



Hungarian University of Agriculture and Life Sciences

Doctoral School of Plant Science

The Thesis of the Ph.D dissertation

**EFFECT OF WATER SUPPLY ON PHYTOCHEMICALS OF
PROCESSING TOMATO (*LYCOPERSICON ESCULENTUM MILL.*)**

By

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1. INTRODUCTION

1.1. Preface

The explosive increase in world population and evidence that global climate is changing, and that this change is accelerating, has become clear in recent years. In this case, global agricultural producers should provide healthy and high-quality vegetables for of the increasing population.

The global climate change is the main reason to bring down the rise of temperature. Light (photosynthesis), temperature and water are considered the most important abiotic stress, which limits crop productivity.

Tomato (*Lycopersicon esculentum* Mill.) is the most popular produced vegetable in the world and one of the most important fruit crops. The nutritional benefit of tomato-based products has been attributed to them being rich in bioactive compounds such as carotenoids and antioxidant vitamins (vitamins E and C). Tomato's nutrients play a main role both in the human and animal diets (Gould, 1992).

Recently, the most serious effect of high temperatures is a reduction or prevention of fruit set and water deficit is another main factor affecting yield and quality of tomato.

1.2. Research purpose and objectives

The main purpose of our research was to better understand how different water supply levels, and environmental factors, biofertilizer and precipitation, influence the fruit quality and quantity in a tomato crop production system.

The aim of our study was to establish the effects of water supply on the growth of processing tomato to apply three different irrigation treatments and determine the efficiency of some environmental factors on tomato processing, to determine which treatment has effects on tomato crop and fruit quality, and to define correlation between treatment and phytonutrient content.

We used processing tomato H1015, considering its economic importance to answer the following research questions:

- Which factors affected the tomatoes' yield and fruit quality?
- Which treatment influenced tomatoes' fruit quality?
- Is there correlation between treatment and the composition of tomato fruits?

This dissertation covers two years of open field and randomized block experiment studying in depth physiological, phytochemical, and production responses of processing tomato (*Lycopersicon esculentum Mill.*) to both biofertilizer inoculation, and water supply each at three levels.

Scientific experiment was conducted as field experiments and laboratory measurements.

- ❖ Field experiment included: Soil water content, canopy temperature, chlorophyll content, chlorophyll fluorescence and photosynthesis.
- ❖ Laboratory measurement included: Soluble solid content (°Brix), total carotenoids, lycopene, β -carotene, lutein, and ascorbic acid were determined in fruits.

2. MATERIALS AND METHODS

2.1. Experimental site and design

Open field experiments were conducted during the years 2018 and 2020, in the Institute of Horticulture's farm at the Hungarian University of Agricultural and Life Sciences, Gödöllő, Hungary (47.577131N, 19.379739E) (Figure 1a). The soil was brown forest soil, which was loamy in texture (consisting of 41% sand, 47.5% silt, and 11.5% clay).

2.2. Plant material and crop management

The cultivar H1015 hybrid of processing tomato (*Lycopersicon esculentum* Mill.) was used for the experiments. The tomato cultivar distributed by Heinz was H1015 hybrid with early ripening (114 days) and had resistance to *Verticillium* race 1, *Fusarium* races 1 and 2, root-knot nematode and bacterial speck. H1015 processing tomato can be grown under both arid and humid conditions. The plants were transplanted on 17 May 2018 and 14 May 2020, in randomized complete block design in four replications. The planting was a single row arrangement with a plant density of 3.5 plants m⁻². The planting was in 140 cm x 20 cm, where the length of rows was 25 m. Before transplanting seedlings were inoculated with 1% liquid solution of the biofertilizer with a drip irrigation system before planting out.

Three bacteria treatments and no bacteria treated treatment were used: **B1** (containing *Pseudomonas putida* B5, *Chryseobacterium* sp. B8/1, *Acinetobacter* sp. PR7/2, *Aeromonas salmonicida* PR10, *Variovorax* sp. BAR04), **B2** (containing *Alcaligenes* sp. 3573, *Bacillus* sp. BAR16, *Bacillus* sp. PAR11), **B3** (containing *Pseudomonas* sp. MUS04, *Rhodococcus* sp. BAR03, *Variovorax* sp. BAR04) and non bacteria treated **B0**. The bacteria were given by BAY-BIO Division for Biotechnology (Bay Zoltán Nonprofit Ltd. for Applied Research, Szeged, Hungary) for the experiments. Seedlings

were soaked in 20 litres of water containing 2 dl of bacteria suspension for 5 minutes before planting in every treatment (Figure 1).



B3 biofertilizer	B3 biofertilizer	B3 biofertilizer
B2 biofertilizer	B2 biofertilizer	B2 biofertilizer
B1 biofertilizer	B1 biofertilizer	B1 biofertilizer
B0 non biofertilizer	B0 non biofertilizer	B0 non biofertilizer

Figure 1. Experimental field location and design in 2018 and 2020

2.3. Meteorological data

Weather forecasts from the National Meteorological Institute were used to calculate plants daily water demand depending on the daily average air temperature and precipitation. The following meteorological variables were recorded daily throughout the plant growing season: maximum and minimum air temperature, air relative humidity, rainfall. Maximum temperatures during the growing period (May-August-September) ranged from 18.8 to 33.7 °C in 2018 and from 12.0 to 33.9 °C in 2020, that minimum from 8.0 to 22.1 °C and from 4.3 to 20.7 °C in the first and second year of the experiment, respectively. The air relative humidity ranged from 57.5 to 91.3% in 2018 and from 50.6 to 92.4% in 2020. Total rainfall was 285.8-305 mm in 2018 and 357-362 mm in 2020, but it's not enough water to grow during vegetation period. Therefore, each plot should use irrigation.

