Water deficit irrigation strategy and arbuscular mycorrhizae application in field crop production

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Abstract: Most of tomato production areas are limited in water during its growing season, therefore irrigation is needed to optimize the productivity, but higher yield results in Brix losses and adversely affect fruits quality. In this field-based study processing tomato (*Lycopersicum esculentum* Mill. 'Uno rosso F1') seedlings inoculated with arbuscular mycorrhizae (AM) compared to non-inoculated (Control) were subjected to three irrigation regimes: Amply irrigation (IR_{100}), water deficit irrigation (IR_{50}), and non-irrigated (IR_0) depending on crop daily water requirement and by adjusting the irrigation water amount. Effects of irrigation water amount, AM inoculation, and their interaction on plant growth, water use efficiency (WUE), fruits quantity, and fruits quality were determined. AM plants performed better growth and increased the WUE remarkably compared to Control plants. In Control plants marketable yield decreased by (10%) when irrigation water reduced by half, and by (30%) in unirrigated compared to the potential yield in amply irrigation. Mycorrhizal inoculation improved marketable yield by (11%) in IR_0, (9%) in $IR_{100,}$ and the highest yield increase was by (59%) in $IR_{50.}$ More mycorrhizal contribution (37%) to fruit production was observed in IR_{50} water regime. Water supply increased the yield from (65 t ha^{-1}) to (85 t ha^{-1}) and resulted in a (0.5) °Brix lose, but mycorrhizal inoculation slightly enhanced the °Brix in marketable fruits.

Keywords: *Lycopersicum esculentum* M., water use efficiency, yield, carotenoids, °Brix.

Changes in climate patterns disturbed the global water cycle especially precipitation aspects, where regional differences in precipitation amount is evidently observed and many areas faced more intense drought (Trenberth et al., 2014). Water restriction especially in water limited environments affects the production of processing tomato negatively, due its summer growing season and high water demand (Patanè et al., 2011). The fact that higher tomato paste from processed tomatoes, and lower energy cost for volatilization during the processing as a result of high soluble solid content in produced fruits makes °Brix the most important ingredient in processing tomatoes (Barrios-Masias and Jackson, 2014). Carotenoids are organic pigments that human body cannot create them and they are playing an important role in cell function.

Arbuscular mycorrhizal fungi (AMF) settle symbiosis relationship with most plant families (Smith and Read, 2008), and processing tomato can establish this symbiotic relationship with AMF too. This symbiont relationship backs up tomato plants to avoid water lack stress (Bakr et al., 2017; Candido et al., 2015), while the host plant supplies the fungus with photosynthates and an ecological niche. The improvement direct and indirect water uptake by AMF (Ruth et al., 2011), is also accompanied by enhancement in nutrient acquisition and uptake especially phosphorus (P), leading to better water retention in plant, enhanced physiological functions, and better growth (Augé, 2001; Smith and Read, 2008) more efficiently under dry soil conditions (Neumann and George, 2004). Further inoculation with commercial inoculum did enhance crop productivity and colonization rate, with no quality improvement (Bakr et al., 2017; Candido et al., 2015). In tomato, antioxidants composition is affected by abiotic factors such as irrigation, temperature, and light (Pék et al., 2014), but mycorrhization could improve lycopene and β-carotene in fruits (Ulrichs et al., 2008).

This field based experiment is to evaluate effects of the commercial bio-inoculant Symbivit®, and different irrigation regimes on growth, production, fruit nutritional content, agronomical water use efficiency of Uno Rosso processing tomato.

Materials and methods

Seeds of processing tomato UNO ROSSO F1 (United Genetics Seeds Co. CA, USA), were sown on April 13 in (Klasmann TS3) substrate and inoculated with the arbuscular mycorrhizae (AM) or not (Control). The commercial inoculum Symbivit® (mixture of G. mosseae, G. etunicatum, G. claroideum, G. microaggregatum, G. geosporum, and R. irregularis) produced by Symbiom Ltd. (Czech Republic, www.symbiom.cz) was used by adding 25 grams of the inoculum to each litter substrate.

