by the phospho-vanadomolybdate complex. The presence of phosphorus is detected thanks to a photocolorimeter (Thermo Scientific Genesys 20) with a cadmium blue filter (ƛ = 468 nm). The reading values of the samples were compared and determined by using a standard range of phosphorus at multiple points: 0, 2, 4, 6, 8, 10, 12 µg of P/mL and previously treated under the same conditions.

4) Biochemical Parameters: Determination and Evaluation of Proline

Contents

To assess the water stress tolerance levels of the two tomato varieties, the average proline levels accumulated by the plants were determined. The protocol described by [32] was used to extract and assay proline. The extraction was performed from a composite mixture of 100 mg of leaf segments from three plants per treatment. The proline concentrations were determined with a Spectrophotometer (Evolution 300 UV-VIS, precision: ± 0.15 nm) by measuring the optical density (OD) at ƛ = 520 nm. The proline contents were calculated and determined by the equation deduced from the calibration curve *i.e.* standard calibration curve, constructed from a range of known and increasing proline concentrations from 0 to 800 µmoles.

2.4.3. Statistical Processing and Data Analysis

The collected data were subjected to a multiple comparison of the means and to an analysis of variance with three factors (inoculum x variety x water deficit) by the Student-Newman-Keuls test (SNK). The analyses were carried out according to a general linear model by the R-4.0.5 software using the "*Agricoleae*" package. The differences between the means were compared using the Student-Newman and Keuls test, and the significance was determined at 95% confidence limits, *i.e.* the significantly different means were discriminated by the SNK test at the p-value of 5%.

3. Results

3.1. Influence of Water Stress on the Mycorrhization Parameters of Tomato Plants

3.1.1. Effect of Water Regimes on the Frequency of Mycorrhization

The results of statistical analyzes on the impact of water regimes and AMF strains on the frequency of mycorrhization of plants revealed a strong significance of the interaction water regimes x AMF strains x varieties ($F = 171$; $P < 2 \times 10^{-16}$). The frequency of mycorrhization of the plants decreases significantly with the reduction in the quantity of irrigation water (*Lady Nema*: $F = 1145$ and $P < 2 \times 10^{-16}$; *Xewel*: $F = 2019$ and $P < 2 \times 10^{-16}$) (**Figure 2**). The un-inoculated plants showed no root colonization by AMF hyphae.

The highest frequencies are obtained in plants of the *Xewel* variety inoculated with *R. fasciculatus*: 95.24%, 88.35%, and 13.64% and in plants of the *Lady Nema* variety inoculated with *C. etunicatum*: 95.12%, 87.01%, and 15.25%, respectively at T100, T70, and T30 water regimes. However, with T70, the frequency of mycorrhization decreases slightly between 9% and 12% in plants of the *Lady Nema* variety and between 7% and 13% in plants of the *Xewel* variety. At T30, this decrease is much higher between 84% and 90%, and between 86% and 88%, respectively in plants of the *Lady Nema* and *Xewel* varieties. The plants of the *Lady Nema* variety inoculated with *F. mosseae* and those of the *Xewel* variety inoculated with *C. etunicatum* were the most affected by water stress.

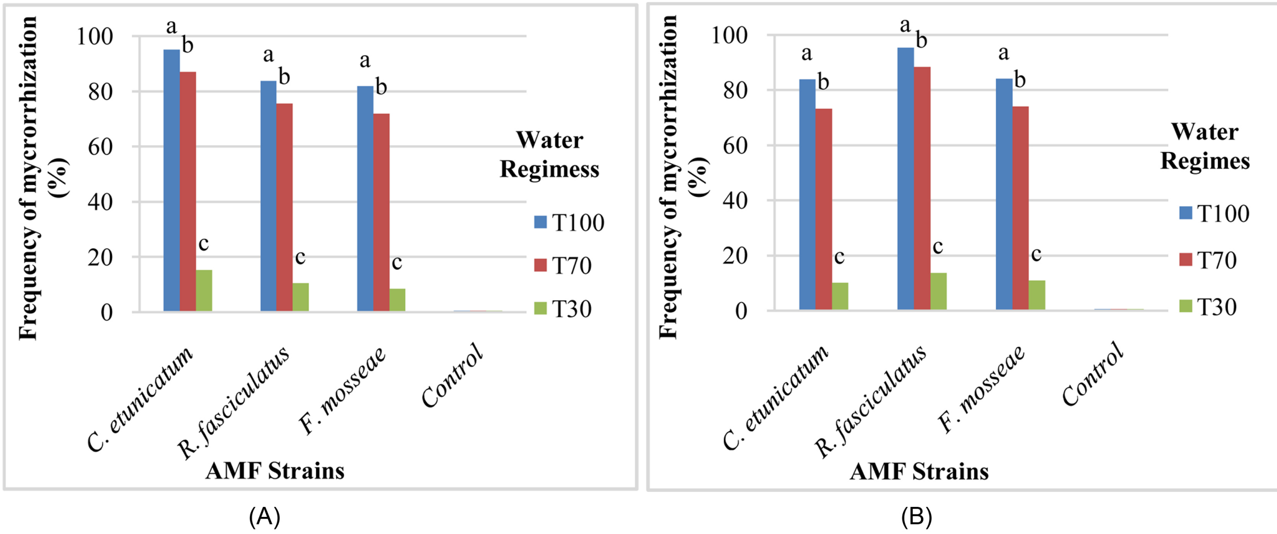

Figure 2. Effect of AMF strains on the mycorrhization frequency of plants of *Lady Nema* (A) and *Xewel* (B) varieties under water stress. For each inoculation condition, the letters a, b and c denote homogeneous groups for the comparison of means according to the Student-Newman-Keuls test at the 5% threshold.

3.1.2. Effect of Water Regimes on the Intensity of Mycorrhization

The results of the statistical analyses on the intensity of mycorrhization of the plants revealed a strong significance of the interaction water regimes x AMF strains x varieties ($F = 81.67$; $P < 2 \times 10^{-16}$). Indeed, the intensity of mycorrhization of the plants decreases significantly with the reduction in the quantity of irrigation water (*Lady Nema*: $F = 962.4$ and $P < 2 \times 10^{-16}$; *Xewel*: $F = 993.6$ and $P < 2 \times 10^{-16}$) (**Figure 3**). The intensity of mycorrhization of the plants decreases more in the plants of the *Lady Nema* variety between 7% and 23% at T70, and between 72% and 84% at T30, while this decrease is between 7% and 17% (T70), and between 72% and 81% (T30) in plants of the *Xewel* variety. The highest intensities are obtained in the plants of the *Lady Nema* variety inoculated with the *C. etunicatum* strain, *i.e.* 40.66%, 37.99%, and 11.42%, respectively at T100, T70, and T30. Under the same water stress conditions, the plants of the *Xewel* variety inoculated with *R. fasciculatus* come second with 40.52%, 37.52%, and 11.22%. Plants inoculated with *F. mosseae* had better mycorrhization intensities compared to those inoculated with *C. etunicatum* in the *Xewel* variety while in the *Lady Nema* variety these plants registered the lowest intensities.

