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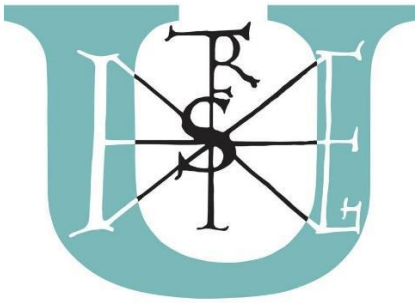

Szent István University

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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ئەم تێزە پێشکەشە بە دایك

و

باوکی خۆشەویستم

1. INTRODUCTION

1.1. Foreword

Wise water use in the agriculture is becoming crucial all around the World. Water scarcity can threaten not only originally arid regions but also areas where people developed a flourishing horticulture based on sufficient water resources, but ongoing climate change can ruin the so far achieved results. There is an urgent need to find solutions for problems caused by irregular water availability. Tomato is the most popular vegetable crop in the World with relatively high water demand, which makes it suitable to be a test plant for the examination of the effect of water scarcity. Arbuscular mycorrhizal fungi play an important role in plant communities enhancing the nutrient and water uptake and these facts infiltrate into the horticulture. Therefore the selected topic is of high importance from horticultural production worldwide.

This dissertation covers two years of open field experiments on two different field sites characterized by different soil types (a sandy loamy soil in growing season 2015, and a loamy soil in 2016 growing season) under different weather conditions. Shifting to another experimental field in the second season due to an administrative procedure by the municipality authority was counted as a weak point by some reviewers, but enabled the assessment of mycorrhizal symbiosis efficiency under two different pedo-climate conditions.

The two years randomized block experiment studied in depth physiological, biochemical, and production responses of processing tomato (*Lycopersicon esculentum* Mill) to both mycorrhizal inoculation, and water supply each at three levels.

For better scientific understanding many field measurements and laboratorial works were conducted:

- Field measurements: Soil volumetric water content, transpiration, canopy temperature, chlorophyll content, photosynthesis, and leaf water potential.
- Laboratorical works:
    - Soil texture, organic matter, field capacity, pH, electrical conductivity, element concentrations in soil.
    - Root staining, soil microbial activity, proline estimation.

- Soluble solid content (ºBrix), total carotenes, lycopene, β-carotene, lutein, and ascorbic acid were determined in fruits.
- Elements concentration in shoot.

1.2. Objectives and outlines

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?

The thesis is a conclusion of cumulative work of published manuscripts from my publications listed in related publications. Moreover, this is to clarify that, from the three levels of mycorrhizal inoculation (Non-inoculated, pre-transplant inoculation at sowing, and field-inoculation at transplant) only non-inoculated (Control), and field-inoculated at transplant (AM++) will be included. Pre-transplant inoculation at sowing (AM+) (***Appendices***: Growth, yield, and water use efficiency ***Appendix 1***; ^{o}Brix ***Appendices 2 & 3***; Leaf water potential ***Appendices 5 & 6***; Proline **Appendix 7**; Yield impact on soluble solid ***Appendix 8***) did not give promising results compared to non-inoculated neither in the first season, nor in the second season, in addition to the large amount of data obtained, the focus of this dissertation will be only on mycorrhizal field inoculated at transplant (AM++) and non-inoculated (Control) plants in three water supply regimes: Full water supply (WS100), deficit water supply (WS50), and no water supply (WS0).

2. LITERATURE REVIEW

2.1. Climate change impact on drought and water scarcity

Climate is continuously changing under the influence of natural and anthropogenic forces, both directly and indirectly (Wagner et al., 2010). Manmade negative impact on climate change through greenhouse gas emissions is a major cause exacerbating the global warming. Due to fossil fuel combustion and changes in land use, the atmospheric concentration of CO_2, N_2O, and CH_4 has increased by 31, 45, and 249% respectively (IPCC, 2001). In 2014 the group of Intergovernmental Panel on Climate Change (IPCC, 2014) reported a warming of 0.85^oC in the global averaged combined land and ocean temperature at the surface between 1880-2012, which intensifies the hydrological cycle worldwide (Milly et al., 2002). Anthropogenic direct effects on hydro cycle causes acute reduction of water availability by surface and groundwater abstraction, diverting of water resources, and reservoirs constructions. The direct influence of human activity on water scarcity is increasing due to population increase and changing in life style (Van Loon & Van Lanen, 2013) and the impacts of drought will become more severe in a number of regions (Seneviratne et al., 2012).

Despite the fact that impacts of climate change on droughts has not been fully understood yet, but alongside the hemispheric changes decreasing soil moisture in eastern Hungary over the last century has been reported (Szep et al., 2005), in addition to changes in drought severity and frequency due to climate change scenarios in the Indian Kansabati river basin when compared to historical droughts (Mishra & Singh, 2009)

Among the environmental factors, drought stress (DS) is one of the most fateful abiotic factor that limit agricultural production. Worldwide up to 45% of the agricultural lands have faced drought stress (Bot et al., 2000). Severe drought stress negatively affects plant physiology, growth, and reproduction and causes considerable losses in crop yield (Barnabas et al., 2008). From studies over the past 35 years, it has been estimated that, worldwide drought caused yield reduction by 21% in wheat and 40% in maize production (Daryanto et al., 2016).

2.2. Drought and Water Scarcity

Water is vital for all kinds of life on our planet. It moves continuously through the water cycle of Eva-transpiration, condensation, precipitation, and runoff. Changes in climate disturbed the water cycle and changed precipitation aspects in amounts, time and place; causing the so called desertification in some regions and flooding in some others.

Although, the intensity of drought differs from region to region and from time to time, but drought is a worldwide phenomenon, and more disastrous drought events occurred in the twentieth century starting from the one which hit China and Russia in the twenties of the last century, resulted in a food crisis, and left millions dead behind (EM-DAT, 2012), followed by a decade of rain deficit in the USA causing the dust bowl in the 1930s (Schubert et al., 2004).

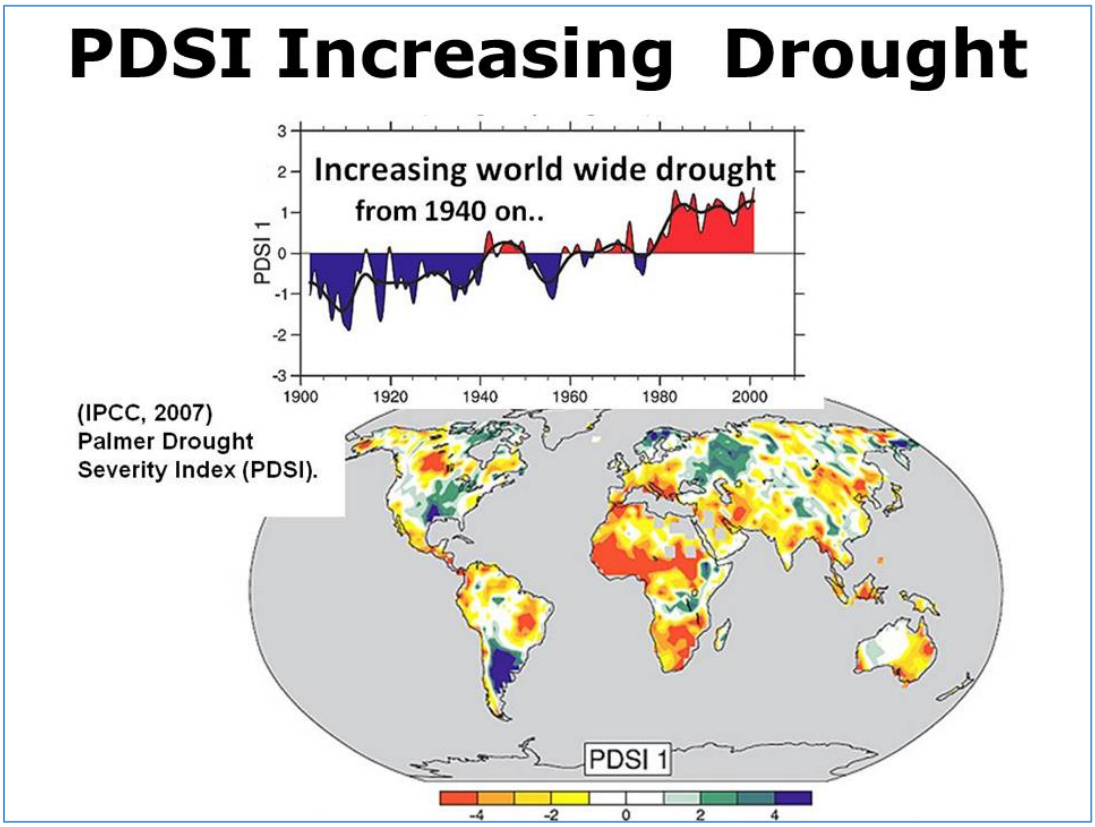

Figure 1. Global changes in dry and wet areas during the 20th century.
Ref: Intergovernmental Panel on Climate Change group (IPCC, 2007)

Twenty first century started with a series of severe drought events hitting different territories worldwide: In 2003 and 2006, Europe faced heat waves caused by low soil moisture and resulted in crop failure (Rebetez et al., 2009), followed by years of drought in the Iberian peninsula starting from 2008 reducing water ground levels and water reservoirs (Andreu et al., 2009), then the lack of precipitation during 2009-2010 in southwest of China hampering the crop production (Lu et al., 2011), and the long lasting drought in 2010 that hit the horn of Africa and triggered a famine in 2011 (Viste et al., 2013).

According to the Intergovernmental Panel on Climate Change group (IPCC, 2007), globally more dry areas have more than doubled over the last 40 years (Palmer Drought Severity Index, PDSI ≤ -3.0) due to El Niño-Southern Oscillation (ENSO) events accompanied by surface warming (Figure 1.).

Drought phenomenon has no uniform definition because of its complexity, but Sheffield and Wood (2011) defined it in the simplest way as a deficit of water compared to normal conditions. Thus, arises a major question about the normal condition and what is considered to be a normal condition. We can determine the normal condition depending on the usage of water and considering the water only in the part of hydrological cycle that is related to the water usage. To answer this question, we have to take in to consideration the component of the hydrological cycle, in addition to the intensity and duration of the water deficit. Drought studies focus on both terrestrial and atmospheric part of the water cycle and their interaction (Sheffield & Wood, 2011).

Drought has four categories: Meteorological, Soil moisture, Hydrological, and Socioeconomic (Sheffield & Wood, 2011; Mishra & Singh, 2010). Mid-term deficit precipitation in wide area causes metrological drought, while soil moisture drought is the reduction of soil moisture to a degree that cannot supply the vegetation with required water for a normal growth. Drought reaches the hydrological level when meteorological drought extended for longer period and low water supply became evident especially in ground water, reservoirs, streamflow, river discharge, lakes, and declining in wetland area. Socioeconomic drought is related with the impacts of the previous mentioned types of drought and occurs when weather related shortage in water supply negatively impacts both sociological and economic sectors.

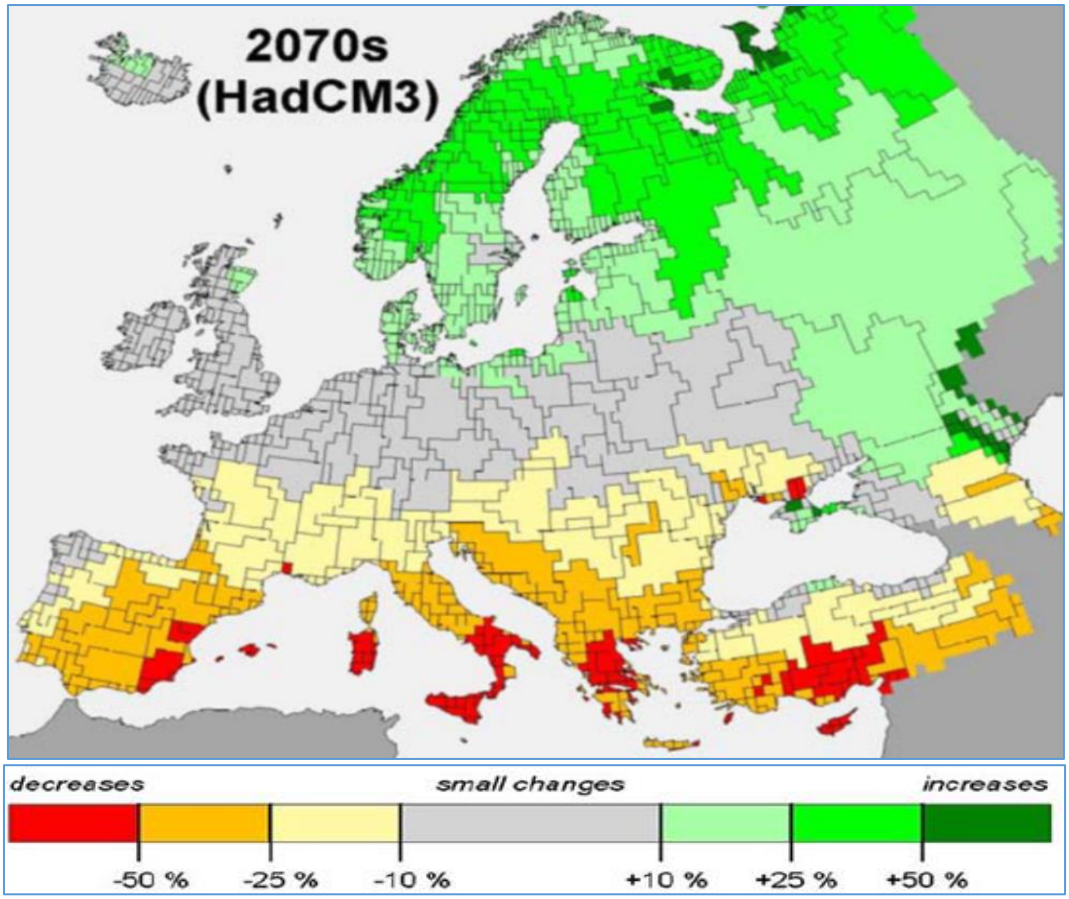

Figure 2. Changes in water availability across Europe.
Ref: © Center for Environmental Systems Research, University of Kassel, June 2001 - WaterGAP 2.1C

Water scarcity is a long term imbalance situation between the available water and the water consumption (EU, 2007), which is caused totally or partially by human beings (Seneviratne et al., 2012). Moreover, drought can exacerbate water scarcity condition (EU, 2007) and differs from drought phenomenon, but they are often used interchangeably, and no sufficient indicators have been illustrated yet to distinguish them (EU, 2012). In Europe, number of river basins effected by water scarcity in summer and all year round are expected to increase by 50% by 2030, and the availability of water is likely to become a major problem across Europe by 2070 with a reduction reaching 30 to 50% according to Hadley Centre Coupled Model, version 3 (Figure 2.).

Regardless to different phrases and definitions used, this study is focusing on low water availability and its impact on the field crop production, therefore both drought and water scarcity will be used.

2.3. Soil Moisture Drought impact on plants

Soil moisture drought is broader than meteorological and hydrological drought, because it can be due to different factors rather than an environmental condition, therefor its definition links various attributes from metrological drought such as lack of precipitation, rainfall departure, and evapotranspiration to soil moisture impacts.

Soil moisture is water in the unsaturated zone of the soil, and most of it returned back to the atmosphere through evatransporation including plant taranspiration and evaporation from the bare soil surface. Soil water availability is not only depending on soil water amount, but also how tightly water is bound to the soil particles, which is affected by soil texture, organic matter content, and the water conductivity.

Soil moisture content has to be determined volumetrically and proportionally to soil volume (Equation 1), due to the fact that only a fraction of soil moisture can be measured and releavant. Volumetric water content (θ) is the volume of water in the soil volume V [cm^3 H_2O/cm^3 soil], defined as:

Equation 1. Volumetric Water Content (VWC)

$$\theta = [\frac{volume\ of\ V}{V}]$$

Depending on the trail application and the method of the measurement, VWC can be applied on multiple levels. Volumetric water content (θ) expressed as [$mm_{\mathbf{water}}/ mm_{\mathbf{soil}}$], since field soil is divided in separate layers. At permanent wilting point, no moisture is remaining that can be extracted by plant roots, while at saturation all soil pores will be filled with water (Figure 3.).

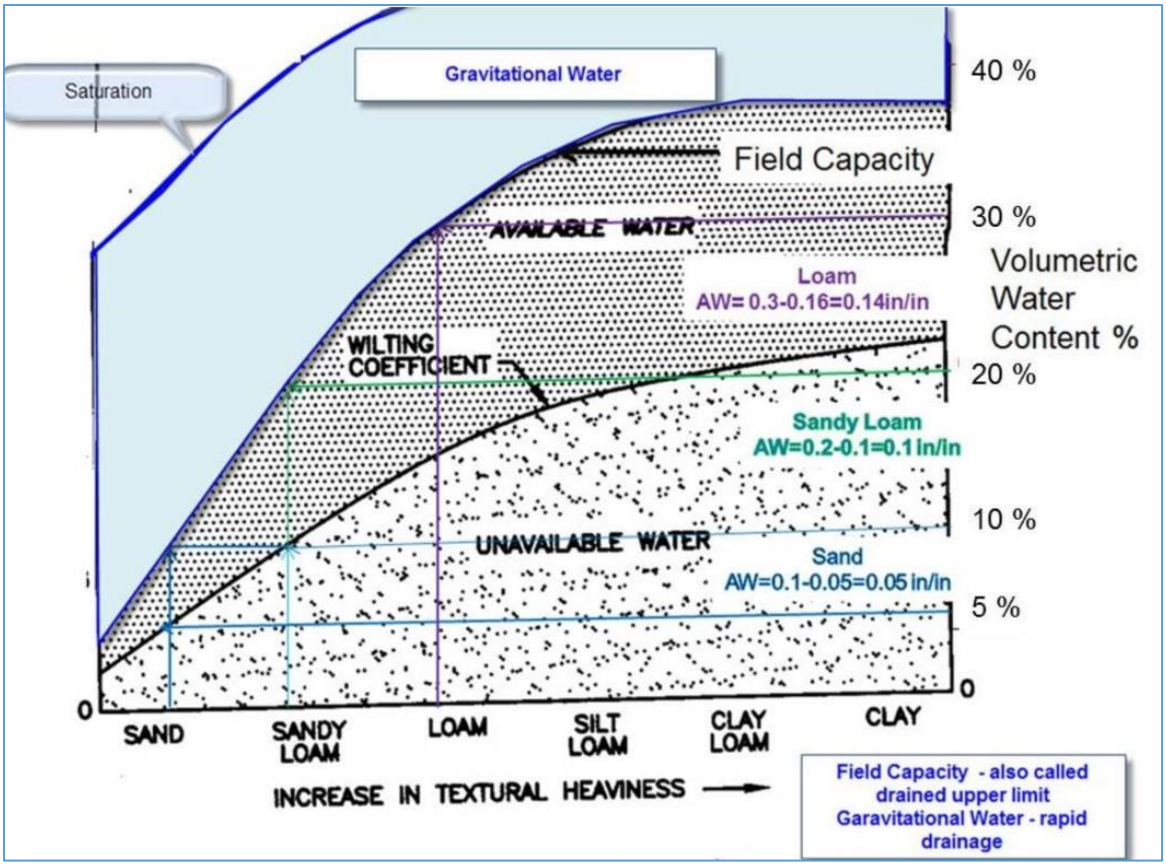

Figure 3. Soil moisture levels and measurement units.
Ref: USDA, NRCS, Engineering Field Manual, by Steve A Miller, Michigan State University.

During the early growth stage, subsoil moisture deficit has less impact on the seedlings growth and the crop yield at harvest, if the topsoil has sufficient moisture. The intensity of agricultural drought can be determined as the difference between water demand by plants and the available soil moisture. Plant root and microorganism activities exist mostly on the upper 20 cm of the soil, in addition to the nutrient supply (Kulik, 1962), therefore soil moisture is the most crucial in the upper soil layer.

Soil moisture has a direct impact on water cycle, and considerably constrained crop production through leaf transpiration and photosynthesis in most regions. Declining of the soil moisture to a level that cannot provide plants water demand depends on many factors involving meteorological and hydrological droughts in addition to differences between actual- and potential evapotranspiration. Plant water demand depends on the biological characteristics of the plant and its growth stage (Demirevska et al., 2009) in addition to the prevailing weather

condition, and physical and chemical properties of the soil. Factors such as soil capacity in holding water, rainfall patterns, and evapotranspiration complicate drought severity prediction.

Drought damages mitosis and cell elongation (Hussain et al., 2008), and causes loss of turgor (Taiz & Zeiger, 2006), resulting in poor growth. Loss in turgor accompanied by lower photosynthesis, limits the expansion of leaves under water stress condition (Rucker et al., 1995). Lack of water in plants leads to low xylem water flow to the adjacent cells that affects negatively cell elongation (Nonami, 1998).

As complex integration of different physiological processes, yield loss under water stress condition depends on growth of the plant and the intensity of the drought. Yield reduction under drought is a consequence of disturbance in several physiological functions including photosynthesis rate (Flexas et al., 2004), assimilation partitioning (Farooq et al., 2009), and flag leaf development (Rucker et al., 1995).

Water stress disturbs many water related physiological regulations including canopy temperature, leaf transpiration, and stomatal conductance but the last is the most affected (Farooq et al., 2009). Plants use water more efficiently through better regulation of stomata and less transpiration and water loss.

Plant roots take up most of nutrients (Ca^{++}, Mg^{+}, and N) via diffusion along with water, therefore lack of soil moisture impairs their mobility and retards the growth as a result (Barber, 1995). Changing root architecture and increasing root length and surface area is a strategy by plants to capture less mobile nutrients (Lynch & Brown, 2001), but soil moisture deficit weaken the root growth and reduce root ability to uptake less mobile nutrients especially phosphorus (Garg, 2003).

The interaction between plant roots and microbes in the rhizosphere is considered the key importance in mobilizing nutrients, while drying the rhizosphere will negatively affects the composition and activity of microbial colonies in the soil and disturbs plant nutrient relation in turn (Schimel et al., 2007).

Photosynthetic process is limited by other factors that are negatively affected by drought stress including, disturbance in photosynthetic machinery, leaf expansion, and leaf senescence (Wahid et al., 2007). Plants close their stomata in response to soil moisture deficit that reduces CO_2 in leaves that may lead to photo damage in plants (Lawlor & Cornic, 2002). Water stress

reduces chlorophyll contents in leaves (Din et al., 2011), and damages thylakoids and photosynthetic membranes (Anjum et al., 2011).

When soil moisture is reduced plant roots produce abscisic acid (ABA) as it is shown in figure 4. signaling the stomatal guards to close the stomata (Turner et al., 2001), therefore stomatal closure is more affected by soil moisture status rather than leaf water potential and the atmospheric humidity. Under soil dry conditions, plants translocate assimilates to the root to improve water uptake (Leport et al., 2006), decreasing the sucrose concentration in the leaves (Komor, 2000).