The experimental farm arranged in a randomized block design with three irrigation regime blocks: Amply irrigation (IR_{100}), water deficit irrigation (IR_{50}), and unirrigated (IR_0) depending on crop daily water requirement and by adjusting the irrigation water amount through a dripping system. Seedlings were arranged in double (twin) rows with 1.1 m and 0.4 m inter rows distance and 0.2 m between plants. Depending on air temperature (data taken from the National Metrological institute) plants daily water requirement calculated (daily water demand mm = average daily Temperature ºC x 0.2 mm $ºC^{-1}$) after Pék et al. (2014). The experimental site had a brown forest soil, loamy in texture (41% sand, 47.5% silt, and 11.5% clay) with a bulk density of 1.49g cm^{-3}, 25% field capacity, neutral in pH, free from salinity (0.212 dS m^{-1}), low in organic matter 1.4%, and contains [(NO_3^- -N (8.6 g kg^{-1}), P_2O_2 (8 g kg^{-1}), K_2O (56.7 g kg^{-1})] super elements. After one month of growth and during transplanting AM seedlings were re-inoculated by adding 20 grams of Symbivit® to the planting hole. According to Helyes and Varga (1994) plant nutrition requirements and plant protection were regulated throughout the growing season. After 100 days of growing the seedlings in the field, fruits and total biomass were harvested on August 23.

Plants roots were stained by Trypan Blue after Phillips and Hayman (1970). A stereomicroscope at × 100 magnification was used to determine internal fungal structures (hyphae, arbuscules), and gridline intersect method (Giovannetti and Mosse, 1980) was used to calculate root length colonization in percentage.

Water use efficiency (WUE) was calculated depending on total marketable fruit (WUE = kg marketable fruit per hectare/ water consumed per hectare m^{-3}).

Extraction of carotenoids and ºBrix determination: The pigments from raw tomato were extracted according to a previously described procedure with slight modification (Abushita et al., 2000). A Hitachi Chromaster HPLC instrument, which consists of a Model 5110 Pump, a Model 5210 Auto Sampler, a Model 5430 Diode Array detector, and a Model 5440 Fluorescence detector, was used for the determination of all compounds. Digital Refractometer Krüss DR201-95 (Küss Optronic, Hamburg, Germany) was used to

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estimate the soluble solid content (°Brix).

Statistical analysis: SPSS Version 22.0. (IBM Hungary, Budapest, Hungary) was use to perform statistical analyses. Effects of mycorrhizae, irrigation level, and their interaction were determined by two-way ANOVA. Means (n=4) with different letters are significantly different at ($P<0.05$) as determined by Tukey test. Capital letters represent mycorrhizal inoculation effect; small letters represent irrigation effect.

Results and discussion

The first month of the growing season experienced regular with sufficient amount of rain, therefore irrigation induced after 5 weeks of transplanting. Throughout the growing season the experimental field supplied 296 mm of rain with couple heavy rain events in the mid of July. Irrigation resulted in 480 mm in IR100 and 388 mm in IR50 including 296 mm of rain, while IR0 block received only 296 mm of rainfall (Fig. 1).

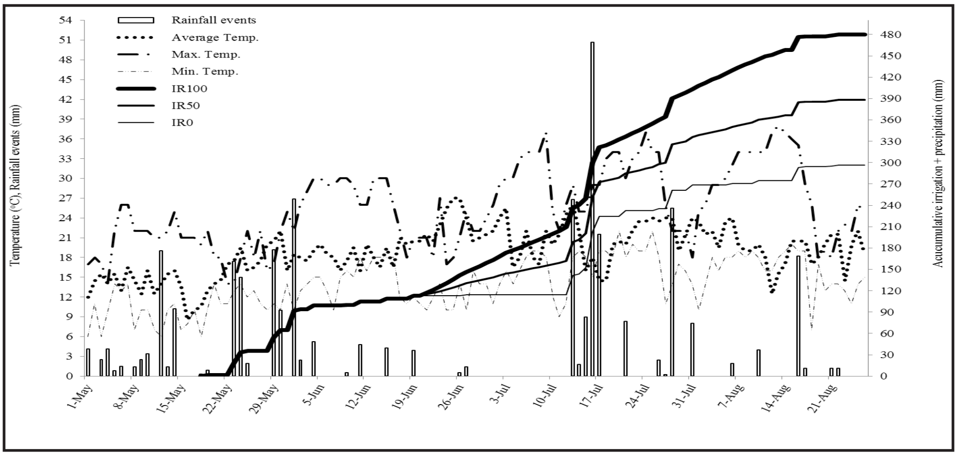

Figure 1. Average daily temperature, precipitation, and accumulative irrigation amount in 2016.

