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Biomass and Nutritional Status of Melon Hybrids Induced by Arbuscular Mycorrhizal Fungi Application Under Varying Irrigation Stress

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Abstract: Water scarcity is a major challenge in northeastern Brazil, where efficient water management strategies are essential for sustainable agriculture. This study aimed to evaluate the performance of melon hybrids in terms of biomass production and nutritional status under varying irrigation levels and mycorrhizal fungi (AMF) inoculation. The experiment was conducted in a greenhouse at the State University of Bahia (Juazeiro, BA, Brazil) using a randomized block design with a 4 × 2 × 4 sub-subdivided plot scheme. The treatments included four irrigation levels (50%, 75%, 100%, and 125% of crop evapotranspiration—ETc), two melon hybrids (Juazeiro and Mandacaru), and four AMF inoculation treatments (non-inoculated with AMF, *Entrophospora etunicata*, *Acaulospora longula*, and their combination), with 10 replications. The results indicated that the inoculation with *A. longula* significantly improved biomass production and plant nutrition, particularly for the Juazeiro hybrid. The most significant improvements were observed in biomass production and nutritional status when this mycobiont was used, highlighting the potential of AMF inoculation as a strategy to enhance water use efficiency and plant tolerance under water-limited conditions. Root colonization in melon plants ranged from 6% to 60%, with an overall average of 36.2%, in Experiment I, and from 6% to 72%, with an average of 40%, in Experiment II. Melon biomass production responded differently to irrigation levels, with Experiment I showing polynomial decreases in biomass as water levels decreased, while Experiment II exhibited linear increases in biomass with higher irrigation, likely influenced by supplementary fertilization. When evaluated, the levels of macronutrients present in the aerial part of the plants did not show significant differences for the treatments concerning the levels of P, K, and Mg, except for Ca. These findings suggest that *A. longula* is a suitable mycobiont for optimizing melon plant performance in regions with limited water resources, like northeastern Brazil.

The study also emphasizes the importance of selecting appropriate Mycorrhizal fungi to maximize symbiotic benefits in melon cultivation under deficit irrigation systems.

Keywords: water stress; Mycorrhizal fungi inoculation; water efficiency in melon cultivation

1. Introduction

Water management technologies in irrigation, designed to maximize water use efficiency in agricultural production, are crucial in arid and semi-arid regions, which are characterized by water limitations. As freshwater availability declines and competition for water increases, finding additional water resources and improving the effective use of available soil water has become a central theme in innovative research on irrigation and optimized water management strategies [1,2]. In regions experiencing water scarcity, irrigation has become a fundamental technological strategy, not only for the sustainability of agricultural production but also as an adaptive measure for managing reduced water availability [3,4].

The challenge of producing underwater scarcity requires integrated strategies to enhance crop tolerance to agricultural drought, which results from the imbalance between environmental demands for evapotranspiration and the capacity of the soil–root system to transport water [5]. Various technologies, such as deficit irrigation, have been tested to improve irrigation water use efficiency, alongside the use of drought-tolerant crop varieties [6,7] and the association of plants with beneficial microorganisms, including bacteria and fungi, which are applied as bioinputs in agriculture [3,8–12].

Studies indicate that the ability of plants to survive with critical water levels in the soil increases when associated with Arbuscular Mycorrhizal Fungi (AMF). These fungi can provide enhanced tolerance to both water scarcity and excessive soil moisture, depending on the severity and duration of the stress [11,13]. A key mechanism behind this effect is AMF's ability to alter the hydraulic properties of roots, increasing water uptake and improving the efficiency of photosynthesis under stress conditions [14]. AMF associations often result in improved stomatal conductance even under conditions of low soil moisture [15].

Moreover, research has demonstrated that AMF can positively influence various physiological, biochemical, and productive traits in plants under water stress. Studies on crops such as tomato and eggplant revealed that Mycorrhizal symbiosis can improve crop performance under severe water stress, sometimes outperforming fully irrigated conditions [3,16]. In this context, AMF plays a crucial role in improving plant responses to environmental stress, especially in sandy soils and semi-arid regions. Additionally, AMF symbiosis with melon has been shown to significantly enhance biomass production and improve plant physiology under varying water stress conditions [17].

Further studies have emphasized the role of AMF inoculants in improving melon growth, promoting earlier flowering, and increasing concentrations of sugars and carotenoids in the fruit pulp, which enhances both yield and quality [18]. These results underscore the potential of AMF as an effective tool for improving melon cultivation, contributing to more sustainable agricultural practices.

Melon cultivation, particularly in regions such as Brazil, often takes place in arid to semi-arid conditions during hot and dry seasons, exposing the crop to extreme drought and high temperatures [19,20]. This renders melons particularly vulnerable to water stress, necessitating the development of strategies that optimize water use efficiency. Studies on melon grown under regulated deficit irrigation and AMF inoculation have shown promising results, with inoculated plants exhibiting a 12% and 9% increase in shoot dry

weight under 75% and 50% crop evapotranspiration deficit, respectively, compared to fully irrigated plants [17]. This highlights the importance of combining deficit irrigation with AMF inoculation to enhance growth and physiological traits under water stress.

Despite the promising results, there is limited evidence regarding the effects of inoculation with specific species or combinations of different AMF species. As water-limited conditions can affect AMF propagation differently across species, it is recommended to select isolates better suited to the prevailing environmental conditions [21,22]. Additionally, the effectiveness of AMF in promoting plant development is influenced by factors such as the crop's Phosphorus (P) needs and the P content available in the soil [23], as well as the compatibility between the plant and the fungal symbionts [11]. In some instances, the plant–fungus association may not be beneficial due to factors like poor compatibility or unfavorable ecological conditions [15,24].

Given these factors, the objective of this study was to assess the biomass production and nutritional status of melon hybrids under varying irrigation levels and AMF inoculation in a protected environment in the semi-arid region of northeastern Brazil.

2. Materials and Methods

The study consisted of two experiments conducted in a greenhouse located in a semi-arid region (9° 24′ S; 40° 30′ W; 368 m altitude) with a hot semi-arid climate (BSh) according to the Köppen classification. The greenhouse was of the arch type, screened, and shaded by 45%, with an east–west orientation, measuring 3.5 m in height, 10 m in width, and 30 m in length.

The experiments used a Fluvisol soil type collected from the 0–40 cm layer. The experimental design was a randomized block with a 4 × 2 × 4 factorial arrangement, organized in a split–split–plot structure, with 10 replications. The plots corresponded to the applied water levels, which were 50%, 75%, 100%, and 125% of crop evapotranspiration (ETc). The subplots represented the melon hybrids Frog skin (*Juazeiro*) and Yellow gold (*Mandacaru*). The sub-subplots were defined by the AMF inoculation treatments, including the isolates *Entrophospora etunicata* (W.N. Becker & Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno (identification in research collections—Univasf 09) and *Acaulospora longula* Spain & N.C. Schenck (identification in research collections—URM-FMA 07), used either alone or combined, as well as the treatment without inoculation (control). These AMF species were chosen because they are commonly found in soils of the Brazilian semi-arid region [25–27].

The evapotranspiration of the melon crop was determined using weighing lysimetry (Figure 1). Six lysimeters were used, with three replicates per hybrid. Each lysimeter consisted of a pot containing soil and a plant, placed on a weighing platform (electronic scale, model 2098, Toledo, 30 kg capacity, 0.05 kg precision, stainless steel 375 × 425; Figure 1B—calibration), positioned in the experimental field on a masonry base (Figure 1C) and connected to a data collector and storage device (Datalogger, CR800, Campbell Sci.—815 West 1800 North Logan, UT 84321-1784. USA) through channel multiplexers (AM 1632 Relay Multiplexer, Campbell Sci.).

The weighing platforms were calibrated to convert the electrical signal (mV) per excitation volt detected by the data collector and storage device, through connection with the analytical balance (weighing platform), into mass data (expressed in kg). This conversion was necessary to compute water loss through the crop evapotranspiration process by measuring the total mass variation of the lysimetric system.