Abiotic stresses including drought are subsequently causing oxidative damage in plants by formation of Reactive Oxygen Species (ROS), causing serious damages to lipids and proteins in cells. ROS generation in plant tissues is either enzymatic or non-enzymatic (Apel & Hirt, 2004). In the same way plants also follow both enzymatic and non-enzymatic mechanisms to withstand the oxidative damage, but the first is most effective (Farooq et al., 2008).

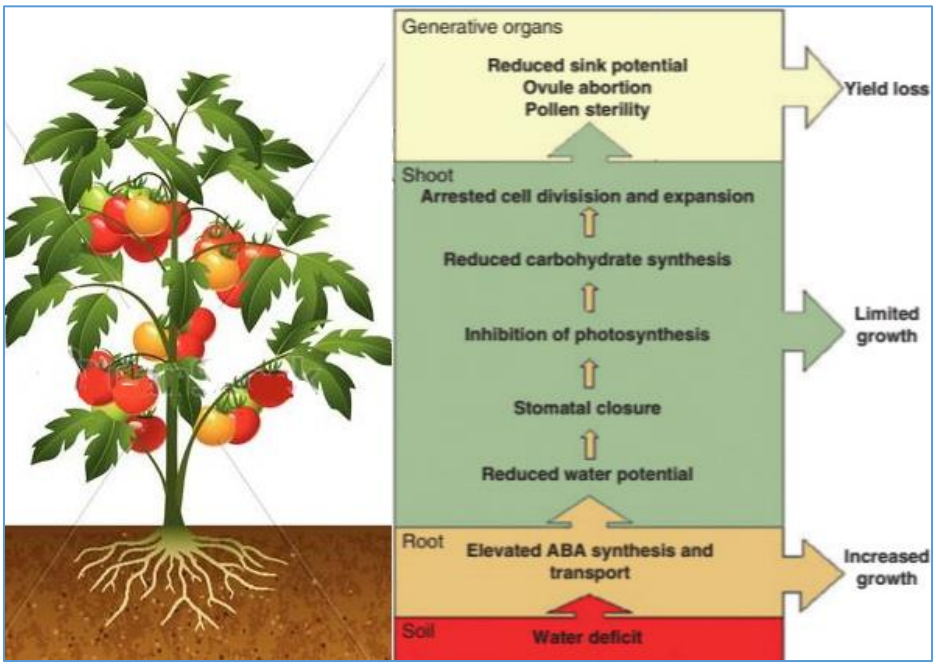

Figure 4. Drought-induced abscisic acid (ABA)-dependent plant responses
(from Barnabas et al., 2008) modified.

2.4. Arbuscular mycorrhizae fungi

Mycorrhizal fungi are the most vital component within the soil microbial community, interfacing between the soil and the plant carbon photosynthates through their association with plant roots. Mycorrhizae can be divided into five groups: the ectomycorrhiza, the arbuscular mycorrhiza, the orchid mycorrhiza, the ericoid mycorrhiza and the ectendomycorrhiza fungi, but the first two groups are the majors.

The extra cellular fungal growth in the root cortex is differentiating the Ecto-mycorrhizae from the Endo-mycorrhizae that have a distinctive inter and intra cellular fungal growth in the root cortex (Bücking et al., 2012) of the hosted plant (Figure 5.).

Arbuscular mycorrhizae (AM) are the most common type of this symbioses (Smith & Read, 2008), forming one-fourth of the total microbial biomass in agricultural soils (Krishnakumar et al., 2013). Arbuscular mycorrhizae fungi evolved more than 400 million years ago (Ordovician) (Redecker et al., 2000), consisting with the hypothesis that they were involved in colonizing our planet by the ancient plants (Simon et al., 1993).

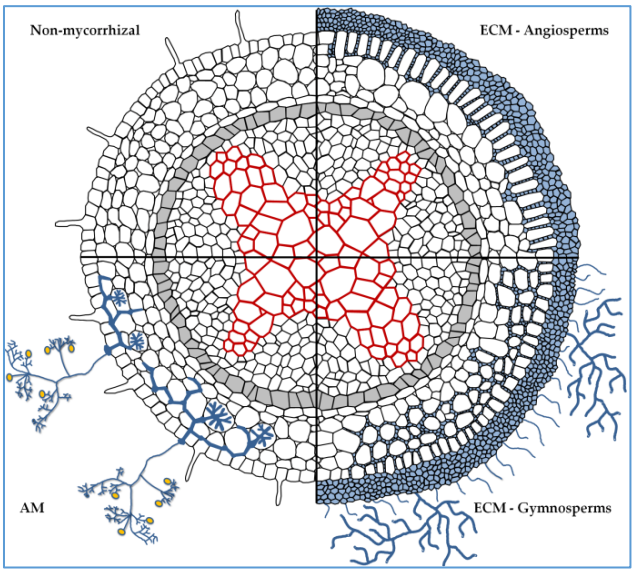

Figure 5. Structural characteristics of arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) roots of gymnosperms or angiosperms.

(from Bücking et al. 2012)

AMF were separated in a new fungal phylum: the Glomeromycota (Schüssler et al., 2001). The Glomeromycota are divided into four orders (Glomerales, Diversisporales, Archaeosporales and Paraglomerales). These orders are further subdivided into 12 families and 33 genera.

About 80% of land plant species are able to form a symbiotic relation at least with one type of mycorrhizae (Wang & Qui, 2006). Arbuscular mycorrhizae fungi (AMF) are obligate symbionts, and the symbioses between AMF and host plants are biotrophic and normally mutualistic. The long-term relationship between both symbionts depends mainly on the mutual bidirectional nutrient transfer and supplemented by other factors such as drought (Smith & Read, 2008).

The characteristic structures (arbuscules) within the cortical cells of the root (Figure 6a.), and the vesicles within or between cells, considered diagnostic for this symbiotic relationship, in addition to the intracellular hyphal coils (Figure 6b.) especially in the absence of arbuscules. Quantitative variations in root colonization may be related to: Simultaneous challenge of roots by many different potential AM colonizers, field environment complexities, and seasonal and site-related differences (Smith & Read, 2008).

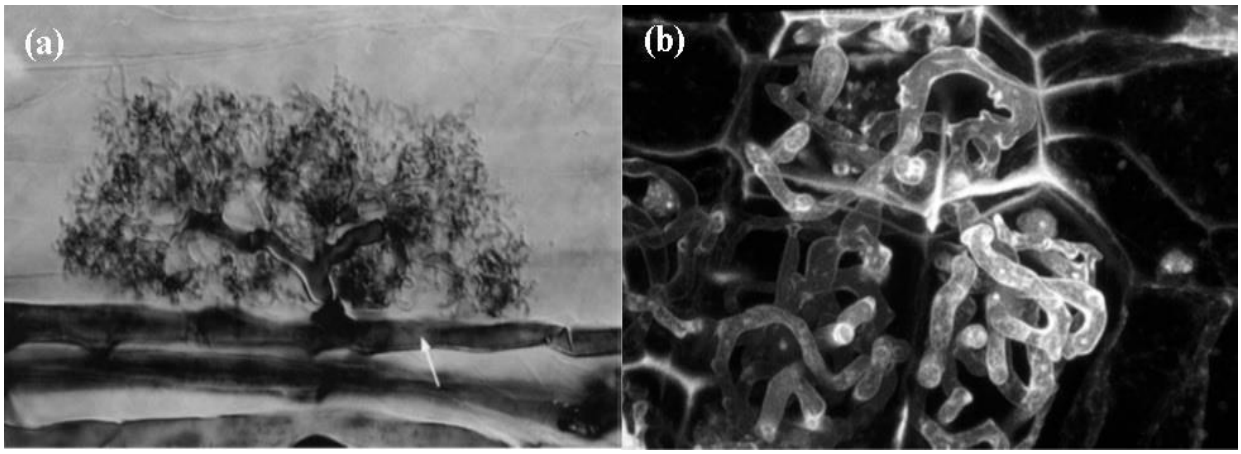

Figure 6. Arum-type arbuscule of *G. mosseae* within a cortical cell of *A. porrum* (a), Paris-type intercellular coils of *G. intraradices* in cortical cells of *Panax quinqui* (b)

(from Smith & Read, 2008)

Many factors can affect the root colonization, but the most important is to know whether the fungi belonging to one species or combination of fungi can colonize which plant under field condition as well as the functionality of the symbioses formed with respect to bidirectional commodities transfer (i.e. phosphorus for carbon) and fitness of partners (Smith & Read, 2008).

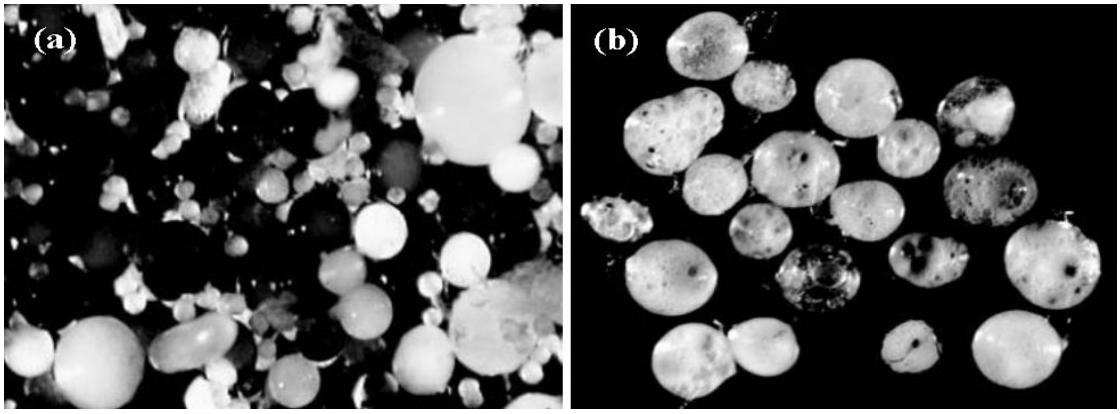

Figure 7. Spores of nine pecies of AM fungi, isolated from a grassland that had developed from an abandoned agricultural site (a), a group of *G. mosseae* spores (b) *(from Smith & Read, 2008).*

In general, AMF colonize plant roots by three means: spores, infected plant root fragments, and the hyphae; all together called propagules. Large spores (Figure 7.) have thick resistant walls and numerous nuclei are enabling them to survive and overcome dispersing by wind, water, and animals (Smith & Read, 2008).

In many habitats and despite the persistent of remarkable spore populations, hyphal networks in soil (Figure 8a.), and fragments of colonized roots (Figure 8b.) are palying an important role in colonizing plant roots (Smith & Read, 2008). Thus when seedlings grow in such established community, their roots will be linked into a complex myceliun network of a variety fungal

species and root of different species of plants. Even when the field has been left bore for years and under harsh environmental conditions where host plants are not exsisting, it is evident that mycelial network can colonize new generations of plants.

Under field conditions, it is difficult to determine different type propagules relative contribution to root colonization, but the spore density can correlate with root colonization, which is certainly not always the case.

Plant traps can be used to determine the root colonization by most probable numbers (MPN) method that mixes and dilutes samples and destroy the hyphal network (Jasper et al., 1992). Moreover, bioassay can be used to assess the contribution of hyphal networks in undestroyed soil cores from standard plants (Braunberger et al., 1994). Both methods are good because they depend on root colonization of trap plants in order to determine the presence of viable propagules, but none of them has the ability to specify the effectiveness of different sorts of propagules.

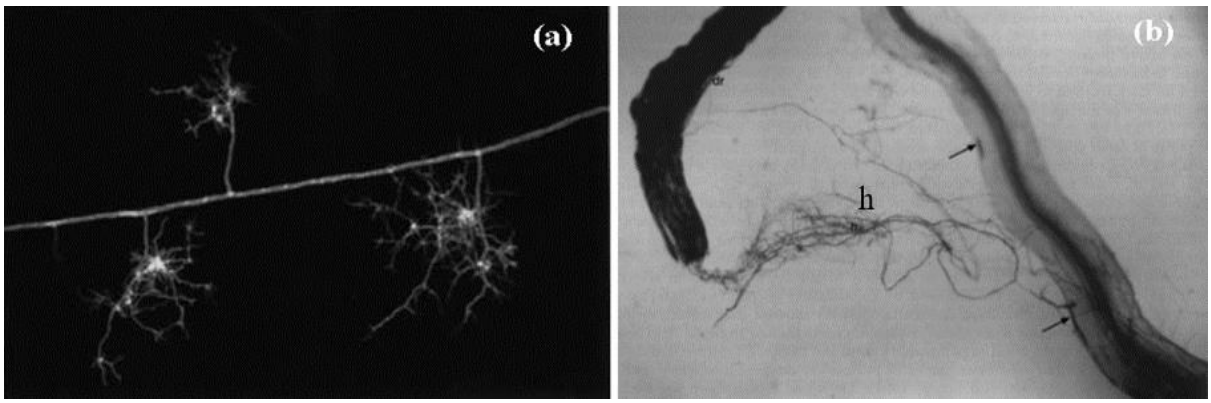

Figure 8. *G. intraradices* an external mycelium on tomato roots grown in monoxenic culture(a), Growth of mycorrhizal hyphae (h) from dead root fragment (b)Extended focus confocal image constructed of 40 optical slices taken at 1 μm intervals on the z-axis *(from Smith & Read, 2008).*

2.5. AM fungi role in sustainable agriculture

Sustainable agriculture production system involves any environmental friendly method of farming that preserves the ecological system, avoids the depletion of the natural resources, and lasts for long term. Unlike conventional farming, sustainable agriculture imposes the use of biological processes to guarantee plant nutrition and crop protection and substitute the use of inorganic fertilizers, herbicides, and pesticides that have been used for many decades. Thus, raising the necessity of using the soil microbial populations for the prosperity of a sustainable production system.

Among the microbial communities of the soil, AMF are the most vital component that maintain soil fertility, enhance soil structure, improve soil water retention, and degrading organic pollutants. Therefore, the role of AMF in sustainable agriculture system is discussed in more detail.

2.5.1. Soil stability and structure

AMF colonize the roots of the host plant, spreading at the same time their hyphae into the surrounding soil. They develop a complex network, which can grow from 5 up to 7.5m in a week (Giovannetti et al., 2001), reaching up to 30 meter per gram of soil (Cavagnaro et al., 2005). The hyphae of arbuscular mycorrhizae fungi produce glomalin (Rillig et al., 2001); its production is proposed as a mechanism by AMF to promote the growth of their partner plant in the natural habitat (Rillig & Steinberg, 2002). The glomalin is a glycoprotein that binds nitrogen, carbon, and several other biological elements in the soil to the minerals, and soil particles enhancing the carbon reserve in the soil and the organic matter as well (Six et al., 2000).

The fact that glomalin contains 30-40% carbon, depositing glomalin on soil particles improves soil ability to withstand drying out (Krishnakumar et al., 2013). Therefore, the AM symbioses play a critical role in developing soil aggregation and maintaining the plant-soil system, leading to a meaningful improvement in the soil texture and the soil water relations (Bethlenfalvay & Shuepp, 1994).

2.5.2. Nutrient uptake and availability

Phosphorus is the major constituent of carotenes and gibberellins of the membrane. The P concentration in plant mature leaves dry matter is between 0.2-0.5 percent with higher rates in younger leaves reaching 0.5-1.0 percent (Keller, 2010). It is one of the super elements that has a crucial role in plant growth and largely applied in crop production. Its deficit may cause crop production failure, meanwhile the excess of phosphorus application in form of phosphate in inorganic fertilizers causes eutrophication to water resources.

In addition to the low availability of Phosphorus (≈1.55% of the total P) due to its extreme immobility in soil, it turns in organic P and becomes immobilized soon after its application in soluble form. Moreover, most inorganic P is adsorbed to the soil particle surface or precipitated in the form of Ca and Mg phosphates under alkaline condition and in calcareous soils, or in the form of Fe and Al phosphate under acidic conditions (Krishnakumar et al., 2013).

To capture less mobile nutrients, plants increase the root length and surface area of the root (Lynch & Brown, 2001), but not under deficit soil moisture conditions that prevent the root growth (Garg, 2003). Under such circumstances plants associate with the AMF for more efficient phosphate acquisition.

The fact that extra-radical mycelium of the fungus are two folds thinner than the thinnest plant root, and they have the ability to adjust the hyphal diameter depending on the soil pore size (Smith et al., 2010), may make AMF more potent to access nutrient in drier and more compacted soils. More efficient contribution of AM in plant P uptake in dry soil (Neumann & George, 2004), may be specially important when plant P availability is limited in soil under dry conditions (Owen et al., 2015). AMF transfer phosphorus more efficiently from the extra-radical mycelium to the intra-radical mycelium by storing the internal phosphorus in the form of polyphosphate and keeping the internal Pi concentration low (Bücking et al., 2012). Additionally, the hyphae of AMF can release phosphate from the low soluble inorganic complexes (Finlay, 2008), and mineralize the organic phosphate (Feng et al., 2003). The positive effects of AMF on other beneficial microorganism such as phosphorus solubilizers and nitrogen fixers in the mycorrhizosphere has been reported, thus possibly related to the positive influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth, and the synergistic interactions between AMF and rhizosphere microbiota (Barea et al., 2002).

Hampers such as high cost of production and application of fertilizers, low outcome of organic production (Jakobsen et al., 2005), and declining phosphate deposits (Smith & Reads, 2008), make the contribution of AMF in nutrients dynamic more promising especially in reusing of phosphorus has been accumulated for decades.

Nitrogen is mostly found in old roots and photosynthesizing leaves (Jackson, 2008), because it is an essential component of nucleotides and amino acids (Jackson, 2008; Benton, 2005), chlorophyll (Jackson, 2008; Resh, 1987) and several growth regulators (Jackson, 2008). Therefore nitrogen has a critical role in plant growth, and the range of its deficiency to its surplus level significantly affects plant growth as well as fruit quantity and quality (Benton, 2005). Nitrogen is known for the high mobility in the soil that facilitate its uptake by plant root especially under humid condition. The high mobility of nitrogen leads to leaching from the upper layer of the soil under wet conditions and limits its availability under dry soil moisture conditions.

Although, the mechanism by which AMF improve the nitrogen uptake in colonized root is not clearly addressed but the possible involvement of an ammonium transporter in nitrogen uptake by the extra-radical hyphae characterized by Lopez-Pedrosa and co-workers in 2006. AMF contribution to ammonium and nitrate acquisition is also proposed (Smith & Read, 2008). On top of that, AMF participates in organic material decomposition (Miransari, 2011) results in releasing big amounts of nitrogen to the soil solution.

The same as for some macro nutrients, trace elements such as iron, copper, zinc, and manganese are slow mobile nutrients. The uptake of these micro elements is limited due to their slow mobility (Tisdale et al., 1995), leading to the depletion in rhizosphere especially when they were not supplied to the soil. Colonized plant roots with AMF have larger surface area created by the extra-radical hyphae reducing the diffusion distance and improving the nutrients especially the immobile metals (Jakobsen et al., 1992). In a pot experiment and by using nutrient solution and substrate (Vermiculite and expanded clay) Balliu and co-workers (2015) recorded an enhancement in relative uptake rate of macro nutrients (N, P, Mg, and Ca) and micro elements (Mn and Fe) of tomato seedlings inoculated with the commercial inocula consisting of 5 Glomus strains. The mycorrhizal contribution to uptake and translocation of copper in *Trifolium repens* has been also demonstrated (Li et al., 1991).

2.5.3. Protection against biotic stressors

In Nature, the rhizosphere is the habitat for many microbial communities acting either as soil borne pathogens toward plants or beneficial microbes establishing a mutualistic relationship with plant roots. Plant pathogens including soil borne part cause massive destructions in crop production (Ramegowda & Senthil-Kumar, 2015) and mainly attracted to exudates excreted by plant roots. Most plants change their gene expression to overcome biotic stresses, but that is not always the case. The production of ROS by plant is also another tactic to tolerate biotic stresses (Miller et al., 2010).

In sustainable agriculture, the negative effects of the pesticides and fertilizers on the soil, and there high cost made the use biological control of pests more urgent. Thus, plant growth promoting rhizo-microorganism (PGPR) became an environmental friendly and cost-effective agent in controlling pests and enhancing plant nutrition.

Arbuscular mycorrhizae fungi are forming the most important component of PGPR, that can protect their partner plants from soil borne pathogens, harmful fungi, and nematodes (Veresoglou & Rillig, 2012), besides enhancing the nutrient uptake (Smith & Reads, 2008), that strengths the host plant against pathogens. Up to date no direct plant protection approach by AMF has been confirmed, but several indirect mechanisms are addressed: AMF can prevent other pathogens from penetrating the root of their host plants either by the direct competition through using the root of the host plant as their ecological niece and prevent the use of the root by nematodes (Cordier et al., 1996), or by lignifying the cell wall of the host roots that hardens the root penetration by other pathogens. Additionally, the AMF can alter the microbial population composition in the mycorhizosphere (Marschner et al., 1997), through changing the respiration of the colonized roots, and modifying both quantity and quality of exudates excreted by roots.

2.5.4. Alleviating abiotic stresses

Many abiotic stresses (such as drought, salinity, heat, cold, and mineral deficiency and toxicities) cause extensive damage to the agriculture sector. The reduction in plant production by abiotic stressors is estimated to reach 70% (Saxena et al., 2013), threatening the agricultural production worldwide.

The symbiotic relationship between the arbuscular mycorrhizae fungi and plant roots meaningfully improves the resistance and the growth of host plants under various abiotic stresses (Abdel Latef et al., 2016). Moreover, Abdel Latef and co-workers (2016) underline mechanisms by which the AMF improves plant resistance to abiotic stresses as: alleviating the oxidative stress in mycorrhizal plants, up taking water and absorbing nutrients rapidly, and changing genes on the transcriptional levels that are involve in stress response or signaling pathway.

Drought is the most vital abiotic factor that, depresses plants growth, causes physiological disorders, and unbalancing plant nutrition. Thus, the role and mechanisms by which the AMF ameliorates the drought impact on their partner plants are discussed in more detail.

- Drought stress

The enhancement of plant growth performance, and plant water relations by AM symbioses under water stress has been well documented (Ruíz-Lozano & Aroca, 2010; Augé, 2001). Paths leading to mitigate water stress impact on host plants by AMF can be indirect such as changes in plant root architecture and growth, soil plant nutrition and soil structure, stomatal regulation, root hydraulic conductivity, and water transport pathways, in addition to the direct contribution of water uptake.