2.4. Water supply

The plants were irrigated two times per week generally, depending on the volume of precipitation. The irrigation water was pre-calculated according to the weather forecast (provided by the National Meteorological Institute) and supplied ahead for 3 or 4 days. After receiving the actual meteorological data, the AquaCrop was used for calculating the crop evapotranspiration for the days since last irrigation, and the next irrigation depth were calculated with the consideration of how much water was actually used by the plants according to the evapotranspiration (Battilani et al., 2012, Allen et al., 1998). Three different irrigation treatments based on crop evapotranspiration (ET_c), meaning optimum water supply (RI), and half of this, $0.5 \times ET_0 \times K_c$; deficit irrigation (DI), and a non-irrigated treatment (NI), were studied in a randomized complete block experimental design with four replicates (Table 1). A drip irrigation system was used for irrigation. This last was applied following the evapotranspiration (ET_c) method according to soil water balance as proposed by Doorenbos et al. (1992) and FAO (2020).

$$\text{Equation 1. Evapotranspiration (ET}_c\text{)} = \text{ET}_0 \times \text{K}_c$$

Where ET_c is the crop evapotranspiration (mm),

ET_0 is the reference of evapotranspiration (mm),

K_c is crop coefficient.

Table 1. Seasonal irrigation volume for each irrigation treatment

Irrigation treatment	Description	Total water supply, mm		Number of irrigations		Irrigation water saving, %	
		2018	2020	2018	2020	2018	2020
NI	Non irrigated	305	362	1	1	34.4	21.2
DI	50% ET_c restoration	385	411.8	20	9	17.2	10.4
RI	100% ET_c restoration	465	459.7	20	9	0	0

ET_c -maximum crop evapotranspiration.

The amount of water to supply with irrigation was that required to fill soil up to field capacity in the 0–60 cm of depth, where most of the roots are expected to develop in processing tomato (Machado and Oliveira, 2005; Marouelli and Silva, 2007; Zotarelli et al., 2009)

During the experimental years, the deficit irrigation treatment was able to save 17.2 percent water in 2018 and 10.4 percent in 2020 (Table 1).

2.4.1. Water use efficiency (WUE)

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

$$\text{Equation 2. Water Use Efficiency (WUE)} = \frac{\text{marketable yield, kg ha}^{-1}}{\text{Water, m}^3 \text{ ha}^{-1}}$$

Water use efficiency (kg m^{-3}) was calculated as the ratio between total marketable fruit weight (kg ha^{-1}) and total water used ($\text{m}^{-3} \text{ ha}^{-1}$).

2.5. Harvesting

The harvest date was 27 August 2018 and 1 September 2020. Plants were harvested at once after 103-110 days of growing. The 25 meters row plots allowed us to randomly mark 4 replicates per treatment. From each replicate the above ground part of 10 tomato plants were cut off at the soil surface. For quantitative and qualitative parameters sampling of 10 plant from each replicate (subplot), guaranteed high precision, and lessened sampling error. At the time of harvest, the total biomass and yield were recorded, then it was classified into marketable (ripe), green and non-marketable (rotten and diseased) fruits and measured. Total fruit yield was determined, and marketable yield was measured considering red fresh fruits. Ripened fruits (approximately 2 kg per treatment) were sampled at harvest for laboratory analyses (AOAC, 1990).

2.6. Experimental field measurements

Measurements of physiological traits were performed every week from the beginning of flowering.

2.6.1. Soil moisture

The soil moisture was measured with PT-1 (Kapacitív Kkt., Hungary). Measurements were taken with three (bottom, medium and head side of field) replications in each treatment.

2.6.2. Chlorophyll content

Chlorophyll content of leaf was measured by SPAD 502 (Minolta, UK) portable chlorophyll meter and it was given as SPAD values. Three readings per plant and four plants were detected in each subplot with 4 replications in each treatment from flowering to fruit development stages. 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 also for nitrogen content in leaves (Martínez et al., 2015).

2.6.3. Chlorophyll fluorescence

Chlorophyll fluorescence was measured by portable fluorimeter PAM 2500 (Walz-Mess und Regeltechnik, Germany). From four plants as four replications tagged for photochemical analysis, a fully developed top leaf was induced to 35 min dark adaptation by leaf clips. PamWin 3.0 software 37 was used to calculate the photochemical quantum yield of PSII from Fv/Fm ratio by fast kinetics method (Van Goethem et al., 2013).

2.6.4. Canopy temperature

The infrared remote thermometer (Raytek Raynger MX4, Santa Cruz, CA, USA) was used to record the canopy temperature (Bócs et al., 2009). The new laser technology takes noncontact temperature measurement from any distance, easy to use, accurate ($\pm 1\%$ in readings), and can read from -30 to 900°C . We measured ten leaf surface temperature in each treatment from flowering to fruit development stages of tomato.

2.7. Phytochemical analysis

The analytical investigations were done at the Regional Knowledge Centre of the Hungarian University of Agriculture and Life Sciences.

2.7.1. Chemicals used for chemical analysis

All analytical grade chemicals and HPLC grade organic solvents were purchased from Merck Group Ltd (Budapest, Hungary). Standard lycopene, lutein, β -carotene, 8- β -apo-carotenal, ascorbic acid and tocopherols were purchased from Sigma-Aldrich (Budapest, Hungary).

2.7.2. Fruit sampling

Tomato fruits were harvested randomly from each treatment at the red ripe stage. A sample of at least 2 kg of visually selected injury free red ripe tomato fruits were chosen and delivered quickly to the laboratory. Tomato fruits were washed with running water to remove dirt and cut into small pieces. They were analyzed for 5-10 fresh fruits weight (g), then all samples were grinded for

total soluble solids, vitamin C and homogenized. The obtained homogenates were immediately frozen at -20°C and used to determine the carotenoids.