3.1.3. Effect of Water Regimes on the Relative Mycorrhizal Dependency

The results of the analyses of variance on relative mycorrhizal dependency of the plants revealed a highly significant effect of the interaction of water regimes x AMF strains x varieties ($F = 41.8$; $P < 2 \times 10^{-16}$. Indeed, mycorrhizal dependency varies significantly with increasing water stress (*Lady Nema*: $F = 411.11$ and $P < 2 \times 10^{-16}$; *Xewel*: $F = 496.43$ and $P < 2 \times 10^{-16}$) (**Figure 4**).

At T100, the plants of the *Lady Nema* variety are almost not dependent on *F. mosseae* and *R. fasciculatus* with respective mycorrhizal dependency of 1.66% and 8.59%, while they had a low dependency on *C. etunicatum* (19.27%). In the

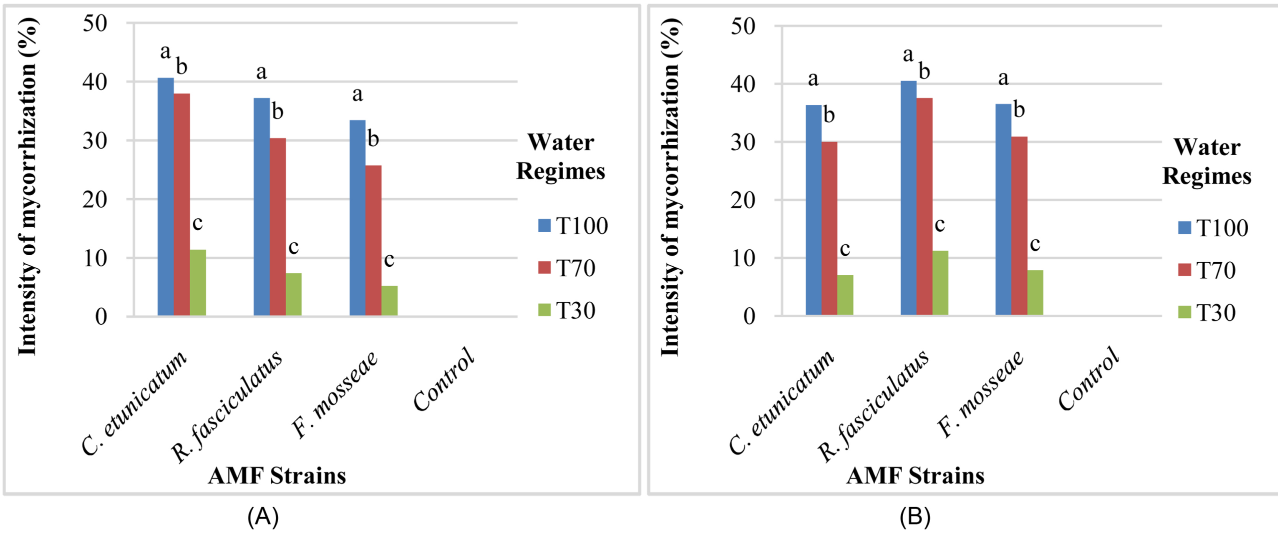

Figure 3. Effect of AMF strains on the mycorrhization intensity of plants of *Lady Nema* (A) and *Xewel* (B) varieties under water stress. For each inoculation condition, the letters a, b and c denote homogeneous groups for the comparison of means according to the Student-Newman-Keuls test at the 5% threshold.

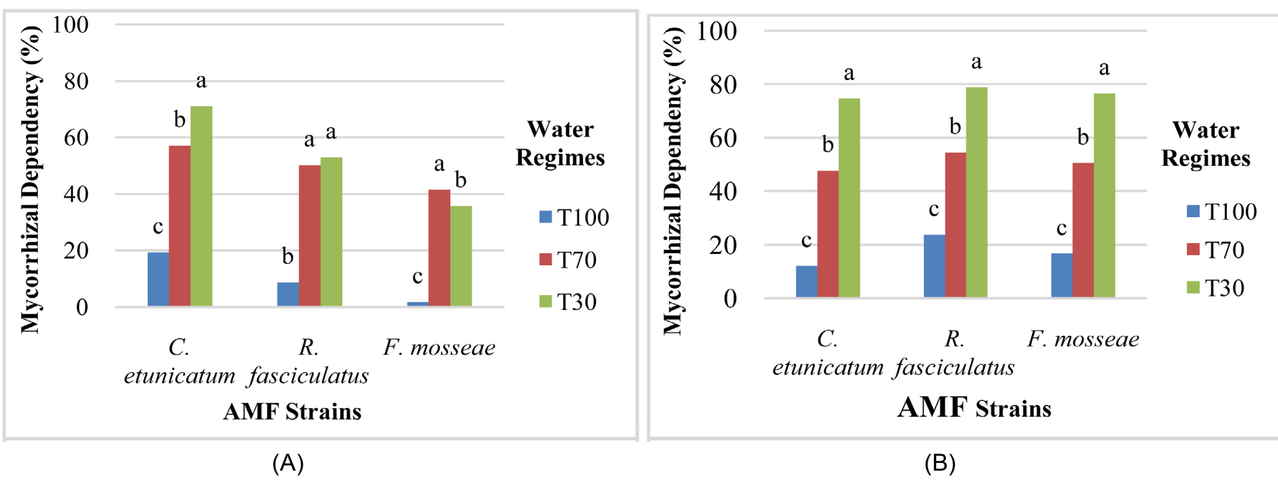

Figure 4. Effect of AMF strains on relative mycorrhizal dependency of plants of *Lady Nema* (A) and *Xewel* (B) varieties under water stress. For each inoculation condition, the letters a, b and c designate homogeneous groups for the comparison of the means according to the Student-Newman-Keuls test at the 5% threshold.

same water condition, the plants of the *Xewel* variety are weakly dependent on *C. etunicatum* (16.61%), *F. mosseae* (12.03%), and *R. fasciculatus* (23.70%). At T70, plants of the *Lady Nema* variety became moderately dependent on *F. mosseae* (41.51%), *R. fasciculatus* (50.22%), and *C. etunicatum* (57.01%). The same is true for those of the *Xewel* variety, which are also moderately dependent on AMF strains but to a lesser degree compared to those of *Lady Nema*. Indeed, they record dependencies of 47.74% (*C. etunicatum*), 50.49% (*F. mosseae*), and 54.40% (*R. fasciculatus*). However, plants of the *Lady Nema* variety are very dependent on *C. etunicatum* at T30 with 70.98%, while they remained moderately dependent on *R. fasciculatus* (52.87%), and *F. mosseae* (35.65%). At this very severe water stress (T30), the plants of the *Xewel* variety are very dependent on all AMF strains, with 78.82% (*R. fasciculatus*), 76.54% (*F. mosseae*), and 74.69% (*C. etunicatum*).