Better root growth from dry soil due to nutrient absorption enhancement especially phosphorus (Neumann & George, 2004), can be counted as an indirect mechanism by AMF to remove the negative effect of soil water deficit on the root growth. Additionally, enhancing soil water retention properties by AMF hyphal network and creating water stable aggregates is previously reported (Rilling & Mummey, 2006; Augé, 2001), which makes the soil water more available for plant roots. Better stomatal regulation by AMF is another proposed mechanism mitigating the water shortage stress (Augé et al., 2015), enhancing water use efficiency (Candido et al.,

2015; Patanè et al., 2014) and reducing oxidative damages (Wu et al., 2006; Ruiz-Lozano, 2003).

Studies suggested the potential water transport via AMF to their host plants depending on two aquaporin genes of the fungi (Chitarra et al., 2016), reinforcing the direct involvement of AMF in plant tolerance to drought. The complex extra-radical hyphal network explores water in soil pores not available for plant roots. The direct contribution of extra-radical hyphae to the total water uptake by plants may reach 4% (Khalvati et al., 2005), and both direct and indirect hyphal contribution to the total water uptake is estimated to be about 20% (Ruth et al., 2011) in separated plant-hyphal chambers. Two more strategies are implemented by AM fungi in order to increase the direct water uptake: increasing the root hydraulic conductivity (Koide, 1985), that intensify the water absorption by the extra-radical hyphae (Ruiz-Lozano et al., 1995; Farber et al., 1991). More efficient shifting between water-transport pathways in mycorrhizal roots (Bárzana et al., 2012) improves the water uptake mechanism under drought conditions.

2.6. The experimental plant

Solanaceae is a large and diverse family with about 90 genera including *Lycopersicon* genus. Despite being small compared to the other genera within the family, *Lycopersicon* is the most important and valuable horticultural crop (Taylor, 1986).

For research purposes and as a model plant, tomato is one of the most studied plant and it is getting more attention due to several characteristics:

- The economic importance of tomato crop.
- It bears fleshy fruits, can grow easily in a wide range of environments, and explores its characteristics (Schwarz et al., 2014).
- Being phylogenetically far from the other model plants such as *Arabidopsis*, maize, and rice (Rick & Yoder, 1988).
- The existence of many mutants and large stock collections (Schwarz et al., 2014).
- Tomato has many wild species with diverse phenotypes and desirable traits important for breeding and evolutionary research important to cross with domestic tomatoes (Kimura & Sinha, 2014).
- It can be hybridized and regenerated in in vitro cell culture, allowing efficient gene transfer by *Agrobacterium tumefaciens* (Rick & Yoder, 1988).
- The possibility of applying the knowledge gained from studies on tomato to many other commercially important crops within the Solanaceae family such as tobacco, peppers, eggplants, and potato (Kimura & Sinha, 2014).

2.6.1. *Lycopersicon esculentum* M.

Tomato belongs to Solanaceae family and it is cultivated as an annual crop despite being perennial. The proposed name *Lycopersicon esculentum* by Miller in 1768 replaced the previous name *Solanum lycopersicum* by Carl Linnaeus (Kriemhild et al., 2000).

Most of the commercial tomato seeds are genetically identical F_1 with hybrid vigour from crossing two inbred tomato lines (Schwarz et al., 2014). Processing tomato UNO ROSSO F_1 is

a common representative cultivar that has appropriate growth habit and morphological features in addition to its economical values (UniGen Seeds, 2017).

Proper fruit setting and development is important to achieve high yield in tomato that has to be accompanied by high quality especially in processing tomato the soluble solid content (ºBrix), and carotenoids to guarantee the marketing. Soil moisture content restricted growth and yield of tomato under low soil moisture content (Abdelhafeez & Verkerk, 1969) is partially related to lower availability of some nutrients.

Despite its high expense, using F_1 hybrid seeds in both protected and field production systems guarantees complete, fast and uniform germination. In addition, water and nutrition supply in the rhizosphere, have profound effects on growth of plants (Atherton & Rudich, 1986).

In most tomato cultivars, day-night temperature higher that 34-20°C or facing four hours of 40°C will cause blossom drop, depending also on humidity and soil moisture condition (Atherton & Rudich, 1986). Abdalla and Verkerk (1970) reported an increase in flower incidence due to nitrogen deficiency in tomato plants subjected to water stress and high temperature. The same is for N-deficiency has been observed by Besford and Maw (1975) for potassium.

For profitable tomato production gaining high yield and good fruit quality is essential, therefore nutrient supply manipulation is of key importance, although tomato can growth over arrange of each nutrient (Atherton & Rudich, 1986). Both the size and the total soluble solids content of tomato fruit are strongly influenced by the solar radiation received acting on the supply of leaf assimilates. In June and July, when the solar radiation is highest both dry matter and sugar content of the fruit are at their highest levels (Atherton & Rudich, 1986).

Reponses to Phosphorus application depends on soil phosphorus status, but its availability depends on the pH of the soil; significant increases in processing tomato yield have been achieved in fields with low available phosphorus in response to superphosphate application (800-2400 kg ha^{-1}). Moreover, Nitrogen supplies strengthen plants in general, but moderate nitrogen grantees high yield both under protected and in field production system (Atherton & Rudich, 1986).

2.6.2. Tomato (*L. esculentum*) nutritional and economical value

Worldwide tomato production reached 177,042,359 tons in 2016 (FAOstat, 2016), of which 41,384,000 are processed (WPTC, 2016), making tomato one of the most important vegetable crops. Processed tomato forms about 23% of the whole consumption 20.5 kilogram per capita diet worldwide (Garming, 2014), becoming major source of carotenoids (181-437 mg), lycopene (95-273 mg), β-Carotene (5.4-17.2 mg), and ascorbic acid (236-418 mg) per kilogram fruit fresh weight (Bakr et al., 2017). Moreover, tomato supports human body with remarkable dietary nutrients including sodium, potassium, phosphorus, iron, magnesium, riboflavin and thiamine (Fraser & Bramley, 2004). Unlike the other foods, processing the tomato for making (ketchup, tomato sauce, tomato paste, etc...) rises lycopene availability by breaking down the cell wall fruits and releasing more lycopene isomers due to heating up during the volatilization process (Philippine Herbal Medicine© 2005-2018; Shi et al., 1999).

Tomato can supply 20-40% of ascorbic acid for adults according to the US daily allowances, mostly because of the large amount consumed rather than its average content, making tomato an important source of vitamins (Atherton & Rudich, 1986).

Nutrient composition and quality are affected by many pre-harvest, and post-harvest factors including genetic and environmental factors. Environmental factors affecting the fruit quality and nutritional composition can be climatic such as precipitation, temperature, and light or cultural practice (soil type fertigation, and chemicals) (Atherton & Rudich, 1986).

Fruits sugar content can be increased by soil moisture reduction and increased at high nitrogen doses (Atherton & Rudich, 1986), while lycopene synthesis lessened at low temperature (Koskitalo & Ormrod, 1972) and inhibited above 30°C (Tomes, 1963). Tomato plants induced to temperatures above 30°C faces irregular ripening, but carotenoids synthesis may be inhibited at 40 °C or higher (Atherton & Rudich, 1986).

Unlike other nutrients potassium is a necessity for good quality of tomato fruits exceeding that required for maximum yield. Positive response of growth and flowering to potassium levels has been reported (Besford & Maw, 1975) in sand culture but more than 100-150 ppm K (moderate level) in the nutrient solution is not efficient.

Calcium reduction in the nutrient solution depressed the growth of tomato, but more than 40 mg l^{-1} ppm did not enhance the growth further (Kalra, 1956). On a sandy field with 5.5 pH increasing lime application from 0.5 to 1.5 kg m^{-2} depressed the yield slightly (Martens, 1963).

Both yield (Adatia & Winsor, 1971) and growth (Shi et al., 1999; Hipp & Gerard, 1969) of tomato may be affected by inadequate supply of magnesium.

Low moisture levels decreased the nitrification in the soil, reduced calcium content in leaves, with no effect on potassium and calcium in fruits, and increased dry matter in fruits (Atherton & Rudich, 1986). Harvesting was delayed by 2 to 3 weeks by increasing the soil moisture through applying 60% more nutrient solution (Kafkafi & Bar-Yosef, 1980).

Carotenes are ubiquitous organic molecules and they cannot be produced by the human body, therefore humans should take carotenes. Carotene group pigments are accumulated during the ripening process (Pék et al., 2010; Helyes et al., 2006a). In Hungary Helyes and co-workers (2002) measured total carotenes in range of 39 to 171 mg/ kg^{-1} in 16 different cultivars of tomatoes.

As for carotenoids, ascorbic acid should be taken, since human body cannot produce it. High levels of the ascorbic acid are needed to maintain the human body and for an effective functioning as antioxidant, but its instability, poor absorption in the intestines, and fast excretion from the body reduces the availability of ascorbic acid (Li & Schellhorn, 2007). Thus, making tomato an important source for vitamin C, due to the daily and large amount consumption of tomatoes.

3. MATERIALS AND METHODS

3.1. 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. Seeds were sown into plastic trays (one seed each hole) in the greenhouse in the middle of April using the Klasmann TS3 substrate (consisting of white sphagnum peat 80%, frozen black sphagnum-peat 20%) for one month. Seedlings were transplanted into the field on May 17 in both seasons.

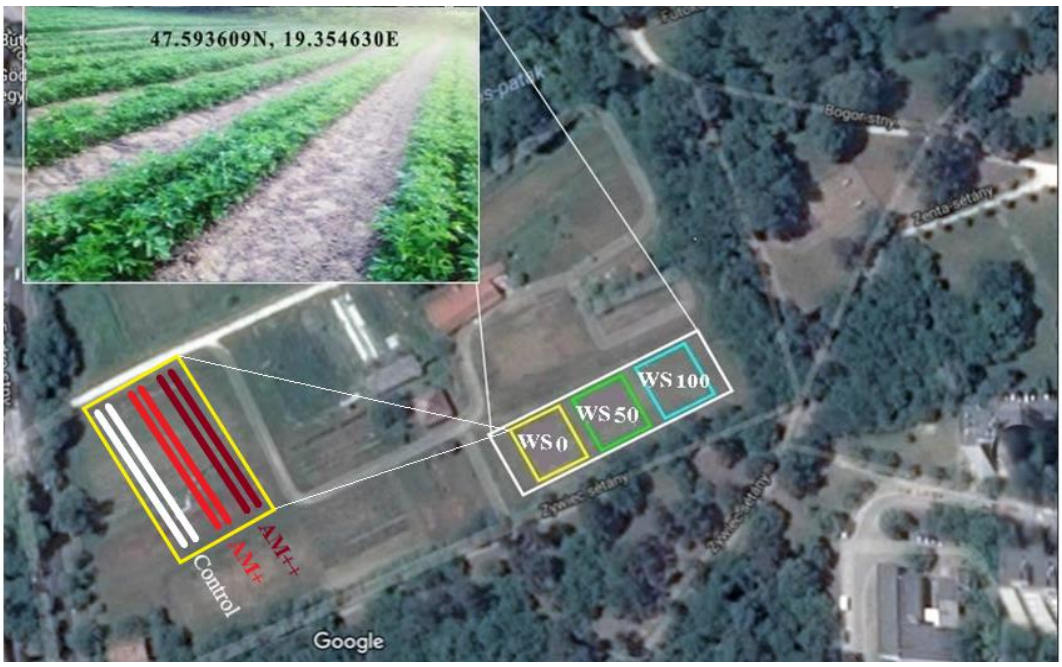

Figure 9. Experimental farm location and design scheme of growing season 2015.

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 in both growing seasons 2015 (Figure 9.) and 2016 (Figure 10.).

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. Soil nutrient content and chemical properties are represented in Table 1.

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. Soil nutrient content and chemical properties are represented in Table 1.

Figure 10. Experimental farm location and design scheme of growing season 2016.

Table 1. Soil chemical properties of the experimental farms, and tomato crop nutrients requirements.

Chemical Properties	**pH**	**EC (mS/ cm)**	**Organic matter (%)**	**NO_3^- (N)**	**P_2O_5**	**K_2O**	**Ca^{+2}**	**Mg^{+2}**	**Fe**	**Cu**	**B**	**SO_4^{-2} (S)**	**Cl^-**	**HCO_3^-**	**Na^+**
				mg kg^{-1}											
2015 Farm	6.6	0.124	1.1	6.0	14	26	53	5.4	0.2	0.1	0.3	5.2	0.0	134	4
Tomato Crop Nutrients Requirment	5.0 – 0.7	Max. 2.5	-	200-250	175-250	400- 600	600-800	100-150	40-50	3-10	1-2	50-120	Max. 175	-	Max. 115
2016 Farm	7.5	0.212	1.4	8.6	8	57	200	12	**122**	4	0.0	0.0	0.0	610	20

3.2. Mycorrhizae materials

3.2.1. 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 (spores, mycelial fragments and small fragments of mycorrhizal plant roots) of six different AMF species mixed with an inert substrate and amended with bio-additives promoting the symbiosis.

According to the producer information, the product contains more the 150,000 propagules per litre (assessed according to the Most Probable Number test) from a mixture of six different AMF species naturally occurring in European soils: *Claroideoglomus etunicatum* (*Glomus etunicatum*), *Rhizophagus microaggregatum* (*G. microaggregatum*), *Rhizophagus intraradices* (*G. intraradices*), *Claroideoglomus claroideum* (*G. claroideum*), *Funneliformis mosseae* (*G. mosseae*), *Funneliformis geosporum* (*G. geosporum*). Between parentheses the names of AMF species are written according to the old nomenclature, as reported in the product information.

3.2.2. 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.

3.2.3. 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.

3.3. Metrological data

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. During the first growing season precipitations were relatively well distributed and the four heavy rain events were occurred. Unlike growing season 2015, the field faced more heavy rain events (9) and cooler temperature during the growing season 2016 (Figure 11.).

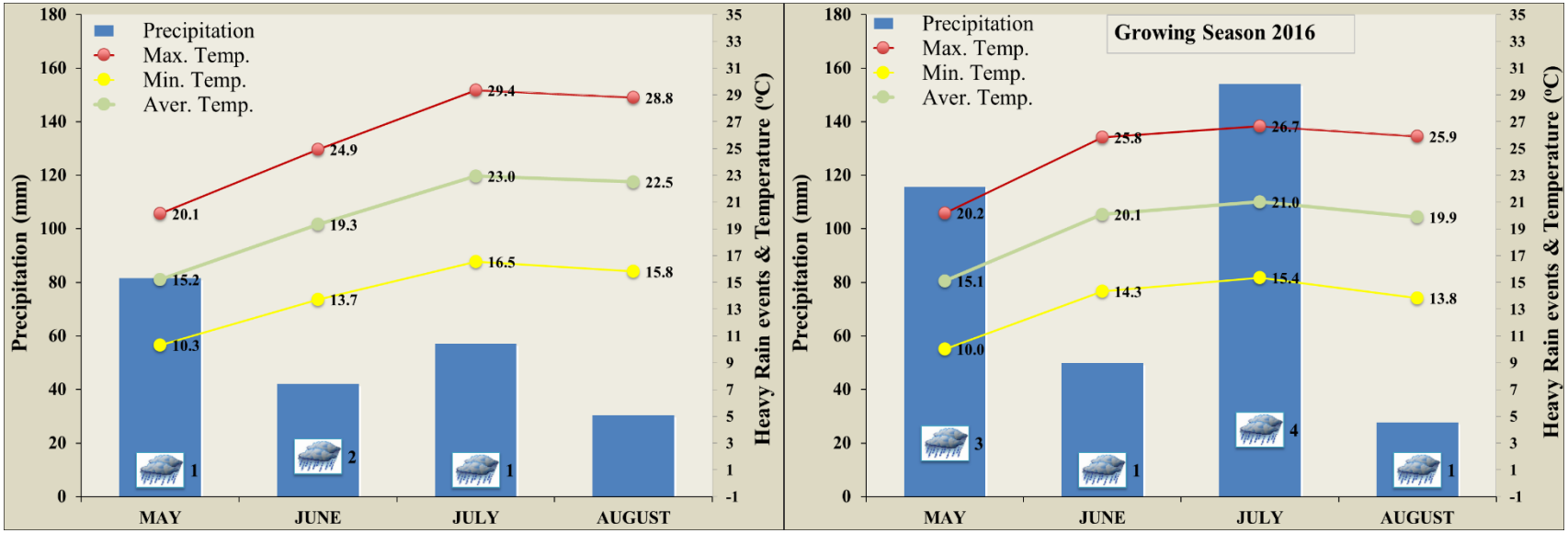

Figure 11. Monthly precipitation, heavy rain events, average temperature, minimum temperature, and maximum temperature.

3.4. Water Supply

Weather forecast data from the national metrological institute were used to calculate the air temperature. 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: no water supply (WS0), deficit water supply (WS50), and full water supply (WS100).

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 (Figure 14.).

The same procedure was followed in 2016 growing season; water supply amount was calculated depending on the air temperature (Pék et al., 2014). Drip irrigation system was used to implement three water supply levels: No water supply (WS0) block received only 296 mm of rainfall, deficit water supply (WS50) block received 50% of the calculated water demand a sum of 388 mm including the rainfall, and fully irrigated (WS100) block received a sum of 480 mm including the rainfall (Figure 15.).

3.5. Fertigation

Throughout both growing seasons plant nutrition requirements and plant protection were regulated after Helyes and Varga (1994).

Weekly fertigation has been done through the drip irrigation system by adding 5 grams of the Ferticare 14-11-25 to each square meter of the cultivated area. Ferticare 14-11-25 is a complex granulate chlorine-free fertilizer produced in Hungary by YARA company and contains both micro and macro elements. The Ferticare 14-11-25 Complex fertilizer has been added 7 times throughout each growing season. -Doses, calculation, and nutrients amount per plant shown in Table 2.

Table 2. Fertigation, Macro-nutrients (mg plant^{-1}), and Micro-nutrients (µg plant^{-1}).

Nutrients Content	Weekly Macro-Nutrients (**mg** plant^{-1})	Total Macro-Nutrients (**mg** plant^{-1})
14% Total N	113	791
11% P_2O_5	88	616
25% K_2O	201	1407
4.5% SO_3^{-2}	34	238
2.3% MgO	18	126
Nutrients Content	Weekly Micro-Nutrients (**µg** plant^{-1})	Total Micro-Nutrients (**µg** plant^{-1})
0.1% Fe	806	5642
0.1% Mn	806	5642
0.02% B	161	1127
0.01% Cu	80.6	564
0.01% Zn	80.6	564
0.002% Mo	16.1	112

An extra dose of the NPK complex fertilizer was added to the experimental field in 2015 in order to compensate for nutrient differences between both experimental fields. Prior to transplanting the field was fertilized with NovaTec® premium 15+3+20(+3+10) produced by COMPO EXPERT from Germany. According to the product information, the NPK complex fertilizer NovaTec® supported with DMPP (3,4-dimethylpyrazolphosphate), that can inhibit the nitrification and reduces N-leaching through delaying the transformation of ammonium to nitrate by 4 to 10 weeks depending on soil temperature and soil humidity (http://www.compo-expert.com/en/home/products/stabilized-fertilizers-with-dmpp.html).

For each square meter of the experimental field 25 grams of NovaTec® was added and the amount of Macro- and Micro- nutrients were calculated per plant as it is shown in Figure 12.

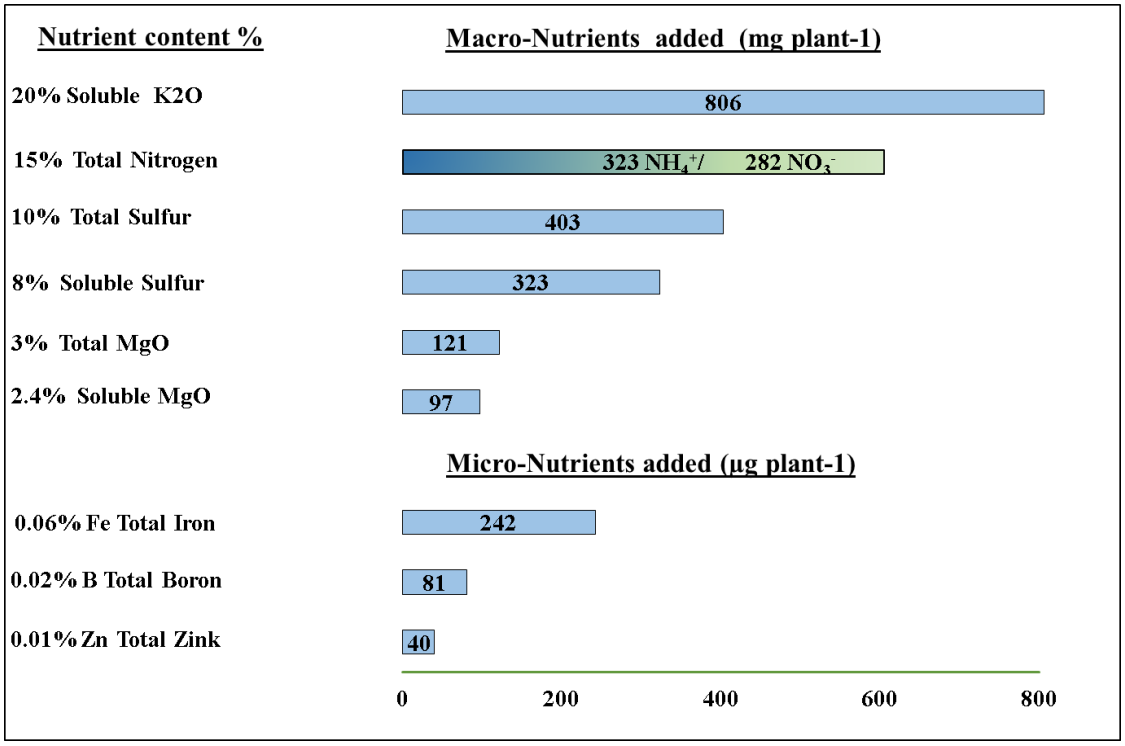

Figure 12. Fertilization, Macro-nutrients (mg plant^{-1}), and Micro-nutrients (**µg** plant^{-1}).

3.6. Harvesting

In the first season, 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 the first season, in 2016 season, plants were harvested at once after 100 days of growing.

The 20 meters twin row plots allowed us to randomly mark 4 subplots (replicates) per treatment. From each replicate the above ground part of 10 tomato plants were cut off at the soil surface and the total fresh biomass were weighted immediately. Fruits were separated from the shoots, then the shoot weight of all subplots and treatments were recorded, followed by separation of marketable, green, and rotten fruits. Weight and number of total, marketable, green, and rotten fruits were recorded.

For quantitative and qualitative parameters sampling of 10 plant from each replicate (subplot), guaranteed high precision, and lessened sampling error.

3.7. Field measurements

Field measurements and their repetition are weather dependent, in addition to which extend water stress was properly induced in our field experiment. Thus, measurements were taken during the water stress period in both growing seasons, but due to longer water stress period field measurements were taken more frequently in the first growing season as following:

- Volumetric water content (VWC) measurements were taken continuously throughout the growth period and prior to irrigation.

