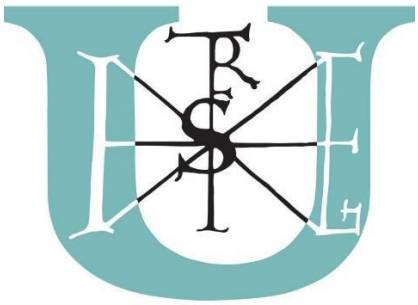

Szent István University

Doctoral School of Environmental Sciences

Ph.D. Dissertation

ARBUSCULAR MYCORRHIZAE FUNGI ROLE IN TOMATO (*L. esculentum* Mill) PRODUCTION UNDER WATER SCARCITY CONDITIONS

By

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Title: Arbuscular mycorrhizae fungi role in tomato (*L. esculentum* Mill) production under water scarcity conditions.

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1. BACKGROUND AND OBJECTIVES

According to the Food and Agriculture Organization (FAO) about 69% of the whole water worldwide are for the agriculture sector (Aquastat, 2014), three folds more than 50 years ago and expected to increase by further 19% by 2050. Changes in climate disturbed precipitation aspects and more intense droughts anticipated (Trenberth et al., 2014), which is considered the most curtail abiotic factor negatively affecting agricultural production, especially in irrigated field cropping systems as freshwater is evidently limited (Farooq et al., 2009).

There is a general understating that droughts are threatening climate sensitive economic sectors such as agriculture and particularly field crop production, since field crop production is totally water dependent and consumes most water withdrawn for the agriculture sector (Aquastat, 2014). This rises the necessity to improve the irrigation water use efficiency and saving water must be the priority of any future planning in water supply, in addition assessing the potential impacts of climate change on crop production at various ways in order to reduce agricultural vulnerability to water scarcity and drought.

Biological processes and arbuscular mycorrhizae fungi (AMF) contribution in nutrient dynamics are getting more attention, due to high costs of fertilizer production and application, in addition to the minimum input of organic production (Jakobsen et al., 2005; Plenchette et al., 2005). According to Smith and Read (2008), reserves for good quality fertilizer production could run out before the end of the century, since phosphate (P) deposits are limited. Thus increasing the urgency to search for plant adaptation for more efficient use of P accumulated in the soil.

Less attention has been paid to sustainable production system establishment or its maintenance through preserving soil resources, meantime increasing the yield was the main purpose in applying commercial inocula (Plenchette et al., 2005). The fact that, arbuscular mycorrhizae (AM) pathway operates in P uptake in colonized roots, makes AMF an integral part of the root functional system and should not be ignored in plans aiming improvement of nutrient use in soil, even when there are no net benefits in term of yield (Smith & Read, 2008)

AMF role in plant growth performance, nutrient absorption enhancement, root architecture improvement, and abiotic stress tolerance is evident (Pozo et al., 2015). Previous studies

confirmed the role of the AMF symbiosis (Augé & Moore, 2005; Augé, 2001), but the mechanism of alleviating water stress on mycorrhizal plants is still a controversy.

Most studies that addressed physiological aspects of mycorrhizal plants were pot-based under standardized environmental conditions (Augé et al., 2015), where plants rhizosphere is restricted and AMF contribution to water and nutrients uptake is limited. Unlike studies under controlled conditions, field-based experiments are substantially different, where more than one environmental factor may interact (Suzuki et al., 2014). Existing autochthonous inoculants alleviate water stress effects on host plants (Ortiz et al., 2015) and protect both native plants and field crops (Armada et al., 2015), therefore inoculation under field conditions is essential to evaluate AMF effects, since the interaction among different AMF is not always synergistic (Suzuki et al., 2014).

The main purpose of this thesis is to better understand of how different timing of inoculation, water supply levels, and environmental factors, such as site geography, soil properties, and precipitation, influence the efficiency of arbuscular mycorrhizal inoculation in a crop production system. We used processing tomato UNO ROSSO F_1, considering its economic importance to answer the following questions:

- Which inoculation is more effective in alleviating water stress impact on plants, pre-transplant inoculation at sowing or field-inoculation at transplant?
- To what degree of the prevailing drought stress do AMF alleviate water stress impact on plants?
- What is the mechanism in which the AM symbiosis back up host plants to overcome water stress; is it drought stress avoidance or drought stress tolerance?
- Do AMF reserve and/or increase plant production under different soil moisture conditions?
- What is the role of AMF in preserving and enhancing fruit quality under different soil moisture conditions?
- What are AMF inoculation effects on the performance of certain physiological and biochemical processes of host plants under different soil moisture conditions?
- Could AMF be used as a mitigation practice tool in facing water scarcity from the agricultural and ecological point of view?

2. MATERIALS AND METHODS

2.1. Experimental site, design and plant material

The experimental farm arranged in a randomized block design with three water supply regime blocks: Full water supply (WS100), deficit water supply (WS50), and no water supply (WS0) depending on the crop daily water requirement and by adjusting the water supply amount through a drip irrigation system. A two way factorial experimental design with three levels of mycorrhizal inoculation, and three levels of water supply was used. Processing tomato UNO ROSSO F_1 seeds (United Genetics Seeds Co. CA, USA) were used in both growing seasons 2015, and 2016. Treatments split to three blocks with four repetitions per treatment, and seedlings were arranged in double (twin) rows with 1.2 m and 0.4 m inter rows distance and 0.2 m between plants.

Growing season 2015: The experiment was carried out on the old farm of the Horticulture institute in Szent István University (SIU), Gödöllő**,** Hungary (47.593609N, 19.354630E). The farm had brown forest soil, sandy loam in texture consists of 69% sand, 22% silt, and 9% clay. The bulk density of the soil was 1.25 g cm^{-3}, with 19% of field capacity, and the water table was bellow 5m, which could not influence the water turnover.

Growing season 2016: in the second growing season the experiment was conducted on the new experimental farm of the Horticulture institute in Szárítópuszta, Gödöllő, Hungary (47.577131N, 19.379739E), where the soil of the farm was loamy in texture (consisting of 41% sand, 47.5% silt, and 11.5% clay) with a bulk density of 1.49g cm^{-3}, and 25% of field capacity.

2.2. Mycorrhizae materials

2.2.1. Commercial inoculum Symbivit®

The commercial inocula Symbivit®, produced in the Czech Republic (https://www.symbiom.cz/), has been used in both growing seasons for the mycorrhizal inoculation. Symbivit® contains propagules of six different AMF species (*G. etunicatum, G. microaggregatum, G. intraradices, G. claroideum, G. mosseae, G. geosporum)* mixed with an inert substrate and amended with bio-additives promoting the symbiosis.