(**A**) (**B**) (**C**)

Figure 1. Weighing lysimeter installed in the experimental area (**A**), calibration of the weighing platform (**B**), and positioning in the experimental field on a masonry base (**C**).

To calibrate the platforms, programming was carried out using PC200W (Version: 4). software, a CR800 data logger, an AM 16/32 multiplexer, and the analytical weighing balances, which served as the weighing platforms. Additionally, soil-filled bags with known masses of 0.05, 0.1, 0.25, 0.50, 1, 2.5, and 5 kg were prepared using an analytical balance.

The known soil masses were applied to the weighing platforms (Figure 1B), which were connected to the datalogger. After the voltage reading stabilized, approximately 30 s later, the electrical signal was recorded. Using the corresponding mass and voltage data, a calibration equation was obtained through regression analysis for each platform composing the lysimeters. The results are presented in Table 1

Table 1. Calibration equations for the lysimeter weighing platforms.

Weighing Platform	Calibration Equation	R^2
1	$y = 25.617x - 9.0081$	1
2	$y = 25.564x - 8.3323$	1
3	$y = 26.02x - 9.0559$	1
4	$y = 25.736x - 8.6371$	1
5	$y = 25.595x - 8.5333$	0.9998
6	$y = 25.042x - 9.0651$	1

Six random locations were selected for the installation of the lysimetric systems. Subsequently, the soil was excavated to a depth of 12 cm, and a masonry base (39.5 cm × 44.5 cm) was constructed and leveled at the bottom (Figure 1C). On each base, the weighing platform was installed (Figure 1B), which, along with the soil and plant, formed the lysimetric system (Figure 1A).

After the installation of the lysimeters in the experimental area, they were connected to a Campbell CR800 datalogger and an AM 16/32 multiplexer. The system was programmed to record electrical signal readings at 1 s intervals, with average values stored every 15 min.

Irrigation was applied to replenish the total amount of water lost through crop evapotranspiration (100% ETc) up to 15 days after transplanting (DAT). After this period, irrigation depths were differentiated according to the programmed irrigation treatments.

The irrigation depths were determined based on the ETc (crop evapotranspiration) value, which was measured individually for each melon plant using weighing lysimetry. This approach involved quantifying daily water losses by summing the mass variations of the lysimetric system from 5:00 AM to 6:00 PM. Calculations were performed using

Equations (1) and (2), where the first equation was used to calculate hourly variations and the second for daily variations.

$$\Delta MT = \mathrm{MT_{i-1}} - \mathrm{MT_i} \quad (1)$$

$$\mathrm{ETc} = \sum_{5{:}00\ AM\ to\ 6{:}00\ PM} (\Delta MT) \quad (2)$$

where

ΔMT = variation in the total mass of the lysimetric set, expressed in kg and to L^{-1} of the water;

$\mathrm{MT_{i-1}}$ = total mass of the lysimetric set from the previous hour, expressed in kg and converted to L^{-1} of the water;

$\mathrm{MT_i}$ = total mass of the lysimetric set from the current hour, expressed in kg and converted to L^{-1} of the water;

ETc = crop evapotranspiration, L plant^{-1} h^{-1}.

To apply a water stress condition, which is necessary to meet the objectives of the study with melon crops and FMAs, irrigation was performed to replenish 50%, 75%, and 125% of ETc, imposing a water deficit of 50% and 25%, as well as a surplus condition of 25% above the water required for the crop's ETc.

The irrigation depths were applied using a drip irrigation system, with online emitters having a flow rate of 2.1 L· h^{-1}, operating at a service pressure of 10 mca. The irrigation was automated, and the irrigation time (IT) was programmed for each treatment.

IT was calculated using the total required volume (*TRV*), considering the efficiency of the irrigation system (Ei = 95%) and the emitter flow rate (Fr = 2.1 L h^{-1}), as shown in Equation (3) below:

$$IT = \frac{TVR}{\mathrm{Ei} \times \mathrm{Fr}} \quad (3)$$

where

IT = irrigation time in hours;

TVR = total volume of water required to replenish the crop's ET;

$\mathrm{MT_i}$ = total mass of the lysimetric set from the current hour, expressed in kg and converted to L;

ETc = Crop evapotranspiration, L plant^{-1} h^{-1}.

The *TRV* represented the amount of water lost through ETc for each melon hybrid, obtained through weighing lysimeter, multiplied by a factor for water replenishment to the soil (ks) of 0.5, 0.75, 1, and 1.25, respectively, to meet treatments 50%, 75%, 100%, and 125% *ETc* (Equation (4)), as follows:

$$TVR = \frac{ET_c \times Ks}{Ei} \quad (4)$$

where

TVR = total volume of water required to replenish the crop's ET;

ETc = crop evapotranspiration of the melon in L plant^{-1} day^{-1};

Ks = soil water availability factor to meet the treatments, decimal;

Ei = irrigation system efficiency, in L.

The experiments were conducted during two distinct periods: the first experiment was carried out from October to December 2015, during the period of the highest water demand (Experiment I), and the second experiment was carried out from April to June 2016, during the period of lower water demand (Experiment II). Complementarily, it was divided into two experiments for methodological adjustment. Through leaf analysis, it was observed that there was a need for more nutrients for the plant's supply.

In Experiment I, the seedlings were formed by sowing the melon hybrids *Mandacaru* (Yellow gold type) and *Juazeiro* (Frog skin type) in trays containing pine substrate and sterilized soil at a 1:1 ratio (*v*/*v*). Ten days after sowing (DAS), the seedlings were transferred to plastic cups with a 0.25 L capacity and, after 15 days, transplanted into 5 L pots previously filled with sterilized soil in an autoclave, subjecting the soil to high pressure and temperature (typically 121 °C at 15 psi). In Experiment II, the seedlings were sown directly into 0.25 L plastic cups containing vermiculite and sterilized soil at a 1:1 ratio (*v*/*v*). The seedlings were transplanted into pots at 10 DAS.

The AMF isolates were propagated for 90 days using *Sorghum bicolor* (L.) Moench as the host plant. The inoculation was performed at the time of seedling transplantation into plastic cups (10 days after sowing, DAS) in both experiments. Inoculation was carried out with AMF species (*A. longula*, *E. etunicata*, and mix AL and EE) via soil–inoculum (containing spores, colonized root fragments, and AMF hyphae), deposited directly on the roots and standardized to 100 glomerospores in each pot.

The plants were grown without pruning and were staked to facilitate cultural practices. The different stages of development were classified according to FAO 56 [28] to identify the duration of each phenological phase of the melon plants.

Plant fertilization was performed weekly using the Hoagland nutrient solution [29], with quantitative adjustments to meet the demands of the melon crop in Experiment II. Both nitrogen (N) and P were applied from inorganic sources (through the Hoagland solution) to minimize the risk of microbial contamination, ensuring stricter control of experimental conditions and preventing the introduction of potentially undesirable organisms. This also considered soil analysis, and the dry shoot biomass was estimated to recalculate the nutrients used (Table 2). For the second experiment, a new setup was implemented using the same soil type, but replacing the soil used in the previous experiment's pots.

Table 2. Initial soil fertility characterization and amount of fertilizers applied in both experiments using melon hybrids (*Mandacaru* and *Juazeiro*). Protected environment, lower-middle São Francisco Valley.

Fertility of the Soil Used in Experiments with Melon Hybrids													
EC dS m^{-1}	pH -	P mg dm^{-3}	K	Ca	Mg	Al	H + Al $cmol_c$ dm^{-3}	SB	CTC	Cu	Fe	Mn mg dm^{-3}	Zn
0.68	7.0	17.3	0.12	2.2	1.4	0.0	0.0	3.7	3.7	0.97	85.3	39.4	8.61
Amount of Fertilizers Applied in Both Experiments													
		Nutrient Solution A								Nutrient Solution B			
		$Ca(NO_3)_4$ *		MAP **			KNO_3 ***			$MgSO_4$ ****		Conmicros *****	
Experiment 1		1 L (Dilution of 5 mL L^{-1})								400 L (Dilution of 2 mL L^{-1})			
		34.0 g		208.0 g			110.0 g			49.0 g		50.0 g	
Experiment 2		340.0 g		10 L (Undiluted) 7.0 g			---			400 L (Undiluted) 49.0 g		50.0 g	

* $Ca(NO_3)$—Calcium Nitrate, ** MAP—Monoammonium Phosphate, *** KNO—Potassium Nitrate, **** $MgSO_4$—Magnesium Sulfate, ***** Conmicros—Micronutrient Fertilizer. Source: Research Data.