Control plants showed relatively high colonization rates (51, 58, and 53%), but mycorrhizal inoculation raised the root colonization in AM plants to (70, 73, and 71%) in IR0, IR50, and IR100 respectively (Fig. 2). 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), and the fact that even the agronomical practices such as winter tillage and bar fallow did not reduce AM colonization potential (Bakr et al., 2017). Mycorrhizal inoculation increased the above ground total biomass in AM plants by 4%, 34%, and 9% in IR_0, IR_{50}, and IR_{100} respectively compared to Control plants (Fig. 2), and this in compatible with (Lekberg et al., 2005; Ortas et al., 2013) additional mycorrhization enhances the growth in field crops, and in processing tomato (Bakr et al., 2017; Candido et al., 2015). The growth enhancement is related to better nutrient (Augé, 2001) and water (Ruth el at., 2011) uptake from the soil.

Better water uptake in AM plants improved the water use efficiency slightly in IR_0, and IR_{100} with most efficient use of water (29.6 kg/m^3) in IR_{50} (Fig. 3); more efficient water use by mycorrhized tomatoes was also observed in previous field grown tomatoes (Bakr et al. 2017; Bowles et al. 2016).

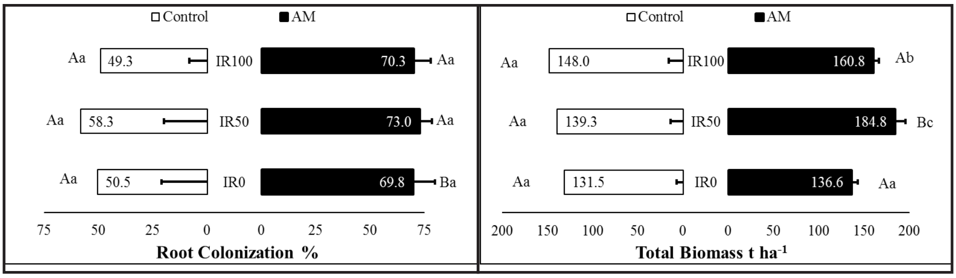

Figure 2. Root colonization rate (%), and total biomass (ton per hectar) of Control plants (empty bars), and AM plants (filled bars) under different irrigation regimes.

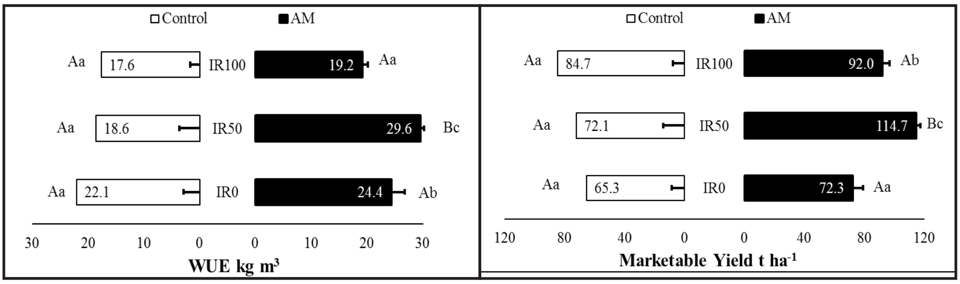

Figure 3. Water use efficiency (WUE) (yield kg / water consumption m3), and total biomass (ton/ hectar)

Irrigation did increase marketable yield from 65 to 72, and 85 tons per hectare in IR_0, IR_{50}, and IR_{100} in non-inoculated plants, but not reaching significant levels statistically. AM plants raised the yield by 9% when fully irrigated, and by 59% under deficit irrigation regime similar to that of Bakr et al. (2017) on a sandy loam field using the same commercial inoculum *Symbivit*®, and exceeding results of Bowles et al. (2016), who recorded an increase of 28% in mycorrhized tomatoes on a very fine sandy loam field under deficit water conditions.