- Transpiration (gs.) was recorded during the fruit setting within 3 consecutive weeks (on June 19, June 26, and July) in 2015, and once (on July 13) in 2016.
  Four readings per plant and four plants in each subplot with 4 replications in each treatment (4 leaves * 4 plants * 4 replications).

- Single-Photon Avalanche Diode (SPAD) readings were taken once in both seasons (on July 3 in 2015, and July 13 in 2016) Four readings per plant and four plants in each subplot with 4 replications in each treatment (4 leaves * 4 plants * 4 replication).

- Chlorophyll fluorescence measurements were taken every week for 5 times (on June 19, June 26, July 3, July 30, and August 6 in 2015), and 3 times (June 29, July 6, and July 13 in 2016) consecutively.
  One reading per plant, for one plant in each subplot with 4 replications in each treatment.(1 leaf * 1 plant * 4 replications).

- Leaf water potential (ψ_L), measured in 3 consecutive weeks (on June 19, June 26, and July) in 2015, and once (on July 13) in 2016.
  One reading per plant and four plants in each subplot with 4 replications in each treatment (1 leaf * 4 plants * 4 replications).
- Canopy temperature was taken every week for 6 times in 2015 (on June 12, June 19, June 26, July 3, July 30, and August 6), and 4 times in 2016 (June 22, June 29, July 6, and July 13) consecutively.
  Four readings per plant and four plants in each subplot with 4 replication in each treatment (4 leaves * 4 plants * 4 replications).

3.7.1. Volumetric water content of soil

The soil water content can be measured based on the permittivity, since small changes in its water content results in significant shifts in the permittivity (permittivity of water is 80, and about 2.5 in dried soil). Soil moisture sensors determines the moisture in soil depending on soil sample permittivity. 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. The Volumetric water content (VWC) of the soil in the root zone, soil texture, and organic matter were used to calculate the field capacity as an important agriculture drought factor to determine soil water holding ability to buffer plants during water deficit periods.

3.7.2. Transpiration

Porometer Delta-T, type AP4 from UK, was used to measure the water loss from the leaves of the plants. The device can readout the stomatal conductance directly or as stomatal resistance depending on the diffusion conductance by comparing the humidification within the chamber to readings from the calibration plate. Unlike the other porometers and gas analysis devices, Delta-T Porometer type AP4 can be simply calibrated in the field

3.7.3. Relative chlorophyll index

As it is reported by Etsushi et al. (2009), chlorophyll content in plant leaves is significantly correlated with Single-Photon Avalanche Diode (SPAD), therefore SPAD values can be used for Nitrogen content in leaves (Martínez et al., 2015). As a non-destructive tool, chlorophyll meter SPAD-502 (Konica Minolta, Japan) was used to measure relative chlorophyll index as SPAD units at fruit setting stage.

3.7.4. 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).

3.7.5. Leaf water potential

Pressure bomb (PMS Instruments Co., Corvallis, OR, USA) was used to determine leaf water potential (ψ_L) at midday (Gonzalez, 2001), by cutting a newly mature leaf from each plant, four replications per treatment and for three consecutive weeks.

3.7.6. Canopy temperature

The infrared thermometer (Raytek Raynger MX4, Santa Cruz, CA, USA) was used to record the canopy temperature (Bőcs et al., 2009). The new laser technology takes noncontact temperature measurement from any distance, easy to use, accurate (± 1% in readings), and can read from -30 to 900°C (http://www.farnell.com/datasheets/44260.pdf).

3.7.7. Water use efficiency

Water use efficiency (WUE) was calculated depending on the total above ground fresh biomass as it is shown in Equation 2.

Equation 2. Water Use Efficiency (WUE)

$$WUE = \frac{Total\ biomass\ ton\ per\ hectare}{Quebic\ meter\ water\ consumed\ \ per\ hectare}$$

3.7.8. Relative field mycorrhizal contribution (RFMC%)

In crop production systems plants response to mycorrhizal inoculation is important to indicate to what extend the mycorrhizal inoculated plants got benefit from the inoculation. RFMC% was calculated using the formula (Equation 3) according to Plenchette et al. (1983).

Equation 3. Relative Field Mycorrhizal Contribution (RFMC%)

$$RFMC\% = \frac{Total\ biomasss\ of\ (AM) plants - Total\ biomasss\ of\ (Control)\ plants}{Total\ biomasss\ of\ (AM)\ plants}\ X\ 100$$

3.8. Laboratorial Analyses

3.8.1. Proline estimation

Leaves were taken during the water stress at fruit setting stage (on July 3 in 2015, and July 13 in 2016) from 4 previously marked tomato plants representing one subplot (repetition) and 4 replications per treatment. Proline concentration was estimated based on the acid-ninhydrin method (Bates et al., 1973) modified (Claussen, 2005). Samples of 0.5 g fresh weight (a mixture of 4 leaves in each subplot) were ground in a mortar using a small spoon of quartz sand and 5 mL of sulfosalicylic acid (3% weight/ volume) was added for leaf crude extraction. Leaf crudes were centrifuged at 3000 rpm for 10 min, and to the supernatant 2 mL of acid-ninhydrin, 2 mL of ortho-phosphoric acid (6 Molar), and 2 mL of glacial acetic acid were added, followed by one-hour water bath incubation at 100ºC. Incubated tubes were left for 5 min at room temperature to terminate the reaction. 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 (Figure 13.) based on the fresh weight (microgram proline per gram leaf).

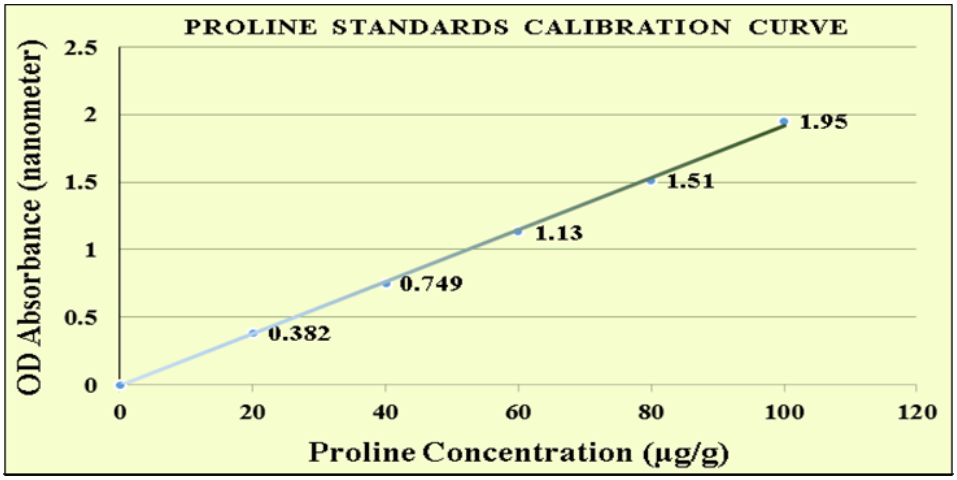

Figure 13. Proline standards calibration curve.

3.8.2. Inorganic elements concentration

At fruit setting stage eight weeks after transplantation, leaflets next to most recent fruiting cluster from 4 plants in each replication were taken, then after leaves were dried at 65°C for 48 hours. Dried shoots were ground with mortar and pestle. 250 mg of 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.

3.8.3. Soil microbial activity

Fluorescein diacetate (FDA) hydrolysis is widely accepted as an accurate and simple method for measuring total microbial activity in a range of environmental samplesincluding soil (Greena et al., 2006). The activity of fluorescein diacetate hydrolase (FDAH) was assessed as described in previous protocols (Adam & Duncan, 2001; Tabatabai & Bremner, 1969).

3.8.4. Mycorrhizal root colonization

Four plants were dug out randomly from repetitive plots from the same treatment, then after a representative subsample regarding different treatments was cut to 10 mm pieces and then five randomly selected pieces from each sample were stained by Trypan Blue (Phillips & Hayman, 1970). A stereomicroscope at × 100 magnification was used to determine internal fungal structures (hyphae, arbuscules), and gridline intersect method (Giovannetti & Mosse, 1980) was used to calculate root length colonization in percentage.

3.8.5. Extraction and counting spores of endogenous AMF

Wet sieving technique (Gerdemann & Nicolson, 1963) has been used for the extraction and counting of spores of endogenous AMF in the experimental soil. In both growing seasons, samples were gathered from the top soil prior to seedlings transplantation from 10 points randomly chosen using the soil sampling auger bit.

Soil samples were air dried and well mixed. About 50 grams of the soil is placed into a conical flask and 500 mL of tap water was added. A glass rod was used to mix the solution vigorously. Large particles settled down after several seconds pause, the suspension was

poured through two sets of 600 µm and 40 µm sieves respectively. Spores remained on the 40 µm sieve were washed slowly into a petri dish. Under a dissecting microscope and by using a peptide spores were caught and counted per gram of soil.

3.8.6. 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). Five-gram samples from tomato fruits were taken in triplicate (at least) and disintegrated in a crucible mortar with quartz sand. Extraction was started with addition of 20 mL methanol to bind the water. The methanol phase was decanted into 100 mL conical flask and the residues were further crushed. The pigments were extracted by a step-wise addition of 60 mL of 1:5 methanol-dichloroethan. The supernatant was quantitatively transferred to the same conical flask containing methanol fraction. To separate the different phases 1 mL water was added and the mixture was shaken for 15 min by a mechanical shaker. The two phases were separated in a separator funnel, and the lower dichloromethane phase containing fat-soluble pigments was dried over anhydrous sodium sulphate. Finally, the organic solvent was evaporated under vacuum by rotary evaporator at not higher than 40°C. 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

The separation of carotenoids was performed on Accucore (Thermo Scientific) C-30, 2.7 µ, 150 x 4.0 mm column with gradient elution of (A) tetra-butyl-methyl-ether (TBME) and (B) methanol. The gradient elution stated with 100% B, changed to 30% A in B in 25 min, stayed isocratic for 5 min and finally turned to 100% A in 5 min (new HPLC protocol, under publication).

Peak identification was based on comparison of spectral properties and retention time of carotenoids of the samples with those of available standard materials like Lycopene, β-carotene and zeaxanthin, which were purchased from Sigma-Aldricht (Budapest, Hungary). In

case of compounds with no available standards, the peaks were tentatively identified according to their spectral characteristics and chromatographic retention as compared to literature data (Borsarelli & Mercadante, 2009; Liaaen-Jensen & Lutences, 2008; Bauernfeind, 1981). The cis isomers of lycopene were identified on the basis of appearance of an extra absorption wavelength at 340 and 361nm. The 9-Z and 13-Z cis isomers were identified according to II-value, which equals to absorbance at 361nm over absorbance at the maximum wavelength of cis lycopene (Liaaen-Jensen & Lutences, 2008).

The column effluents were detected and integrated at their maximum absorption wavelength for quantitative determinations. They were quantified as either lycopene- or β-carotene-equivalent (μg g^{-1}, which is equal to g kg^{-1}) according to their spectral characteristics (Rodriguez-Amaya, 2001).

- Extraction and determination of Ascorbic Acid

Five grams of well homogenised sample were disrupted in a crucible mortar with quartz sand. To the macerate 50mL of meta-phosphoric acid (analytical grade) was gradually added and the mixture was then transferred to a 100mL Erlenmeyer flask with stopper and then filtered. The filtrate was further purified by passing through a 0.45mm Whatman cellulose acetate syringe filter before injection on HPLC column.

The HPLC determination of ascorbic acid was performed on C18 Nautilus, 100-5, 150 × 4.6mm (Macherey-Nagel, Düren, Germany) column with gradient elution of 0.01M KH2PO4 (A) and acetonitrile (B). The gradient elution started with 1% B in A and changed to 30%B in15min, then, turned to 1%A in B in 5min. The flow rate was 0.7 mL/min. The highest absorption maximum of ascorbic acid under these conditions was found to be 265 nm. For quantitative determination of ascorbic acid standard materials Sigma-Aldrich (Budapest, Hungary) were used. Stock solutions and then working solutions were prepared for L-ascorbic acid at level of 0-120 μg/mL to make the calibration between concentration and peak area.

- HPLC equipment and conditions

A Chromaster liquid chromatograph in (Hitachi, Japan) consisting of a Model 5110 Gradient pump, a Model 5210 auto sampler and a Model 5430 photodiode array detector was used. Operation and data processing were performed by EZChroma Elite software.

3.8.7. Soluble solid content determination

According to Johnstone et al. (2005) refractive index is considered the most common tool to estimate the soluble solid content, and its values are reported as percentage. To estimate the ºBrix digital Refractometer Krüss DR201-95 (Küss Optronic, Hamburg, Germany) was used.

3.9. 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 (herein referred as AM), AM with three levels (Control, AM+, AM++) and Water supply (herein referred as WS) 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: (ºBrix content, and marketable yield), (Soluble solid yield, and marketable yield), (proline content, and leaf water potential), (Canopy temperature, and Stomatal conductance) variables.

4. RESULTS AND DISCUSSIONS

Worldwide, field crop production faces water stress that limits crops productivity, and mycorrhizal symbiosis is considered as a key component backing up host plants to overcome water lack stress as it is addressed in numerous studies (Candido et al., 2015; Ruiz-Sánchez et al., 2010; Smith & Read, 2008; Augé, 2001). In these two years field-based trials, mycorrhizal field-inoculation at transplant, boosted yield, and enhanced growth and water use efficiency under both deficit water supply and full water supply levels compared to non-inoculated and pre-transplant at sowing inoculated plants (***Appendices 1 & 2***) as it was also found by others (Di Cesare et al., 2012).

As it is mentioned in the fertigation sector (see 3.1.3. Fertigation), to fulfil plants nutritional requirements fertilizers were added weekly through the drip irrigation system. In each growing seasons plants were fertigated seven times, as a result each plant has been supplied with the same amounts of different macro nutrients: 791 mg of total N (323 NH_4^+/ 282 NO_3^-), 616 mg of P_2O_5, 1407 mg of K_2O, 238 mg of SO_3^-, and 126 of MgO. In addition to six different micro nutrients: 5642 µg of Fe, 5642 µg of Mn, 1127 µg of B, 564 µg of Cu, 564 µg of Zn, and 112 µg of Mo (Table 2).

The experimental field used in growing season 2015 has been used for many years for field studies compared to the 2016 growing field that was left fallow for several years. Moreover, differences in texture, field capacity, and lower holding water capacity of the 2015 experimental farm caused more depletion of nutrients especially the super elements, therefore the farm supplied with a supplementary dose of NovaTec® fertilizer at the rate of 25 grams for each square meter prior to transplanting. Thus, plants of the first season have additionally received these elements: 605 mg of total N, 403 mg of P_2O_5, 806 mg of K_2O, 323 mg of SO_3^-, and 97 of MgO. In addition to three different micro nutrients: 242 µg of Fe, 81 µg of B, 40 µg of Zn (Figure 12.).

4.1. Water stress induction and soil water condition

During the first growing season (2015) the experimental farm has received 186.6 mm of rain, and watering through the drip irrigation system resulted in supplying 436.3 mm and 316.3 mm to both WS100- and WS50-blocks respectively including the precipitation. Soil water content was ranging between 0.14-0.17, 0.11-0.14, and 0.07-0.10, corresponding to 73-89%, 58-73%, and 37-52% of field capacity in WS100, WS50, and WS0 blocks respectively. The relative well distribution of the rain events during the first two months, the last three weeks of no rain (Figure 14.), and the low water holding capacity of the experimental soil allowed proper water stress induction to WS0- and WS50- plants. Based on the midday leaf water potential 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.

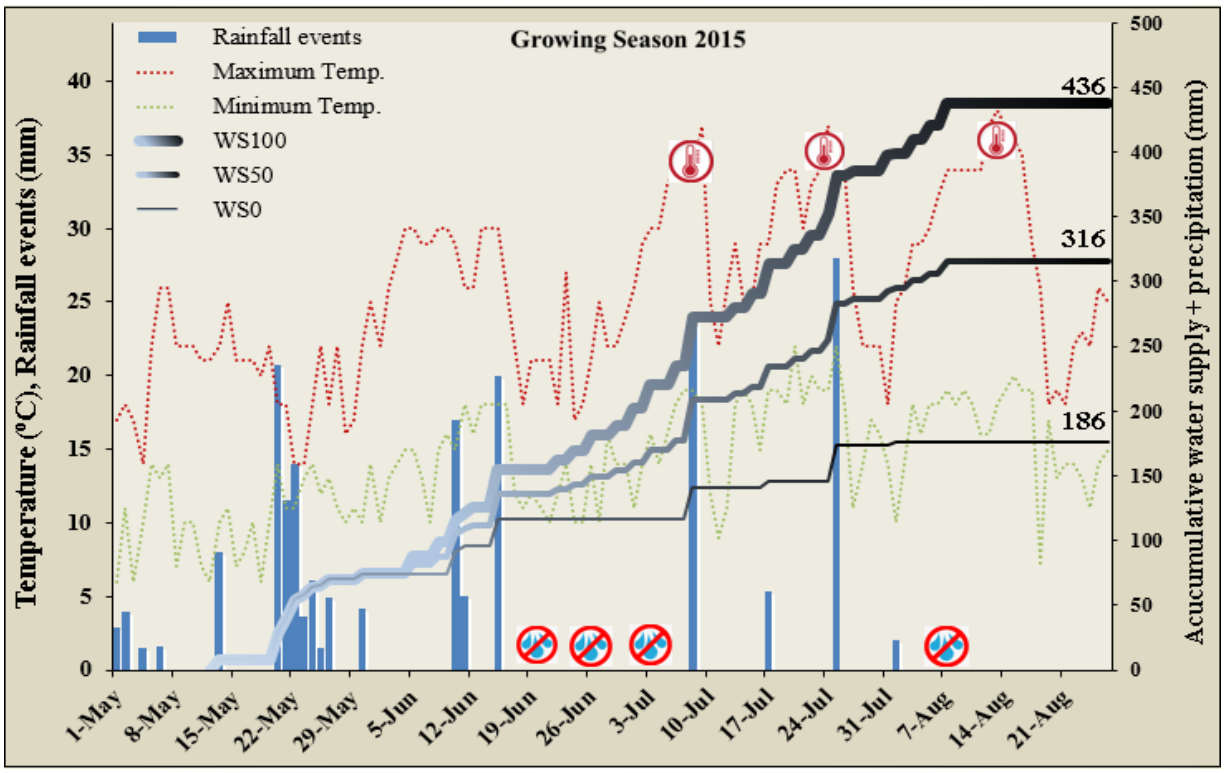

Figure 14. Daily temperature, precipitation, and accumulative water supply amount in growing season 2015.

The first month of the second growing season (2016) experienced regular with sufficient amount of rain, therefore water supply was started after 5 weeks from transplanting. Throughout the growing season precipitations supplied the experimental field with 296 mm of rain with couple heavy rain events in the middle of July. Water supply resulted in 480 mm in WS100 and 388 mm in WS50 including 296 mm of rain, while WS0 block received only 296 mm of rainfall (Figure 15.). Throughout the second growing season field capacity was ranging between 84-108%, 60-76%, and 52-68% calculated depending on the volumetric water content (21-27, 15-19, and 13-17%) in WS100, WS50, and WS0 blocks respectively. Both high precipitation level during the growing season 2016, and the high water holding capacity of the soil compared to the previous season did not allow plants to face real water stress neither in no water supply nor in deficit water supply. The three weeks of no rain in mid growing season (Figure 15.) did cause a mild stress in unirrigated plants depending on plants leaf water potential, thus 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.

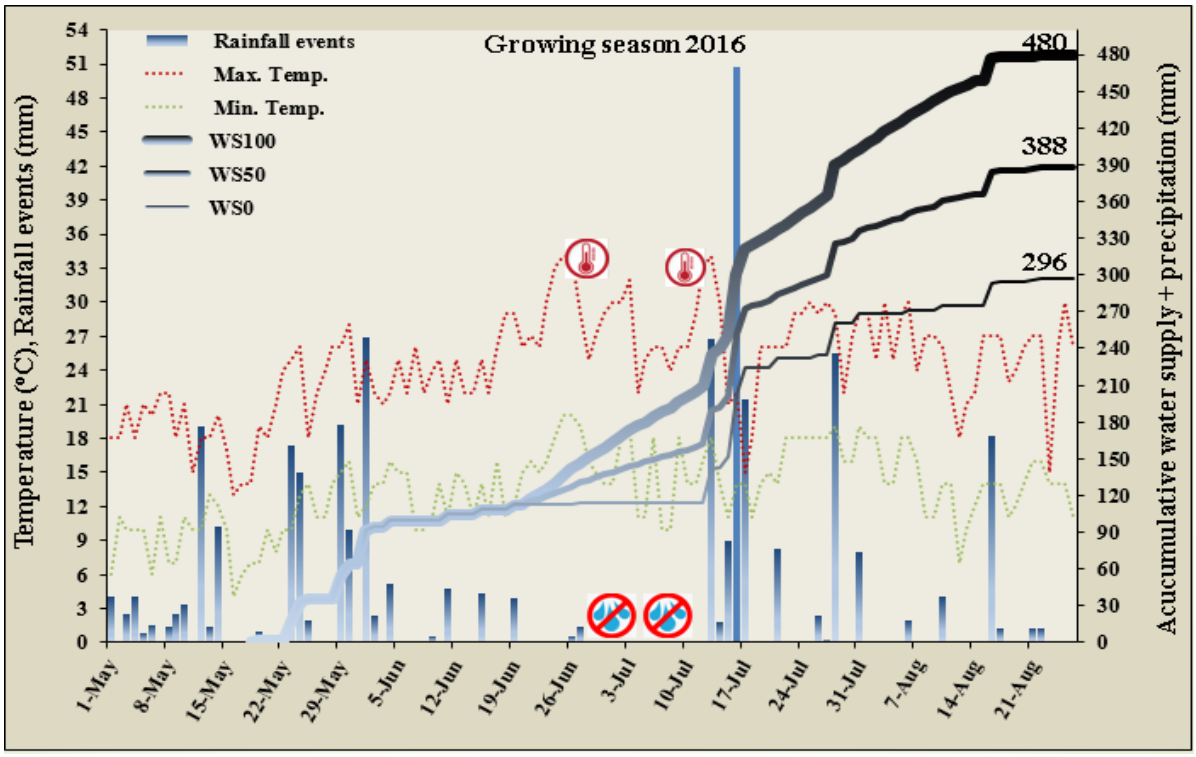

Figure 15. Daily temperature, precipitation, and accumulative water supply amount in growing season 2016.