For macro and micronutrient analysis, the samples (shoot dry biomass) were sent to the Soil and Leaf Analysis Laboratory at the Brazilian Agricultural Research Corporation *Semiárido* campus, and the analyses followed the methodology described by [30].

Both experiments received periodic phytosanitary treatments at three-day intervals through preventive applications with neutral detergent and cottonseed oil without the use of systemic products.

At 42 days after transplanting (DAT) for Experiment I and 49 DAT for Experiment II, the plants were harvested, and the fresh and dry shoot biomass was determined using a semi-analytical balance. To obtain the dry shoot biomass, the plant material samples

were dried in a forced-air circulation oven at 60 °C for 48 h and weighed to determine the biomass.

The relative increase in shoot dry biomass of plants inoculated and non-inoculated with AMF was calculated using Equation (5) below:

$$IR\ (\%) = (X - Y)/Y \times 100, \quad (5)$$

where IR = relative increase in dry biomass (%);

X = dry shoot biomass of inoculated plants in g·plant^{-1};

Y = dry shoot biomass of non-inoculated plants in g·plant^{-1}.

In both experiments, Mycorrhizal colonization was determined. For this, 0.5 g of roots were separated from the soil, washed, and preserved in 50% ethyl alcohol until processing. The roots were then cleared and stained with trypan blue in lactoglycerol using the method of [31], and Mycorrhizal colonization was determined using the quadrant intersection method [32].

Inside the protected environment, air temperature and humidity data were collected with a thermohygrometer, global solar radiation with a pyranometer, and wind speed with an anemometer. The daily averages during Experiment I, conducted from October to December 2015, were minimums of 38.8% humidity, 27.8 °C temperature, 0.12 kW m^{-1} solar radiation, and 0.03 m s^{-1} wind speed, and maximums of 58.7% humidity, 32.3 °C temperature, 0.18 kW m^{-1} solar radiation, and 0.42 m s^{-1} wind speed. For Experiment II, conducted from April to June 2016, the minimums were 45.4% humidity, 25.3 °C temperature, 0.025 kW m^{-1} solar radiation, and 0.018 m s^{-1} wind speed, and the maximums were 75.1% humidity, 30.2 °C temperature, 0.18 kW m^{-1} solar radiation, and 0.20 m s^{-1} wind speed.

The results were initially subjected to an analysis of variance (ANOVA) to determine if there were any statistically significant differences among the treatments. For the qualitative factors that showed significant differences, a post-hoc mean comparison was performed using the Tukey test at a 5% probability level to identify which specific treatments differed from one another. For the quantitative factors, regression analysis was applied to determine the relationship between the variables and the optimal model that best fits the data. The most appropriate regression model was selected based on the significance of the variables and the goodness of fit. All statistical analyses were conducted using Excel spreadsheets for data organization and the "Assistat" 7.6 software for more complex statistical procedures, ensuring accurate and reliable results.

3. Results

During the first experiment, the water consumption of melon was 12.6 and 14.2 L·plant^{-1}, respectively, for the *Juazeiro* hybrid (Frog skin type) and *Mandacaru* hybrid (Yellow gold type) at 42 DAT (days after transplanting). In Experiment II, the water consumption was 16.4 and 17.4 L·plant^{-1}, respectively, for the *Juazeiro* and *Mandacaru* hybrids at 49 DAT.

There was a significant effect at the 1% probability level in both experiments for the isolated factors (irrigation levels, hybrids, and type of inoculation) on the fresh aerial biomass (FAB) and dry aerial biomass (DAB) of the melon plants. In both studies, the *Juazeiro* hybrid showed higher FAB and DAB than the *Mandacaru* hybrid (Table 3).

In Experiment I, inoculation with Arbuscular Mycorrhizal Fungi (AMF) did not result in a significant increase in dry biomass. For plants inoculated with EE and EE + AL, the reductions were 24.12 and 15.22% of FAB and 11.36 and 7.57% of DAB, respectively, in relation to the control (free of AMF) (Table 3). The *Mandacaru* hybrid, irrigated with 125% of ETc, especially in the treatment with the mixed AMF inoculum, showed the lowest dry biomass increase (Figure 2). Nevertheless, the highest percentage of root

colonization was observed in melon plants with this inoculation treatment compared to other treatments (Table 4).

Table 3. Analysis of variance and mean values for Mycorrhizal colonization, fresh biomass, and dry biomass of the aerial part, as a function of different irrigation levels (50%, 75%, 100%, and 125% of crop evapotranspiration—ETc), hybrids (*Juazeiro* and *Mandacaru*) of melon, and inoculation with Arbuscular Mycorrhizal Fungi, for both experiments.

Sources of Variation		Experiment I			Experiment II		
		Mycorrhizal Colonization	Production		Mycorrhizal Colonization	Production	
			FAB	DAB		FAB	DAB
Water levels (WL)		54.4 **	14.1 **	7.7 **	115.4 **	70.5 **	130.0 **
Melon hybrids (MH)		0.7 ^{ns}	87.3 **	50.5 **	6.4 *	43.0 **	20.2 **
AMF (A)		340.5 **	23.2 **	16.0 **	210.1 **	4.9 **	5.2 **
WLxMH		0.9 ^{ns}	0.5 ^{ns}	2.4 ^{ns}	2.9 ^{ns}	0.4 ^{ns}	0.9 ^{ns}
WLxA		22.5 **	1.4 ^{ns}	0.8 ^{ns}	14.4 **	3.1 **	1.5 ^{ns}
MHxA		1.5 ^{ns}	0.7 ^{ns}	1.8 ^{ns}	0.5 ^{ns}	0.5 ^{ns}	0.3 ^{ns}
Interaction WLxMHxA		2.4 *	1.1 ^{ns}	0.8 ^{ns}	1.1 ^{ns}	0.8 ^{ns}	1.2 ^{ns}
Factors		Mycorrhizal Colonization	Production		Mycorrhizal Colonization	Production	
		(%)	FAB (g)	DAB (g)	(%)	FAB (g)	DAB (g)
Hybrids	*Juazeiro*	24.5 a	45.3 a	13.6 a	31.4 a	117.0 a	24.3 a
	Mandacaru	25.3 a	31.3 b	11.8 b	28.5 b	96.0 b	21.7 b
C.V.		23.9	27.0	13.7	18.3	17.0	14.0
Inoculation	NI	0.0 -	42.7 a	13.2 a	0.0 -	97.1 b	21.5 b
	EE	36.6 a	32.4 c	11.7 b	38.3 a	108.4 a	23.5 ab
	AL	37.4 a	41.9 a	13.6 a	40.2 a	111.6 a	24.4 a
	EE + AL	25.5 b	36.2 b	12.2 b	41.3 a	110.0 a	22.6 ab
C.V.		24.0	18.4	12.1	22.5	16.9	13.4

NI—non-inoculated, EE—*E. etunicata*, AL—*A. longula*, EE + AL—*E. etunicata* + *A. longula*. The significant is for $p < 0.01$ (**), $p < 0.05$ (*), and ^{ns} not significant. Means followed by the same letter in the column do not differ statistically from each other according to Tukey's test at a 5% probability level.

In Experiment II, plants inoculated with AMF showed significant increases in dry biomass. The *Juazeiro* hybrid melons, inoculated with *A. longula* and with mixed AMF inoculum, both under water deficit conditions and full irrigation, demonstrated a higher rate of increase compared to plants inoculated with *E. etunicata* (Figure 2). In Experiment I, the *Mandacaru* hybrid, subjected to an irrigation level corresponding to 125% of crop evapotranspiration (ETc), did not show a significant increase in dry biomass (Figure 2). The irrigation level of 125% of ETc was not sufficient to cause continuous waterlogging, which did not result in spore death but may have reduced the activity of some AMF species used. The excess water applied (25% of ETc) was drained from the pot but remained in trays underneath, undergoing evaporation and/or being utilized through capillary rise by the plants. Consequently, soil aeration occurred after these processes. However, in Experiment II, this same treatment resulted in a slight increase, suggesting that, despite excessive water, a more adequate nutritional status may enhance the development and effectiveness of AMF.