2.7.3. Extraction of phytonutrients

Extraction of carotenoids

The pigments from raw tomato were extracted according to a previously described procedure with slight modification (Abushita et al., 2000). To extract the carotenoid pigments, 5 grams of the whole tomato or pumas and 10 grams of juice were taken and crushed in a crucible mortar with addition of 1 g of ascorbic acid and quartz sand. To the macerate 20 ml of methanol were added to bind the water. The methanol fraction was decanted into 100 ml Erlenmeyer flask with stopper. The residues were further crushed and extracted by a step-wise addition of 50 ml of a mixture of 1:6 methanol-1,2-dichloroethan. The extract was pooled with the methanol fraction. To increase solubility of pigments in the less polar solvent 1 ml of water was added that assisted in separating the two phases. After mechanical shaking for 15 min the two phases were separated in a separating funnel. The lower phase containing pigments dissolved in the less polar solvent was dried on anhydrous sodium sulphate and passed to a round bottom flask. The solvent was then evaporated under vacuum at 40°C to dryness using vacuum-controlled evaporator (Ingos RVO-400). The residues were re-dissolved in 10 ml HPLC grade acetone before injection onto the HPLC column (Daood et al., 2013).

Extraction of ascorbic acid (Vitamin C)

To extract vitamin C 5-10 grams of different tomato fractions were disintegrated in a crucible mortar with quartz sand. To the macerate 30-50 ml of 3% metaphosphoric acid solution were gradually added with continuous crushing after each addition. The supernatant was quantitatively transferred to an Erlenmeyer flask with stopper and subjected to ultrasonic force in a water bath ultrasonic device (Raypa, Turkey) for 2 min followed by mechanical shaking for 15 min and filtration through a Hahnemühle DF 400-125 type

filter paper. The filtrate was further cleaned up by passing through a Whatman 0.22 μm cellulose acetate syringe filter before injection on the HPLC column.

2.7.4. HPLC instrument and conditions

Hitachi Chromaster HPLC instrument consisting of a Model 5110 Pump, a Model 5430 Diode Array detector, a Model 5440 Fluorescence detector, and a Model 5210 autosampler was used. The separation and data processing were operated by EZChrom Elite software.

Carotenoids were simultaneously separated on a core C-30, 2.6 μm , 150x4.6 mm (Accucore Thermo Scientific, USA) with gradient elution of tert-butyl methyl ether (TBME) (A) in methanol containing 2% water (B) according to Daood et al. (2013). The gradient elution started with 100% B and turned to 30% A in B in 25 min, stayed isocratic for 5 min and turned to 100% B in 5 min. The eluted carotenoids compounds were detected by Diode Array detector between 190 and 600 nm (Liaaen-Jensen and Lutnes, 2008). Identification of carotenoids was based on comparison of retention time and spectral characteristics with those of available standards such as lutein, β -carotene and lycopene. In case of no standard materials available, the compounds were identified on the basis of their mass determined by LC-MS/MS, spectral characteristics and retention behaviour as previously described in details (Daood and Biacs, 2005). Quantitative determination of carotenoids was based on using β -8-apocarotenal as internal standard spiked with the samples. For quantification, the area of each compound was integrated at the maximum absorbance wavelength.

Vitamin C (L-ascorbic acid) was separated on aqua Nautilus (Macherey Nagel, Düren, Germany), 3 μm , 150 x 4.6 mm column with gradient elution of acetonitrile (A) in 0.01M KH_2PO_4 (B). The separation started with 2% A in B, changed 30% A in B in 15 min stayed isocratic for 5 min and finally turns to 2% A in B in 5 min. The separated compounds were detected by Diode Array detector between 190 and 400 nm. Identification and quantification of L-

ascorbic acid was based on using of calibration curve of standard solutions. Under the used conditions L-ascorbic acid had an absorption maximum at 262 nm, at which the area was integrated.

2.7.5. Determination of soluble solids content (°Brix)

The fruit soluble solids content (°Brix) was determined by a digital manual refractometer KRÜSS DR201-95 (KRÜSS Optronic, Hamburg, Germany), tested samples were expressed by the Brix of fresh juice. The soluble solids yield (t/ha) was calculated using the average soluble solids content of fruits and yield data. 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.

2.8. Data analysis

Data of yield and quality traits were statistically analyzed by a one and two-ways analysis of variance (ANOVA). The analysis of variance was conducted separately within each year, considering water treatment as fixed factor. Two-ways analysis (PGPR x irrigation) were used in each year. Means were compared using the Tukey HSD and the significant difference was detected at $P < 0.05$ level. Correlation analysis was also performed, in order to define possible relationships among WUE and Brix. All calculations were performed using SPSS and Excel version 2010 (Microsoft Corporation, Redmond, WA).

3. RESULTS AND DISCUSSIONS

3.1. Water stress induction and soil water condition

During the first growing season (2018) the experimental farm has received 304.6 mm of rain, and watering through the drip irrigation system which resulted in supplying 464.8 mm in regular irrigation (RI) and 384.8 mm in deficit irrigation (DI) treatment blocks respectively including the precipitation. Soil moisture in the blocks was ranging between 24.8-27.8%. The relative well distribution of the rain events during the development stages of tomato, and the low water holding capacity of the experimental soil allowed proper water stress induction NI and DI treatment plants.

During the development stages of tomato, the maximum temperature ranged from 24.6 to 31.5°C and the minimum temperature ranged from 13.6 to 18.3°C. During fruit development stage there was high temperature (31.5 °C) and during beginning of flowering stage there was low temperature (13.6 °C). In 2020, total precipitation was 357 mm during the growing season of tomato. If we divide it by vegetation stages, precipitation was 181 mm in the growing stage, 26 mm in the beginning of flowering stage, 34 mm in the flowering stage, 35 mm in flowering to fruit setting, 20 mm in fruit development, 45 mm in fruit ripening and 16 mm at harvesting. The drip irrigation system resulted in supplying 459.7 mm in regular irrigation (RI) and 411.8 mm in deficit irrigation (DI) treatment blocks respectively including the precipitation. The non-irrigated treatment's water supply was 102.7 lower than the regular irrigated treatment and 54.8 lower than the deficit irrigated treatment. Soil moisture ranged between 21-26.4% blocks. The total precipitation was 54.2 mm higher than compared to 2018.

The air maximum temperature ranged from 18.9 to 33.1°C and the minimum temperature ranged from 3.4 to 14.9°C during the development stages of

tomato. Maximum temperature was 33.1°C in the fruit development and fruit ripening stages and minimum temperature was 3.4°C in the growing stage.