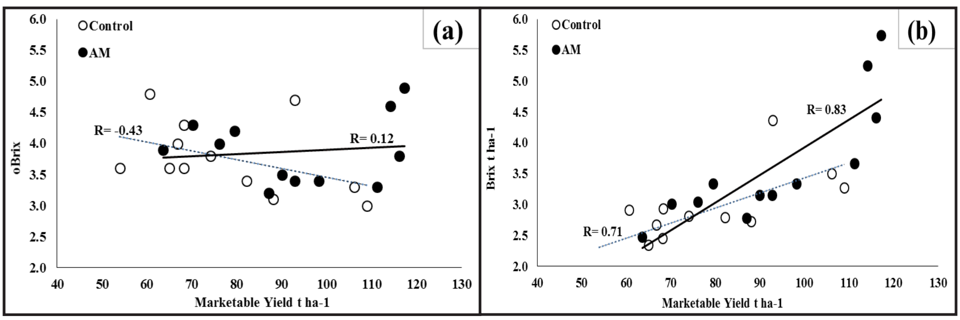

Figure 4. Yield impact on (a) soluble solid (ºBrix) content, and (b) soluble solid production per unit area.

In Control plants a loss of a half unit of °Brix was registered when marketable fruit raised from (65 t ha^{-1}) to (85 t ha^{-1}) due watering increase to optimal level (Fig. 4a). Despite losses in soluble solid content in non-inoculated plants, yield increase recompensed this loss and soluble solid was increased as a mass production per area, while this trend was more pronounced in AM fruits with a very strong positive relation (r = 0.83) between soluble solid production and yield production per unite area (Fig. 4b). Unlike Control plants, mycorrhizal inoculation slightly enhanced the °Brix content in marketable fruits (r = 0.12) along with the yield increase.

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Table 1. Fruit and antioxidant concentrations (µg g^{-1})

Irrigation	Treatment	ºBrix	Total Carotene	Lycopene	β-Caroten	Ascorbic Acid
IR_0	Control	3.7^{Aa} ±0.1	313^{Ac} ±32	205^{Bb} ±10	13.5^{Bc} ±2	331^{Aa} ±23
	AM	4.1^{Ba} ±0.2	282^{Aa} ±32	165^{Aa} ±23	9.7^{Aa} ±1	293^{Aa} ±61
IR_{50}	Control	4.5^{Bb}±0.4	233^{Ab}±26	188^{Ab}±26	10.1^{Ab} ±1	418^{Aa}±39
	AM	4.2^{Ba} ±0.7	281^{Aa} ±42	185^{Ab} ±23	9.7^{Aa} ±.2	374^{Ab} ±45
IR_{100}	Control	3.2^{Aa} ±0.2	181^{Aa} ±11	95^{Aa} ±19	5.4^{Aa} ±1	334^{Aa} ±74
	AM	3.4^{Aa} ±0.1	437^{Bb} ±50	273^{Bb} ±30	17.2^{Bb} ±4	236^{Aa} ±72
Significant of Source of variation			(ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)			
Mycorrhizae (M)		**	***	***	**	**
Irrigation (IR)		***	*	ns	ns	*
M * IR		ns	***	***	***	ns

In control plants, decreasing irrigation positively affected and increased significantly the total carotenoids concentration in marketable fruits from 181 µg g^{-1} in IR_0 to 233 µg g^{-1} in IR_{50}, and 313 µg g^{-1} in IR_{100}. The same was observed for lycopene, and β-Carotene and along gradients of decreasing irrigation amount lycopene, and β-Carotene concentrations were increased gradually, but this enhancement is accompanied by a decrease in marketable fruit (Table 1). Despite, slight changes within the same treatment and between the treatments, ascorbic acid levels have not shown a clear trend.

In addition to the increase in fresh fruit biomass by 11% and 59% in both IR_0, and IR_{50} regimes, mycorrhization could preserve the high antioxidant levels. Moreover, beside the yield improvement in AM plants, mycorrhizal inoculation did increased the total carotenoids by 2.5 folds, and tripled both lycopene and β-Carotene content in ripened fruits in IR_{100} compared to Control plants (Table 1). This scored for the mycorrhizal fungi effects in optimizing the yield with respect to the quality that not achieved in a previous study (Bakr et al., 2017). The enhancement in nutrient acquisition from the soil due to mycorrhization may resulted in better accumulation of the antioxidants in tomato fruits which also reported by Ulrichs et al. (2008).

Conclusion

This study illustrated field based evident that water deficit irrigation and arbuscular mycorrhizae field inoculation can be followed as an effective strategy in field crop mass production especially in limited water environments. AM enhanced the water uptake and did make considerable improvement in water use efficiency, production, and guarantee high quality when it is combined with deficit irrigation. More studies needed to standardize the application of AMF, and irrigation strategy as well especially under field condition where many environmental and biological aspects are interacting.

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