4.2. Mycorrhizal development and rhizosphere microbial activity

The natural presence of AMF in the soil, and the negative impact of pesticides on the non-targeted organisms challenge producing non-mycorrhizal plants under field conditions. In these field studies, long term bar fallow was not able to isolate the mycorrhization totally (Douds et al., 2011). Moreover, molecular analyses have not been used to determine whether the positive effects were related to the native AMF or the commercial strains, but the growth of mycorrhizal inoculated plants under different environmental stressors can be used to measure AMF inoculation efficiency (Ruiz-Lozano et al., 1995). Incompatible to what was surveyed by Lekberg and Koide (2005), usual agricultural practices such as bare fallow and winter tillage followed in the experimental field could not decrease colonization potential in the soil and relatively high colonization rate by the endogenous mycorrhizal fungi in Control plant roots was registered (Table 3).

Using the wet sieving technique from many samples taken randomly from the upper 20 cm of the field experiments soils, we estimated 3 to 4 spores in each gram soil in the first season and 4 to 5 spores per gram soil in the second growing season. The relatively high root colonization levels in control plants is due to natural occurrence of AMF in most of the agricultural soils in Hungary, where Glomeraceae family is dominating the mycorrhizal community (Magurno et al., 2015). Although, the existence number of endogenous AMF spores were not high, but the hyphal networks and fragments of colonized roots in the soil playing an important role in colonizing new cultivated plants even when the field has been left bore for several years (Smith & Read, 2008).

Root colonization estimated at harvest, which was similar to colonization rate in a previous study (Candido et al., 2015), and much higher than that of Subramanian and co-workers (2006) who recorded only (5%) in non-inoculated and (45-50%) in inoculated roots after one month of inducing plants to different drought intensities. The field mycorrhizal inoculation did increase the root colonization in inoculated plants significantly with no effects of water supply levels on mycorrhizal colonization, indicating high adaptation ability of arbuscular mycorrhizae strains introduced to the field; the ability of AMF strains to colonize plant roots differs due to their functional and physiological characteristics (Fitter, 2005).

Table 3. 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.

Despite the high colonization in control plants, mycorrhizal inoculation improved growth, water use efficiency, stomatal conductance, photosynthetic efficiency, and leaf water potential in AM++ plants, this may be related to the displacement of native AMF assemblage by potential invasive AMF especially under dry condition (Symanczik et al., 2015). Thus, proves better efficiency of exogenous mycorrhizae inoculated, in enhancing root water uptake, since fungal species differ in their effectiveness to enhance plant water uptake (Baum et al., 2015).

In the first season, fluorescein diacetate (FDA) hydrolysis indicated higher soil microbial activity in the rhizosphere of inoculated plants under no water supply block with a slight increase not reaching significant levels under deficit water supply block; higher microbial activity in mycorrhizosphere under drier soil conditions (Table 3), may related to the positive interaction between the AMF and several microorganisms including plant-growth promoting bacteria (Kasim et al., 2013), phosphate-solubilizing microorganisms, and AMF mycelial net ability to mineralize, solubilize, and transport nutrient complexes to the root (Owen et al., 2015). Unlike 2015, in 2016 season mycorrhizal inoculation did not enhance the

microbial activity in the mycorhizosphere (Table 3); the only explanation is the high microbial activity (from 1.04 to 1.14) in all inoculated and non-inoculated treatments, and at all water level compared to results in 2015 growing season (from 0.62 to 0.85) with the same commercial inocula and under similar water supply intensities.

Relative field mycorrhizal contribution (RFMC %) to root colonization positively affected the biomass production at all water supply levels (Table 3) reaching (42%) in WS50 water regime and for 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.

4.3. Quantitative parameters of tomato fruits

4.3.1. Total and rotten non-marketable fruits

Irrespective of mycorrhizal inoculation and water supply levels, in general fruit production was higher in growing season 2016 compared to growing season 2015, mostly related to higher capacity in holding water of the second season soil, higher precipitation and cooler season in 2016 (Figure 15.), and higher soil microbial activity (Table3); resulting in better performance of physiological processes.

Table 4. Total yield (t ha^{-1}), rotten fruits (t ha^{-1}), and rotten/total yield ratio (%)

Water supply	Mycorrhizal Inoculation	Total Yield (t ha^{-1})		Rotten fruits (t ha^{-1})		Rotten/Total %	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
WS0	Control	19.8^{Aa} ±4	114.1^{Aa} ±10	1.1^{Ba} ±0.4	43^{Ba} ±03	5.5^{Ba} ±2	38^{Ba} ±2
	AM++	21.2^{Ba} ±1	116.5^{Ba} ±08	0.8^{Aa} ±0.2	36^{Aa} ±11	3.8^{Aa} ±1	31^{Aa} ±8
WS50	Control	68.1^{Ab} ±2	121.4^{Aa} ±15	7.9^{Bb} ±3.1	42^{Aa} ±11	12^{Bb} ±4	35^{Ba} ±6
	AM++	110.8^{Bc} ±4	167.0^{Bc} ±03	5.4^{Ab} ±1.7	48^{Ba} ±04	4.8^{Aa} ±2	29^{Aa} ±2
WS100	Control	87.0^{Bc} ±3	129.9^{Aa} ±15	15.7^{Bc} ±3.4	42^{Ba} ±15	18^{Bb} ±1	33^{Ba} ±13
	AM++	89.7^{Bb} ±3	136.4^{Bb} ±02	11.6^{Ac} ±3.3	40^{Aa} ±07	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 (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, small letters represent water supply effect.

In 2015, 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 (Table 4), 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 4). 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.

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 4). The fact that in 2016 AM++ plants in WS50 gave the highest yield (167 t ha^{-1}) may explain also higher rotten fruits (Figure 16.). For better understanding of how mycorrhizal inoculation lessened the rotten fruits, the ratio of rotten to total yield is calculated proportionally [(rotten fruit t ha^{-1}/ total yield t ha^{-1})/100]. In general, high percent of the total yield has rotten due to last season heavy rains during the ripening period in 2016 compared to 2015 growing season (Table 4). Mycorrhizal inoculation affected fruit quality positively including less rotten fruits in both seasons and at all water supply levels; better calcium uptake by AM++ plants led to better translocation of Ca^{+2} into the fruits, lessened blossom-end rot disorder, and minimized losses due to fruit cracking. In previous studies (Shafshak & Winsor, 1964), liming resulted in firmer fruits, and an adequate (above 0.12%) calcium concentration in fruits prevented the blossom-end rot disorder (Atherton & Rudich, 1986).

Figure 16. Blossom-end rot disorder.
(Photo by BAKR, 2016)

4.3.2. Marketable fruits

In non-inoculated plants, water supply did increase marketable fruits in 2015 season 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 (Figure 17.), while these differences were not reaching significant levels statistically. In the 2015 season, 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 growing season 2016.

Mycorrhizal contribution to marketable fruit production was the best under deficit water supply exceeding that obtained by Candido and co-workers (2015), who used a single inoculum *G. mosseae* GP11, and recorded an increase of about 11%, and also results exceeding by Bowles et al. (2016), who registered an increase of 28% in mycorrhizal tomatoes on a very fine sandy loam field under deficit water condition.

As it is mentioned previously (see 4.1. Water stress induction and soil water condition) in 2015 due to the low holding water capacity of the soil and the lack of precipitation, plants faced severe water stress and irrespective of mycorrhizal inoculation unirrigated plants could supply only 20% of the potential fruit biomass. Unlike the first season, in 2016 the loamy texture of the soil and its high water holding capacity allowed only a moderate water stress in no water supply plots and Control plants gave 65.3 t ha^{-1}, while mycorrhizal inoculation did increase the marketable fruits by 10% (Figure 17.).

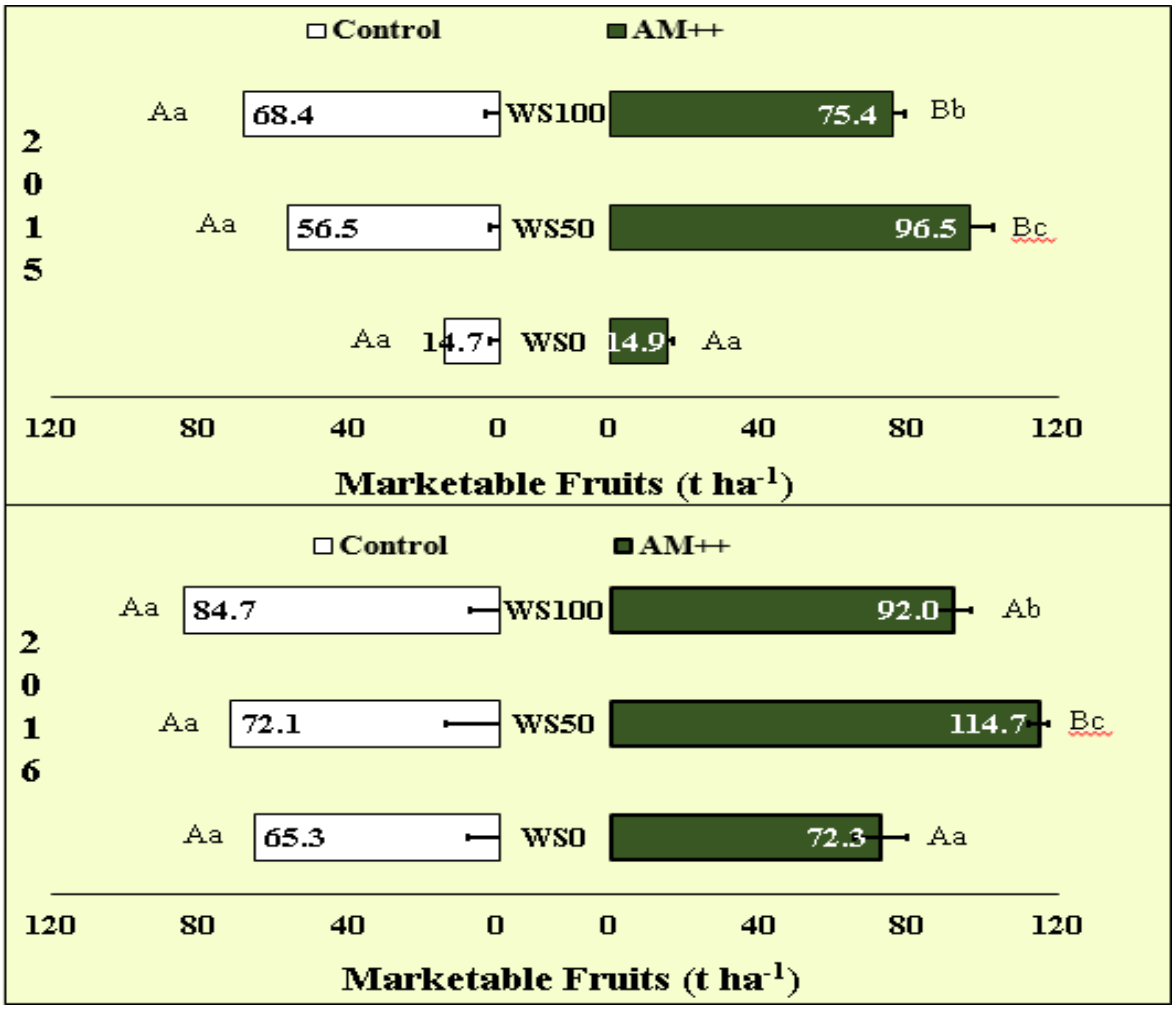

Figure 17. Marketable fruits (t ha^{-1}), of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

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.

4.4. Qualitative parameters of tomato fruits

4.4.1. 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 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 18.). The loss of four units of ºBrix in first season, and a half unit in the second season (Table 5), is due to the fact that, fruits sugar content can be increased by soil moisture reduction (Atherton & Rudich, 1986), and the mycorrhizal contribution in enhancing soluble solid is related to the enhancement of nutrient uptake especially the nitrogen, which also found to increase the sugar content in fruits (Atherton & Rudich, 1986). Despite losses in soluble solid content in non-inoculated plants, yield increase decompensated this loss and soluble solid was increased as a mass production per area, while this trend was more pronounced in fruits of AM++ plants with very strong positive relations (r = 0.87), (r = 0.83) in both 2015 and 2016 growing seasons respectively (Figure 19.).

Table 5. ºBrix (g /100g), and soluble solid production (t ha^{-1}) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	ºBrix (g /100g)		Soluble Solid (t ha^{-1})	
		2015	**2016**	**2015**	**2016**
WS0	Control	8.03^{Ac} ±0.3	3.65^{Aa} ±0.1	1.18^{Aa} ±0.2	2.39^{Aa} ±0.4
	AM++	8.20^{Bb} ±0.4	4.10^{Ba} ±0.2	1.22^{Aa} ±0.1	2.97^{Aa} ±0.4
WS50	Control	5.03^{Bb} ±0.4	4.45^{Ab} ±0.4	2.83^{Aa} ±0.2	3.22^{Aa} ±0.8
	AM++	3.88^{Aa} ±0.1	4.15^{Ba} ±0.7	3.74^{Bb} ±0.3	4.77^{Ba} ±0.9
WS100	Control	3.73^{Ba} ±0.2	3.20^{Aa} ±0.2	2.55^{Aa} ±0.2	2.71^{Aa} ±0.3
	AM++	3.45^{Aa} ±0.3	3.38^{Ba} ±0.1	2.59^{Ba} ±0.2	3.11^{Ba} ±0.2
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)					
Mycorrhizal Inoculation (AM++)		**	***	**	ns
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.

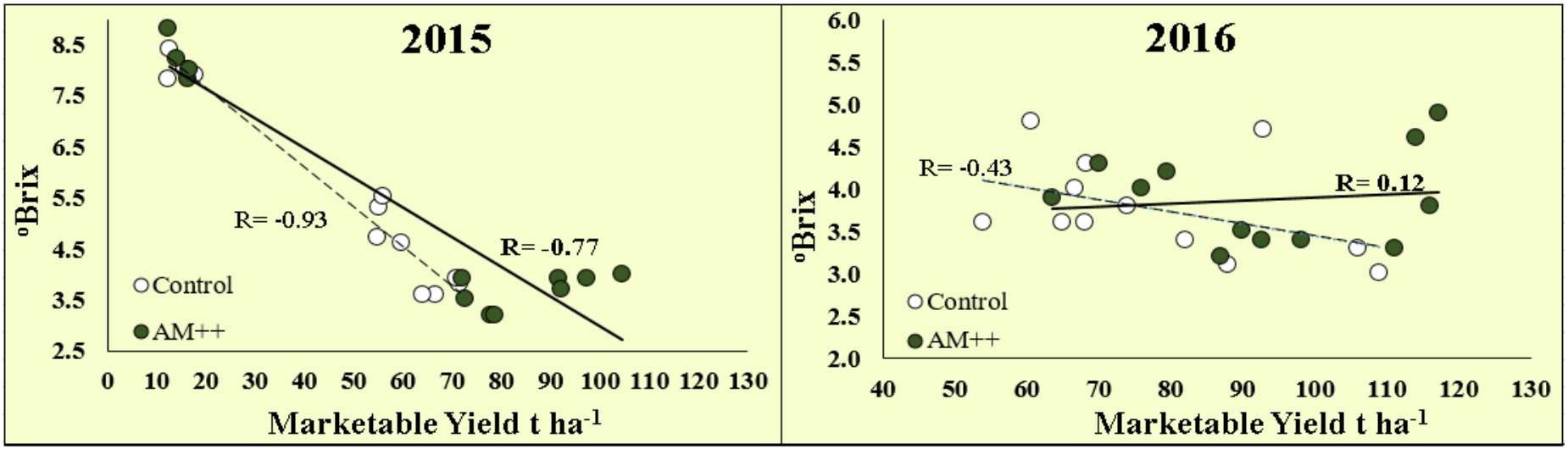

Figure 18. Fruit °Brix content (g per 100 g), and marketable yield (t ha^{-1}) relationship of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

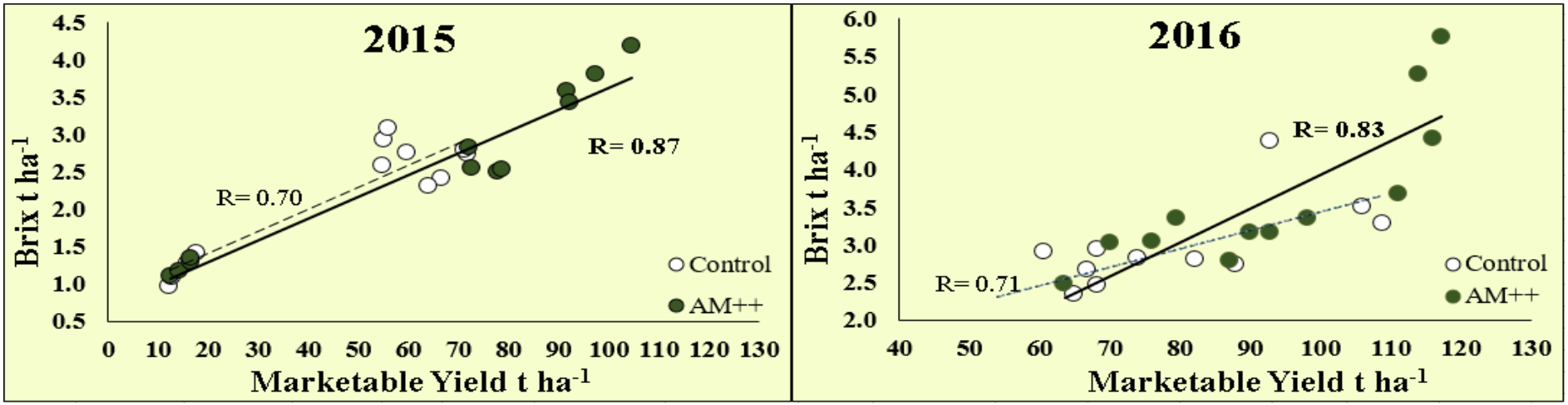

Figure 19. Soluble solid yield (t ha^{-1}), and marketable yield (t ha^{-1}) relationship of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

4.4.2. Carotenoids and ascorbic acid

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. The HPLC profile and spectrum of the main carotenoids showed in Figure 20.

Due to their nutritional importance and biological activities, here we are focusing on β-carotene, lycopene, and total carotenoids.

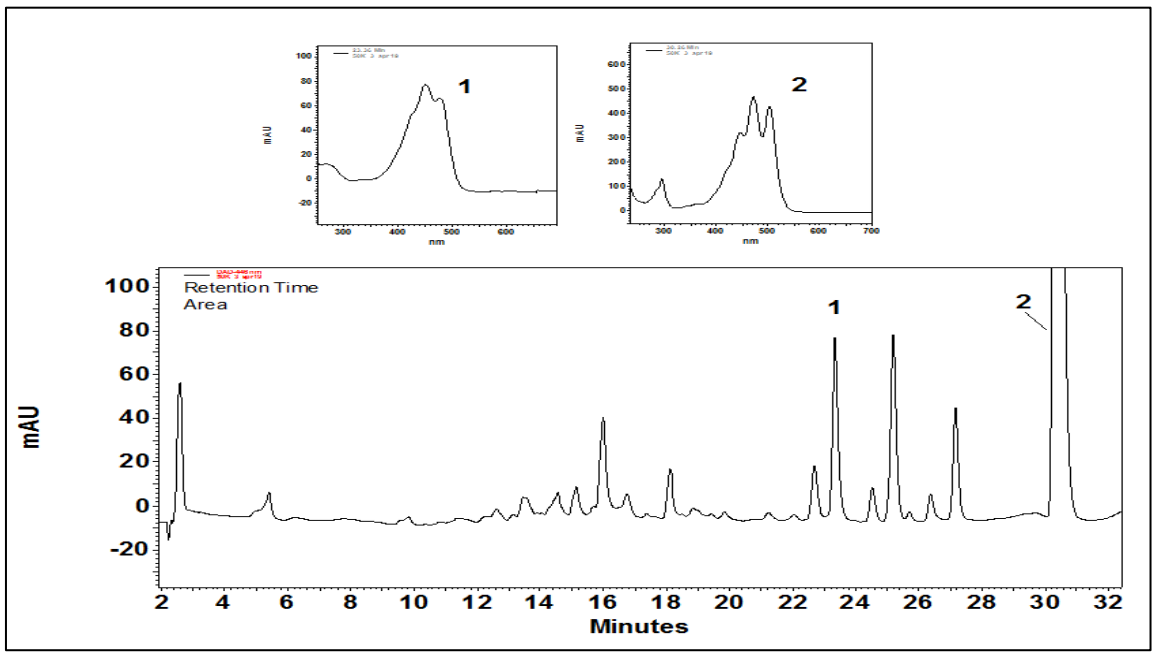

Figure 20. HPLC separation of tomato carotenoids on core C30 column with gradient elution of TBME in Methanol. Peaks: 1: β-carotene, 2: Lycopene.

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 6; Table 7), but higher yield overcame the concentration loss by higher production of antioxidants per unit area (Table 8). More antioxidants accumulation along gradients of the water supply reduction could be due the abiotic factors such as water supply, temperature, and light that affect the natural composition of the antioxidants in tomato fruits (Pék et al., 2014; Helyes et al., 2006a)

Pigments accumulate during the ripening process (Pék et al., 2010; Helyes et al., 2006b), and lycopene synthesis is inhibited above 30°C (Tomes, 1963), explaining the low content of lycopene in tomato fruits in 2015 season, when plants experienced temperatures above 30°C for 2 weeks (Figure 14.), but not in no water supply WS0 block, where plants harvested two weeks earlier and experienced relatively cooler temperatures during the ripening period in agreement with the explanation of Dumas et al. (2003).

In addition to water supply and inoculation factors, seasonal variation played an important role in lycopene accumulation in fruits. Regardless of inoculation and water supply, lycopene content was ranging from 49.0 to 113.4 mg g^{-1}, lower that the range (97.7 to 155 mg g^{-1}) recorded in five different processing cultivars in Hungary (Helyes et al., 2006a). 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 6). Thus, counts for the pedoclimate (Soil- water, -aeration, and -temperature) role and its positive effect on the plant growth performance in general and more particularly on the mycorrhizal efficiency.

Table 6. Total Carotene (µg g^{-1}), and lycopene (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})	
		2015	**2016**	**2015**	**2016**
WS0	Control	136.3^{Bb} ±1.3	313^{Ac} ±32	100.1^{Bb} ±2	205^{Bb} ±10
	AM++	146.5^{Bb} ±15	282^{Aa} ±32	113.4^{Bc} ±12	165^{Aa} ±23
WS50	Control	106.3^{Ba} ±7.7	233^{Ab} ±26	72.0^{Ba} ±6	188^{Ab} ±26
	AM++	90.75^{Aa} ±11	281^{Aa} ±42	67.5^{Ab} ±9	185^{Ab} ±23
WS100	Control	94.27^{Aa} ±19	181^{Aa} ±11	66.1^{Ba} ±7	95^{Aa} ±19
	AM++	80.29^{Aa} ±4.6	437^{Bb} ±50	49.0^{Aa} ±1	273^{Bb} ±30
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		***	***	***	***

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.