An interaction was observed between irrigation levels and AMF inoculation treatments in Experiment II, linear increase in colonization (EE and AL) and positive quadratic polynomial regression for control and negative for EE + AL as a function of different irrigation levels in both experiments (Figure 2C). Although the highest percentages of mycorrhizal colonization were observed in the roots of plants subjected to excessive irrigation (125% ETc), this condition did not result in higher FAB compared to non-inoculated plants (Figure 2B).

Table 4. Mycorrhizal colonization in melon roots, as a function of different irrigation levels (50, 75, 100, and 125% ETc), the *Juazeiro* and *Mandacaru* hybrids, and treatments with Arbuscular Mycorrhizal fungi in a protected environment (Experiment I).

Treatment	Hybrids	Mycorrhizal Colonization			
		Water Levels (% ETc)			
AMF	Melon	50	75	100	125
NI		-	-	-	-
EE	*Juazeiro*	15.6 b	26.6 ab	50.0 a	53.6 a
AL	(Frog skin)	8.6 b	32.8 ab	41.8 a	58 a
EE + AL		6.2 b	17.2 b	35.4 ab	45.2 a
NI		-	-	-	-
EE	*Mandacaru*	11.4 b	31.0 ab	43.8 a	59.8 a
AL	(Yellow gold)	11.4 b	31.0 ab	43.8 a	59.8 a
EE + AL		10.2 b	17.4 b	31.6 ab	41.2 a
CV%-	Water levels = 45.3; Hybrid = 23.9; AMF = 24.0				

NI—non-inoculated, EE—*E. etunicata*, AL—*A. longula*, EE + AL—*E. etunicata* + *A. longula*. Means followed by the same letter within each combination of irrigation levels do not differ statistically from each other according to Tukey's test at 5% probability.

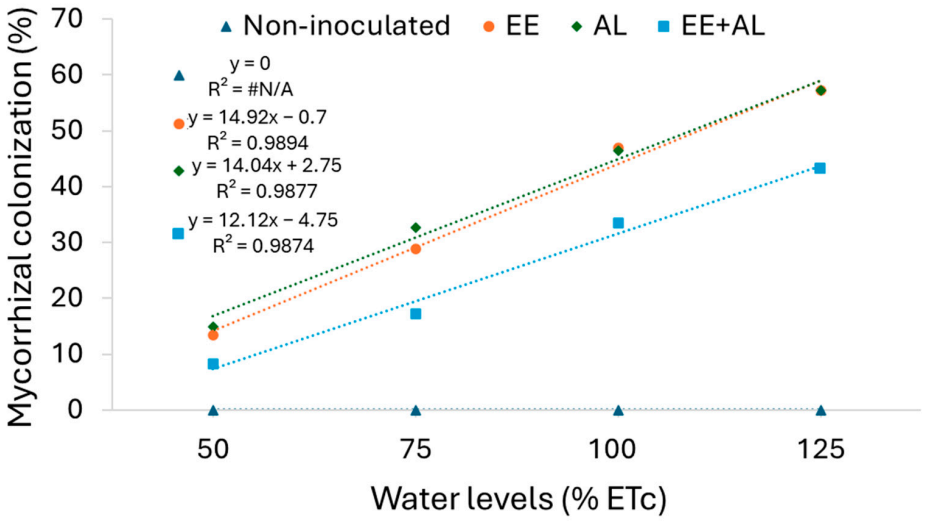
Significant Mycorrhizal colonization was observed, showing a linear increase in colonization as a function of different irrigation levels in both experiments. Root colonization in melon plants ranged from 6% to 60%, with an overall average of 36.2%, in Experiment I, and from 6% to 72%, with an average of 40%, in Experiment II (Figure 2A,B). Typical structures, such as arbuscules, hyphae, and initial colonization within the root cortex, were observed (as exemplified in Figure 3), indicating the successful establishment of a mutualistic symbiosis between the fungi and the melon roots.

No Mycorrhizal colonization was observed in non-inoculated plants in either experiment, indicating the absence of contamination by native AMF in the disinfected soil. The range observed in the colonization percentages across the experiments may be related to several factors, most notably water supply and soil water availability. The lowest irrigation levels resulted in a lower percentage of Mycorrhizal colonization, leading to a linear adjustment.

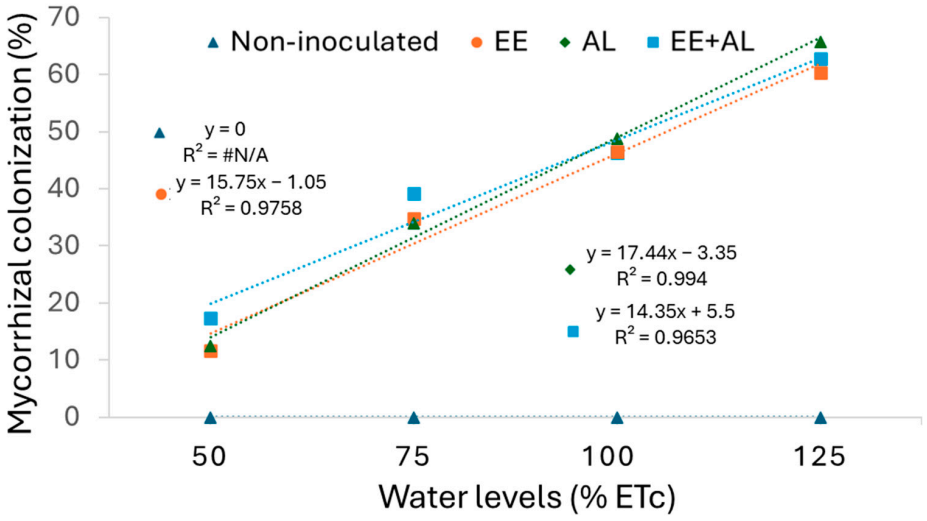
In Experiment I, there was an interaction between irrigation levels, water deficit, and AMF treatment for Mycorrhizal colonization. The results showed that both hybrids exhibited similar behavior, with higher average colonization at the 100% ETc irrigation level when inoculated with *A. longula* and *E. etunicatum* isolates. Additionally, for all inoculation conditions, the 125% ETc irrigation level also resulted in higher average colonization (Table 4).

The fresh and dry biomass of the melon plants in Experiment I decreased polynomially with the reduction in the applied irrigation level (Figure 4). Although both melon hybrids were subjected to waterlogging at the 125% ETc level, this condition did not negatively influence the production of fresh and dry biomass. It was found that the amount of water that maximized the fresh biomass production of the melon was 14.4 L (*Juazeiro*) and 16.1 L (*Mandacaru*). For the melon, regardless of the hybrid, the percentage value of ETc used in calculating the irrigation level is 115.2% and 106.9% for maximum efficiency of fresh aerial biomass (FAB) and dry aerial biomass (DAB), respectively.