In the experimental years, compared to irrigated and non-irrigated treatments, tomato's leaf size was smaller and fruit number and fruit size were lower in the non-irrigated and non biofertilizer treatment.

In 2020, the level of precipitation was 52.4 mm higher than in 2018, which had a positive effect on plant development, especially in the NI treatment. From flowering to fruit ripening stages the air temperature ranged between 27.2 to 33.1 which allowed for fruit ripening and accumulation of fruit nutrients.

3.2. Photosynthetic efficiency and relative chlorophyll content

SPAD's high value was measured in NI treatment than irrigated treatment. It means the plant suffers from water stress and response to it with high SPAD values. Chlorophyll fluorescence was the lowest in NI treatment in 2018 and RI treatment in 2020. Water stress and high temperature are effect on it (Table 2). Canopy temperature level was close to each other and haven't significant difference between treatments and years. Highest total yield was occurred on RI in 2018 and on DI in 2020 (Table 2.).

Table 2. Physiological traits and yield of tomato under different water supply treatment (Mean \pm SD, n=10)

Year	Water supply treatment	Canopy* temperature °C	SPAD	Fv/Fm ^y	Total yield* t ha ⁻¹	Correlation
2018	NI	28.4 \pm 1.6	50.0 \pm 2.2	0.737 \pm 0.05	50.6 \pm 10.5	R ² = 0.26 CT vs TY
	DI	27.7 \pm 1.1	48.8 \pm 2.2	0.749 \pm 0.03	65.0 \pm 15.8	R ² = 0.70 SPAD vs TY
	RI	27.7 \pm 2.0	47.0 \pm 2.6	0.757 \pm 0.03	72.3 \pm 10.2	R ² = 0.82 Fv/Fm vs TY
2020	NI	25.7 \pm 1.9	51.1 \pm 4.3	0.785 \pm 0.02	49.7 \pm 3.6	R ² = 0.80 CT vs TY
	DI	26.4 \pm 2.1	47.5 \pm 4.8	0.773 \pm 0.02	51.1 \pm 8.3	R ² = 0.62 CT vs TY
	RI	26.2 \pm 1.5	46.7 \pm 4.2	0.764 \pm 0.03	48.4 \pm 7.6	R ² = 0.88 Fv/Fm vs TY, R ² = 0.71 SPAD vs TY

* - Canopy temperature (CT), Total yield (TY)

Statistical analysis of physiological traits and total yield in 2020 showed a strong correlation between chlorophyll fluorescence and total yield ($R^2 = 0.88$) in the RI treatment, between the SPAD and total yield ($R^2 = 0.71$) in the RI treatment, and leaf temperature and total yield ($R^2 = 0.62$, $R^2 = 0.80$) in the DI and NI treatments (Table 2). This means physiological traits have a strong effect on yield. According to Horvath et al. (2020) deficit irrigation and mycorrhizal treatments have significantly positive effect on photosynthesis expressed by chlorophyll fluorescence and increased fruit weight. Nemeskeri and Helyes (2019) reported some vegetable's responses to water stress based on their stomatal behaviour, canopy temperature, chlorophyll fluorescence and the chlorophyll content of leaves. These stress markers can be used for screening the drought tolerance of genotypes, the irrigation schedules or prediction of yield.

3.3. Total biomass and water use efficiency

The Brix is the most important factor in tomato yield and is one of the factors influencing the quality (Patanè and Cosentino, 2010; Battilani and Letterio, 2015) and selling price of the fruit production. Brix yield depends on the water supply conditions which determine the yield and accumulation of soluble solid in the fruit of tomato.

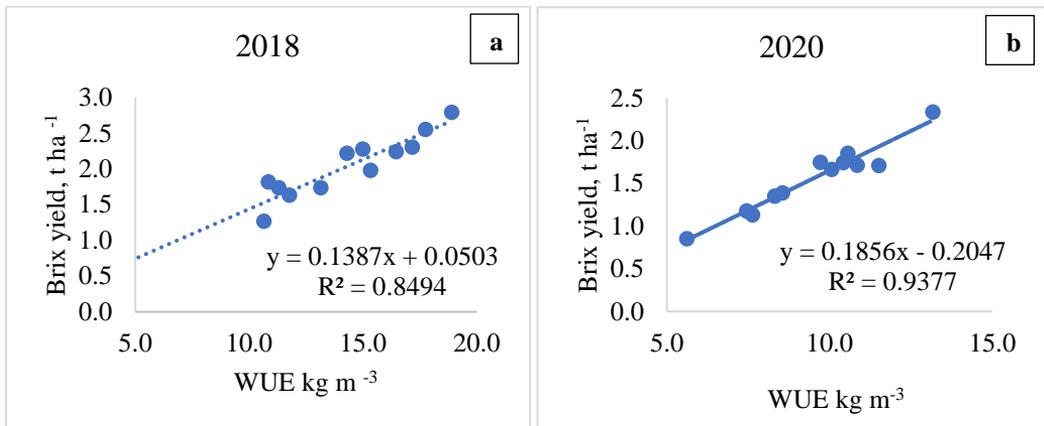


Figure 2. Relationship between Brix and WUE in 2018 and 2020

An analysis of relationship between water use efficiency on the Brix yield of ripe tomato fruits over the years of the study shows that the two factors are strongly correlated, with $R^2=0.84$ (Figure 2a) in 2018 and $R^2=0.93$ in 2020 (Figure 2b). This result also confirmed that of Bócs (2018) who found a higher significant correlation ($R^2=0.8533$) between the Brix yield and water supply conditions in a moderate dry year than in very dry year ($R^2=0.7547$).