The same is true for total carotenes, since Lycopene forms about 83% of all carotenoids according to (López et al., 2001). 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; mainly because of the cooler days (Figure 15.) especially during the ripening period Dumas et al. (2003) and the pedoclimate differences of the experimental sites. In non-inoculated Control plants, decreasing water supply amount positively affected and increased significantly the total carotenoids concentration in marketable fruits, but this enhancement accompanied by the yield reduction (from 68.4 to 56.5 and 14.7 t ha^{-1}) in 2015 and (from 84.7 to 72.1 and 65.3 t ha^{-1}) in 2016, the same is true for pre-transplant inoculated (AM+) plants (***Appendices 3 &4***).

Mycorrhizal inoculation could preserve higher antioxidants (Table 6; Table 7), and this trend was better pronounced in the second season when mycorrhizal inoculation did increased the total carotenoids by 2.5 folds, and tripled both lycopene and β-Carotene content in ripened fruits in WS100 compared to Control plants. In general, in 2015 mycorrhizal inoculation could preserve the antioxidants, but we could not meet both high yield accompanied with high nutrient content. In 2016 along with the yield increase antioxidants in fruits increased too (Table 6; Table 7), which is highly likely related to the enhanced growth and nutrients acquisition in comparable with what was reported by Ulrichs et al. (2008).

Table 7. β-Carotene (µg g^{-1}), and ascorbic acid (µg g^{-1}) in fruits of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	β-Carotene (µg g^{-1})		Ascorbic acid (µg g^{-1})	
		2015	**2016**	**2015**	**2016**
WS0	Control	2.63^{Ba} ±.22	13.5^{Bc} ±2	330^{Ab} ±30	331^{Aa} ±23
	AM++	3.24^{Bb} ±.36	9.7^{Aa} ±1	361^{Ab} ±12	293^{Aa} ±61
WS50	Control	2.23^{Aa} ±.42	10.1^{Ab} ±1	286^{Aa} ±26	418^{Aa} ±39
	AM++	1.89^{Aa} ±.88	9.7^{Aa} ±.2	304^{Aa} ±07	374^{Ab} ±45
WS100	Control	2.42^{Aa} ±.30	5.4^{Aa} ±1	273^{Aa} ±14	334^{Aa} ±74
	AM++	2.63^{Ba} ±.22	17.2^{Bb} ±4	273^{Aa} ±31	236^{Aa} ±72
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)					
Mycorrhizal Inoculation (AM++)		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.

Table 8. Total carotene (kg ha^{-1}), lycopene (kg ha^{-1}), and β-Carotene (g ha^{-1}) of non-inoculated (Control), and field inoculated (AM++) plants.

Water supply	Mycorrhizal Inoculation	Total Carotene (kg ha^{-1})		Lycopene (kg ha^{-1})		β-Carotene (**g** ha^{-1})	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**
WS0	Control	2.00^{Aa} ±0.3	20.46^{Aa} ±3.8	1.47^{Aa} ±0.24	13.33^{Aa} ±1.42	38.5^{Aa} ±6.5	881^{Ab} ±181
	AM++	2.18^{Aa} ±0.3	20.23^{Aa} ±1.4	1.68^{Aa} ±0.22	11.87^{Aa} ±1.40	48.7^{Aa} ±9.9	697^{Aa} ±86
WS50	Control	6.01^{Ab} ±0.7	16.84^{Aa} ±3.7	4.07^{Ab} ±0.49	13.59^{Aa} ±3.18	126.0^{Ab} ±25	726^{Aa} ±166
	AM++	8.71^{Bb} ±0.6	32.19^{Bb} ±4.4	6.48^{Bb} ±0.50	21.20^{Bb} ±2.41	182.2^{Bb} ±19	1111^{Bb} ±49
WS100	Control	6.46^{Ab} ±1.4	15.51^{Aa} ±5.5	4.53^{Ab} ±0.62	8.13^{Aa} ±3.40	165.3^{Ab} ±28	452^{Aa} ±118
	AM++	6.05^{Ab} ±0.4	40.33^{Bc} ±5.6	3.70^{Ab} ±0.24	25.15^{Bb} ±3.31	223.0^{Bb} ±52	1589^{Bc} ±383
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)							
Mycorrhizal Inoculation (AM++)		*	***	*	***	**	***
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, small letters represent water supply effect.

The same as for carotenoids, HPLC method was used to separate the ascorbic acid on Nucleosil C18 aqua (Nautilus) column with the gradient elution of Acetonitrile in 0.01M KH_2PO_4 solution (Figure 21.).

Despite slight changes between treatments and within the same treatment, ascorbic acid contents in marketable fruits have not shown a clear trend. The ascorbic acid content in fruits was slightly lower in the first season (273 to 361 mg g^{-1}) compared to the content range (236 to 418 mg g^{-1}) in the second growing season (Table 7). In general, the ascorbic acid contents were within the normal range compared to the range (286 to 446 mg g^{-1}) extracted from different processing cultivars in Hungary (Helyes et al., 2006a) and this is counted for the mycorrhizal symbioses that could preserve the normal level of ascorbic acid accompanied by yield boosting especially under water deficit conditions.

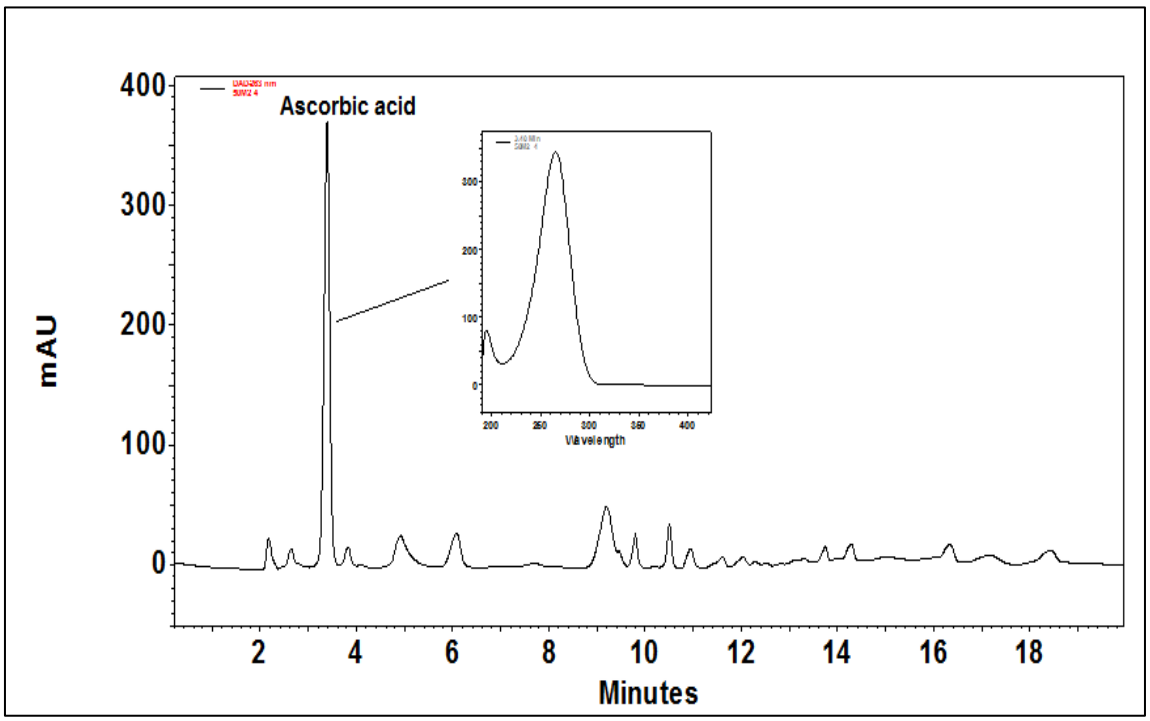

Figure 21. HPLC Separation of Vitamin C on Nucleosil C18 aqua (Nautilus) column.

4.5. Physiological response to mycorrhizal inoculation

4.5.1. 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 9); supporting a previous report by Jezdinsky et al. (2012) on sweet pepper.

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 9), leading to plant growth improvement (Jezdinsky et al., 2012), and indicating less damage to photosynthetic apparatus in AM++ plants under water stress condition (Bárzana et al., 2012). 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.

Table 9. 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	$0.66^{Aa} \pm .05$	$0.74^{Aa} \pm .02$	$47.0^{Aa} \pm 0.5$	$55.6^{Ab} \pm 0.8$
	AM++	$0.74^{Ba} \pm .03$	$0.75^{Aa} \pm .03$	$48.6^{Aa} \pm 2.6$	$55.4^{Ac} \pm 0.6$
WS50	Control	$0.75^{Ab} \pm .02$	$0.74^{Aa} \pm .02$	$46.4^{Aa} \pm 0.7$	$54.0^{Ab} \pm 1.3$
	AM++	$0.78^{Bb} \pm .03$	$0.77^{Bb} \pm .03$	$48.5^{Ba} \pm 1.5$	$53.3^{Ab} \pm 1.5$
WS100	Control	$0.75^{Ab} \pm .04$	$0.76^{Aa} \pm .02$	$47.0^{Aa} \pm 1.7$	$49.8^{Aa} \pm 1.2$
	AM++	$0.77^{Bb} \pm .01$	$0.74^{Aa} \pm .02$	$47.7^{Aa} \pm 1.9$	$50.7^{Aa} \pm 0.5$
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.

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 9); different from results by Zhang et al. (2014), who reported higher chlorophyll content in inoculated plants. Enhancements in relative chlorophyll content accompanied by photosynthetic efficiency improvement. Readings of SPAD significantly correlated with Fv/Fm ratio (Etsushi et al., 2009). 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 9).

Water supply reduction increased the chlorophyll content in leaves in both WS0 and WS50 watering levels, thus should not contradict the previous season outcome since water stress induction has not caused severe stress in WS0 and WS50 blocks in growing season 2016 explaining unclear trend in some physiological aspects.

4.5.2. 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 10) with the exception of the no water supply block in the first season, where vegetative growth period shortened by two weeks due to severe stress caused by decreasing the soil moisture content (see 4.1. Water stress induction and soil water condition). 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 10). The great improvement in the growth performance by mycorrhizal inoculation under moderate deficit water condition, is related to the enhancement of nutrient uptake especially phosphorus (Figure 25.) (Smith & Read, 2008), and the best photosynthetic efficiency of photosystem II (Table 9) which is also reported by Birhane and co-workers in (2012).

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 10). 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; in agreement with previous reports (Candido et al., 2015; Patanè et al., 2014) on processing tomato under field conditions. More efficient water use, is related to better ability of mycorrhizal roots of increasing the water uptake, that possibly related to better ability of mycorrhizal roots to switch between water-transport pathways in response to water deficit stress (Bárzana et al., 2012), higher root hydraulic conductivity (Koide, 1985), and better regulation of the stomata closure especially under water deficit condition (Table11).

Moreover, the hyphae of arbuscular mycorrhizae fungi produce glomalin (Rillig et al., 2001); its production is proposed as a mechanism by AMF to promote the growth of their partner plant in the natural habitat (Rillig & Steinberg, 2002). Glomalin is a glycoprotein that binds nitrogen, carbon, and several other biological elements in the soil to the minerals, and soil particles enhancing the carbon reserve in the soil and the organic matter as well (Six et al., 2000), leading to a meaningful improvement in the soil texture and the soil water relations (Bethlenfalvay & Shuepp, 1994).

Table 10. 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.

4.5.3. Leaf water potential

Leaf water potential (Ψ_L) is the most important index of plant water status showing its potential to resist drought through better water uptake and/or better hydration.

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 22). 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, and pre-transplant inoculated (AM+) plants (***Appendices 5 & 6***) 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 22.).

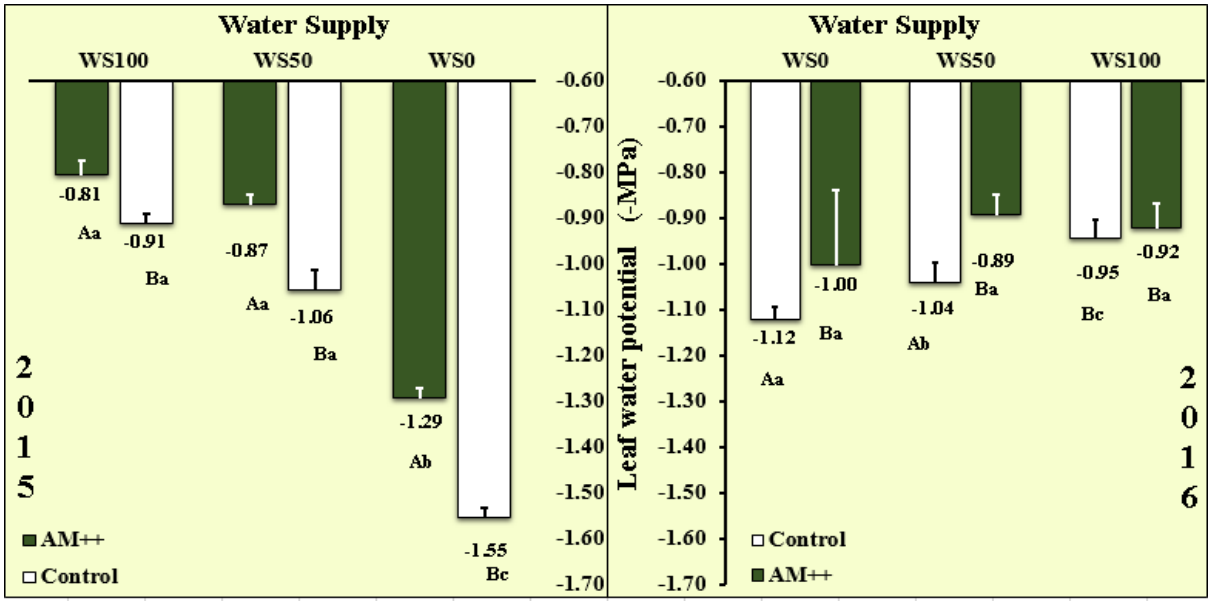

Figure 22. Leaf water potential (-MPa) of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

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.

Our results are confirming recent reports on maize and tomatoes (Bárzana et al., 2012), on snapdragon (Asrar et al., 2010), and soybean (Porcel and Ruiz-Lozano, 2004), illustrated higher leaf water potential in host plants colonized by arbuscular mycorrhizal fungi, and contradicting many previous studies surveyed by Augé (2001) indicated no changes in leaf water potential due to mycorrhizal inoculation. Different results from previous studies probably are due growth conditions (mostly pot-based in optimized environment in greenhouses or growth chambers), and the diversity of study designs.

4.5.4. Stomatal conductance and canopy temperature

Plants regulate the water loss and carbon dioxide assimilation for photosynthesis by the stomata on the leaves, making stomatal conductance an important indicator of water status in plants. Stomatal conductance is in a positive significant relationship with the rate of transpiration. 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 11); the high seasonal precipitation was exceeding plants water requirements in some periods of the growing season in 2016 especially after heavy rains.

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 in WS50 block (Table 11).

In a meta-analysis from hundreds of studies (in greenhouse, growth chamber, and field) published over past decades Augé and co-workers (2015) conducted a quantitative review and concluded higher stomatal conductance in mycorrhizal plants compared to non-mycorrhizal plants under all soil moisture conditions, with greater inoculation effect under moderate drought stress. Moreover, results from the meta-analysis finalized the best AMF contribution to stomatal conductance enhancement under severe water deficit stress, in the contrary our results showed no contribution of mycorrhizal inoculation under severe stress (Table 11).

Table 11. Stomatal conductance (mmol m^{-2} s^{-1}), and canopy temperature (°C) 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 (°C)	
		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.

Being very sensitive to abiotic variables (light, and temperature), stomatal conductance can change within seconds and modify mycorrhizal induced changes in stomatal conductance (Augé et al., 2004). In our study, the stomatal conductance measurements have been taken within three consecutive weeks from the middle of June and the first week of July, when the daily temperature were ranging between 25-30°C and the light intensities were higher than (300 μmolm^{-2} s^{-1}), which are necessary to avoid any unexpected changes in the pyrometer readings. Further support is the leaf water potential of severe stress plants in 2015 in no water supply block (Ψ_L decreased by 70%) and the high organic osmolyte proline (Table 12) in addition to the very strong negative linear correlation (r= -97) between the stomatal conductance and the canopy temperature (Figure 23.).

Canopy temperature has changed reversely to stomatal conductance in both seasons; it correlated very strongly and negatively (r = -0.97) with the stomata conductance in 2015 season, and related to stomatal conductance in a weak negative (r = - 0.31) relationship with stomatal conductance (Figure 23.) in 2016 growing season.

The gradual decrease in canopy temperature in Control plant stands along with water supply increasing in 2015; from 34.1ºC in WS0 to 30.6ºC in WS50 and 28ºC in WS100, and more efficient decrease (from 30.6 to 29.0 ºC) under WS50 due to mycorrhizal inoculation (Table 11), emphasizing the finding by (Yu et al., 2015), being the canopy temperature inversely proportional to stomatal conductance. Stomatal conductance determines vapour loss and CO_2 assimilation in plant leaves, and along with canopy temperature have a direct influence on hydration, photosynthesis, and biomass production. Same as photosynthetic efficiency did change only in deficit water supply WS50 blocks due to mycorrhizal inoculation.

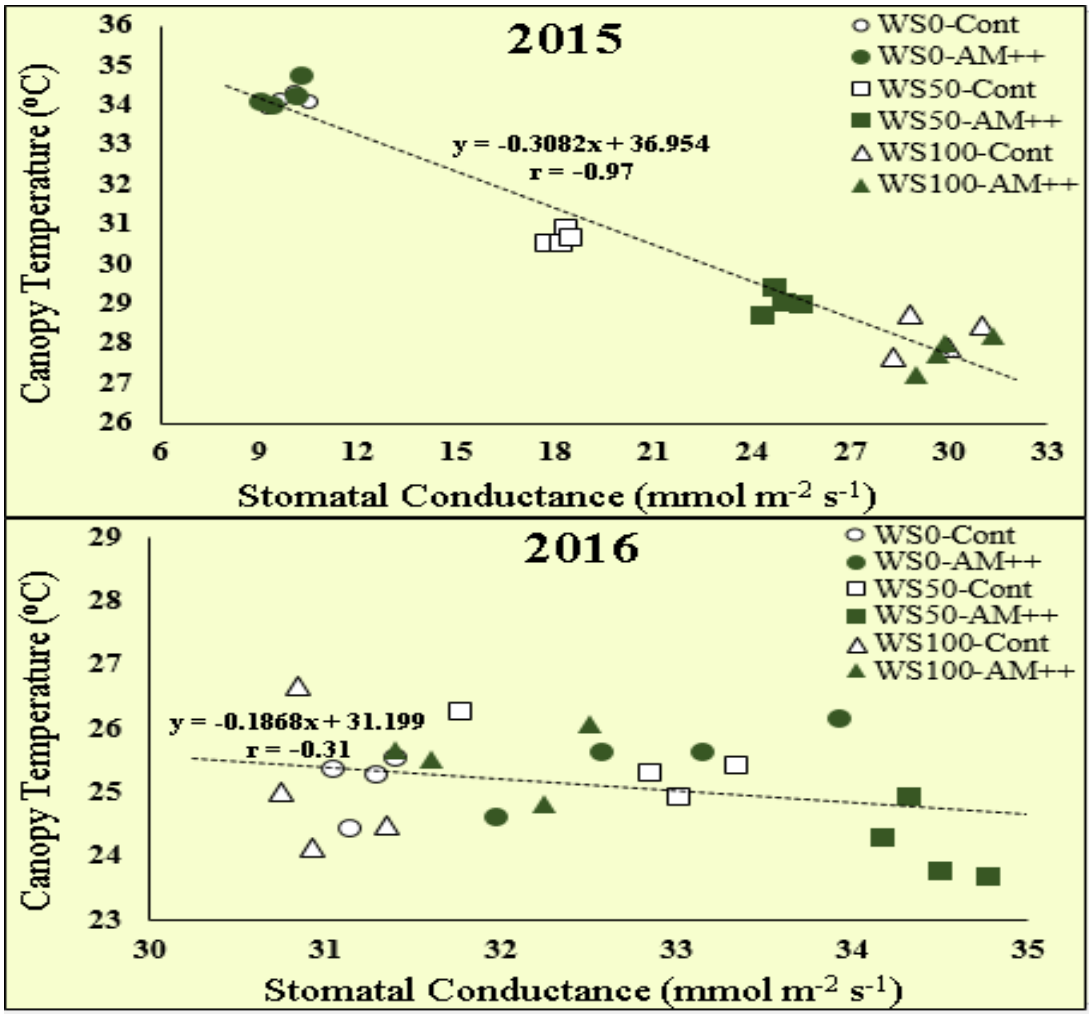

Figure 23. Canopy temperature and Stomatal conductance relationship of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

4.5.5. 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. In both seasons, the concentration of the phosphorus in shoot was negatively affected by the water supply; in non-inoculated Control plants, the P concentration in shoots decreased by 8% in WS50, and further reduced by about 25% when plants fully irrigated in WS100 compared to plant not irrigated in WS0 (Figure 24.). To explain this result, we have to recall the data for the total yield, since leaflets were taken for these element measurement at fruit setting and plants translocate elements and pump them for fruit setting; decreasing fruit setting (total yield Table 4) along water reduction from (87 t ha^{-1}) in WS100, to (68 t ha^{-1}) in WS50, then to (20 t ha^{-1}) in WS0 in 2015 followed by a reduction from (130 t ha^{-1}) in WS100, to (121 t ha^{-1}) in WS50, then to (114 t ha^{-1}) in WS0 in 2016 growing season explains higher P content in plants that produced less yield. In the first season, mycorrhizal inoculation did enhance the P shoot concentration in WS50, higher efficiency of mycorrhizal symbiosis under deficit water condition with no change under optimum water supply in WS100, in agreement with results by Conversa et al. (2013) in 2 years field experiment on processing tomato.