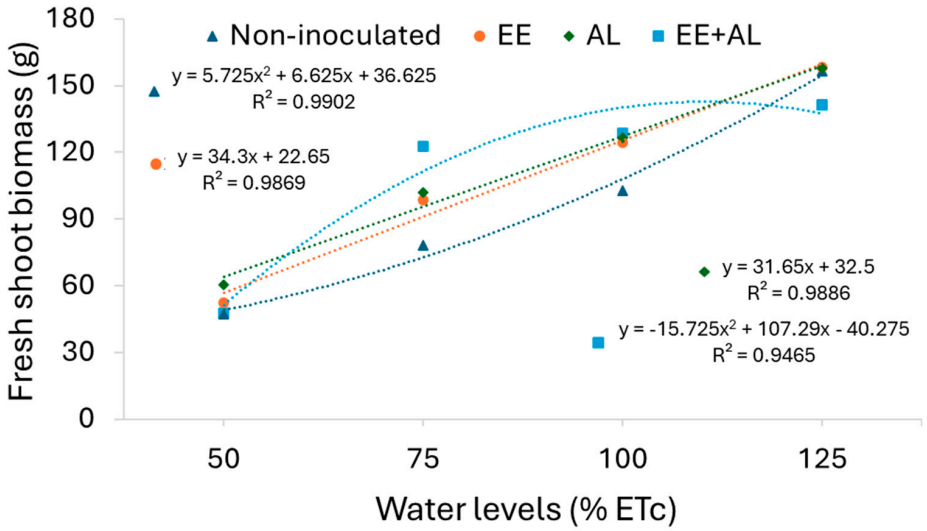
In Experiment II, it was observed that increasing the applied water level resulted in a linear increase in the values of dry and fresh biomass of the melon's aerial part (Figure 4). Additionally, the fresh and dry biomass of the aerial part of the melon in Experiment II showed higher values compared to those obtained in Experiment I. This fact may be related to the supplementary fertilization used to correct the nutrition of the plants in Experiment II. In this experiment, the fresh and dry biomass of the aerial part showed linear growth as the applied irrigation level increased.



(**A**)



(**B**)



(**C**)

Figure 2. Mycorrhizal colonization—Experiment I (**A**) and Experiment II (**B**), fresh shoot biomass—Experiment II (**C**) as a function of different irrigation levels (50, 75, 100, and 125% ETc) and treatments with Arbuscular Mycorrhizal Fungi, in melons cultivated in a protected environment.

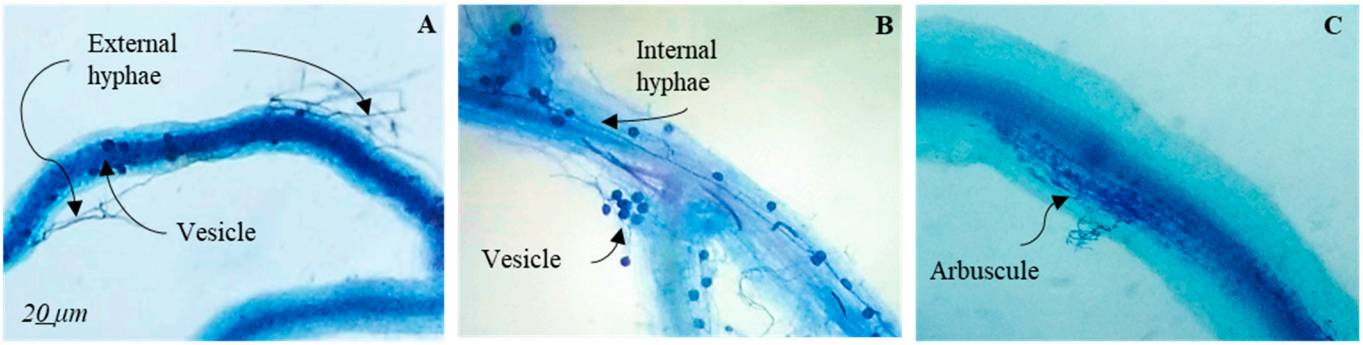


Figure 3. Root colonization of melon plants subjected to inoculation with AMF in melon roots (*Juazeiro* hybrid) after 45 days in a greenhouse/netted house. Arrows indicate (**A**) external hyphae and vesicles; (**B**) internal hyphae and vesicles; and (**C**) intraradical colonization.

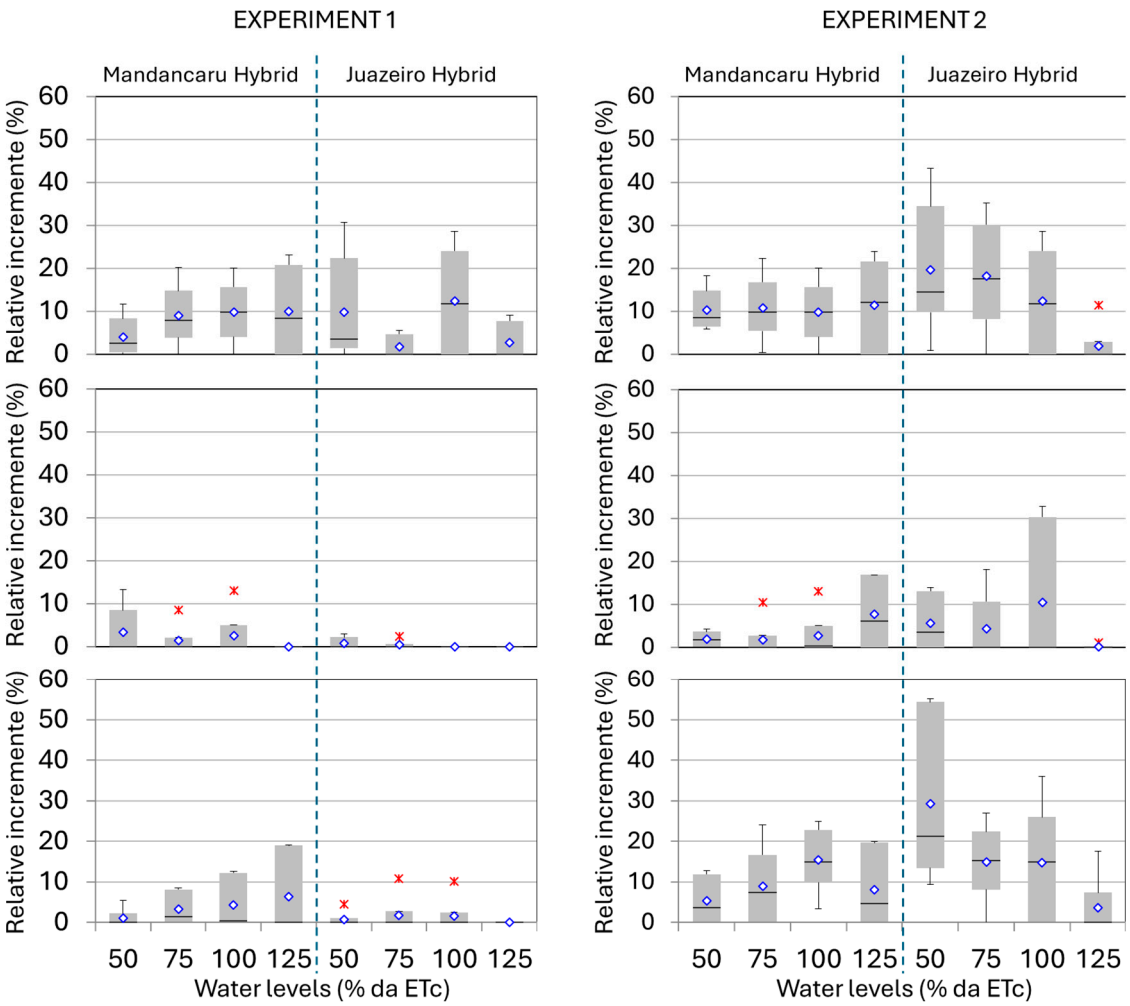


Figure 4. Boxplot representing relative increment values (IR) in %, for the hybrids *Juazeiro* and *Mandacaru* based on different irrigation levels (50, 75, 100, and 125% of ETc). Response capacity in terms of percentage difference in the increase of aerial biomass. EE—*E. etunicata*, AL—*A. longula*, EE + AL—*E. etunicata* + *A. longula*. Key: '◊' average; '-' median; '□' 25% to 75% probability; '⊤' maximum; '⊥' minimum; '*' outlier.

Regarding the rate of increase in dry biomass, in Experiment I, it was observed that plants inoculated with *A. longula* showed a notable symbiosis with the Juazeiro melon, particularly when irrigated with deficit levels (50 and 75% ETc) and full irrigation (100% ETc), the reduction in Mycorrhizal colonization was 79.42% and 21.53% under water deficit of 50

and 25% (use of 50 and 75% ETc), compared to full irrigation. This was not the case for the Mandacaru hybrid, where the mixed inoculum stood out (Figure 5), whose Mycorrhizal colonization values were reduced by 44.93% under a water deficit of 25% and by 67.72% when compared to the control (100%ETc) (Table 4).