3.4. Effect of water deficiency on yield of tomato

Tomato fruit yields of irrigated treatments were higher than that of non-irrigated treatments. The total yield was divided into three categories: marketable, green and non-marketable (rotten). In 2018, the total yield was 50.6 t ha⁻¹ in the NI, 65 t ha⁻¹ in DI, and 72.3 t ha⁻¹ in the RI treatment, and in the marketable fruit category yield were ranged between 45.4-59.7 t ha⁻¹, in

green yield category 4.4-9.9 t ha⁻¹, and in non-marketable yield category 0.9-2.7 t ha⁻¹. Statistical analysis showed the non-marketable yield of RI treatment was higher than other NI and DI treatments. And there was a significant difference in the three different irrigation treatments (Table 3). According to our research, the results of the 2018 brix value were higher in DI treatment than other treatments. This means the use of deficit irrigation treatment in tomato production and get quality fruit yield and can be kept at a stable average fruit yield (Table 3).

Table 3. Tomato yield in three different irrigation treatments in 2018 and 2020

Year	Treatment	Biomass, t ha ⁻¹	Total fruit yield, t ha ⁻¹	Marketable fruit yield, t ha ⁻¹	Green fruit yield, t ha ⁻¹	Non marketable fruit yield, t ha ⁻¹	Brix yield, t ha ⁻¹
2018	NI	66.2±14.1	50.6±10.5	45.4±8.9	4.4±1.9	0.9±0.3b	1.9±0.5
	DI	81.0±17.1	65.0±15.8	59.2±13.3	4.6±3.3	1.1±0.9b	2.2±0.6
	RI	87.4±10.5	72.3±10.2	59.7±9.7	9.9±4.7	2.7±1.4a*	2.0±0.3
2020	NI	60.3±4.2	49.7±3.6	40.2±5.7	2.5±1.1	7.1±1.6	1.9±0.3a
	DI	62.7±10.3	51.1±8.3	40.4±6.5	2.1±1.2	8.7±2.9	1.6±0.3ab*
	RI	64.5±6.3	48.4±7.6	34.6±6.1	1.7±1.0	12.2±6.3	1.2±0.2b

*- significantly difference (P<0.05) by Tukey HSD calculation. NI= non-irrigation, DI= deficit irrigation, RI= regular irrigation

In the wet year (2020) the marketable yield was low and a higher ratio of non-marketable yield was measured than in drier year which was due to the flooding water stress. In 2020, the level of precipitation was 52.4 mm higher than in 2018, which had a positive effect on the total fruit yield, in the NI treatment. The total fruit yield ranged from 48.4 to 51.1 t ha⁻¹ in the three different irrigation treatments. In the marketable fruit category yields ranged between 34.6-40.4 t ha⁻¹, in green yield category 1.7-2.5 t ha⁻¹, and in non-marketable (rotten) yield category 7.1-12.2 t ha⁻¹. Out of the three treatments, the DI treatment was observed to have a higher yield and lower rotten fruit

yield than the RI treatment. According to others (Ozbahce and Tari, 2010; Helyes et al. 2012), the optimum irrigation (RI) treatment increases the yield, however in our experiments due to the higher amount of precipitation in 2020, it negatively affected the yield, reducing the total yield and increasing the amount of rotten yield (Table 3). As statistical analysis showed, Brix value of DI treatment was significantly different ($P < 0.05$) from the RI treatment (Table 3).

Comparison of two years marketable yield, highest yield 59.2-59.7 t ha⁻¹ was observed in DI and RI treatments, in 2018 and 40.2-40.4 t ha⁻¹ was observed in DI and NI treatments, in 2020 (Figure 3). According to Bakr (2019) the optimum irrigation (RI) and deficit irrigation (DI) treatments increases the yield.

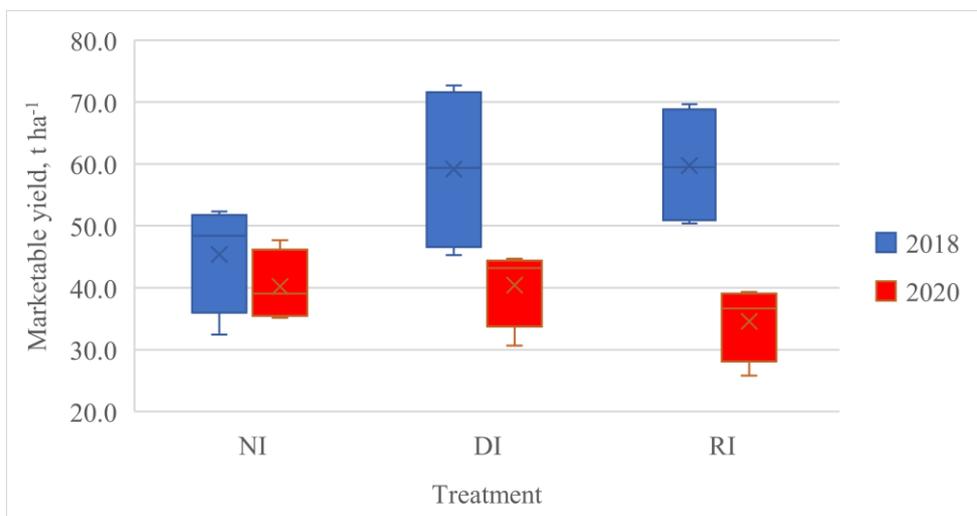


Figure 3. Marketable yield under different irrigation treatments in two years. The 2018 yield was similar to that of other researchers (Helyes et al., 2012, 2019), with the irrigated treatment marketable yield higher than the non-irrigated treatment. However, due to heavy precipitation in 2020, the total yield was higher in the DI treatment in comparison with RI treatment, but the marketable fruit yield decreased and rotten yields increased (Figure 3, Table 3).

3.5. Effect of water deficiency on nutritive value of tomato fruit

In wet year (2020) β carotene and 13 cis lycopene content of tomato fruit was significantly higher than a drier (2018) year.

In the two experimental years, lycopene content was highest in the NI treatment, which is in agreement with Liu et al. (2011). Lowest content was in the RI treatment (Figure 4). However, the lycopene content in the DI treatment was close to that in the NI treatment, which showed that the amount of lycopene in the variety decreased slightly even when the deficit irrigation was used. This shows that we can reduce water consumption and produce a quality fruit yield by using deficit irrigation.