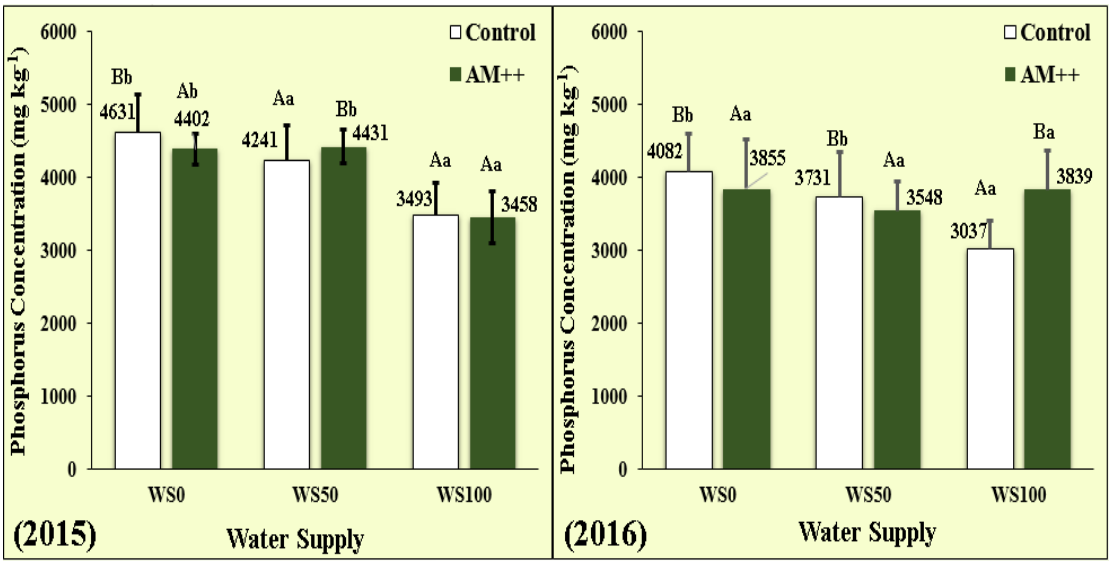

Figure 24. Phosphorus concentration (mg kg^{-1}) in plant shoots of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

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.

Unlikely in the second season, lower P content in plant shoots in WS50 block, may be related to the fact that in 2016 season plants in WS50 block were only slightly stressed (depending on Ψ_L), in addition to P depletion in plant shoots (Krishnakumar et al., 2013) due to the highest fruit setting (Table 4. total yield 167 t ha^{-1}).

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 25.) in 2015. Higher contribution of the field mycorrhizal inoculation to the total P uptake compared to control plants is possibly related to the plant and fungal species dependency; in tomato plants, *G. mosseae* was less effective compared to *G. intraradices* that contributed by almost 100% of the P uptake by host plant roots (Bücking et al., 2012).

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 (Figure 25.), indicating moderate water deficit the best condition for mycorrhizal inoculation to promote nutrient uptake (particularly P) in the host plants.

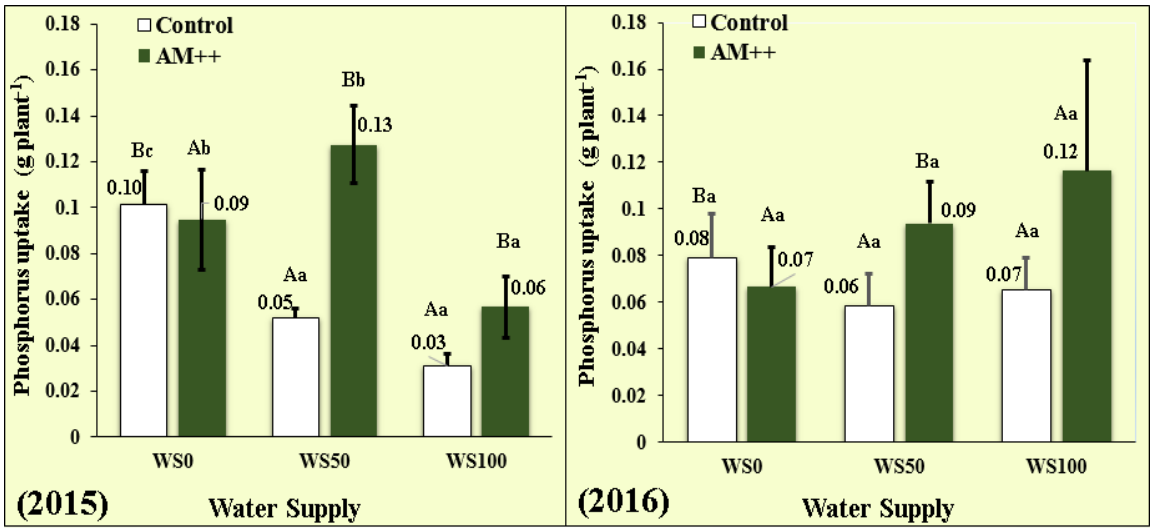

Figure 25. Phosphorus uptake (g plant^{-1}).

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.

4.5.6. Shoot organic and inorganic osmolytes

Organic solutes such as proline and soluble sugar accumulation in plant tissues under water stress conditions is an important strategy followed by plants to cope and recover from short periods of drought stress. In both seasons and regardless to mycorrhizal inoculation, Control and AM++ plants in no water supply blocks increased proline accumulation by more than two folds (Table 12) 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 (from 29.2 to 15.7 mg kg^{-1}), and (25.0 to 11.8 mg kg^{-1}) in both growing seasons respectively 2015 and 2016 (Table 12) compared to Control plants. In our study we propose that mycorrhizal inoculation ameliorated the water status of host plants, and lessened proline production, in agreement with explanation was given by Ruiz-Sánchez et al. (2010) in a pot study, showing the ability of mycorrhizal plants to avoid water stress and accumulated less osmolytes in shoots.

Table 12. Proline (mg kg^{-1}), and K^+ (mg kg^{-1}) concentrations in shoots of non-inoculated (Control), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	Proline (mg kg^{-1}) Fresh weight		K^+ (mg kg^{-1}) Dry weight	
		2015	**2016**	**2015**	**2016**
WS0	Control	84.8^{Bb} ±2	37.4^{Ba} ±9	35852^{Bb}	23614^{Ba}
	AM++	76.2^{Ac} ±5	29.1^{Ab} ±9	28215^{Aa}	22588^{Ab}
WS50	Control	29.2^{Ba} ±2	25.0^{Ba} ±9	32319^{Bb}	21778^{Aa}
	AM++	15.7^{Aa} ±1	11.8^{Aa} ±4	30344^{Ab}	24301^{Bb}
WS100	Control	32.6^{Aa} ±7	15.6^{Ba} ±5	23601^{Ba}	17980^{Aa}
	AM++	31.8^{Ab} ±1	13.5^{Aa} ±3	20563^{Aa}	18680^{Ba}
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		**	***	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.

These findings disagreed with reports by (Yooyongwech et al., 2013; Fan & Liu, 2011), who illustrated an increase of proline concentration in leaves of mycorrhizal plants as osmoprotectant in response to water stress conditions.

Very strong negative correlations (r = 91 in 2015, and r = 86 in 2016) between leaf water potential and proline content in shoots (Figure 26.) 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. Numerous studies on different plant species concluded similar results but in pot trials: Padmavathi et al. (2016) on tomato and bell pepper, Doubková et al. (2013) on field scabious, Asrar et al. (2012) on snapdragon, and Ruiz-Sánchez et al. (2010) on rice.

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 12). 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). Potassium has an important role in CO_2 assimilation and osmotic regulation in leaves of plants (Römheld & Kirkby, 2010), our results implied as for proline, less need for potassium in AM++ plants in response to water stress, due to better water uptake and more efficient use of water.

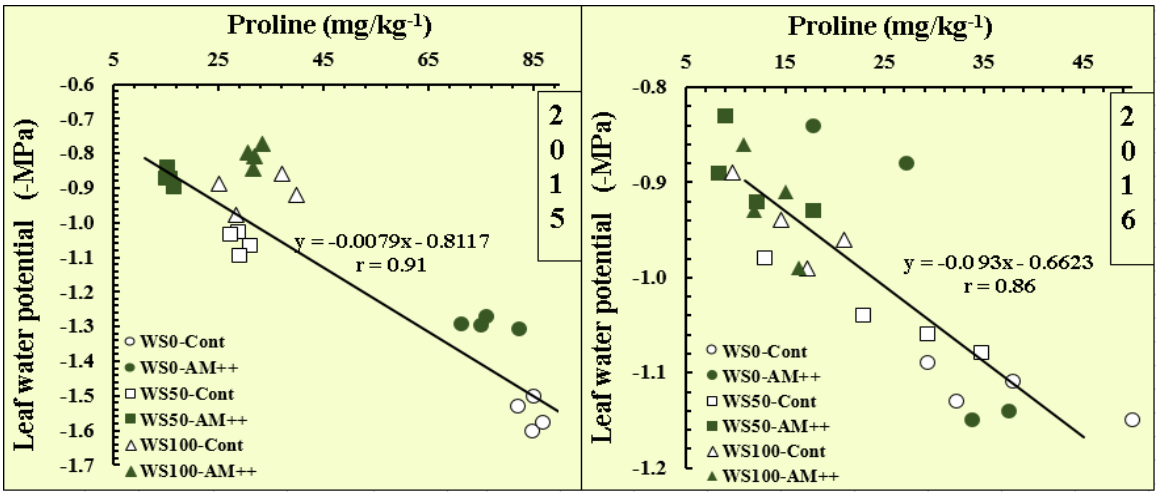

Figure 26. Leaf proline concentration and leaf water potential relationship.

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 13) emphasizing higher water stress in the first season as it was also assessed depending on leaf water potential and explained previously. 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 13); 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 (see 4.3.1. Total and rotten fruits).

Table 13. Mg^{2+} (mg kg^{-1}), and Ca^{+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	Mg^{2+} (mg kg^{-1}) Dry weight		Ca^{+2} (mg kg^{-1}) Dry weight	
		2015	**2016**	**2015**	**2016**
WS0	Control	6153^{Ba}	5081^{Aa}	34608^{Aa}	39383^{Aa}
	AM++	5810^{Aa}	6691^{Bb}	38598^{Ba}	47947^{Ba}
WS50	Control	6338^{Ba}	5372^{Ba}	41275^{Aa}	46141^{Aa}
	AM++	5199^{Aa}	4903^{Aa}	42582^{Ba}	56260^{Ba}
WS100	Control	6068^{Ba}	5374^{Aa}	48385^{Bb}	56152^{Ab}
	AM++	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 (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.

4.6. 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.

6. SUMMARY

Agriculture production is facing many problems including: limitation of the natural resources; changes in climate aspects; fertilizers expenses, limit, and negative impacts on the agro-ecosystem; abiotic and biotic stresses; degrading of the arable lands and water resources. Thus, the conventional agriculture production cannot guarantee and secure food production for the world population that grows exponentially. Water and nutrients (especially P) availability are main factors restricting the crop production, which is totally water dependent. Human activities (urbanization, population increase and higher living standards, conflicts for water, and pollution) in addition to changes in climate pattern aggravating water scarcity. Within the soil microbial populations arbuscular mycorrhizal fungi (AMF) play the strategic role in the sustainable use of natural resources namely (water, soil, and nutrition).

Many studies investigated eco-physiological aspects of this relationship and demonstrated enhancing in drought stress resistance in host plants, but mostly pot and container-based studies in greenhouses and growth chambers where the environment is optimized. Unlike pot experiments, in the field, the rhizosphere is not restricted and soil texture, structure, water and nutrient content, temperature, aeration, and chemical and microbial properties are playing important role in the efficiency of the AM symbiosis. Moreover, many other environmental factors (most important: precipitation, heat, cold, and light) are interacting which are not synergistic always. Due to the beneficial role of the AM symbiosis in the sustainable agriculture system, and their economic importance in crop production, systematic quantitative analyses are necessary to optimize the field-inoculation at transplant of AMF to get the benefit from the symbiosis in most efficient way.

In this work, the AM inoculation resulted in efficient root colonization to the same magnitude regardless of inoculation timing, field site, and water supply levels. We illustrated more efficient association of the AM symbiosis at transplant (AM++) compared to at sowing pre-transplant inoculation (AM+) depending on the relative field mycorrhizal contribution (RFMC %) to the growth; in IR50 water supply regime (the most proper water supply level) AM+ contributed by 18 and 15% to the growth, much less to that of field-inoculation at transplant AM++ contribution 42 and 25% in both growing seasons respectively. Higher RFMC, in addition to better performance of most of the physiological processes concluded more potential impact of AMF field-inoculation at transplant.

Our results supported irrefutable field-based and laboratorial evidences that AM symbiosis could not help their host plants to resist (neither avoid nor tolerate) the drought stress under sever deficit water conditions lasted for more than two weeks. In another word the AM inoculation is not the magic tool and cannot save plants under severe water deficit condition as it is suggested in many previous works, therefore irrigation strategy is the most important factor in AM symbiosis association efficiency.

The field-inoculation at transplant with multi-species inoculum increased the biomass by 73 and 43% in both seasons, reflects better water and nutrient up take. Better water uptake support by more efficient use of water (41% and 13%), higher leaf water potential (22% and 17%), and lower canopy temperature (by 1.5ºC and 1.3ºC), and better regulation of stomatal conductance in field inoculated plants under moderate water deficit in both seasons. In both seasons, less need for inorganic osmolytes (K^+, and Mg^{++}) and less proline (86%, and 119%) in shoots of AM++ plants in WS50 block supported our conclusion that the AM symbiosis alleviated the water deficit stress by increasing the water uptake and avoiding the water shortage instead of enhancing water stress tolerance in their host plants.

Higher water uptake led to better nutrient uptake by mycorrhizal inoculation, the P uptake increased (from 0.5 to 1.3 g $plant^{-1}$) in 2015 and (from 0.6 to 0.9 g $plant^{-1}$) under deficit water supply regime, which is in turn affected positively the fruit setting and boosted the total yield compared to non-inoculated (from 68.1 to 110.8 t ha^{-1} in 2015, and from 121.4 to 167.0 t ha^{-1} in 2016). Moreover, the enhancement of the calcium uptake in AM plants enhanced the quality and decreased the blossom-end rot disorder of the fruits.

Beside the seasonal effect, the pedoclimate imposed a great effect on the mycorrhizal efficiency, resulted in higher yield on the loamy field, higher total carotenes (from 106 to 333 mg kg^{-1}), lycopene (from 77 to 208 mg kg^{-1}), and β- carotene (from 3 to 12 mg kg^{-1}) compared to old sandy loam farm calculated as an average across all water levels. In addition to high water holding and field capacity of the loamy farm, higher microbial activity (1.05-1.12 in the loamy rhizosphere) compared to (0.62-0.85 in the sandy loamy rhizosphere) is due more stable soil moisture resulted in better mycorhizosphere microbial interaction between plant roots and microbes that enhanced mobilizing nutrients.

We recommended the use of the field-inoculation at transplant multi-species inoculum as an integrative method in the sustainable field production system. Our results confirmed the key role importance of the irrigation strategy in the AM- crop symbiosis efficiency, therefore

scheduling and regulating water amount based on soil characteristics and crop development stage are necessary to reach compromise results. The complex relationship between the AMF and the host plant on one hand, and the AMF other microbial communities in the mycorhizosphere on the other hand propose further investigation to maximize benefits from the symbiosis relationship between arbuscular mycorrhizae fungi and field crops.

7. ÖSSZEFOGLALÁS

Korunk mezőgazdasága számos nehézséggel küzd - így a természeti források kimerülése, a klímaváltozás negatív hatásai, a tápelemutánpótlás problematikája (műtrágyák árának növekedése, negatív hatásai), biotikus és abiotikus stresszhatások, a talajdegradációs folyamatok, vízforrások szűkössége-, hogy csak néhányat említsünk. Mindezek ismeretében a hagyományos mezőgazdálkodás nem tudja garantálni a nagymértékben növekedő emberiség élelmiszer ellátását. A víz és tápelemek (elsősorban a P) megfelelő szintű biztosítása az élelmiszer ellátás alapját képező növénytermesztés alapja, melyek fenntartása kulcsfontosságú. Az emberi hatások (urbanizáció, populációnövekedés, nagyobb életszínvonal, szennyeződések, vízért folytatott harc) a klímaváltozással együtt még inkább súlyosbítják a vízzel összefüggő, vízhiány okozta problémákat, melyek megoldása mára égető vált.

A talaj mikrobiális alkotóelemei közül az arbuszkuláris mikorrhiza gombák (AMF) fontos szerepet játszanak a természeti források (víz, talaj, tápelemek) fenntarthatóságának biztosításában. Több tanulmány ismert, melyek a mikorrhiza-kapcsolat kedvező ökofiziológiai hatásait mutatják be, így például a növények szárazságtűrésének fokozását mikorrhiza gombák jelenlétekor. Az eredmények túlnyomó többsége azonban tenyészedényes vizsgálatokban, optimalizált körülmények között született. Szabadföldön, a tenyészedények rögzített méretéhez képest, a gyökerek által átjárható terület nem limitált, és a talaj, struktúrája, oxigén ellátottsága, víz és tápelem tartalma, hőmérséklete, kémiai és mikrobiológiai paraméterei is fontos szerepet játszanak a mikorrhiza szimbiózis kialakításában, annak hatékonyságában. Mindemellett több más környezeti tényező is - köztük a csapadék, hideg, meleg és a fény, hogy csak a legfontosabbakat említsük - befolyásolják ezt a mindkét fél számára kedvező kapcsolatot. Az arbuszkuláris mikorrhizának a fenntartható mezőgazdaságban betöltött szerepe, gazdasági jelentősége miatt, igen fontos a pontosabb megismerésük, szisztematikus vizsgálatuk, hogy optimalizálni tudjuk a mikorrhizaoltás hatékonyságát, szántóföldi körülmények között is a növénytermesztésben.

Vizsgálataimban az arbuszkuláris mikorrhiza(AM) oltás szántóföldi körülmények közötti tesztelésekor eredményes kolonizáció volt megfigyelhető, függetlenül az oltás időpontjától, a szabadföldi kísérleti terület minőségétől és a vízutánpótlás mértékétől. Ugyanakkor eredményeink alapján a palánták szántóföldi kiültetéskor alkalmazott mikorrhiza (AM++) sokkal hatásosabbnak bizonyult, mint a magvetéskor alkalmazott (AM+) oltás. Mindez jól nyomon követhető volt a mikorrhizának a növények növekedésére gyakorolt hatása -relative

field mycorrhizal contribution (RFMC%) - alapján. Az 50%-os vízellátásnál (IR50) a magvetéssel együtt történő mikorrhiza oltás RFMC értéke 2015-ben 18%, míg 2016-ban 15% volt. A kiültetéskor alkalmazott AM oltás ennél sokkal kedvezőbb, 42% illetve 25% eredményt mutatott. A számított RFMC értékek illetve a növényben mért fiziológiai paraméterek alapján megállapítható, hogy adott körülmények között a szabadföldön történő mikorrhiza oltás a leghatékonyabb, de a mikorrhiza nem valami csodaszer. Amennyiben több mint két héten át tartó jelentős mértékű vízstressz jelentkezik, akkor a mikorrhiza kapcsolat már nem képes a gazdanövény élettani folyamataiban bekövetkező negatív változásokat eliminálni vagy kivédeni. A mikorrhiza oltás kedvező hatásainak a biztosítása érdekében így egy meghatározott mértékű vízellátottság (öntözés) kulcsfontosságú, nem várhatunk pozitív eredményeket extrém, nagymértékű visszafordíthatatlan változásokat előidéző aszályos periódus után.

A szántóföldi körülmények között alkalmazott, több mikorrhiza fajt tartalmazó mikorrhiza oltás (AM++) a legígéretesebb eredményeket az optimális vízellátottság 50%-át biztosító öntözésnél adta, amikor a biomassza mennyiségét 73 %-kal növelte meg 2015-ben illetve 43%-kal 2016-ban. Eredményeink, a mikorrhizált növényállomány kedvezőbb víz- illetve tápelem felvételét mutatták a nem oltott növényekhez képest. A vízfelvétel elősegítését a mikorrhizált növények a vízhasznosítás növelésével (41% 2015-ben, 13% 2016-ban), nagyobb lombfelület vízpotenciállal (22% 2015-ben és 17% 2016-ban), és alacsonyabb átlagos levélfelületi hőmérséklettel (1,5 °C 2015-ben és 1,3 °C 2016-ban) valamint a sztóma-konduktancia jobb szabályozása útján éri el. Mindehhez hozzájárul még, a kisebb mennyiségű szervetlen ozmolit (K^+ és Mg^{++}) valamint prolin (86% 2015-ben 119% 2016-ban) jelenléte, a mikorrhizált növények lombjában. Eredményeink megerősítik hipotézisünket, mely szerint az AM szimbiózis az adott körülmények között, vízhiányos időszakban a vízfelvétel fokozásával, a vízhiány elkerülésével, nem pedig a növény víztűrőképességének a növelése útján éri el hatását.

A mikorrhiza oltás nem csak a víz-, hanem a növények tápelemfelvételét is megnövelte, mely a P-vonatkozásában 0,5 g P növény^{-1} értékről 1,3 g P növény^{-1} értékre nőtt 2015-ben, és 0,6-ról 0,9 g P növény $^{-1}$ koncentrációra 2016-ban. Mindez pozitív hatással volt a termés érésére és mennyiségre is, 2015-ben a mikorrhiza oltás 68,1 t ha^{-1} -ról 110,8 t ha^{-1} –ra, illetve 2016-ban 121,4 ha^{-1} –ról 167,0 t ha^{-1} –ra növelte meg a termésátlagot. Mindemellett a mikorrhiza oltás által előidézett, megnövekedett Ca-felvétel, a termés minőségi javulását eredményezte.

A mikorrhiza hatását a szezonalitás mellett jelentős mértékben befolyásolja a pedoklíma, melynek eredményeképpen az oltás agyagos területen jelentkező nagyobb terméshozam mellett a karotin tartalom 106 mg kg^{-1} –ról 333 mg kg^{-1}-ra, a likopin koncentráció 77-ről 208 mg kg^{-1}-ra, valamint a β- karotin 3-ról 12 mg kg^{-1} értékére történő növekedését idézte elő átlagosan, a homoktalajon mért értékekhez képest. Mindebben szerepet játszik az agyagtalajnak a homoktalajnál lényegesen nagyobb víztartó képessége, valamint a nagyobb mikrobiális aktivitása.

Összefoglalva, javasoljuk a paradicsom palánták szántóföldi kiültetésekor kivitelezett, több mikorrhiza gombát tartalmazó oltóanyag használatát a fenntartható szabadföldi ipari paradicsomtermesztésben. Eredményeink az AM oltás mellett az adott talaj tulajdonságaihoz, valamint a termesztett növény fejlődési stádiumának megfelelő szintű és mértékű öntözési technológia kiválasztásának fontosságára is rávilágítanak. A mikorrhiza gomba és gazdanövény komplex rendszerének pontosabb megismerése mellett igen fontos még a talaj mikroba-közösségének más tagjaival való kapcsolatának a feltárása, mely segíthet a szimbiózis által nyújtott maximális haszon elérésében.