3.2. Influence of Mycorrhizal Inoculation on Agro-Morphological Parameters of Plants Cultivated under Water Stress

3.2.1. Effect of Mycorrhizal Inoculation on Survival Rates of Plants Cultivated under Water Stress

The results of the statistical analyzes revealed a significant interaction of water regimes x AMF strains x varieties ($F = 2.144$; $P = 0.0306$). The survival rate of plants decreases significantly with the increase in the level of water stress depending on the inoculation conditions (*Lady Nema*: $F = 24.15$ and $P < 2 \times 10^{-16}$; *Xewel*: $F = 50.11$ and $P < 2 \times 10^{-16}$) (Figure 5). However, the inoculated plants had a better survival rate compared to the control ones. Indeed, the best survival rates for the plants of the *Lady Nema* variety were obtained with *C. etunicatum* (100%, 84% and 4%, respectively in T100, T70, and T30) and for the plants of the *Xewel* variety with *R. fasciculatus* (100%, 85%, and 40%, respectively at T100, T70, and T30). At T100, the different AMF strains did not have much effect on improving the survival rates. However, when applying water stress, the best improvement in survival rates for plants of the *Lady Nema* variety were obtained with the *C. etunicatum* strain, *i.e.* 20% at T70 and 74% at T30 whereas in plants of the *Xewel* variety, it is *R. fasciculatus* which better improved survival rates (19% at T70 and 76% at T30). However, the decrease in the survival rate of inoculated plants is not very different from one variety to another. Thus, in the *Lady Nema* and *Xewel* varieties, at T70, respective reductions in the survival rate of 16% and 10% (*C. etunicatum*), 21% and 15% (*R. fasciculatus*), 25% and 20% (*F. mosseae*), and 28% and 27% (controls) were registered. At T30, the decreases recorded in the *Lady Nema* and *Xewel* varieties were, respectively, 59% and 64%

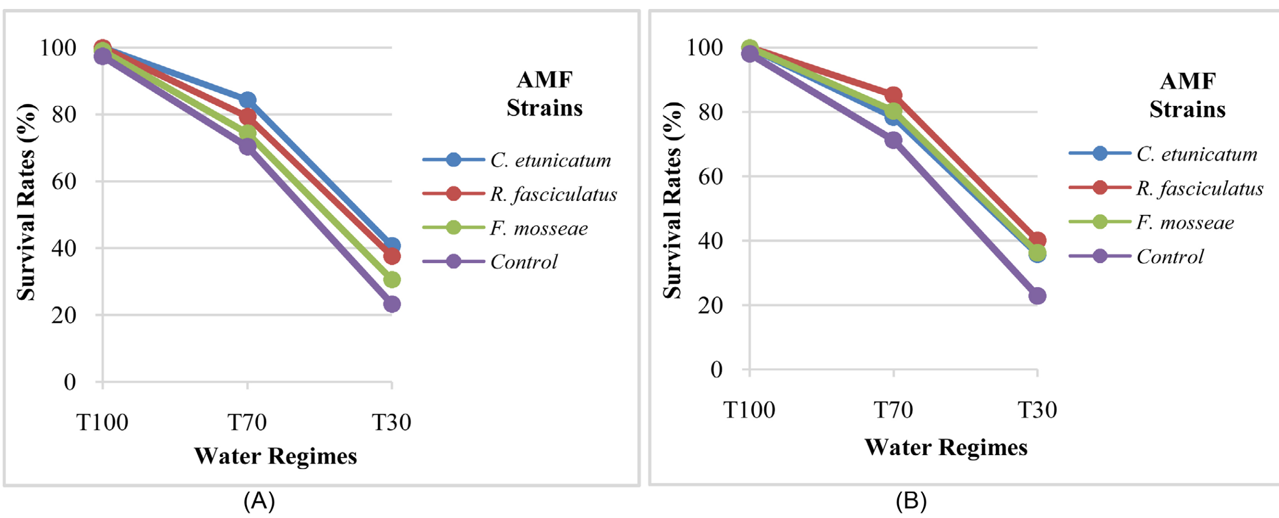

Figure 5. Effect of AMF strains on the evolution of plant survival rates of *Lady Nema* (A) and *Xewel* (B) varieties under different water stress regimes.

(*C. etunicatum*), 62% and 60% (*R. fasciculatus*), 69% and 64% (*F. mosseae*) compared to those of the control plants (76% and 77%).

3.2.2. Effect of Mycorrhizal Inoculation on the Fresh and Dry Weights of the Aerial and Root Parts of Plants Grown under Water Stress Conditions

The results of the variance analysis revealed a significant interaction between water regimes x AMF strains x varieties regarding the average fresh and dry weights of the aerial part (Fresh weight: F = 22.14 and $P = 2 \times 10^{-16}$; Dry weight: F = 6.745 and $P = 7.09 \times 10^{-7}$), those of the root part (Fresh weight: F = 07.742 and $P = 2.41 \times 10^{-10}$; Dry weight: F = 2.841 and $P = 1.96 \times 10^{-4}$) and also the total dry weights (F = 2.102 and $P = 6.89 \times 10^{-6}$) of the plants. These different weights varied significantly with the increase in water stress (Table 4).

Table 4. Comparison of the means of the fresh and dry weights of the aerial and root parts of the plants as a function of the water regimes and of the AMF strains in the *Lady Nema* and *Xewel* varieties.