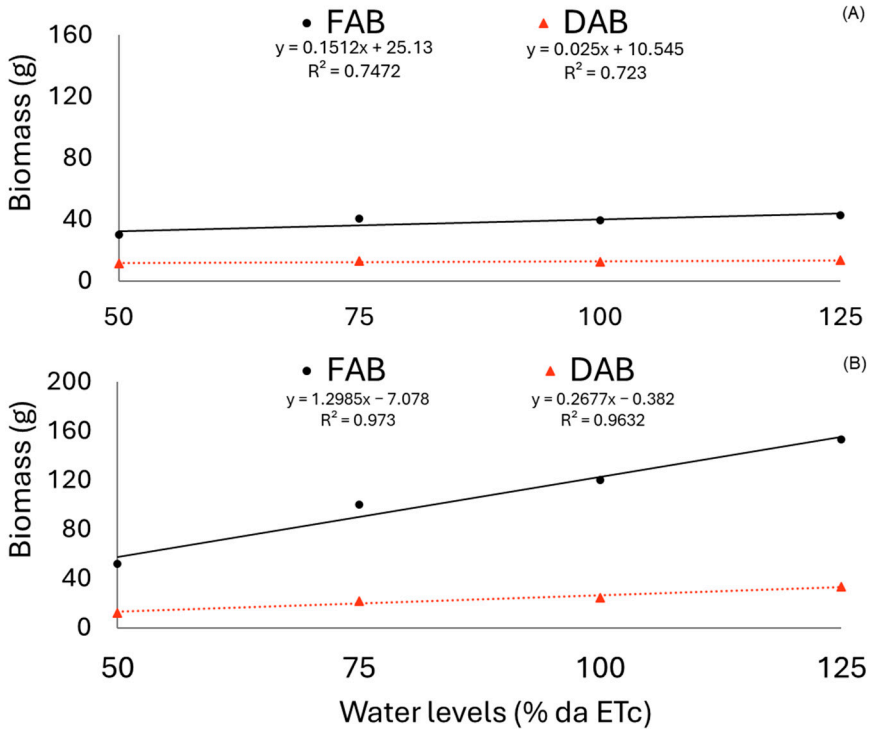


Figure 5. Variation of fresh and dry biomass of the aerial part of the melon for Experiment I (**A**) and Experiment II (**B**), based on different irrigation levels (50, 75, 100, and 125% ETc), *Juazeiro*, BA. 2015 and 2016.

In both experiments conducted with melon plants, the levels of macronutrients present in the aerial part of the plants did not show significant differences for the treatments in isolation concerning the levels of Phosphorus (P), Potassium (K), and Magnesium (Mg), except for calcium (Ca) in Experiment II, as detailed in Table 5.

The reduced results when the fungi are combined may involve competitive interactions or interference with the Mycorrhizal functions of these fungal species.

The accumulation of calcium varied based on the differentiation of water levels, hybrids, and inoculation with AMF individually in Experiment II. It was observed that there was less accumulation in the treatments without inoculation and with inoculation with the species *E. etunicata*. Regarding the water levels, the Ca content increased as the availability of water increased, following a linear model. Thus, it was noted that the greater the applied water level, the higher the absorption of Ca by the crop (Figure 6).

In Experiment I, the average values of Ca accumulated in the leaves of the melon showed statistically significant differences in response to the applied treatments, varying according to the irrigation levels, hybrids, and inoculation with Arbuscular Mycorrhizal Fungi (AMF). The lowest accumulation of Ca was observed in the treatment where the *Juazeiro* hybrid was inoculated with *E. etunicata* and subjected to full irrigation (100% of the crop's evapotranspiration—ETc). This treatment was the only one that showed significant differences compared to the treatment with the mixed inoculum but did not differ from the other treatments (Table 5).

Table 5. Analysis of variance and mean values for macronutrients contained in the aerial part of the melon in function of different irrigation levels (50, 75, 100, and 125% ETc), hybrids *Juazeiro* and *Mandacaru*, and treatments with Arbuscular Mycorrhizal Fungi for Experiments I and II.

Sources of Variation		Experiment I				Experiment II			
		Macronutrients				Macronutrients			
		P	K	Ca	Mg	P	K	Ca	Mg
Water levels (WL)		1.024 ^{ns}	0.017 ^{ns}	0.242 ^{ns}	0.018 ^{ns}	0.0767 ^{ns}	0.5485 ^{ns}	179.324 **	0.161 ^{ns}
Melon hybrids (MH)		0.063 ^{ns}	0.038 ^{ns}	1.947 ^{ns}	0.280 ^{ns}	1.8438 ^{ns}	1.9796 ^{ns}	0.057 ^{ns}	0.923 ^{ns}
AMF (A)		0.065 ^{ns}	1.355 ^{ns}	0.873 ^{ns}	0.719 ^{ns}	1.6418 ^{ns}	1.1816 ^{ns}	26.289 **	1.238 ^{ns}
WLxMH		0.345 ^{ns}	0.007 ^{ns}	0.398 ^{ns}	0.273 ^{ns}	0.5152 ^{ns}	0.6032 ^{ns}	1.877 ^{ns}	0.693 ^{ns}
WLxA		0.880 ^{ns}	1.266 ^{ns}	2.020 *	0.504 ^{ns}	0.1974 ^{ns}	1.3334 ^{ns}	1.326 ^{ns}	0.919 ^{ns}
MHxA		2.032 ^{ns}	1.748 ^{ns}	1.550 ^{ns}	0.608 ^{ns}	1.3939 ^{ns}	2.1361 ^{ns}	2.415 ^{ns}	2.172 ^{ns}
Interaction WLxMHxA		0.308 ^{ns}	1.390 ^{ns}	2.096 *	0.651 ^{ns}	1.2967 ^{ns}	0.0651 ^{ns}	0.567 ^{ns}	0.460 ^{ns}
Factors		P	K	Ca	Mg	P	K	Ca	Mg
		g Kg^{-1}				g Kg^{-1}			
Hybrids	*Juazeiro*	2.20 a	20.0 a	25.8 a	4.19 a	2.81 a	16.1 a	23.4 a	1.66 a
	Mandacaru	2.19 a	20.1 a	26.7 a	4.29 a	2.48 a	14.9 a	23.3 a	1.82 a
C.V.		12.92	19.24	16.77	23.31	53.74	29.28	13.15	56.63
Inoculation	NI	2.20 a	19.9 a	26.6 a	4.27 a	2.74 a	15.9 a	20.2 c	1.86 a
	EE	2.20 a	20.7 a	25.3 a	4.17 a	2.65 a	15.2 a	23.4 b	1.72 a
	AL	2.19 a	19.7 a	26.3 a	4.16 a	2.88 a	16.3 a	27.6 a	1.79 a
	EE + AL	2.21 a	19.9 a	26.5 a	4.36 a	2.36 a	14.4 a	22.0 bc	1.59 a
C.V.		7.49	11.19	13.34	14.64	35.94	28.33	14.74	33.71

NI—non-inoculated, EE—*E. etunicata*, AL—*A. longula*, EE + AL—*E. etunicata* + *A. longula*. The significant is for $p < 0.01$ (**) and $p < 0.05$ (*), and ^{ns} not significant. Means followed by the same letter in the column do not differ statistically from each other according to Tukey's test at a 5% probability level.