8. RELATED PUBLICATIONS

- ✓ Bakr J, Pék Z, Helyes L, Posta K. (2018): Mycorrhizal inoculation alleviates water deficit impact on field grown processing tomato. Polish Journal of Environmental Studies. 27 (5): 1949–1958. DOI:10.15244/pjoes/78624
- ✓ Bakr J, Daood H, Helyes L, Posta K. (2017): Water deficit irrigation strategy and arbuscular mycorrhizae application in field crop production. Columella: Journal of Agricultural and Environmental Sciences. 4 (1): 69-74. DOI: 10.18380/SZIE.COLUM.2017.4.1.suppl.
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11. APPENDICES

Appendix 1. Season 2015: Total biomass, marketable fruits, water use efficiency (WUE), Root Colonization (%), and relative field mycorrhizal contribution of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes

Water supply	Treatments	Total Biomass t ha^{-1}	Marketable Fruits t ha^{-1}	WUE kg m^{-3}	Root Colonization (%)	RFMC (%)
WS0	Control	$33.5^{Aa} \pm 2.2$	$14.69^{Aa} \pm 2.6$	$18.0^{Aa} \pm 2.4$	$54^{Aa} \pm 6$	
	AM+	$32.6^{Aa} \pm 0.4$	$15.16^{Aa} \pm 1.4$	$17.5^{Aa} \pm 0.4$	$67^{Ba} \pm 8$	-3
	AM++	$34.6^{Aa} \pm 1.4$	$14.92^{Aa} \pm 2.0$	$18.6^{Aa} \pm 0.4$	$70^{Ba} \pm 6$	3
WS50	Control	$74.3^{Ab} \pm 1.4$	$56.45^{Ab} \pm 2.3$	$24.3^{Ab} \pm 0.9$	$49^{Aa} \pm 6$	
	AM+	$91.2^{Bb} \pm 1.8$	$63.91^{Ab} \pm 6.1$	$29.8^{Bc} \pm 1.2$	$64^{Ba} \pm 9$	18
	AM++	$128.9^{Cc} \pm 1.5$	$96.47^{Bc} \pm 6.0$	$42.1^{Cc} \pm 1.0$	$63^{Ba} \pm 8$	42
WS100	Control	$92.6^{Bc} \pm 1.2$	$68.41^{Bc} \pm 3.7$	$21.7^{Bc} \pm 0.6$	$55^{Aa} \pm 6$	
	AM+	$74.3^{Ac} \pm 1.5$	$57.64^{Ab} \pm 5.8$	$17.4^{Ab} \pm 0.7$	$73^{Ba} \pm 6$	-25
	AM++	$100.0^{Cb} \pm 2.6$	$75.38^{Bb} \pm 3.4$	$23.5^{Cb} \pm 1.2$	$71^{Ba} \pm 6$	7
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)						
Mycorrhizal Inoculation (AM)		***	***	***	***	
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.

Appendix 2. Season 2016: Total biomass, marketable fruits, water use efficiency (WUE), Root Colonization (%), and relative field mycorrhizal contribution of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.

Water supply	Mycorrhizal Inoculation	**Total Biomass t ha^{-1}**	**Marketable Fruits t ha^{-1}**	**WUE kg m^{-3}**	**Root Colonization (%)**	**RFMC (%)**
WS0	Control	$131.5^{Aa} \pm 7$	$65.3^{Aa} \pm 8$	$44.4^{Ab} \pm 2.5$	51 ± 21	
	AM+	$138.4^{Aa} \pm 4$	$67.0^{Aa} \pm 10$	$46.7^{Ac} \pm 1.3$	51 ± 19	5
	AM++	$136.6^{Aa} \pm 7$	$72.3^{Aa} \pm 7$	$46.2^{Ab} \pm 2.3$	70 ± 10	4
WS50	Control	$139.3^{Aa} \pm 14$	$72.1^{Aa} \pm 14$	$35.9^{Aa} \pm 3.6$	58 ± 20	
	AM+	$163.7^{Bb} \pm 6$	$94.1^{Bb} \pm 10$	$42.2^{Bb} \pm 1.5$	70 ± 11	15
	AM++	$184.8^{Bc} \pm 11$	$114.7^{Cc} \pm 3$	$47.6^{Bb} \pm 2.9$	73 ± 5	25
WS100	Control	$148.0^{Aa} \pm 16$	$84.7^{Aa} \pm 8$	$30.8^{Aa} \pm 3.3$	49 ± 6	
	AM+	$157.3^{Ab} \pm 9$	$86.2^{Ab} \pm 6$	$32.8^{Aa} \pm 1.7$	67 ± 13	6
	AM++	$160.8^{Ab} \pm 6$	$92.0^{Ab} \pm 5$	$33.5^{Aa} \pm 1.2$	70 ± 6	8
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)						
Mycorrhizal Inoculation (AM)		***	***	***	**	
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.

Appendix 3. Growing season 2015: ^{o}Brix (g /100g), total carotene, lycopene, β-Carotene, ascorbic acid, (mg kg^{1}) of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.

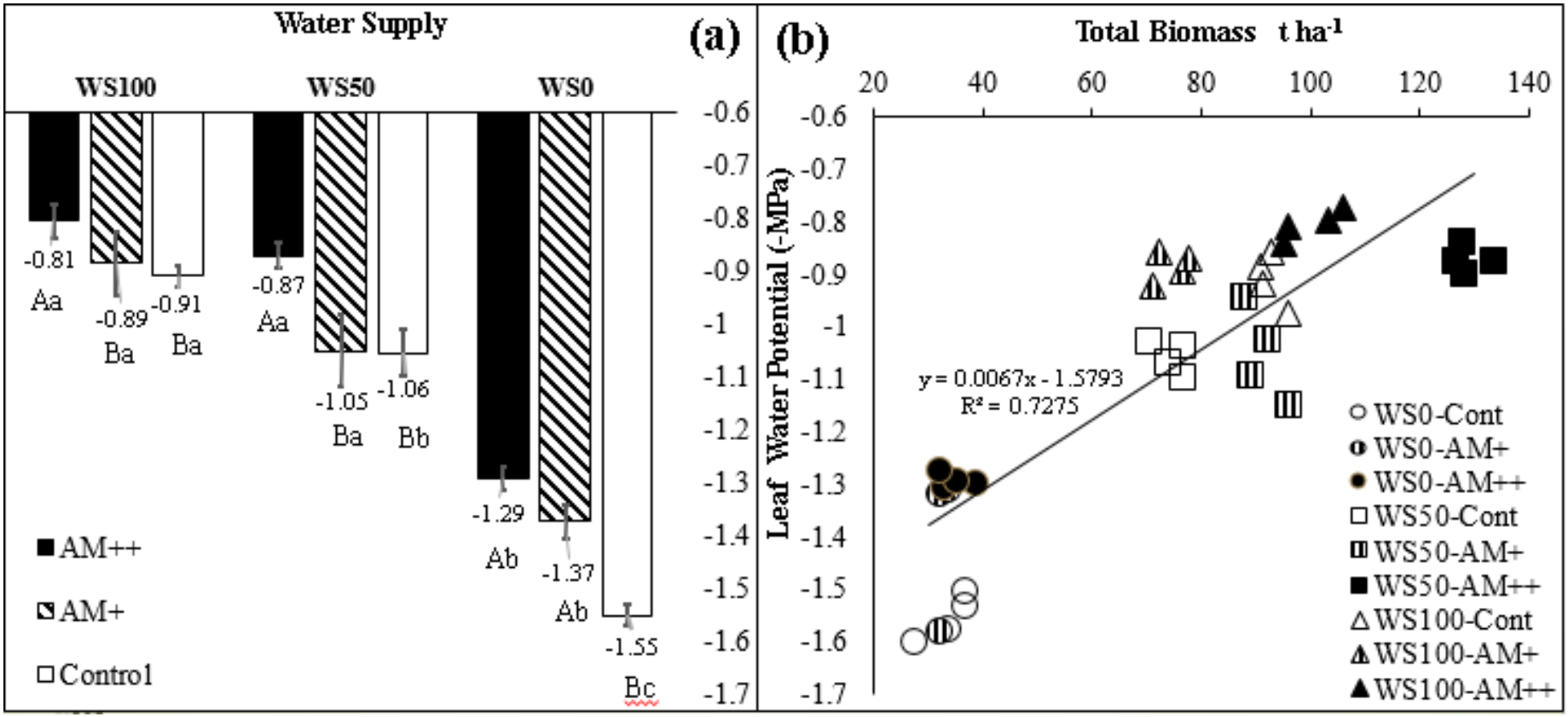
Water supply	Mycorrhizal Inoculation	**^{o}Brix (g /100g)**	**Total Carotene (mg kg^{-1})**	**Lycopene (mg kg^{-1})**	**β-Carotene (mg kg^{-1})**	**Ascorbic acid (mg kg^{-1})**
WS0	Control	8.03^{Cc} ±0.3	136.3^{Bb} ± 1.3	100.1^{Bb} ± 2	2.63^{Ba} ± .22	330^{Ab} ± 30
	AM+	7.80^{Cc} ±0.2	76.01^{Aa} ± 5.2	57.4^{Aa} ± 5	1.46^{Aa} ± .14	311^{Aa} ± 32
	AM++	8.20^{Cb} ±0.4	146.5^{Bb} ± 15	113.4^{Bc} ± 12	3.24^{Cb} ± .36	361^{Ab} ± 12
WS50	Control	5.03^{Bb} ±0.4	106.3^{Ba} ± 7.7	72.0^{Ba} ± 6	2.23^{Aa} ± .42	286^{Aa} ± 26
	AM+	5.10^{Bb} ±0.7	119.5^{Bb} ± 19	91.3^{Bb} ± 15	2.50^{Bb} ± .26	293^{Aa} ± 58
	AM++	3.88^{Aa} ±0.1	90.75^{Aa} ± 11	67.5^{Ab} ± 9	1.89^{Aa} ± .88	304^{Aa} ± 07
WS100	Control	3.73^{Aa} ±0.2	94.27^{Aa} ± 19	66.1^{Ba} ± 7	2.42^{Aa} ± .30	273^{Aa} ± 14
	AM+	3.80^{Aa} ±0.2	93.83^{Aa} ± 6.3	67.7^{Ba} ± 7	2.32^{Ab} ± .50	289^{Aa} ± 10
	AM++	3.45^{Aa} ±0.3	80.29^{Aa} ± 4.6	49.0^{Aa} ± 1	2.70^{Ab} ± .76	273^{Aa} ± 31
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)						
Mycorrhizal Inoculation		**	**	ns	ns	ns
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.

Appendix 4. Growing season 2016: ^{o}Brix (g /100g), total carotene, lycopene, β-Carotene, ascorbic acid, (mg kg^{-1}) of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.

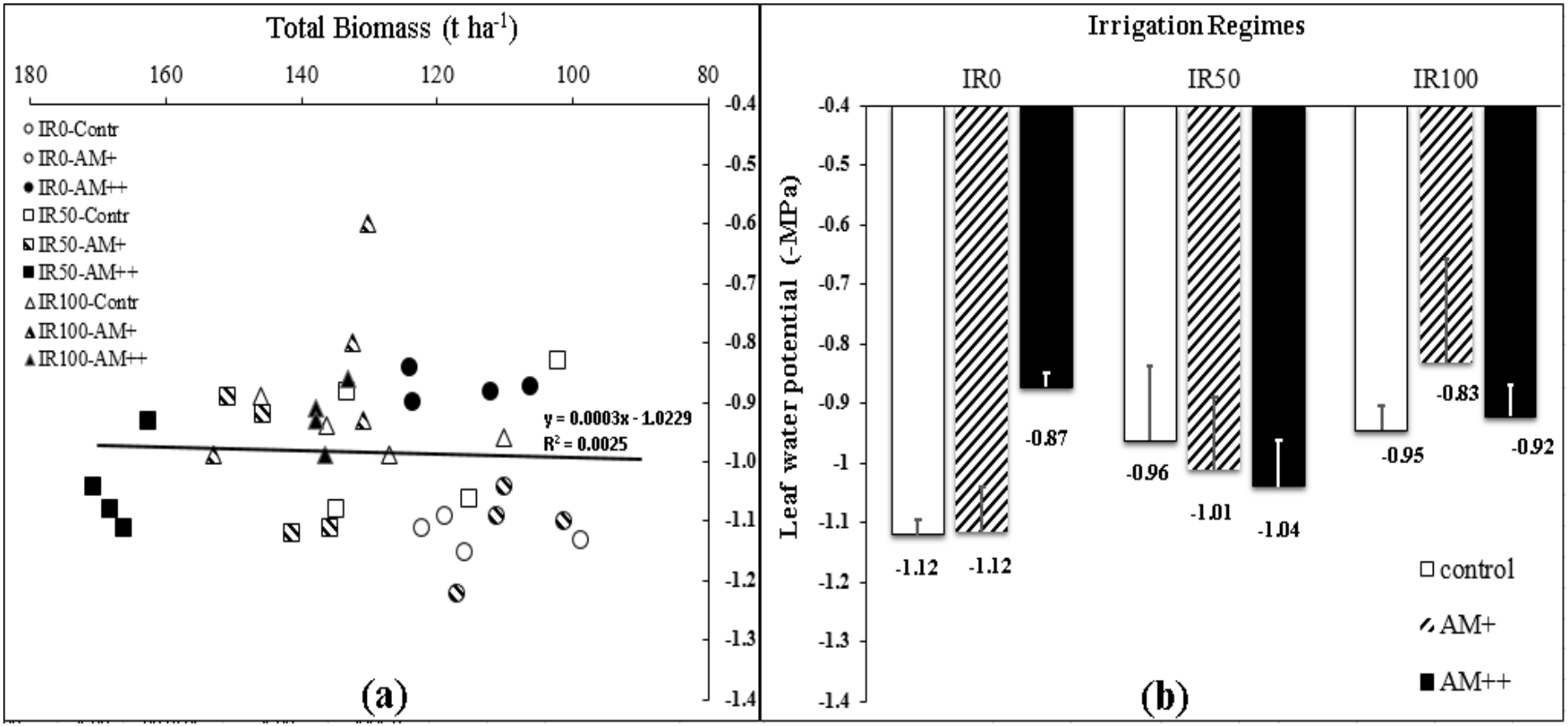
Water supply	Mycorrhizal Inoculation	^{o}Brix (g /100g)	Total Carotene (mg kg^{-1})	Lycopene (mg kg^{-1})	β-Carotene (mg kg^{-1})	Ascorbic acid (mg kg^{-1})
WS0	Control	3.65^{Aa} ±0.1	313^{Ac} ±32	205^{Ab} ±10	13.5^{Bc} ±2	331^{Aa} ±23
	AM+	4.65^{Ba} ±0.5	264^{Aa} ± 55	167^{Ab} ± 41	9.0^{Aa} ± .4	274^{Aa} ± 66
	AM++	4.10^{Ba} ±0.2	282^{Aa} ±32	165^{Aa} ±23	9.7^{Aa} ±1	293^{Ab} ±61
WS50	Control	4.45^{Ab} ±0.4	233^{Bb} ±26	188^{Bb} ±26	10.1^{Ab} ±1	418^{Aa} ±39
	AM+	4.10^{Aa} ±0.4	216^{Aa} ± 24	106^{Aa} ± 13	10^{Aa} ± 1	336^{Ab} ± 44
	AM++	4.15^{Aa} ±0.7	281^{Ba} ±42	185^{Bb} ±23	9.7^{Aa} ±.2	374^{Ab} ±45
WS100	Control	3.20^{Aa} ±0.2	181^{Aa} ±11	95^{Aa} ±19	5.4^{Aa} ±1	334^{Ba} ±74
	AM+	3.28^{Aa} ±0.6	402^{Bb} ± 35	250^{Bc} ± 11	16^{Bb} ± 3	204^{Aa} ± 71
	AM++	3.38^{Aa} ±0.1	437^{Bb} ±50	273^{Bb} ±30	17.2^{Bb} ±4	236^{Ba} ±72
Significant of Source of variation (ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)						
Mycorrhizal Inoculation (AM)		***	***	***	*	**
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.



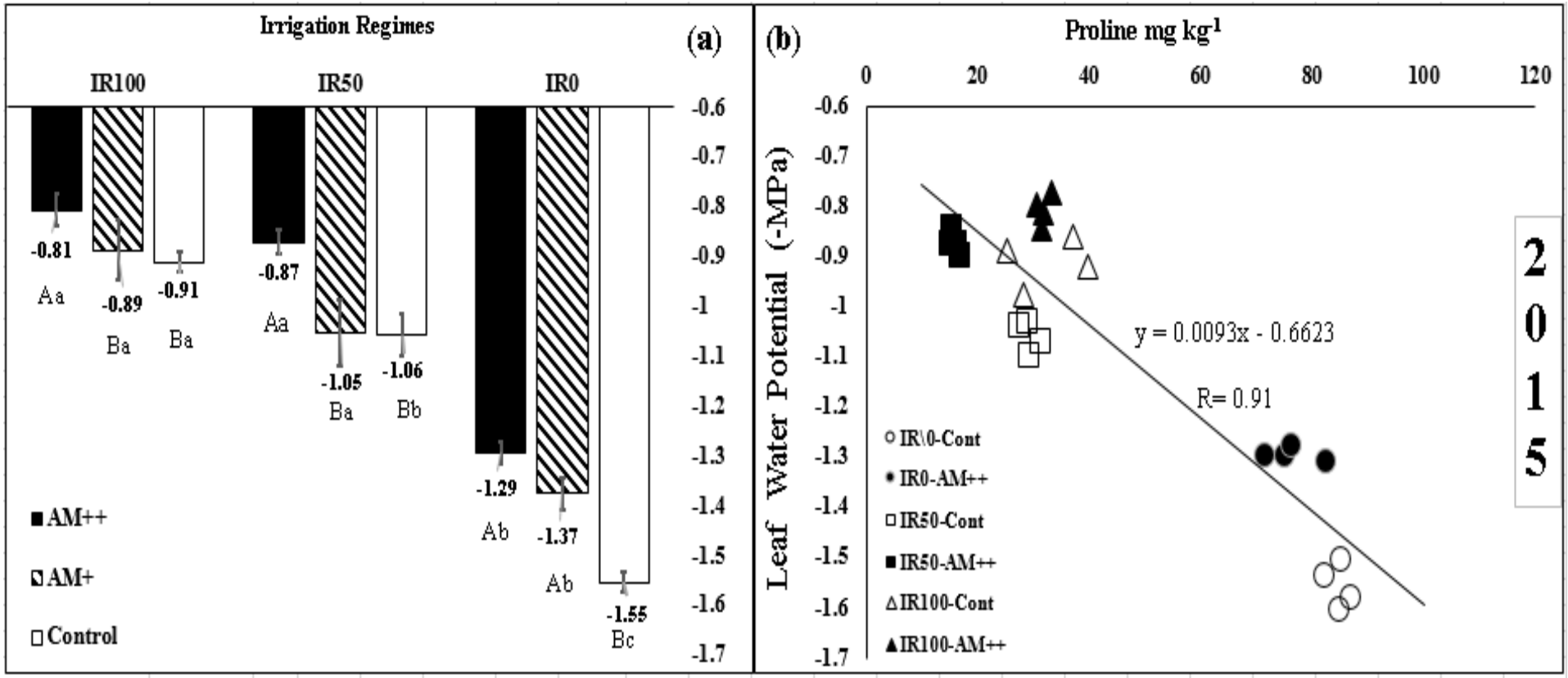
Appendix 5. Growing season 2015: Leaf water potential (a), total biomass and leaf water potential relationship (b) of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.

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.



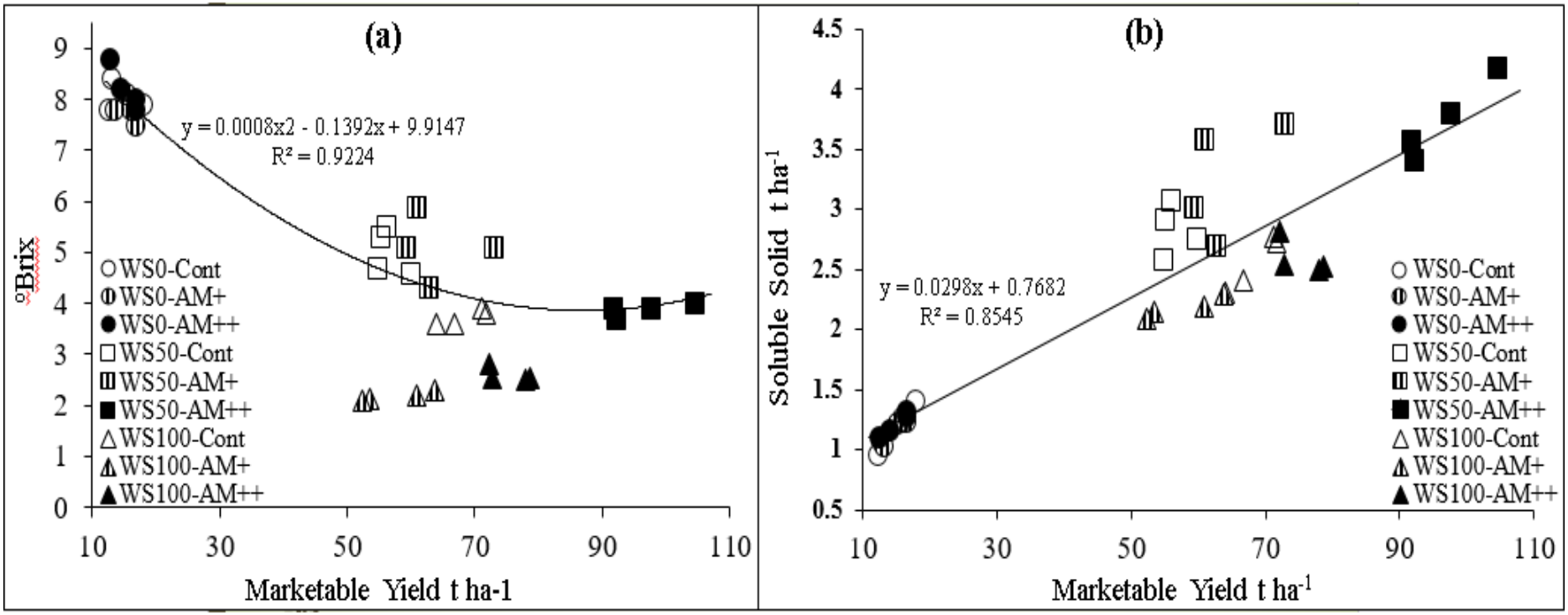
Appendix 6. Growing season 2016: Total biomass and leaf water potential relationship (a), leaf water potential (b) of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.

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.



Appendix 7. Growing season 2015: Leaf water potential (a), proline and leaf water potential relationship (b) of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.

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.



Appendix 8. Growing season 2015: Yield impact on soluble solid (ºBrix) content (a), and soluble solid production (b) of non-inoculated (Control), pre-transplant inoculated (AM+), and field inoculated (AM++) plants in three water supply regimes.