Varieties	AMF Strains	Water regime	AFW (g)	RFW (g)	ADW (g)	RDW (g)	TDW (g)	(ADW/TDW) ×100	(RDW/TDW) ×100	red/inc Rate ADW (%)	red/inc Rate RDW (%)
Lady Nema	*Claroideoglomus etunicatum*	T100	**17.31a**	**4.81a**	**4.62a**	1.97b	**6.59a**	**70**	30		
		T70	15.12b	**4.94a**	3.12b	**2.09a**	5.21b	60	**40**	**−32**	**+6**
		T30	07.52c	2.04b	2.01c	0.54c	2.55c	**79**	21	**−56**	−73
	Rhizophagus fasciculatus	T100	**16.74a**	**4.7a**	**3.88a**	1.94b	**5.82a**	67	33		
		T70	13.26b	**4.72a**	2.48b	**2.02a**	4.5b	55	**45**	−36	**+4**
		T30	05.09c	2.09b	1.08c	0.49c	1.57c	**69**	31	−72	−75
	Funneliformis mosseae	T100	**14.02a**	**4.5a**	**3.49a**	**1.92a**	**5.41a**	65	35		
		T70	10.11b	**4.51a**	1.89b	**1.94a**	3.83b	49	**51**	−46	**+1**
		T30	04.26c	1.41b	0.75c	0.4b	1.15c	65	35	−79	−79
	Control	T100	**13.86a**	**4.1a**	**3.44a**	**1.88a**	**5.32a**	65	35		
		T70	09.11b	2.55b	1.05b	**1.19a**	2.24b	47	**53**	−69	−38
		T30	03.45c	1c	0.52c	0.22b	0.74c	**70**	30	−85	−76
Xewel	*Claroideoglomus etunicatum*	T100	**16.01a**	**4.49a**	**3.81a**	**2.01a**	**5.82a**	65	35		
		T70	12.89b	**4.51a**	2.81b	**2.05a**	4.86b	58	**42**	**−26**	**+2**
		T30	04.35c	2.94b	1.67c	0.74b	2.41c	**69**	31	**−56**	−63
	Rhizophagus fasciculatus	T100	**16.35a**	**4.99a**	**4.32a**	**2.39a**	**6.71a**	64	36		
		T70	14.84b	**5.04a**	2.96b	**2.61a**	5.57b	53	**47**	−31	**+9**
		T30	07.03c	3.34b	1.85c	1.03b	2.88c	64	36	−57	−57
	Funneliformis mosseae	T100	**16.15a**	**4.61a**	**4.02a**	**2.12a**	**6.14a**	65	35		
		T70	13.05b	**4.64a**	2.99b	**2.14a**	5.13b	58	42	−26	**+1**
		T30	04.96c	2.98b	1.72c	0.88b	2.6c	66	34	−57	−58
	Control	T100	**13.27a**	**4.24a**	**3.41a**	**1.71a**	**5.12a**	**67**	33		
		T70	08.88b	**4.24a**	1.01b	**1.53a**	2.54b	40	**60**	−70	−11
		T30	02.84c	1.23b	0.41c	0.2b	0.61c	**67**	33	−88	−88

AMF: Arbuscular Mycorrhizal Fungi; AFW: Aerial Fresh Weight, ADW: Aerial Dry Weight, RFW: Root Fresh Weight, RDW: Root Dry Weight, TDW: Total Dry Weight; red: reduction; inc: increase. For each variety, the values on the same column followed by the same letter are not significantly different according to Student-Newman-Keuls test at the 5% threshold.

The aerial fresh and dry weights were greater than the root weights under all conditions, in both varieties, while the root parts were the most impacted at T30. The inoculated plants revealed the best results compared to the controls. Thus, an improvement in root dry weight of up to 9% was noted in inoculated plants for both varieties submitted to a moderate water stress (T70). Plants of the *Lady Nema* variety inoculated with *C. etunicatum* recorded the best weights with a 6% increase and a 73% decrease in root dry weight, respectively at T70 and T30. These plants also recorded the lowest rates of reduction in aerial dry weight with 32% and 56%, respectively at T70 and T30. For the plants of the *Xewel* variety, the best weights are obtained with *R. fasciculatus* and *C. etunicatum* strains. Indeed, inoculation with *R. fasciculatus* brought the largest increase with 9% and the smallest decrease with 57% of root dry weights at T70 and T30, respectively. For aerial dry weights, inoculation with *C. etunicatum* provoked the smallest decreases, namely 31% and 57% at T70 and T30, respectively.

3.3. Influence of Mycorrhizal Inoculation on the Biochemical Parameters of Plants Grown under Water Stress Conditions

3.3.1. Effect of Mycorrhizal Inoculation on the Mineral Element Contents of Plants Grown under Water Stress

The analysis of variance revealed a significant effect of the interaction of water regimes x AMF strains x varieties regarding carbon ($F = 51.19$; $P < 2 \times 10^{-16}$), nitrogen ($F = 140.17$; $P = 1.88 \times 10^{-3}$) and phosphorus ($F = 774.04$; $P = 1.03 \times 10^{-3}$) (**Table 5**). The analysis of variance also revealed a very significant AMF effect and water regime effect ($P < 2 \times 10^{-16}$). Indeed, under all inoculation conditions, the carbon, nitrogen and phosphorus contents of the aerial part of the plants in both varieties decreased significantly when the intensity of water stress increased. In addition, these contents are higher in inoculated plants compared to un-inoculated control plants.

Plants of the *Xewel* variety inoculated with *R. fasciculatus* recorded the highest carbon content with 30.59% and 21.55%, respectively at T70 and T30, while those of the *Lady Nema* variety inoculated with *C. etunicatum* recorded the highest content at T100, with 24.72%. Regarding nitrogen, plants of the *Lady Nema* variety recorded the highest levels with 3.91%, 3.51%, and 3.20%, respectively at T100, T70, and T30. Plants of the *Xewel* variety inoculated with *R. fasciculatus* registered the highest phosphorus contents with 0.19%, 0.18%, and 0.17%, respectively at T100, T70, and T30.

3.3.2. Effect of Mycorrhizal Inoculation on the Proline Levels of Plants Grown under Water Stress

Figure 6 shows the average proline content of plants of *Lady Nema* and *Xewel* varieties under water stress as a function of inoculation conditions. The results of the variance analysis revealed that the interaction water regimes x AMF strains x varieties is very significant ($F = 4.544$; $P = 1.32 \times 10^{-03}$). Indeed, the average proline levels of the plants increase significantly under all inoculation

Table 5. Comparison of the means of Carbon, Nitrogen and Phosphorus contents of the plants aerial parts submitted to different water regimes and AMF strains for *Lady Nema* and *Xewel* varieties.