Mycorrhizal pre-transplant inoculation at sowing*:* To produce pre-transplant at sowing inoculated seedlings (AM+), half of the seedling trays were inoculated at sowing by adding 25

g of the commercial inoculum Symbivit® to each litre of the substrate (Klasmann TS3). The other half of the trays were sown without any type of mycorrhizal inoculation and later used as non-inoculated (Control) treatment.

Mycorrhizal field-inoculation at transplant: Seedlings were kept for a month in the greenhouse under controlled conditions and then bedded out in the open field. During transplantation of the seedlings to the field half of the non-inoculated (Control) seedlings were inoculated (AM++) in the field by adding 20 g of the Symbivit® inoculum into the planting hole for each seedling

2.3. Water supply

Weather forecasts from the National Metrological institute (http://www.met.hu/en/idojaras/) were used to calculate plants daily water demand depending on the daily average air temperature and precipitation. Water supply was calculated depending on the air temperature (daily water demand mm = average daily temperature ºC x 0.2 mm $ºC^{-1}$) according to Pék and co-workers (2014). Drip irrigation system was used to implement three watering regimes.

During the first growing season in 2015, the field received 186.3 mm of precipitation. Thus, the no water supply block received only 186.3 mm from rainfalls, water deficit (WS50) block received 50% of the calculated water supply demand a sum of 306.3 mm including the rainfall, and fully irrigated (WS100) block received a sum of 426.3 mm including the rainfall In growing season 2016, WS0 block received only 296 mm of rainfall, WS50 block received 50% of the calculated water demand a sum of 388 mm including the rainfall, and WS100 block received a sum of 480 mm including the rainfall.

2.4. Fertigation

Plant nutrition requirements and plant protection were regulated after Helyes and Varga (1994). Weekly fertigation has done using drip irrigation system and by adding 5 grams of the Ferticare 14-11-25 to each aquare meter of the cultivated area.

2.5. Harvesting

In 2015, plants in no water supply regime faced severe water deficit stress that shortened their growth period by 2 weeks, therefore total biomass and fruits of WS0 tomato plant stands were

harvested first on August 11, and then followed by both WS50, and WS100 on August 25. Unlike 2015, in 2016 plants were harvested at once after 100 days of growing.

2.6. Field measurements

2.6.1. Volumetric water content of soil

In the field, digital soil moisture meter PT1 (Kapacitív Kkt. Budapest, Hungary) was used to estimate volumetric soil water content (VWC), records were taken at six different soil depths (5, 10, 15, 20, 25, and 30 cm) just prior to watering.

2.6.2. Leaf transpiration

Porometer Delta-T, type AP4 from UK, was used to measure the water loss from the leaves of the plants and the up taken CO_2 for photosynthesis.

2.6.3. Relative chlorophyll index

As a non-destructive tool, chlorophyll meter SPAD-502 (Konica Minolta Hungary Business Solutions Ltd., Budapest, Hungary) was used to measure relative chlorophyll index as SPAD units at fruit setting stage.

2.6.4. Chlorophyll fluorescence

Chlorophyll fluorescence was measured by portable fluorimeter PAM 2500 (Walz-Mess und Regeltechnik, Germany). From four repetitive plants tagged for photochemical analysis, a fully developed top leaf was induced to 35 min dark adaptation by leaf clips. PamWin 3.0 software was used to calculate the photochemical quantum yield of PSII from Fv/Fm ratio by fast kinetics method (Van Goethem et al., 2013).

2.6.5. Leaf water potential

Pressure bomb (PMS Instruments Co., Corvallis, OR, USA) was used to determine leaf water potential (ψ_L) at midday after Gonzalez (2001).

2.6.6. Canopy temperature

The infrared thermometer (Raytek Raynger MX4, Santa Cruz, CA, USA) was used to record the canopy temperature (Bőcs et al., 2009).

2.7. Laboratorial Analyses

2.7.1. Proline estimation

Proline concentration was estimated based on the acid-ninhydrin method (Bates et al., 1973). Spectrophotometer (Hitachi U-2900, Tokyo, Japan) was used to read the absorbance of the extracts at 520 nm. The concentration of proline in the extracts was calculated using the calibration curve for proline standards based on the fresh weight (µg proline / g leaf).

2.7.2. Inorganic elements concentration

250 mg of dried milled leaves were digested in CEM MARS 5 (Magne-Chem Ltd., Budapest, Hungary) device using microwave pressure digestion method for elemental analyses. ICP-OES spectrometer (HORIBA Jobin Yvon ACTIVA-M, Edison, NJ, USA) was used to quantify shoot element concentrations.

2.7.3. Soil microbial activity

The activity of fluorescein diacetate hydrolase (FDAH) was assessed as described in previous protocols (Tabatabai & Bremner, 1969; Adam & Duncan, 2001).

2.7.4. Mycorrhizal root colonization

Samples were stained by Trypan Blue (Phillips & Hayman, 1970). Root colonization percentage calculated by gridline intersect method (Giovannetti & Mosse, 1980) Extraction and counting spores of endogenous AMF

2.7.5. Extraction and counting spores of endogenous AMF

Wet sieving technique (Gerdemann & Nicolson, 19630) has been used for the extraction and counting spores of endogenous AMF.

2.7.6. Analysis of carotenoid components and ascorbic acid

Extraction of carotenoid: The pigments from raw tomato were extracted according to a previously described procedure with slight modification (Abushita et al., 2000). The residues were re-dissolved in HPLC acetone, as the best organic solvent that ensure high solubility of most of carotenoids before injection onto HPLC column (Daood et al., 2013).

HPLC analysis of tomato: Chromaster Hitachi HPLC instrument consisting of a Model5430 diode-array detector, a Model 5210 auto-sampler and a Model5110 gradient pump was used for HPLC analysis.

Extraction and determination of Ascorbic Acid: The analytical determination of ascorbic acid was performed on C18 Nautilus, 100-5, 150 × 4.6 mm (Macherey-Nagel, Düren, Germany) column with gradient elution of 0.01M KH2PO4 (A) and acetonitrile (B). For quantitative determination of ascorbic acid standard materials (Sigma-Aldrich, Budapest, Hungary) were used.

2.7.7. Soluble solid content determination

Refractive index is considered the most common tool to estimate the soluble solid content as percentage (Johnstone et al., 2005). To estimate the ^{o}Brix digital Refractometer Krüss DR201-95 (Küss Optronic, Hamburg, Germany) was used.