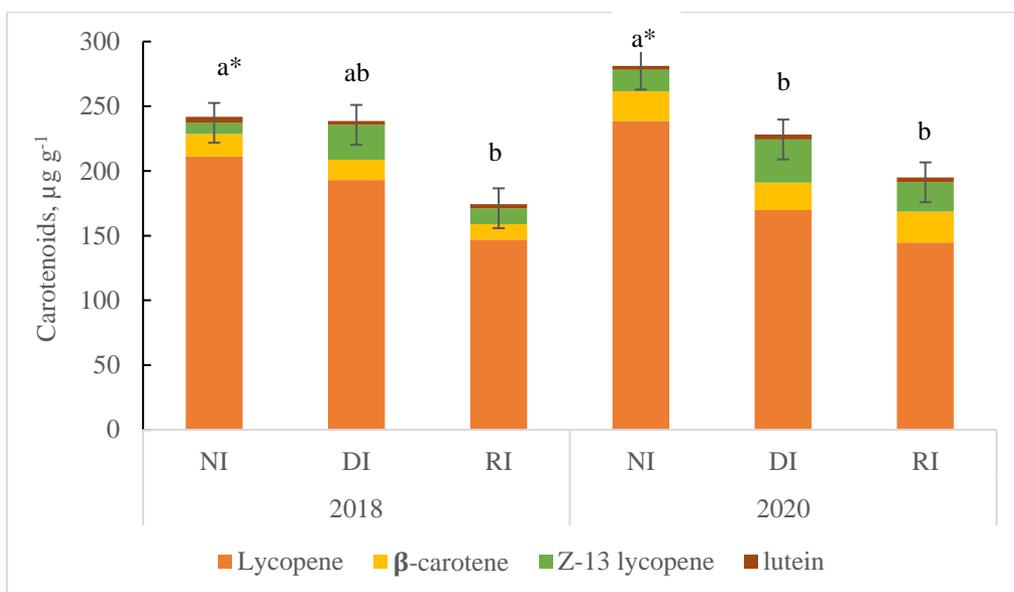


Figure 4. Effect of water supply on nutritional values of ripening fruit of tomato in 2018-2020. Different letters are significant different at $P < 0.05$ level using Tukey's test.

The highest lycopene content of tomato fruit was detected under non-irrigated conditions in comparison with the regularly irrigated condition in both drier (2018) and wet (2020) years (Figure 4). Significantly high lutein content of

fruit was only measured under non irrigated condition in 2018 while it was low in 2020 and no difference between the water supply conditions.

In experimental years, we have studied how the water supply and biobacteria combined treatment affects the nutritional value of tomato fruits. Our study showed Brix value and vitamin C content was the highest in the non-irrigated treatment (Table 4).

In 2018, the Brix value was 4.52 for the NI and bacteria combined treatment and the vitamin C content was $52.3 \mu\text{g g}^{-1}$, while in 2020, the Brix value was 4.53 for the NI and bacteria combined treatment and the vitamin content was $33.7 \mu\text{g g}^{-1}$ (Table 4). The Brix and vitamin C content was significantly different ($P < 0.001$) in the three different irrigation treatments. The 2018 and 2020 studies of Brix showed that there was significant difference ($P < 0.05$) between different biofertilizer treatments. Among the different biofertilizer treatments, the B1 bacterium had a positive effect on the Brix value (Table 4). In dry years using deficit irrigation B1 treatment increased significantly the marketable and green yield and decreased the ratio of diseased yield (data not shown).

The highest total yield was found in the biofertilizer treatments under deficit irrigation treatment (79.8 t ha^{-1}), in 2018 and biofertilizer treatments under non-irrigated treatment (53.2 t ha^{-1}), in 2020. It was the highest in B3 treatment under all different irrigation treatments in 2018, and B2 under all different irrigation treatments in 2020. In statistical analysis, total yield was the highest in DI treatment of all different treatments, with a significant difference $P < 0.01$. Also, there was significant difference between the different treatments of PGPRs (Table 4). It means PGPRs and irrigation treatment has a significant effect on total yield, in 2018.

In 2020, the level of precipitation was 52.4 mm higher than in 2018, which had a positive effect on the total fruit yield, in the NI treatment. The total yield of 2020 was not significantly different in the three different irrigation

treatments. However, results showed that there was significant difference ($P<0.05$) between different biofertilizer treatments.

Liu et al. (2011) found that irrigation increased marketable and total fruit yield by 66-127%, while it decreased soluble solids content by 19% which was also reported by Favati et al. (2009).

Table 4. Effect of water supply (WS) and PGPRs on Brix and vitamin C content of H-1015 F1 tomato

Water supply/ years	PGPRs	Total yield, t ha ⁻¹		BRIX		Vitamin C, µg g ⁻¹	
		2018	2020	2018	2020	2018	2020
NI	B0	50.7 b	49.7 b	4.25 b	4.64 a	51.84 a	35.85 a
	B1	50.9 b	54.8 a	5.07 a	4.72 a	56.02 a	35.83 a
	B2	68.6 a	56.7 a	4.36 b	4.55 a	50.72 a	32.18 b
	B3	68.8 a	51.5 b	4.39 b	4.20 b	50.62 a	31.05 b
<i>effect of NI</i>		59.8 B	53.2 A	4.52A	4.53A	52.30A	33.73A
DI	B0	65.0 c	51.1 a	3.65 c	4.00 b	43.34 b	37.68 a
	B1	77.4 b	51.3 a	3.51 c	3.62 b	40.97 b	31.03 b
	B2	83.5 a	55.7 a	3.61 c	3.80 b	45.36 b	33.13 b
	B3	93.2 a	47.0 b	3.69 c	3.88 b	44.93 b	31.45 b
<i>effect of DI</i>		79.8 A	51.3 A	3.62B	3.82B	43.65B	33.32A
RI	B0	72.4 b	48.4 b	3.37 c	3.40 c	39.64 b	33.15 b
	B1	61.6 c	50.1 b	3.46 c	3.24 c	43.66 b	28.25 c
	B2	76.4 b	56.2 a	3.33 c	3.42 c	42.51 b	26.93 c
	B3	85.0 a	43.5 c	3.22 c	3.04 c	42.57 b	28.58 c
<i>effect of RI</i>		73.9 A	49.5 A	3.35C	3.27C	42.10B	29.23B
PGPRs	B0	62.7 c	49.7 b	3.76 b	4.01 a	44.94 a	35.56 a
	B1	63.3 c	52.1 ab	4.02 a	3.86 ab	46.88 a	31.70 b
	B2	76.2 ab	56.2 a	3.76 b	3.92 a	46.20 a	30.74 b
	B3	82.3 a	47.4 b	3.77 b	3.70 b	46.04 a	30.36 b
Significance	WS	**	ns	***	***	***	**
	PGPRs	*	*	*	*	ns	**
	WS x PGPRs	ns	ns	**	*	ns	ns