Varieties	AMF Strains	Water regimes	Carbon (%)	Nitrogen (%)	Phosphorus (%)
Lady Nema	*Claroideoglomus etunicatum*	T100	**24.724a**	**3.913a**	**0.188a**
		T70	**24.252a**	**3.508a**	**0.173a**
		T30	19.11b	3.196a	0.173a
	Rhizophagus fasciculatus	T100	**23.91a**	**2.802a**	**0.171a**
		T70	**21.51a**	**2.47ab**	**0.152ab**
		T30	15.94b	2.021b	0.138b
	Funneliformis mosseae	T100	**16.24a**	**2.49a**	**0.152a**
		T70	**14.467ab**	**2.164a**	0.107b
		T30	12.954b	1.858b	0.089b
	Control	T100	**15.683a**	**2.262a**	**0.14a**
		T70	**12.196ab**	1.823b	0.058b
		T30	9.797b	1.575b	0.022b
Xewel	*Claroideoglomus etunicatum*	T100	**19.11a**	**1.919a**	**0.171a**
		T70	12.298b	**1.904a**	**0.147ab**
		T30	15.297b	1.549b	0.117b
	Rhizophagus fasciculatus	T100	**20.782a**	**2.201a**	**0.191a**
		T70	**17.594ab**	**2.187a**	**0.175a**
		T30	15.553b	**2.102a**	**0.174a**
	Funneliformis mosseae	T100	**20.524a**	**1.937a**	**0.174a**
		T70	17.011b	**1.725a**	**0.159a**
		T30	18.296b	**1.909a**	**0.148a**
	Control	T100	**18.253a**	**1.884a**	**0.13a**
		T70	11.998b	1.479b	0.051b
		T30	12.683b	1.141b	0.02c

For each variety and AMF Strains, the values on the same column followed by the same letter are not significantly different according to Student-Newman-Keuls test at the 5% threshold.

conditions with increasing water stress (*Lady Nema*: $F = 521$; $P < 2 \times 10^{-16}$; *Xewel*: $F = 411$; $P < 2 \times 10^{-16}$).

The increase in proline levels is greater in inoculated plants compared to controls. Moreover, this increase is more marked in the plants of the *Lady Nema* variety, compared to the plants of the *Xewel* variety inoculated with *F. mosseae* at T70 and T30 and those inoculated with *R. fasciculatus* at T30. Indeed, the greatest

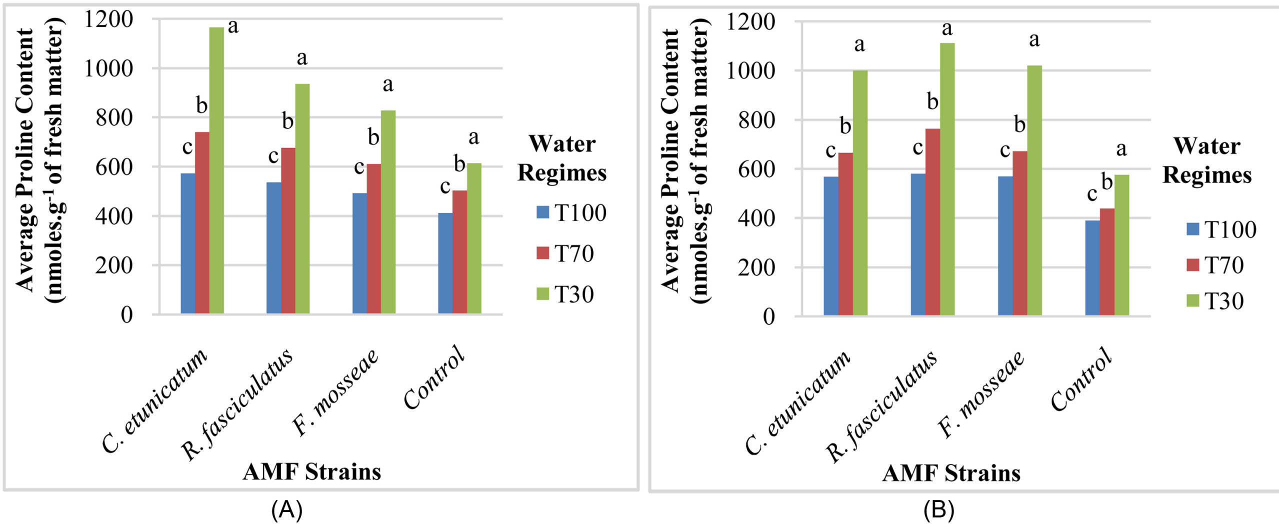

Figure 6. Effect of AMF strains on average proline contents of plants of *Lady Nema* (A) and *Xewel* (B) varieties under water stress. For each inoculation condition of each variety, the letters a, b and c designate homogeneous groups for the comparison of the means according to the Student-Newman-Keuls test at the 5% threshold

increase at T70 was obtained in plants of the *Xewel* variety inoculated with *R. fasciculatus* (32%), followed by those of the *Lady Nema* variety inoculated with *C. etunicatum* (29%). At T30, the latter recorded the greatest increase (104%), followed by plants of the *Xewel* variety inoculated with *R. fasciculatus* (92%). However, the highest average levels of accumulated proline were recorded in plants of the *Lady Nema* variety inoculated with *C. etunicatum* (572.25, 739.44, and 1165 nmoles·g^{-1} of fresh matter) and in those of the variety *Xewel* inoculated with *R. fasciculatus* (580.36, 763.65, and 1112.11 nmoles·g^{-1} of fresh material) at water regimes T100, T70, and T30.

4. Discussion

4.1. Influence of Mycorrhization on Tomato Plants Subjected to Water Stress

AMF are often reported to be beneficial for plant growth and abiotic stress tolerance [33]. In semi-arid regions, mycorrhizal symbiosis has been shown to play an important role in the development of the cultivation of several species, in particular tomatoes [34]. Indeed, these fungi colonize the roots of most plant species and thus improve the acquisition of hydromineral resources from their hosts [7].

The impact of AMF on the tolerance of tomato plants to water stress was studied in order to assess the contribution of mycorrhization in the compensation processes of plants having undergone these water stresses. However, depending on the AMF strains, the mycorrhization parameters of the plants decrease with the intensity of water stress, which results in a reduction in the beneficial effects of mycorrhizal symbiosis. Indeed, the frequency and intensity of mycorrhization of plants significantly decreased under water stress conditions in plants of *Lady*