2.7.8. Water use efficiency (WUE)

WUE calculated depending on total biomass as $WUE = \frac{Total\ biomass\ ton\ per\ hectare}{Quebic\ meter\ water\ consumed\ \ per\ hectare}$

2.7.9. Statistical analyses

Analysis of variances was conducted by two ways ANOVA, the software IBM SPSS Statistics for Windows, Version 22.0. (IBM Hungary, Budapest, Hungary) was used to run statistical analyses. Main effects were: Arbuscular mycorrhizal inoculation, AM with three levels (Control, AM+, AM++) and Water supply with three variants (WS0, WS50, and WS100). As a prerequisite for the statistical test, the assessment of the normality of the data was done by Shapiro-test. Due to our equal variances across groups, the Levene test was conducted to verify the homogeneity assumption. Means of four replications were separated by least significant difference (LSD, $P \leq 0.05$). In case of significant interaction between AM and WS, Tukey's HSD posthoc test was performed to determine significant differences among the treatments.

Before data analyzing percentage values for root colonization were arc-sine [square-root (X)] transformed. Pearson correlation coefficient is used to assess the direction and the strength of the linear relationships between some variables.

3. RESULTS

In this work, mycorrhizal field-inoculation at transplant, boosted yield, and enhanced growth and water use efficiency under both deficit water supply and full water supply levels.

3.1. Mycorrhizal development and rhizosphere microbial activity

Mycorrhizal inoculation significantly increased the root length colonization in inoculated plants (68-79% in 2015, and 70-73% in 2016) compared to non-inoculated (52-58% in 2015, and 49-58% in 2016) with no effects of water supply levels (Table 1), indicating high adaptation ability of arbuscular mycorrhizae strains introduced to the field.

Fluorescein diacetate hydrolase (FDAH) indicated higher microbial activity only under no water supply condition in growing season 2015. In general higher microbial activity (from 1.04 to 1.14) in all inoculated and non-inoculated treatments, and at all water level was recorded in 2016 compared to results in 2015 growing season (from 0.62 to 0.85) with the same commercial inocula and under similar water supply intensities (Table 1). Relative field mycorrhizal contribution (RFMC %) to root colonization positively affected the biomass production at all water supply levels (Table 1) reaching (42%) in WS50 water regime and for-

Table 1. Root colonization (R. Col. %), fluorescein diacetate (µMoles of p-nitrophenol /g of soil/ hr), and relative field mycorrhizal contribution (RFMC %).

Water supply	Mycorrhizal Inoculation	R. Col. (%)		FDA (µm p-nitrophenol g^{-1} hr^{-1})		RFMC (%)	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
WS0	Control	57^{Aa} ±7	51^{Aa} ±21	0.71^{Aa} ±.07	1.04^{Aa} ±.2		
	AM++	78^{Ba} ±9	70^{Aa} ±10	0.85^{Ba} ±.12	1.14^{Aa} ±.4	3	4
WS50	Control	52^{Aa} ±7	58^{Aa} ±20	0.62^{Aa} ±.18	1.14^{Aa} ±.2		
	AM++	68^{Ba} ±11	73^{Aa} ±05	0.68^{Aa} ±.10	1.05^{Aa} ±2	42	25
WS100	Control	58^{Aa} ±7	49^{Aa} ±08	0.64^{Aa} ±.07	1.07^{Aa} ±.2		
	AM++	79^{Ba} ±8	70^{Ba} ±08	0.64^{Aa} ±.12	1.12^{Aa} ±.3	7	8
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)							
Mycorrhizal Inoculation		***	*	**	ns		
Water supply (WS)		ns	ns	*	ns		
AM++ * WS		ns	ns	ns	ns		

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculation, small letters represent water supply effect.

less extend (7% and 3%) in both WS100 and WS0 respectively. Similar trend with less differences between the water supply levels has been observed in season 2016, where mycorrhizal inoculation best contributed to biomass production in WS50 (25%), followed by (8%) in WS100 and (4%) in WS0.

3.2. Quantitative parameters of tomato fruits

3.2.1. Total-, marketable-, and rotten fruits

Irrespective of mycorrhizal inoculation and water supply levels, in general fruit production was higher in growing season 2016 compared to growing season 2015 (Table 2). In 2015 growing season, water supply did increase the total yield in non-inoculated Control plants by 48 ton when plant supplied with half of their water requirement in WS50 block, and by 66 ton in each hectare when fully watered in WS100. Same trend but for less extend has observed in 2016 growing season (Table 2), but not reaching significant levels. Field mycorrhizal inoculation affected the fruit production positively at all watering levels and in both seasons with the best interaction between water supply and mycorrhizal inoculation under water deficit condition reaching 110.8 and 167.0 tons per hectare in both first and second growing seasons respectively (Table 2). Increases in total yield by about 63% in 2015 and 38% in 2016 in AM++ compared to Control plants are due to the enhancement in plants water relations, nutrient uptake, and many physiological processes that be explained in next sections.

Table 2. Total yield (t ha^{-1}), marketable fruits (t ha^{-1}), and rotten fruits (t ha^{-1}).

Water supply	Mycorrhizal Inoculation	Total Yield (t ha^{-1})		Marketable fruits (t ha^{-}		Rotten fruits (t ha^{-1})	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
WS0	Control	19.8^{Aa} ±4	114.1^{Aa} ±10	14.7^{Aa} ±3	65.3^{Aa} ±8	5.5^{Ba} ±2	38^{Ba} ±2
	AM++	21.2^{Ba} ±1	116.5^{Ba} ±08	14.9^{Aa} ±2	72.3^{Aa} ±7	3.8^{Aa} ±1	31^{Aa} ±8
WS50	Control	68.1^{Ab} ±2	121.4^{Aa} ±15	56.5^{Aa} ±2	72.1^{Aa} ±14	12^{Bb} ±4	35^{Ba} ±6
	AM++	110.8^{Bc} ±4	167.0^{Bc} ±03	96.5^{Bc} ±6	114.7^{Bc} ±3	4.8^{Aa} ±2	29^{Aa} ±2
WS100	Control	87.0^{Bc} ±3	129.9^{Aa} ±15	68.4^{Aa} ±4	84.7^{Aa} ±8	18^{Bb} ±1	33^{Ba} ±13
	AM++	89.7^{Bb} ±3	136.4^{Bb} ±02	75.4^{Bb} ±3	92.0^{Ab} ±5	13^{Ab} ±3	29^{Aa} ±4
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)							
Mycorrhizal Inoculation		***	**	***	***	*	***
Water supply (WS)		***	***	***	***	***	***
AM++ * WS		***	**	***	***	***	***

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculation, small letters represent water supply effect.