* $P\leq 0.05$, ** $P<0.01$, *** $P<0.001$, ns=non-significant. Mean values in the column having different letters are significantly different at $P < 0.05$ level using Tukey's test. NI= non-irrigated, DI= deficit irrigation, RI= regular irrigation treatment, B0= without bacterium treatment, Capital letter= significant difference of water supplies, smaller letter=significant difference of bacteria treatments, PGPR= plant growth promoting rhizobacteria.

3.6. Effect of PGPR on nutritive value under different water supplies

Irrigation (DI, RI) increases the yield of tomato but decreased significantly the Brix and vitamin C content in the tomato fruit in comparison with the non-irrigated one however the degree of decrease depends on the year. Plant growth promoting rhizobacteria (PGPR) are believed to promote the drought tolerance of plants grown under water scarcity. The processing industries require the large yield with high Brix value. According to the results, plants treated by B1 treatment produced tomato fruits with higher Brix than non-treated plants and its effect was more pronounced under non-irrigated condition in particular dry year (2018).

The highest lycopene content was found in the combined treatment with irrigation and fertilizer, in 2018. It was the highest in B1 treatment ($268.7 \mu\text{g g}^{-1}$) under non-irrigation, B1- ($208.3 \mu\text{g g}^{-1}$) and B2- ($228.0 \mu\text{g g}^{-1}$) under deficit irrigation, and B3- ($219.9 \mu\text{g g}^{-1}$) under regular irrigation. In statistical analysis, lycopene content was the highest in NI treatment of all different treatments, with a significant difference $P < 0.001$. But no significant difference was found between the different treatments of PGPRs (Table 5). It means PGPRs have no significant effect on lycopene, but irrigation treatment has a significant effect on lycopene content.

β carotene content was highest B1- $6.46 \mu\text{g g}^{-1}$ in NI treatment, while B1- $4.49 \mu\text{g g}^{-1}$, B2- $5.21 \mu\text{g g}^{-1}$ in DI + biofertilizer treatment, and B3- $6.73 \mu\text{g g}^{-1}$ in RI + biofertilizer treatment, but there was no significant difference between irrigation and biofertilizer treatment. This means that two treatments did not affect the β carotene content (Table 5).

Lutein value ranged between 1.33 - $2.04 \mu\text{g g}^{-1}$ in NI + biofertilizer treatment, 0.82 - $1.38 \mu\text{g g}^{-1}$ in DI + biofertilizer treatment and 0.77 - $1.5 \mu\text{g g}^{-1}$ in RI + biofertilizer treatment. The highest value was $2.04 \mu\text{g g}^{-1}$ in the NI + B1 treatment. The Tukey test for statistical analysis found that different irrigation treatment had significant ($P < 0.001$) effected on the lutein content and

biofertilizer ($P < 0.05$) also influenced significantly the lutein content in a dry year (Table 5).

PGPRs have no significant effect on lycopene and beta carotene however they influence the other phytonutrients. It seems B3 treatment increases some carotenoids and phytoene and phytofluene in comparison with B0. Use of B1 treatment under NI and DI conditions increases the lycopene, beta and gamma carotene and lutein content in a drier year (2018) (Table 5).

In 2020, the highest lycopene content was found in the combined treatment with irrigation and fertilizer. It was the highest in B1 treatment ($238.4 \mu\text{g g}^{-1}$) under non-irrigation, B1- ($166.1 \mu\text{g g}^{-1}$) under deficit irrigation, and B3- ($172.2 \mu\text{g g}^{-1}$) under regular irrigation. The highest result was also reported in NI treatment. In statistical analysis, lycopene content was the highest in NI treatment of another different treatment, with a significant difference $P < 0.001$. But no significant difference was found between the different treatments of PGPRs (Table 6). The irrigation treatments had significant effect on lycopene content. β carotene content was highest B1- $6.91 \mu\text{g g}^{-1}$ in NI treatment, B3- $6.68 \mu\text{g g}^{-1}$ in DI + biofertilizer treatment, B1- $8.09 \mu\text{g g}^{-1}$ in RI + biofertilizer treatment, but there was no significant difference between irrigation and biofertilizer treatments. This means that irrigation and biofertilizer treatment did not affect the β carotene content (Table 6).

Lutein value ranged between 0.84 - $1.11 \mu\text{g g}^{-1}$ in NI + biofertilizer treatment, 0.95 - $1.1 \mu\text{g g}^{-1}$ in DI + biofertilizer treatment and 1.04 - $1.42 \mu\text{g g}^{-1}$ in RI + biofertilizer treatment. The highest value was $1.2 \mu\text{g g}^{-1}$ in the RI + B1 treatment. The Tukey test for statistical analysis found that different irrigation treatments had significant ($P < 0.01$) effected on the lutein content, however biofertilizer did not significantly influence the lutein content, in 2020 (Table 6). Results show that B1 treatment increases the cis lycopene, phytoene and phytofluene content of tomato fruit in wet year (2020) in comparison with B0 treatment (Table 6).