Nema and *Xewel* varieties ($P < 2 \times 10^{-16}$). The highest mycorrhization frequencies are recorded in plants of the *Xewel* variety inoculated with *Rhizophagus fasciculatus* with 95.24%, 88.35%, and 13.64% and in those of the *Lady Nema* variety inoculated with *C. etunicatum* with 95.12%, 87.01%, and 15.25%, respectively at water regimes T100, T70, and T30. Regarding the intensities of mycorrhization, the most important are obtained during the inoculation of *C. etunicatum* in the *Lady Nema* variety with 40.66%, 37.99%, and 11.42% and during the inoculation of *R. fasciculatus* in the *Xewel* variety with 40.52%, 37.52%, and 11.22%, respectively at T100, T70, and T30. According to [35], beyond 12% mycorrhization intensity, the benefits derived by the plant symbiont are not negligible. The three AMF strains in this study induced intensities of mycorrhization greater than 30% during moderate water stress (T70). This testifies to the beneficial effect of these strains in the tolerance to water stress. Plant response to a water deficit condition is a function of root-associated arbuscular mycorrhizal fungus. Frequencies (35.96% maximum) and intensities (4.28% maximum) of mycorrhization of tomato plants under water stress conditions lower than ours were recorded by [17]. A low frequency of mycorrhization (28%) is obtained in tomato plants inoculated with *Glomus clarum* under water stress conditions by stopping watering for 72 hours. A 21% decrease in the frequency of mycorrhization is also noted compared to unstressed plants by [36]. Low frequencies of mycorrhization (35%) are recorded in tomatoes without water stress, which suggests that AMF contribute more to the hydromineral nutrition of the plant under stress conditions [37]. Two weeks after sowing, filaments of mycelium within the roots of cultivated tomato are observed, with a frequency of around 18% and a low intensity (less than 5%). Six weeks later, mycorrhization frequencies exceeded 50%. This testifies to the rapidity of mycorrhization in tomato which can establish an early symbiosis with the fungal partner [38]. The results of the statistical analyzes also revealed a significant increase ($P < 2 \times 10^{-16}$) in the relative mycorrhizal dependency of the plants of the two tomato varieties. Thus, the plants of the *Xewel* variety inoculated with *R. fasciculatus* (19% - 57% and 71%) and those of the *Lady Nema* variety inoculated with *C. etunicatum* (24% - 54% and 79%) appeared more dependent on mycorrhization, respectively at T100, T70 and T30. These authors [39] classify degrees of relative mycorrhizal dependency as follows: excessive (RMD > 75%), high (50% < RMD < 75%), medium (25% < RMD < 50%), marginal (RMD < 25%), and independent (RMD ≤ 0%). Taking this classification into account, we can consider the plants of the tomato varieties studied as being marginal at T100. In the presence of water stress (T70 and T30), the *Lady Nema* variety has an average dependency on *F. mosseae* (41.51% and 35.65%), and a high dependency on *R. fasciculatus* (50.22% and 52.97%), and *C. etunicatum* (57.01% and 70.98%). As for the *Xewel* variety, the plants have an average dependency on *C. etunicatum* (47.74%) at T70, while at T30, their dependency becomes excessive on *C. etunicatum* (74.69%), *F. mosseae* (76.54%), and *R. fasciculatus* (78.82%). If the relative mycorrhizal dependency is greater than 50%, tomato varieties can be classified as "obligator mycotrophs" [40]. Some crops such as legumes, *Solanaceae*, fruit trees (citrus, oil palms, essences), have a high mycorrhizal dependency and respond better to inoculation with AMF compared to cereals or certain vegetable crops that have medium mycorrhizal dependency [6]. However, in the absence of constraint, the tomato plants revealed a very low dependency or even a mycorrhizal independency to AMF. This is explained by the architecture of its root system with long and numerous absorbent root hairs [41]. However, according to [42], some tomato varieties remain dependent on AMF. Thus, mycorrhizal dependency depends on the variety/AMF interaction since the selected varieties differ in the morphology of their root system. The relative mycorrhizal dependency (RMD) is a quantity reflecting the effectiveness of the fungus in colonizing the root system of plants in a given environment depending on the availability of phosphorus in the soil [28]. Thus, the differences observed in the responses of inoculated plants may be related to the morphological properties of the plant roots, the effectiveness of AMF, the availability of phosphorus in the soil [40], the fungus/plant or fungus/culture medium compatibility [43]. In this study, there is a correlation between the effectiveness of mycorrhization (stimulation of growth) and the extent of root colonization by AMF. Moreover, the relationship between relative mycorrhizal dependency and agronomic parameters became evident. In fact, the greater the dependency on a fungal strain, the greater the growth and biochemical parameters (chlorophyll and proline contents) of the plants inoculated with the mycorrhizal strain concerned. There is thus a positive correlation between mycorrhizal dependency and the frequency and intensity of mycorrhization, allowing the plants to better tolerate water stress. The beneficial effects of AMF have been proven in several species, particularly in tomatoes [34]. The beneficial effect of AM symbiosis on the ability of tomato plants is to better tolerate water stress, combine physiological and metabolic mechanisms, thus regulating the disturbances or even internal ion imbalances induced by water stress.

4.2. Effect of Mycorrhizal Inoculation on the Agro-Morphological Parameters of Plants Grown under Water Stress Conditions

Water is essential to plants at all levels of their development. At the molecular level, water acts as a matrix for all enzymatic reactions in the photochemical phase of photosynthesis and provides hydrogen and oxygen. At the cellular level, water has a direct impact on the architecture of organs, their elongation and growth. Indeed, the cell turgor pressure, a function of the difference between the internal and external water potentials, is exerted in the form of tension on the cell wall, which allows it to express its plasticity. On the other hand, auxin plays a key role in this cellular plasticity, which represents the engine of growth [44]. Finally, at the plant level, it allows the assimilation of solutes present in the soil and their migration to the aerial parts of the plant, while at the same time ensuring thermal regulation of the tissues exposed to the sun's rays. Consequently, a prolonged water deficit manifests itself progressively on different aspects of the plant and gradually affects several morphological and agronomic parameters, to more or less variable degrees between organs of the same plant and between different varieties of a plant same species [45]. In addition, it is recognized that water stress is an important factor limiting the production of food plants (cereals, vegetables plants, fruit trees, etc.). It affects all aspects of growth. This is manifested in the plant by a series of modifications correlated with morpho-physiological, biochemical, genetic, molecular characters and even the expression levels of genes associated with drought [46].