Marketable fruits did increase in 2015 season in non-inoculated plants due to water supply increase, from (15 t ha^{-1}) in WS0, to (57 t ha^{-1}) in WS50, and (68 t ha^{-1}) in WS100, but in 2016 it is started from (65 t ha^{-1}) in WS0, to (72 t ha^{-1}) in WS50, and (85 t ha^{-1}) in WS100 (Table 2). In 2015, compared to Control plants, mycorrhizal inoculation raised marketable fruits by 9% when fully irrigated, and by 71% under deficit water supply; similarly an increase of 9% in WS100, and 59% in WS50 were recorded in AM++ plants in 2016.

In addition to the efficient contribution in the yield production increase, mycorrhizal inoculation decreased the amount of rotten fruits in both seasons and at all water levels, except in WS50 in 2016 (Table 2). Mycorrhizal inoculation affected fruit quality positively including less rotten fruits in both seasons and at all water supply levels and minimized losses due to fruit cracking.

3.3. Qualitative parameters of tomato fruits

3.3.1. Soluble solid content

In Control plants, the content of soluble solid (ºBrix) showed an adverse relationship with the marketable yield in both growing seasons; very strongly (r = -0.93) in the first growing season, and a moderate downhill (r = -43) in 2016 growing season. Mycorrhizal inoculation could slightly slow down the soluble solid content decrease along with the yield increase (r from -0.93 to -0.77) in 2015 growing season, while in the second growing season mycorrhizal inoculation could not only prevented the brix loss but also enhanced the soluble solid level (from r = -0.43 to r = +0.12) in the marketable fruits (Figure 1).

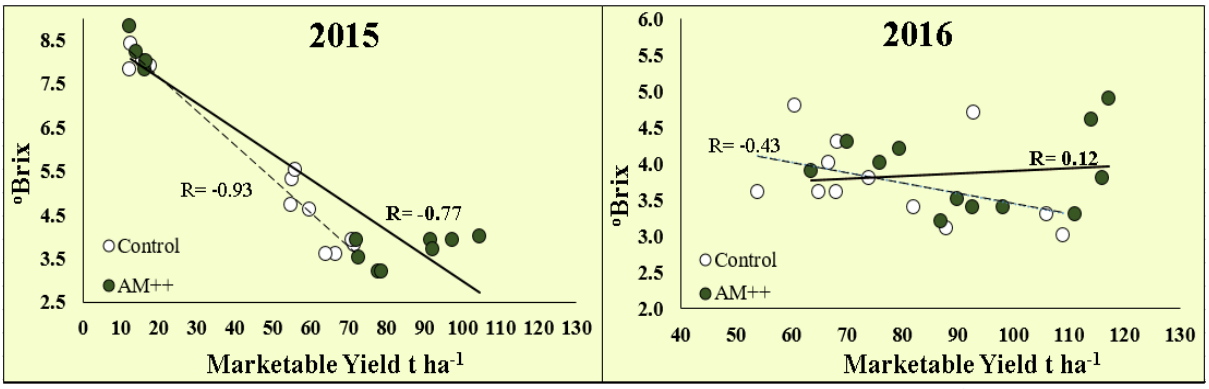

Figure 1. Fruit ºBrix content (g per 100 g), and marketable yield (t ha^{-1}) relationship.

3.3.2. Carotenoids

Tomato carotenoids profile was separated by HPLC method into several compounds including the majors such as lycopene, 13Z-lycopene, lycopene, lycoxanthin, β-carotene, and lutein. Due to their nutritional importance and biological activities, here we are focusing on β-carotene, lycopene, and total carotenoids.

Decreasing watering amount increased the total carotenoids, lycopene, and β-Carotene contents in fruits of Control plants in both growing seasons 2015 and 2016 (Table 3), but higher yield overcame the concentration loss by higher production of antioxidants per unit area. Regardless of inoculation and water supply, lycopene content was ranging from 49.0-113.4 mg g^{-1}. In the second season the lycopene content was ranging from 95 to 273 mg g^{-1}, not only exceeding the normal range (100-155 mg g^{-1}), but almost duplicated and tripled when compared to lycopene contents in fruits with the same watering level and mycorrhizal treatment the first growing season (Table 3).

The same is true for total carotenes, since Lycopene forms about 83% of all carotenoids. In AM++ and Control plants and at all water supply levels, antioxidants in tomato fruits were much higher in 2016 growing season in comparison with 2015.

Table 3. Total Carotene (µg g^{-1}), lycopene (µg g^{-1}), and β-Carotene (µg g^{-1}) in fruits of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	Total carotene (µg g^{-1})		Lycopene (µg g^{-1})		β-Carotene (µg g^{-1})	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
WS0	Control	136.3^{Bb} ±1.3	313^{Ac} ±32	100.1^{Bb} ±2	205^{Bb} ±10	2.63^{Ba} ±.22	13.5^{Bc} ±2
	AM++	146.5^{Bb} ±15	282^{Aa} ±32	113.4^{Bc} ±12	165^{Aa} ±23	3.24^{Bb} ±.36	9.7^{Aa} ±1
WS50	Control	106.3^{Ba} ±7.7	233^{Ab} ±26	72.0^{Ba} ±6	188^{Ab} ±26	2.23^{Aa} ±.42	10.1^{Ab} ±1
	AM++	90.75^{Aa} ±11	281^{Aa} ±42	67.5^{Ab} ±9	185^{Ab} ±23	1.89^{Aa} ±.88	9.7^{Aa} ±.2
WS100	Control	94.27^{Aa} ±19	181^{Aa} ±11	66.1^{Ba} ±7	95^{Aa} ±19	2.42^{Aa} ±.30	5.4^{Aa} ±1
	AM++	80.29^{Aa} ±4.6	437^{Bb} ±50	49.0^{Aa} ±1	273^{Bb} ±30	2.63^{Ba} ±.22	17.2^{Bb} ±4
Significant of Source of variation (ns= not significant, * P<0.05, ** P<0.01, *** P<0.001)							
Mycorrhizal Inoculation		**	***	ns	***	ns	**
Water supply (WS)		***	*	***	ns	***	*
AM++ * WS		***	***	***	***	ns	ns

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculstion effect, small letters represent water supply effect.

3.4. Physiological response to mycorrhizal inoculation

3.4.1. Photosynthetic efficiency and relative chlorophyll index

In our study and in both seasons, the stomatal conductance improvement by mycorrhizal inoculation in WS50 water regime positively influenced the photosynthetic efficiency of PSII system in AM++ plants (Table 4).