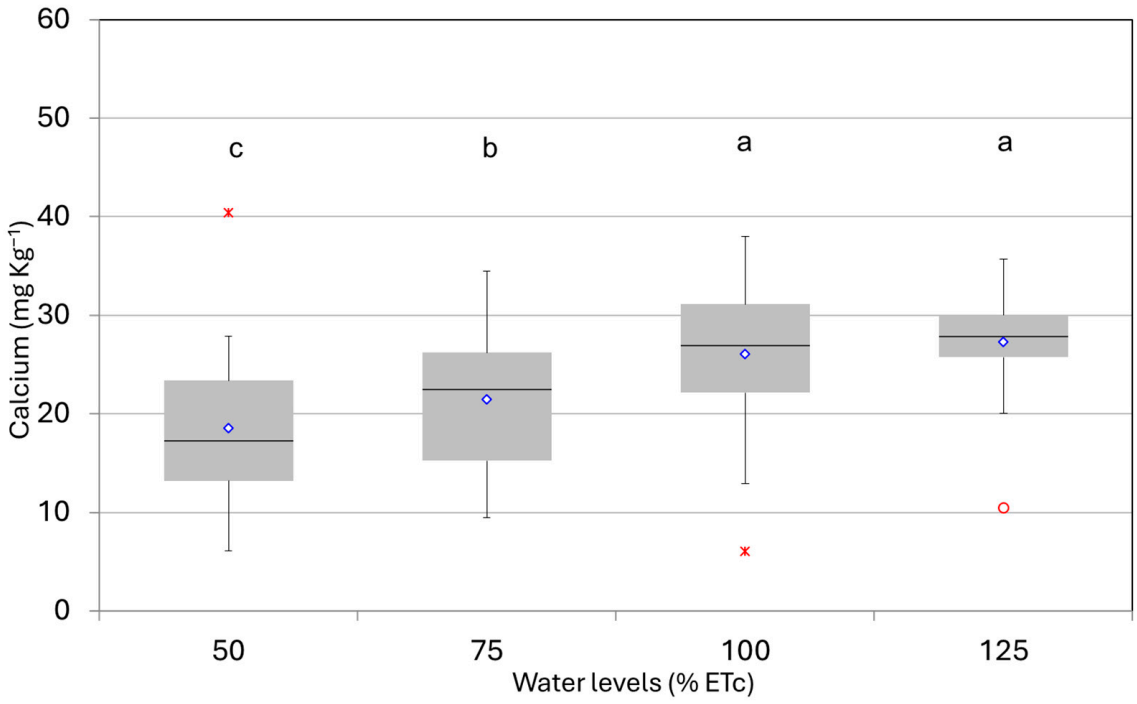


Figure 6. Variation in Ca content in response to different irrigation levels (50, 75, 100, and 125% of ETc) for all treatments, Experiment II. Key: '◊' average; '-' median; '□' 25% to 75% probability; '⊤' maximum; '⊥' minimum; '*' outlier; and '°' extreme. Means followed by the same letter in the column do not differ statistically from each other according to Tukey's test at a 5% probability level.

In the treatments where the *Juazeiro* hybrid was subjected to full irrigation without inoculation, inoculated with *A. longula*, or in the treatments with the *Mandacaru* hybrid irrigated with 75% of ETc, both without inoculation and inoculated with mixed AMF, no statistically significant differences were observed compared to the treatments with lower Ca accumulation (*Juazeiro* hybrid inoculated with *E. etunicata* and irrigated with 100% of ETc), nor with the other treatments that showed higher average Ca accumulation (Table 6).

Table 6. Ca content in the aerial part of melon plants as a function of different irrigation levels (50, 75, 100, and 125% of ETc), the Juazeiro and Mandacaru hybrids, and treatments with Arbuscular Mycorrhizal Fungi, Experiment I.

		Calcium (mg Kg^{-1})			
Treatment	**Hybrid**	**Water Levels (% da ETc)**			
AMF	**Melon**	**50**	**75**	**100**	**125**
NI	*Juazeiro*	27.2 a	24.4 a	26.4 ab	27.2 a
EE		24.1 a	24.7 a	22.2 b	24.5 a
AL		27.2 a	26.4 a	24.5 ab	23.1 a
EE + AL		26.1 a	28.0 a	29.4 a	23.6 a
NI	*Mandacaru*	28.8 a	22.9 ab	32.5 a	22.6 a
EE		26.5 a	29.5 a	26.7 a	23.9 a
AL		26.4 a	29.9 a	26.8 a	25.4 a
EE + AL		26.0 a	24.2 ab	26.6 a	27.5 a

NI—non-inoculated, EE—*E. etunicata*, AL—*A. longula*, EE + AL—*E. etunicata* + *A. longula*. FMA treatment with means followed by the same letter, within each combination of irrigation level and type of fungus, do not differ statistically from each other by the Tukey Test at 5% probability.

However, the treatments where the *Juazeiro* hybrid was subjected to full irrigation without inoculation, inoculated with the species *A. longula*, as well as the *Mandacaru* hybrid irrigated with 75% of ETc—both without inoculation treatment and inoculated with the mixed AMF—did not show statistically significant differences. These were compared to the treatments with the lowest average Ca accumulation (i.e., the treatment where the *Juazeiro* hybrid was inoculated with *C. etunicatum* and subjected to full irrigation) and to the other treatments that obtained higher averages (as detailed in Table 6).

It is worth noting that when analyzing the *Mandacaru* hybrid, when irrigated with 75% of the crop's evapotranspiration and without inoculation treatment, and comparing it to the same hybrid inoculated with the mixed AMF under the same irrigation conditions (75% of ETc), it can be observed that although no statistically significant difference was found regarding the average Ca value in the aerial part of this hybrid, the condition without inoculation resulted in a 5.48% reduction in Ca accumulation.

4. Discussion

The physiological changes resulting from the response to water deficiency have significant impacts on plant growth and development [1]. However, these effects can vary between cultivars, manifesting differently in aspects such as starch storage, sustained growth, and desiccation tolerance. In this context, the "Frog skin" melon (*Juazeiro*) showed higher fresh and dry shoot biomass values compared to the "Yellow gold" melon (*Mandacaru*) while also demonstrating lower water consumption. This characteristic suggests that "Frog skin" exhibits greater water-use efficiency, which is related to osmotic adjustment, contributing to the allocation of photoassimilates and fruit formation. Studies conducted by [2] confirmed this ability of the "Frog skin" melon and highlighted the reduction in transpiration under water stress at 15, 30, and 45 days after transplanting (DAT). This contrasts with the "Yellow gold" melon, which did not show the same transpiration reduction ability at 30 DAT, indicating a lower capacity to adjust its transpiration. This may result in greater

water loss and energy expenditure to maintain turgor, consequently negatively impacting dry matter accumulation [3].

Other studies, such as that conducted by [4], also identified the "Frog skin" cultivar as more tolerant to saline irrigation water, followed by the "Yellow gold" variety, reinforcing the idea that this cultivar excels in adverse environmental conditions.

In Experiment II, treatments with mycorrhizal inoculation demonstrated a positive response, showing higher nutrient supply both in full irrigation and under water deficit conditions. These treatments yielded superior results compared to the non-inoculated treatment, showing greater fresh and dry shoot biomass productivity. Studies by [5,6] also reported increased water absorption by Arbuscular Mycorrhizal Fungi (AMF), a fact associated with increased soil nutrient availability. Additionally, [7] discussed the benefits of AMF application in the production of various crops under water scarcity conditions, emphasizing notable improvements in water-use efficiency in both full and deficit irrigation scenarios. Similar studies on melons subjected to full and deficit irrigation and their association with AMF, conducted by [8], also corroborated the positive effects of Mycorrhizal inoculation. These studies showed that inoculation significantly increased shoot biomass, especially under full and moderate irrigation (100% and 75% of field capacity), as well as under water stress (50% of field capacity), when compared to non-mycorrhized plants.

The reduction in AMF colonization observed under water deficit conditions can be explained by the decrease in spore germination rates of the AMF isolates used in this study. Some studies confirm that when soil water potential drops below field capacity and during a prolonged water deficit stress period (considering the crop cycle duration), spore germination is reduced [9,10]. Since germination is a crucial process for establishing the mycorrhizal association, the absence or delay of this stage can result in lower Mycorrhizal colonization during the studied period, averaging 45 days.