Tomato plants inoculated with different AMF strains show better growth and better survival rate compared to non-inoculated plants, this is maintained even under water stress conditions. Indeed, in this study, the applied water stress led to a decrease in the survival rate of plants in the varieties *Lady Nema* from 59% to 76% and *Xewel* from 60% to 77% at T30. However, inoculated plants revealed significantly higher survival rates ($P < 2 \times 10^{-16}$) than un-inoculated ones. The survival of the plant under water stress conditions depends on its ability to perceive the stress generated and to transmit the signal to the different parts of the plant and to initiate a set of physiological and chemical modifications [44]. The number of leaves also decreased with the reduction in the amount of irrigation water. However, there is a greater number of leaves in plants inoculated with AMF compared to control plants (data not shown). The inoculated plants recorded the best results compared to the control ones. An increase in this improvement in the growth of tomato plants may be due to early inoculation, the benefits of which for the plant are known and have been highlighted by [47], who demonstrated that AMF and pre-inoculation have a positive impact in improving the growth and development of tomato seedlings. The fresh and dry weights of the aerial part of the inoculated plants were reduced by water stress in the *Lady Nema* and *Xewel* varieties. However, inoculation with AMF allowed the plants to have higher weights than the control plants. Similar results were recorded by [48] with increased aerial (60%) and root (24%) weights of tomato plants grown under water stress and inoculated with *Funneliformis mosseae*. A 17% increase in fresh root weight was also obtained in tomato plants mycorrhized with *Glomus clarum* [36]. It is also noted in the presence of low water stress (T70). In a situation of water stress, the plant promotes the development and activity of the root system to explore deeper soil horizons and extract water [3]. Indeed, when tomato plants are inoculated with *Rhizophagus intraradices*, a 62.5% drop in aerial weights is recorded while root weights increase by 12.8% [48]. An increase of 30% to 40% of the stem heights and the fresh and dry biomass of the plants is noted thanks to the inoculation with native mycorrhizae [38]. The AMF, thanks to their mycelial network, help the plant to widen and deepen its prospecting zone. This author [49] concludes that enhanced biomass production in times of drought can be achieved primarily by maximizing soil water uptake while diverting most of the available soil moisture to stomatal transpiration. This is defined as the effective use of water (EUW) and is the main driver of agronomic or genetic improvement in crop production under a limited water regime. However, with severe water stress, these material weights are all affected with a greater reduction in non-inoculated plants. Plants of the *Xewel* variety appeared less affected compared to those of the *Lady Nema* variety regarding biomass. There would then be a better response with respect to the choice of AMF/variety pairs. For example, [50] showed that *F. mosseae* improves root development by supplying and transporting nutrients. Root infection by mycorrhizal fungi leads to the development of mycelial hyphae, which indirectly increase the volume of soil prospected and accessible to the host plant, resulting in better mobilization of nutrients from the rhizosphere [51]. Their impact is essential in all or part of the development cycle of the plant, especially, but not exclusively, for water and mineral nutrition. During an effective mycorrhizal symbiosis, the AMF takes advantage of the carbon resources synthesized by the host plant via photosynthesis and which are essential for its metabolism and fruiting. In return, the fungal hyphae improve the water and mineral nutrition of the host plant thanks to the increase in the volume of prospected soil and the production of various extracellular enzymes (proteinases, phosphatases, etc.) capable of mobilizing nutrients from complex soil compounds [52]. Climate demand, commonly referred to as potential evapotranspiration (Etp), causes water loss from the stomata. In the absence of a sufficient water resource accessible to the roots of the plant, the latter loses part of its internal water and the water potential of the cells is lowered. The consequences for the plant are multiple. There may be a reduction in cell expansion and, subsequently, cell growth due to a drop in cell water potential leading to a reduction in turgor pressure, the driver of cell growth. The cells are then smaller, resulting in smaller organ sizes [3]. To maintain its water status, the plant seeks to increase the osmotic pressure present in its cells by reducing its transpiration and increasing its suction power concerning soil water. It then diverts part of the metabolites intended for the growing organs toward the cells. Thus, they serve as osmolytes to increase osmotic pressure [53]. The reduction in transpiration leads to an increase in the temperature of tissues, leaves in particular [54]. Thus, certain stages are precipitated, because the plant perceives a higher temperature, which translates into a shorter calendar duration of the phenological phases and, therefore, a lesser interception of the light resource [55]. In this study, inoculation with *C. etunicatum* and *R. fasciculatus* gave the best results in terms of growth, respectively for the plants of the *Lady Nema* and *Xewel* varieties.

4.3. Effect of Mycorrhizal Inoculation on the Biochemical Parameters of Plants Grown under Water Stress Conditions

The increase in mineral levels in mycorrhizal plants is one of the most reported major benefits of mycorrhizal symbiosis, although it depends on the partners involved and the experimental conditions. Thus, in our study, a significant increase in nitrogen, carbon, and phosphorus content is detected in mycorrhizal plants. This increase is greater for those who recorded the highest frequencies and intensities of mycorrhization. This improvement can be attributed to a better exploration of the soil by the hyphae, which increases the availability and transport of mineral elements and water to the plant [7]. Similar results were obtained in tomato by [37]. However, mineral contents decrease with increasing water stress intensity.

Nitrogen is a vital component for the AMF and the plant. I1 enters into the formation of phospholipids, co-enzymes and amino acids. A positive impact of AMF as a catalyst for nitrogen biosynthesis has also been described by [56]. The AMF mycelium is able to take up nitrogen in the form of ammonium ions (NH_4^+), in the form of nitrates (NO_3^-) [57] and in the form of amino acids [58], with a clear preference for NH_4^+ ions [59]. It is, therefore, likely that mycorrhizal roots can benefit from organic nitrogen [60]. This is confirmed by the work of [61] who reported a 42% improvement in the nitrogen nutrition of mycorrhizal tomato plants. In our study, at T70, the nitrogen contents increased by 48% and 51%, respectively in plants of the *Lady Nema* variety inoculated with *C. etunicatum* and in those of the *Xewel* variety inoculated with *R. fasciculatus*. This improvement is more marked at T30 with 51% in these two symbiotic couples. Nitrogen-fixing bacteria and mycorrhizal fungi in some cases combine to dissolve minerals, allowing crops to grow in soils low in soluble P and N [62]. An improvement in nitrogen nutrition allows better protein synthesis and, consequently, an increase in chlorophyll content. In addition, it helps to promote the flowering, quality and development of tomato fruits [63]. Nitrogen is considered as the engine of plant growth and contributes to the vegetative development of all aerial parts of the plant, leaves, stems, etc. [62]. This explains why, in agriculture, most chemical fertilizer formulations contain these 3 major macro-elements nitrogen (N), phosphorus (P) and potassium (K).

Mycorrhizal symbioses also play a key role in carbon biosequestration. Since the AMF is heterotrophic for carbon, the carbonaceous substances necessary for its energy needs come from the host plant. The estimate of the carbon transferred from the plant to the mycorrhizal fungus varies from 4% to 20% of the total carbon in the plant [64]. In this study, the carbon contents are higher in the mycorrhizal plants compared to those of the control ones. Thus, an improvement of 50% and 49%, respectively at T70 and T30, is observed for the plants of the *Lady Nema* variety inoculated with *C. etunicatum*. Under the same water stress conditions, plants of the *Xewel* variety, inoculated with *R. fasciculatus*, revealed improvements of 61% and 41%. Indeed, the high activity of fungal structures requires a lot of energy and more carbon from the plant since the fungal partner cannot produce it itself and this mineral is essential for its proper functioning. However, the beneficial effects of symbiosis may well have compensated for this loss of carbon for the plant by better functioning of the internal mechanisms that induce better growth.