In the first season, water supply reduction has lessened the photosynthetic efficiency of photosystem II in non-inoculated Control plants only in no water supply WS0 block, but mycorrhizal inoculation enhanced the photosynthetic efficiency at all watering levels (Table 4). In the second season, although mycorrhizal inoculation did enhance photosynthesis process only in WS50, values of PSII maximum efficiency (Fv/Fm) indicated no photo-oxidative damage neither in full irrigated nor in unirrigated plants.

In the first growing season, mycorrhizal inoculation enhanced Single-Photon Avalanche Diode (SPAD) only under water deficit condition WS50, with no remarkable changes neither in no water supply WS0 nor in fully irrigated WS100 blocks (Table 4). Unlike the first season and regardless of water supply levels, no effects of mycorrhizal inoculation have been found on leaf chlorophyll content SPAD values (Table 4).

Table 4. Maximum efficiency of PSII, and Single-Photon Avalanche Diode (SPAD) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	maximum efficiency of PSII (Fv/Fm)		Single-Photon Avalanche Diode (SPAD)	
		2015	**2016**	**2015**	**2016**
WS0	Control	0.66^{Aa} ±.05	0.74^{Aa} ±.02	47.0^{Aa} ±0.5	55.6^{Ab} ±0.8
	AM++	0.74^{Ba} ±.03	0.75^{Aa} ±.03	48.6^{Aa} ±2.6	55.4^{Ac} ±0.6
WS50	Control	0.75^{Ab} ±.02	0.74^{Aa} ±.02	46.4^{Aa} ±0.7	54.0^{Ab} ±1.3
	AM++	0.78^{Bb} ±.03	0.77^{Bb} ±.03	48.5^{Ba} ±1.5	53.3^{Ab} ±1.5
WS100	Control	0.75^{Ab} ±.04	0.76^{Aa} ±.02	47.0^{Aa} ±1.7	49.8^{Aa} ±1.2
	AM++	0.77^{Bb} ±.01	0.74^{Aa} ±.02	47.7^{Aa} ±1.9	50.7^{Aa} ±0.5
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)					
Mycorrhizal Inoculation (AM++)		***	***	*	ns
Water supply (WS)		***	ns	ns	***
AM++ * WS		ns	*	ns	ns

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculation effect, small letters represent water supply effect.

3.4.2. Total biomass and water use efficiency

Both water supply and mycorrhizal inoculation did create significant differences in the above ground total biomass (fruits, stem, and leaves) in both growing seasons (Table 5) with the exception of the no water supply block in the first season, where vegetative growth period shortened by weeks due to severe stress caused by decreasing the soil moisture content. In non-inoculated plants, decreasing water supply gradually decreased the total biomass by 66% in WS0, and 20% in WS50 in the first growing season, while in second growing season by 11% in WS0, and 6% in WS50 compared to fully irrigated plants in WS100 block.

On the contrary, mycorrhizal inoculation increased the total fresh biomass in the first season by 3% in WS0, 74% in WS50, and 8% in WS100; the biomass increased in the second season by 4% in WS0, 33% in WS50, and 9% in WS100 respectively (Table 5).

In our study, the water use efficiency (calculated from the total biomass) was not enhanced when water supply amount increased to fulfil plants water requirement, despite the increase of the total above fresh biomass (Table 5). Slight increases (8%, and 9%) in both 2015, and 2016 seasons have been achieved when plants fully irrigated, while statistically not reaching significant level in the 2016 season. The most efficient use of water was recorded in deficit water supply blocks WS50 (42.1 kg, and 47.6 kg above ground biomass production per cubic meter water consumed) in 2015 and 2016 seasons respectively.

Table 5. Total biomass (t ha^{-1}), and water use efficiency (WUE) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	Total Biomass (t ha^{-1})		WUE (kg m^{-3})	
		2015	**2016**	**2015**	**2016**
WS0	Control	33.5^{Aa} ±2.2	131.5^{Aa} ±7.4	18.0^{Aa} ±2.4	44.4^{Ab} ±2.5
	AM++	34.6^{Aa} ±1.4	136.6^{Aa} ±6.7	18.6^{Aa} ±0.4	46.2^{Ab} ±2.3
WS50	Control	74.3^{Ab} ±1.4	139.3^{Aa} ±14	24.3^{Ab} ±0.9	35.9^{Aa} ±3.6
	AM++	128.9^{Bc} ±1.5	184.8^{Bc} ±11	42.1^{Bc} ±1.0	47.6^{Bb} ±2.9
WS100	Control	92.6^{Ac} ±1.2	148.0^{Aa} ±16	21.7^{Ac} ±0.6	30.8^{Aa} ±3.3
	AM++	100.0^{Bb} ±2.6	160.8^{Ab} ±5.7	23.5^{Bb} ±1.2	33.5^{Aa} ±1.2
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)					
Mycorrhizal Inoculation (AM++)		***	***	***	***
Water supply (WS)		***	***	***	***
AM++ * WS		***	**	***	**

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculation effect, small letters represent water supply effect.

3.4.3. Leaf water potential

Along gradients of water supply reduction, Ψ_L decreased too (more negative) in Control- plant leaves from (-0.91 MPa) in WS100, to (-1.06 MPa) in WS50, and (-1.55 MPa) in WS0 in 2015 and from (-0.92 MPa) in WS100, to (-1.04 MPa) in WS50, and (-1.12 MPa) in WS0 in 2016 (Figure 2). Thus indicates differences between both seasons in plants water stress due to water stress induction, when plants severely stressed in 2015 and moderately stressed in 2016 in no water supply regime.

Compared to Control plants, mycorrhizal inoculation remarkably increased the Ψ_L in plant leaves by (20, 22, and 12%) in WS0, WS50, and WS100 respectively in the growing season 2015 and by (11, 17, and 03%) in WS0, WS50, and WS100 respectively in 2016 growing season (Figure 2). Based on the midday leaf water potential during the 2015 growing season, Control plants faced severe water stress (Ψ_L decreased by 70%) in WS0, and moderate water stress (Ψ_L decreased by 16%) in WS50 compared to Control plants in WS100 block; in 2016 growing season Control plants moderately stressed (Ψ_L decreased by 19%) in WS0, and slightly stressed (Ψ_L decreased by 10%) in WS50 compared to Control plants in WS100 block.