The benefits provided to the host plant depend on the AMF species used [7,11], as although there is no symbiotic specificity, more favorable combinations between plants and AMF isolates can be found. The isolates *E. etunicata* and *A. longula* are species adapted to semi-arid conditions, and they showed higher colonization values with melons in Experiment I (where there was less nutrient availability). In Experiment II, due to increased nutrient availability in the soil, there were no differences in the average Mycorrhizal colonization percentages or in the fresh shoot biomass (FAB) between the AMFs; however, these increased compared to the non-inoculated treatment and the dry shoot biomass (DAB) of plants inoculated with *A. longula* was higher than that of non-inoculated plants, without differing from the other treatments. This strengthens the positive response of the beneficial effects of AMF in association with melons, especially when soil nutrient availability is present, providing benefits for water deficit tolerance and crop productivity, as highlighted in Experiment II.

On the other hand, the use of the *E. etunicata* (EE) isolates did not benefit the melon, although some studies have reported the effectiveness of this species in enhancing plant growth, development, and resistance to water deficit [12] and saline stress [13]. It is possible that the effectiveness of the *E. etunicata* (EE) isolates in enhancing plant growth, development, and resistance to water deficit or saline stress may be soil-dependent. This result emphasizes the importance of characterizing the efficiency of the AMF isolates used. Although the *E. etunicata* (Univasf 09) isolate was not effective in benefiting melon development, other isolates of this species, such as Univasf 06, were effective in promoting the growth of *Schinopsis brasiliensis* (baraúna) [14], *Ziziphus joazeiro* (juazeiro) [15], and *Pseudobombax simplicifolium* (embiruçu) [16], native plants of the Caatinga biome.

It is known that the use of mixed inocula can yield better results for host plants and may, therefore, be more suitable than introducing a single fungus species, as it contains

species with different symbiotic strategies and abilities [17]. However, in some cases, competition between fungi can occur [18], leading to negative production responses. Ref. [18] worked with a mixture of AMF (50% native fungi and 50% G. *clarum (Rhizoglomus clarum))* in a study with clover subjected to different pH levels and P availability in the soil and found no reduction in production from using the mixture. On the contrary, they also found high effectiveness of the introduced fungus (*G. clarum*). In the present study, no competition between the fungi was observed.

The interaction between macronutrient nutrition and Mycorrhizal colonization is important to explore the complex relationship between the host plant's nutritional status and the functioning of AMF. AMF play a critical role in plant nutrition, especially in facilitating the uptake of nutrients that are otherwise poorly available, such as P, N, and micronutrients. However, the efficiency of AMF colonization and its impact on nutrient absorption can be influenced by the levels of macronutrients available to the plant [19–23].

Macronutrients such as N, P, K, Ca, Mg, and sulfur are fundamental to plant growth and metabolism. The presence of adequate levels of these nutrients can enhance plant growth and development, providing a better environment for Mycorrhizal colonization. For example, P, one of the key nutrients involved in energy transfer within the plant, is often a limiting factor in soil. Mycorrhizal fungi are particularly efficient in acquiring P from the soil and transferring it to the host plant, which leads to an improvement in plant P status. Conversely, high P availability in the soil may reduce the need for Mycorrhizal fungi, as the plant can directly absorb sufficient P, which might lower the extent of fungal colonization [19–23].

This interaction between the mixed inoculum and environmental conditions also influences the absorption of essential nutrients, such as Ca, which plays a vital role in root development. Ca is a macronutrient important for root development, being essential in the translocation and storage of carbohydrates and proteins. Additionally, Ca stabilizes the plant cell wall and membranes, regulates cation–anion balance and osmoregulation, and functions as a secondary messenger [19].

In the context of our study, AMF inoculation did not impact P absorption by melon plants [19]. It is possible that the soil already had sufficient P availability for root absorption. For annual crops in general, based on soil samples collected at a depth of 0 to 20 cm and clay content above 16%, a P content of 17.3 mg dm^3 can be classified as adequate to very high in terms of P availability, depending on the percentage of clay present in the soil [20,21]. Additionally, this availability was supplemented with periodic fertilization throughout the experiment, regardless of the presence of Mycorrhizal fungi. In this scenario, the soil's intrinsic capacity to provide the nutrient may have been sufficient to meet the crop's nutritional demands, and the Mycorrhizal association may not have had an additional effect on P absorption [22].

The absorption of macro and micronutrients can depend not only on the AMF species but also on the host plant [23]. Nutrient absorption also depends on root development and its effects on intracellular root activity, as well as the efficiency with which both partners (plant and fungus) interact and exchange nutrients through the Mycorrhizal interface [24].

The *E. etunicata* (EE) species did not favor the *Juazeiro* hybrid, either in terms of increased dry shoot biomass (relative increase—RI) or Ca absorption, even under full irrigation conditions (100% ETc). The inefficiency of the symbiosis may result from a combination of factors, including species incompatibility, genetic variation in the plants, and functional diversity among AMF strains. These complex elements must be considered when investigating the underlying reasons for the lack of mutual benefits between *E. etunicata* (EE) and the *Juazeiro* hybrid. Further research and experimental studies are essential to deepen our understanding of this interaction and to seek efficient use in AMF inoculation strategies.

Previous studies corroborate these findings. For example, [25] found significant variation in Ca absorption with the use of Mycorrhizal inoculation in melon production using coconut fiber substrate. Meanwhile, [26] observed that AMF inoculation increased P and K content, although this increase was not observed under the conditions studied in this work.

An association that can be made is that the soil already had adequate levels of P and K, and the addition of AMF did not result in a significant increase of these nutrients. Furthermore, soil fertility and nutrient retention capacity influence the effectiveness of symbiosis.

Although the effects of AMF inoculation on plant nutrient uptake are well-documented, discrepancies in results may arise due to various environmental factors, such as soil nutrient availability, water stress conditions, and the specific AMF species or isolates used in the experiment. Studies have shown that soil fertility, the presence of pre-existing nutrients, and plant genotype play crucial roles in determining the success of AMF symbiosis and its impact on nutrient uptake [23]. In some cases, no significant increases in P or K content have been observed, which may be attributed to the already adequate levels of these nutrients in the soil or the limited capacity of the Mycorrhizal fungi to mobilize these nutrients under certain conditions [24–26].

5. Conclusions

The conclusions of this study highlight the effectiveness of the symbiotic association between Arbuscular Mycorrhizal Fungi (AMF) and melon plants, including the *Juazeiro* and *Mandacaru* hybrids. Inoculation with AMF, particularly with the species *A. longula* (AL, isolate URM-FMA 07), showed significant benefits in drought tolerance, resulting in notable increases in the fresh and dry biomass productivity of melon plants.

It was observed that the efficiency of this symbiosis is maximized when there is adequate nutrient availability in the soil, as demonstrated in Experiment II. Under these conditions, the plants exhibited greater Mycorrhizal colonization and a substantial increase in fresh and dry shoot biomass. In contrast, under nutrient-restricted conditions, *A. longula* stood out by providing efficient colonization and biomass increase, even when nutrient levels were low.

Although Mycorrhizal colonization is reduced under severe water deficit, the benefits of the symbiosis are not entirely compromised. The *Juazeiro* hybrid, known as Frog skin, showed greater tolerance to water stress and a higher affinity with the *A. longula* species, resulting in greater Mycorrhizal colonization and significant biomass increases, underscoring its superiority over other varieties in low water availability conditions.

These findings have important implications for agricultural management practices and Mycorrhizal inoculation strategies, offering valuable insights into optimizing crop productivity and resilience in adverse conditions.

Author Contributions: Conceptualization, L.B.M. and J.M.M.d.M.; methodology, J.M.M.d.M., L.B.M., M.D.I.D., A.M.Y.M. and I.L.; software, J.M.M.d.M.; validation, J.M.M.d.M., L.B.M., F.N.V. and L.M.V.; formal analysis, J.M.M.d.M. and I.L.; investigation, J.M.M.d.M., L.B.M., D.K.A.d.S., A.M.Y.M. and I.L.; resources, J.M.M.d.M., L.B.M., A.M.Y.M. and I.L.; data curation, J.M.M.d.M.; writing—original draft preparation, L.B.M., J.M.M.d.M. and I.L.; writing—review and editing, L.B.M., I.L., M.J.M.G. and J.M.M.d.M.; supervision, L.B.M.; project administration, L.B.M.; Valdation, L.M.V.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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