One of the main mechanisms put in place by plants to tolerate abiotic stress is a better absorption of phosphorus [65]. A large difference in phosphorus content between mycorrhizal and non-mycorrhizal plants on the one hand, and a higher efficiency of some fungi compared to others on the other hand, are reported by [66] Phosphorus enters into the synthesis of many molecules such as ATP, nucleotide monophosphates, phospholipids, certain enzymes and co-enzymes [67]. In addition to its essential role in photosynthesis [68], phosphorus also remains essential for energy transfers within the plant [69]. This mineral helps maintain the structure of cell membranes, thus improving the transport of nutrients through them. In tomatoes under water stress, flowering, fruit set and fruit size are improved thanks to better nitrogen nutrition [70]. Unlike many other mineral nutrients, phosphorus is not very mobile in soils [71]. A small proportion, generally less than 1%, is immediately available to plants, which find it difficult to acquire this element when their needs are greater [72]. Thus, under the action of root removal, areas of impoverishment are rapidly created in the rhizosphere around the roots. However, hyphae are able to uptake phosphorus well beyond what would be the depletion zone of roots alone. Some authors suggest that thanks to the hyphae, mycorrhized roots could have easier access to phosphorus from organic matter [63]. Mycorrhizal plants can take up phosphorus by two distinct pathways: the direct uptake pathway through the rhizoderm and root hairs, and the mycorrhizal pathway through fungal extraradical mycelium. When taken up by mycelial hyphae, phosphorus molecules assemble to form polyphosphorus corresponding to chains of phosphorus residues linked by phospho-anhydride bonds *i.e.* high energy phosphate bonds [63]. PolyP chains travel along hyphae to be hydrolyzed to inorganic phosphorus in arbuscules and translocated to cortical cells [73]. Better phosphorus uptake was observed in tomato plants mycorrhized with *R. fasciculatus* [74].

To cope with disturbances or even osmotic imbalances induced by water stress, plants adapt by synthesizing compatible organic solutes acting as osmoticums and called osmolytes. These allow plants to increase turgidity potential as high as possible to maintain osmotic potential [75]. The accumulation of osmolytes, expressed by molecular modifications within leaf organs, such as soluble sugars, glycine betaine, potassium, nitrates and proline, is considered as a biochemical marker of water stress [76]. Thus, many plants belonging to different species accumulate proline under stress [77]. When plants are in a state of stress, it is recognized that proline can perform several functions, namely: osmotic adjustment [78], osmoprotective [79], anti-oxidant [80], cytosolic acidity regulator [81], stress marker [76] and adaptive trait [82].

In this study, the average proline content of the tomato plants increased significantly at all the inoculation conditions with the decrease in the water regime (*Lady Nema*: $F = 521$ and $P < 2 \times 10^{-16}$; *Xewel*: $F = 411$ and $P < 2 \times 10^{-16}$). The highest proline contents are recorded in plants of the *Lady Nema* variety inoculated with *C. etunicatum* with 572.25, 739.44, and 1165 nmoles·g^{-1} of fresh matter and in those of the *Xewel* variety inoculated with *R. fasciculatus* with 580.36, 763.65, and 1112.11 nmoles·g^{-1} of fresh matter, respectively at water regimes T100, T70, and T30. An increase in leaf proline content was previously observed in tomato plants under water stress by [4] and [18]. In our work, proline levels, as well as their increase under water stress, are greater in inoculated plants compared to controls. Indeed, the inoculated plants recorded increases in proline content up to 112 times greater than the controls at T30. An increase of 50% (2/3 of the field capacity) to 500% (1/3 of the field capacity) of the proline content thanks to the AMF was recorded by [83]. A 20% increase in proline content in mycorrhizal tomato plants under water stress conditions was also obtained by [17] while [48] obtained a 900% increase. The increase in proline content would be directly related to the application of water stress [84]. The more the level of applied stress increases, the more the proline levels become marked [85]. This negative correlation between proline accumulation and soil moisture is observed in several species [86]. The amounts of accumulated proline would be linked to the plant's ability to tolerate water stress [87], because proline could play an osmoticum role [88]. The accumulation of proline, induced by stress, can be the result of three complementary processes according to [89]: 1) stimulation of its synthesis, 2) inhibition of its oxidation and/or 3) alteration of protein biosynthesis (glutamate). Proline extensively protects the cell membrane and protein content of plant leaves [90]. It also acts as an osmolyte alongside enzymes and other macromolecules, and therefore protects the plant against low water potential and causes osmotic regulation in plant organs. It acts as a compatible soluble compound without exerting a toxic effect unlike ions [91]. In addition to the osmotic role attributed to proline, it is involved in the detoxification of active forms of oxygen [92] and the stabilization of proteins [53]. However, there is great controversy over proline accumulation, which to some authors appears to be more a symptom of stress vulnerability than an adaptive response [93]. The results of our work suggest that proline accumulation is more a stress tolerance strategy erected by tomato plants. The protection generated by the fungal partner resulted in better mineral assimilation and an accumulation of a proline-type osmotic regulator in greater quantity [94].

5. Conclusion

This study was conducted to evaluate the impact of mycorrhization on the growth of two varieties of tomato plants subjected to increasing water stress in order to determine the best symbiotic couples. A significant interaction, between varieties (*Lady Nema* and *Xewel*), AMF strains (*C. etunicatum*, *R. fasciculatus* and *F. mosseae*), and water stress levels (T100, T70, and T30) is highlighted. Thus, the different levels of water stress applied reduced the parameters of mycorrhization, growth, and some physiological and biochemical characteristics of the plants of the two varieties. However, the inoculated plants performed better than the controls. Indeed, the AMF allowed a gain in biomass, *i.e.* fresh and dry weights of the aerial and root parts of the plants, with greater advantage for the aerial part. In addition, the mycorrhizal symbiosis allowed a significant increase in Carbon, Nitrogen, Phosphorus and Proline contents. For plants of the *Lady Nema* variety, the association with *Claroideoglomus etunicatum* brought the best results followed by that with *Rhizophagus fasciculatus* and finally *Funneliformis mosseae*. For plants belonging to the *Xewel* variety, the *Rhizophagus fasciculatus* strain turns out to be the best symbiotic partner to overcome water stress, followed by *Funneliformis mosseae*, and *Claroideoglomus etunicatum*.

The best symbiotic couples have been determined for each variety, it can therefore be recommended to carry out the mycorrhization of tomato plants in the dry Sahelian zone, which often has water deficits in the cultivation areas, for better and sustainable productivity.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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