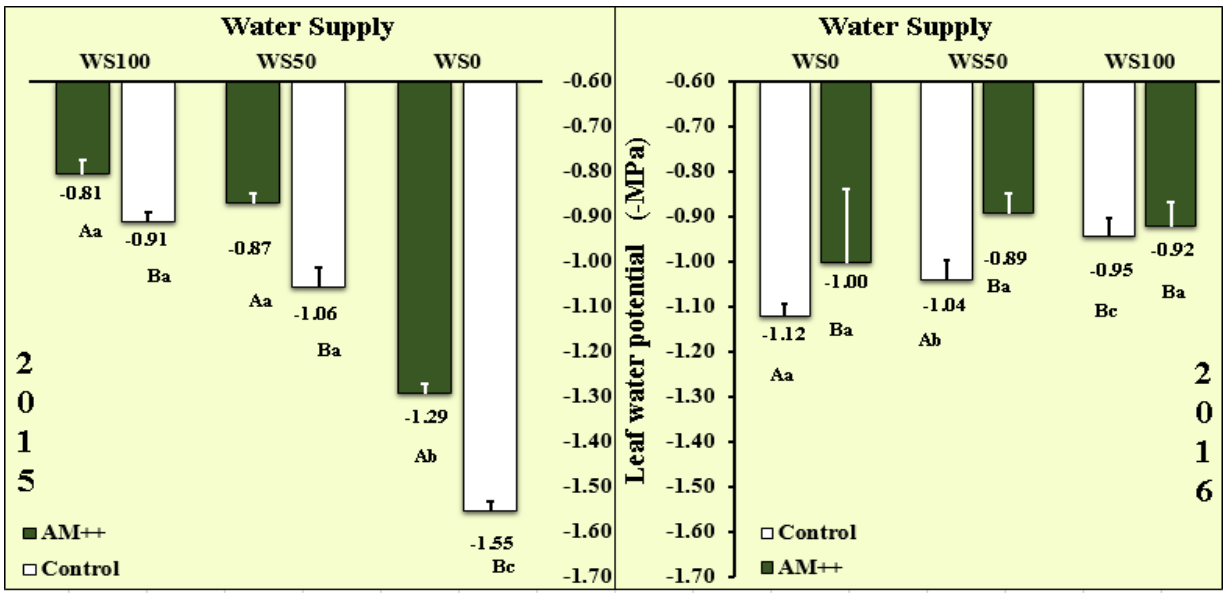

Figure 2. Leaf water potential (-MPa) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

3.4.4. Stomatal conductance and canopy temperature

Differences in water stress levels in plants induced to water deficits reflected also in stomatal conductance; thus in 2015 season Control- plants have lost one third and two thirds of their stomatal conductance along gradients of water reduction in both WS50 and WS0 blocks respectively, while an adverse effect has been recorded in 2016 when water supply reduced to the half in WS50 block with no change in further water reduction in no water supply WS0 block (Table 6). Mycorrhizal inoculation has enhanced the stomatal conductance at all water supply levels in 2016 with no effect in no water supply and full water supply in 2015 season, while a meaningful increases (from 18.2 to 24.9 mmol m^{-2} s^{-1}) in 2015, and (from 32.9 to 34.4 mmol m^{-2} s^{-1}) in 2016 were observed in AM++ plants compared to Control plants stands in WS50 blocks.

A gradual decrease (from 34.1ºC in WS0 to 30.6ºC in WS50 and 28ºC in WS100) in canopy temperature in Control plant stands along with water supply increasing in 2015 was observed, but mycorrhizal inoculation more efficiently decreased the canopy temperature (from 30.6 to 29.0 ºC) in WS50 block (Table 6).

Table 6. Stomatal conductance (mmol m^{-2} s^{-1}), and canopy temperature (ºC) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	Stomatal conductance (mmol m^{-2} s^{-1})		Canopy temperature (ºC)	
		2015	**2016**	**2015**	**2016**
WS0	Control	9.92^{Aa} ±1.1	31.2^{Aa} ±0.1	34.1^{Ac}	25.1^{Aa}
	AM++	9.75^{Aa} ±1.1	32.8^{Ba} ±0.7	34.1^{Ac}	25.5^{Aa}
WS50	Control	18.22^{Ab} ±3.3	32.9^{Ab} ±0.8	30.6^{Ab}	25.5^{Aa}
	AM++	24.88^{Bb} ±2.4	34.4^{Bb} ±0.3	29.0^{Bb}	24.2^{Bb}
WS100	Control	29.52^{Ac} ±2.8	31.0^{Aa} ±0.3	28.2^{Aa}	25.1^{Aa}
	AM++	29.96^{Ac} ±1.9	31.9^{Ba} ±0.5	27.8^{Aa}	25.5^{Aa}
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)					
Mycorrhizal Inoculation (AM++)		***	***	***	ns
Water supply (WS)		***	***	***	ns
AM++ * WS		***	ns	***	*

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4).Capital letters represent mycorrhizal inoculation effect, small letters represent water supply effect.

3.4.5. Plant's phosphorus uptake

The soil of the experimental farms contained only (14 mg kg^{-1}, and 8 mg kg^{-1} P_2O_5) of available phosphorus in both 2015, and 2016 seasons respectively, which is considered low for crop production.

The total P uptake calculated as (P concentration in shoot x shoot mass), was consequently higher in WS0, beside the highest P concentration (4631 mg kg^{-1} in 2015, and 2082 mg kg^{-1} in 2016), higher shoot biomass compensated the lack of fruits. The P uptake in non-inoculated Control plants decreased to the half (0.05 g) in WS50, and to one third (0.03 g) in WS100 (Figure 3) in 2015. Despite the P translocation load on P shoots reserve due to the highest fruit setting (111 t ha^{-1} in 2015, and 167 t ha^{-1} in 2016), mycorrhizal inoculation enhanced the P uptake in plants in WS50 water supply blocks (0.13 g $plant^{-1}$, and 0.09g $plant^{-1}$) in both growing seasons 2015 and 2016 respectively, indicating moderate water deficit the best condition for mycorrhizal inoculation to promote nutrient uptake (particularly P) in the host plants.

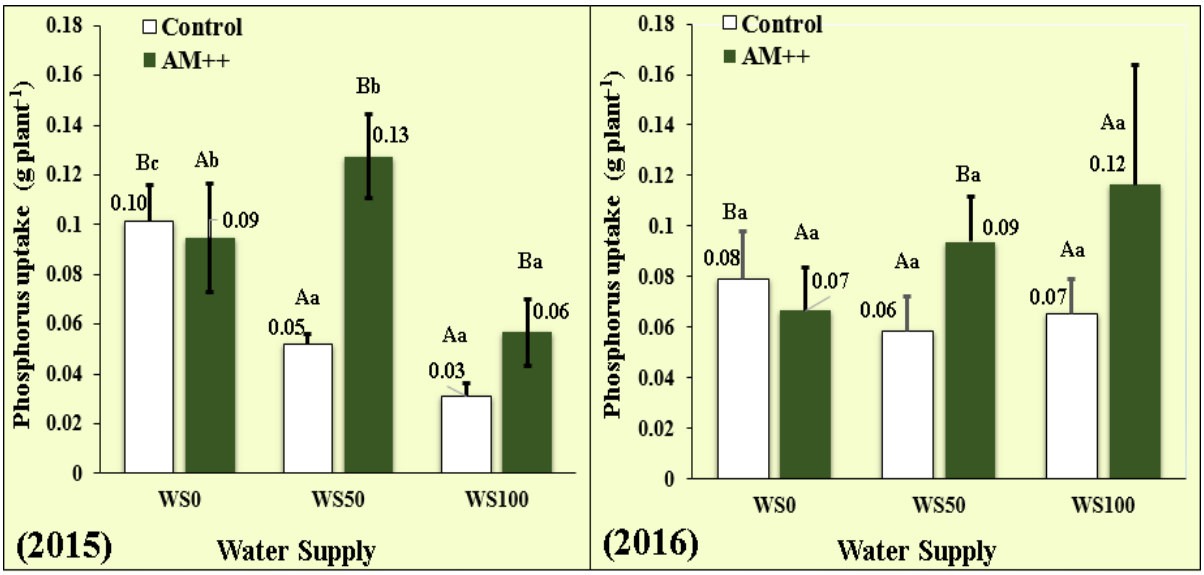

Figure 3. Phosphorus uptake (g $plant^{-1}$) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

3.4.6. Shoot organic and inorganic osmolytes

Control and AM++ plants in no water supply blocks increased proline accumulation by more than two folds in shoots in response to water stress compared to plants fully irrigated. Mycorrhizal inoculation reduced the proline concentration compared to non-inoculated in full water supply blocks, but statistically it was not reaching significant levels in 2015. In AM++ plants shoots, proline accumulation reduced to the half in WS50 in both growing seasons (Figure 4) compared to Control plants.

Very strong negative correlations (r = 91 in 2015, and r = 86 in 2016) between leaf water potential and proline content in shoots (Figure 4) were observed supporting our hypotheses. Lower proline concentration in shoots accompanied by higher leaf water potential in mycorrhizal inoculated plants is a definite proof that AMF alleviated water stress in plants and more effectively under moderate water stress.

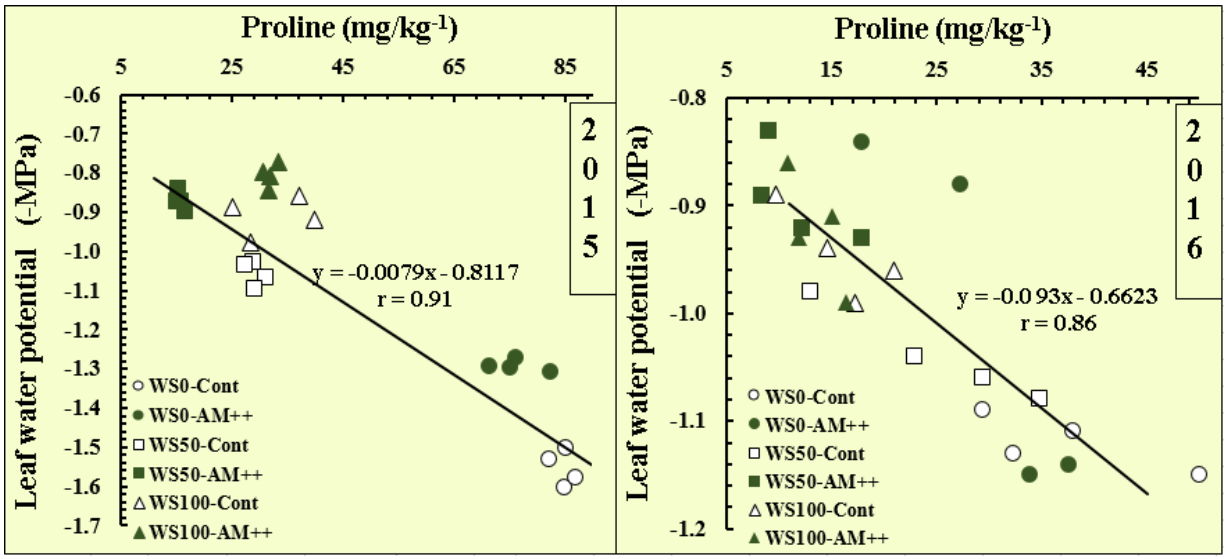

Figure 4. Leaf proline concentration and leaf water potential relationship.

In Control treatments, decreasing water supply amount, did increase K^+ concentrations in plant shoots by (37% in WS50, and 52% WS0) in 2015, and (21% in WS50, and 31% WS0) in 2016 growing season (Table 8). Overall mycorrhizal inoculated AM++ plants accumulated

less K^+ in shoots compared to Control plants, with significant shifts in WS0 levels (from 35852 to 28215 mg kg^{-1}in 2015, and from 23614 to 22588 mg kg^{-1} in 2016).

Control plants showed higher Magnesium concentrations in shoots (6153, 6338, and 6068 mg kg^{-1}) in 2015 growing season compared to 2016 growing season (5081, 5372, and 5374 mg kg^{-1}) in WS0, WS50, and WS100 water supply blocks respectively (Table 8). AM++ plants accumulated less Mg^{+2} in shoots compared to Control plants in all water supply levels in 2015 with no clear trend in 2016.

In general, the calcium concentrations in shoots were higher in 2016, moreover a gradual decrease in Ca^{+2} concentration was observed along water reduction in both season; the mobility, availability, and the uptake of Ca is positively affected by soil moisture content. Mycorrhizal inoculation enhanced Ca^{+2} contents in leaves in both seasons and at all watering levels except for WS100 in 2015 (Table 8); The modulation of Calcium concentration by AM++ appeared to be, therefore, related to a physiological pathway different from drought stress tolerance. Higher Ca^{+2} concentrations in AM++ plant leaves enhanced the fruit quality, firmness, and prevented fruits from blossom-end rot disorder.

Table 8. K^+ (mg kg^{-1}), Ca^{+2} (mg kg^{-1}), and Mg^{2+} (mg kg^{-1}) concentrations in shoots of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	K^+ (mg kg^{-1}) Dry weight		Mg^{2+} (mg kg^{-1}) Dry weight		Ca^{+2} (mg kg^{-1}) Dry weight	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
WS0	Control	35852^{Bb}	23614^{Ba}	6153^{Ba}	5081^{Aa}	34608^{Aa}	39383^{Aa}
	AM++	28215^{Aa}	22588^{Ab}	5810^{Aa}	6691^{Bb}	38598^{Ba}	47947^{Ba}
WS50	Control	32319^{Bb}	21778^{Aa}	6338^{Ba}	5372^{Ba}	41275^{Aa}	46141^{Aa}
	AM++	30344^{Ab}	24301^{Bb}	5199^{Aa}	4903^{Aa}	42582^{Ba}	56260^{Ba}
WS100	Control	23601^{Ba}	17980^{Aa}	6068^{Ba}	5374^{Aa}	48385^{Bb}	56152^{Ab}
	AM++	20563^{Aa}	18680^{Ba}	5450^{Aa}	5978^{Bb}	36719^{Aa}	59504^{Ba}
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)							
Mycorrhizal Inoculation		*	***	*	*	***	**
Water supply (WS)		***	**	***	*	***	***
AM++ * WS		ns	***	***	**	*	***

Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4).Capital letters represent mycorrhizal inoculation effect, small letters represent water supply effect.

4. NEW SCIENTIFIC RESULTS

- ✓ I have indicated that, inoculation timing has a substantial effect on the efficiency of mycorrhizae. Depending on plants physiological responses, biochemical changes, plant production, and fruits quality, I have found that, the field inoculation at transplanting with the commercial inoculum Symbivit® is more efficient than pre-transplant inoculation at sowing in alleviating the water deficit stress impact on field grown *L. esculentum* M.

- ✓ I presented that, mycorrhizal inoculations neither pre-transplant at sowing nor field-inoculation at transplanting, could ameliorate severe water stress impact on the host plants.

- ✓ I proved that, under the field conditions AMF can increase the water uptake and help host plants to avoid the water stress impact particularly under moderate deficit of soil moisture.

- ✓ By measuring the water use efficiency I determined that Mycorrhizal field-inoculation, helped their host plant to overcome the water stress impact through avoidance mechanism by increasing the water and nutrient uptake. Less organic and inorganic osmolytes in plants induced to moderate water deficit stress, supported by most important indices of plant water status (leaf water potential, stomatal conductance, and canopy temperature) are definite field based proofs that the water and nutrient uptake meaningfully increased by the mycorrhizal inoculation. In another word mycorrhizal inoculation protected the plants from the water deficit instead of stimulating them to tolerate the stress. Also, it was found that, the positive effect of the mycorrhizal inoculation on stomatal regulation is partially contributed to the mediation of the water stress by sustaining plant soil water balance.

- ✓ I indicated that depending on seasonal variation, mycorrhizal field-inoculation could enhance the fruit quality (higher Soluble solid-, Carotenoids-, β-carotene-, and lycopene- contents) accompanied by a meaningful increase of tomato yield particularly under moderate water deficit conditions of the soil. The pedo-climate condition played an important role in mycorrhizal efficiency.

5. CONCLUSION AND RECOMMENDATIONS

The two years experiments supports field-based evidence that the exogenous strains of arbuscular mycorrhizae that commercially produced can be used as an integrated application for processing tomato production alleviating moderate water stress impacts and enhancing both production and the quality of the fruits. The AM field-inoculation at transplant can be an effective strategy when combined with a deficit water supply not exceeding plant requirements.

Field-based evidence supported the finding that, AM field-inoculation is more effective than pre-transplant inoculation at sowing in our experiments, but economical aspect should be considered, since more inoculum is required. Despite higher colonization rates, at sowing pre-transplant inoculation, the mycorrhizal inoculation slightly enhanced the plant growth, plant production, carotenoids and lycopene contents, and some physiological processes as well (stomatal conductance, water use efficiency) especially under deficit water condition.

Based on the volumetric water content of the soil and plant water status indices (leaf water potential, stomatal conductance, canopy temperature, and water used efficiency), mycorrhizae-treated plants (pre-transplant at sowing, and field-inoculated at transplant) were severely stressed under severe water deficit condition and the mycorrhizal inoculation lost its efficiency and could not alleviate the water stress impact.

Mycorrhizal field-inoculation at transplant improved the performances of tomato plants compared to the Control treatments, particularly under moderate water deficit stress. Significant differences were recorded in the total upper fresh biomass production, physiological performances (Stomatal conductance, water use efficiency, canopy temperature, leaf water potential, photosynthetic efficiency, leaf chlorophyll content, and phosphate uptake), leading to a partial inhibition of the osmolytes-dependent drought tolerance mechanisms. Inoculated plants required less osmolytes during the moderate water stress supported by most indices of plant water status, indicating that AM symbiosis helped their host plants to avoid the water stress by increasing the water and nutrient uptake. Moreover, better regulation of the stomatal closure in inoculated plants also contributed partially in maintaining soil plant water balance.

The field-inoculation at transplant increased the plant productions more efficiently under moderate water stress. Better fruit setting accompanied by the enhancement of the quality

(higher carotenoids, lycopene, and β-Carotene) only in the second growing season on the loamy soil, while on the sandy loam soil of the first growing season the mycorrhizal inoculation could only preserve the abscisic acid.

The better performance of plant growth and physiology is accounted for the AM symbiosis, but more efficient performance of the mycorrhizal symbiosis was recorded on the loamy soil of the second season. Some soil characteristics (soil texture, higher water holding capacity, lower temperature) played an important role in the AM symbiosis performances.

Our results encourage the use of AM inocula as "bio-enhancers" as a mitigation practice tool in facing water scarcity in industrial scale agriculture systems, and illustrates the high potential for the yield increase and the fruit quality enhancement. We proved the higher efficiency of field-inoculation at transplant in alleviating drought impact, increasing yield and enhancing the fruit quality compared to at sowing pre-transplant mycorrhizal inoculation, but economical aspect should be considered, since more inoculum is required.

AM symbiosis failure under severe water deficit stress, more potential contribution of AM inoculation under moderate, and for less extend under optimum watering is definite proof that the irrigation strategy is playing the key role in the symbiosis efficiency. Under actual agroecosystem conditions many biological and environmental factors are interacting, therefore optimizing AM fungi application is required to reach promising results, and systematic quantitative analyses are needed to determine the crop response to mycorrhizal field-inoculation at transplant. More investigations should be conducted regarding the AMF specificity, commercial inocula composition, and pedoclimates role on AM symbiosis.

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