Table 5. Effect of water supply (WS) and PGPR on phytonutrients of H-1015 F1 tomato (2018)

Water supply	PGPRs	Lycopene μg g ⁻¹	β carotene μg g ⁻¹	Lutein μg g ⁻¹	13 cis lycopene μg g ⁻¹	Phytoene μg g ⁻¹	Phyto- fluene μg g ⁻¹
NI	B0	232.45 b	4.38 c	1.33 c	3.01 g	7.71	4.22
	B1	268.76 a	6.46 a	2.04 a	7.45 d	10.47	5.42
	B2	239.09 b	4.78 bc	1.47 c	6.23 e	9.62	4.77
	B3	248.38 ab	5.24 b	1.75 b	9.00 c	12.00	5.44
<i>effect of NI</i>		247.17A	5.21	1.65A	6.42B	9.94A	4.96A
DI	B0	181.87 c	3.87 d	0.82 e	6.22 e	6.66	3.66
	B1	208.29 b	4.49 c	1.08 d	6.83 e	7.70	3.91
	B2	228.04 b	5.21 b	1.38 c	5.64 f	10.81	5.23
	B3	188.81 cd	3.61 d	1.26 c	25.36 a	9.90	5.03
<i>effect of DI</i>		201.75B	4.29	1.13B	11.01A	8.77B	4.46A
RI	B0	149.50 d	3.65 d	0.89 e	3.60 g	5.81	3.04
	B1	136.87 d	3.63 d	0.77 e	3.48 g	5.31	2.56
	B2	161.81 d	4.73 bc	1.03 d	6.78 e	7.03	3.40
	B3	219.95 b	6.73 a	1.50 c	12.58 b	10.39	4.96
<i>effect of RI</i>		167.03C	4.69	1.05B	6.61B	7.13B	3.49B
PGPRs	B0	187.94	3.97	1.02 b	4.27 b	6.73 c	3.64 b
	B1	204.64	4.86	1.29 ab	5.92 b	7.82 bc	3.96 b
	B2	209.65	4.91	1.29 ab	6.22 b	9.15 ab	4.47 ab
	B3	219.05	5.19	1.50 a	15.65 a	10.76 a	5.14 a
Sign.	WS	***	ns	***	***	**	**
	PGPR	ns	ns	**	***	***	**
	WSx PGPR	*	**	*	***	ns	ns

*P<0.05, **P<0.01, ***P<0.001, ns=non-significant. Mean values in the column having different letter are significantly different at P < 0.05 level using Tukey's test. NI= non-irrigated, DI= deficit irrigation, RI= regular irrigation, B0= without bacterium treatment. Capital letter shows significant difference of water supplies, and smaller letter shows significant difference of bacteria treatments.

Table 6. Effect of water supply (WS) and PGPR on phytonutrients of H-1015 F1 tomato (2020)

Water supply	PGPRs	Lycopene µg g ⁻¹	β carotene µg g ⁻¹	Lutein µg g ⁻¹	13 cis lycopene µg g ⁻¹	Phytoene µg g ⁻¹	Phyto- fluene µg g ⁻¹
NI	B0	233.18	6.45	0.84	4.66 e	9.90	4.75
	B1	238.36	6.91	1.08	5.08 e	12.10	5.71
	B2	208.34	5.62	0.94	8.21 bc	10.24	4.71
	B3	196.62	5.83	1.11	5.56 e	10.20	5.52
<i>effect of NI</i>		219.13A	6.20	0.99B	5.88A	10.61A	5.17A
DI	B0	156.93	5.43	1.04	9.31 b	6.32	2.75
	B1	166.14	5.88	1.07	6.28 d	7.58	3.60
	B2	156.48	6.46	0.95	4.17 e	7.17	3.48
	B3	160.53	6.68	1.10	7.31 c	6.99	3.54
<i>effect of DI</i>		160.02B	6.11	1.04B	6.77A	7.02B	3.34C
RI	B0	141.38	6.72	1.04	6.30 d	6.41	3.21
	B1	168.29	8.09	1.42	15.77 a	9.66	4.79
	B2	143.68	6.45	1.31	3.66 f	7.99	4.18
	B3	172.22	7.95	1.40	3.93 f	9.19	4.80
<i>effect of RI</i>		156.39B	7.30	1.29A	7.42A	8.31B	4.24B
PGPR	B0	177.16	6.20	0.98	6.76 ab	7.54 b	3.57 b
	B1	190.93	6.96	1.19	9.04 a	9.78 a	4.70 a
	B2	169.50	6.18	1.07	5.35 b	8.46 ab	4.12 ab
	B3	176.46	6.82	1.28	5.60 b	8.79 a	4.62 a
Sign.	WS	***	ns	**	ns	***	***
	PGPR	ns	ns	ns	**	*	*
	WSxPGPR	ns	ns	ns	***	ns	ns

*P<0.05, **P<0.01, ***P<0.001, ns=non-significant. Mean values in the column having different letter are significantly different at P < 0.05 level using Tukey's test. NI= non-irrigated, DI= deficit irrigation, RI= regular irrigation, B0= without bacterium treatment. Capital letter shows significant difference of water supplies, and smaller letter shows significant difference of bacteria treatments.

NEW SCIENTIFIC RESULTS

- I showed a close relationship ($R^2=0.82$ and $R^2=0.88$) between chlorophyll fluorescence (F_v/F_m) and yield under 100% water supply (RI), while no strong correlation was found under NI and DI treatments.
- The effect of water supply on the marketable fruit yield depends on seasonal variation. In 2018, the lowest yield was with NI, while lowest yield was with RI in 2020. But in two seasons the DI produced the highest marketable yield. The no irrigation treatment (NI) increased significantly ($p < 0.01$) the total soluble solid and carotenoid.
- I have indicated that biofertilizers have a positive effect on tomato quality components in different climate conditions (dry year (2018) and rainy year (2020)). The best results were obtained with B1 biofertilizer (contains *Pseudomonas putida* B5, *Chryseobacterium* sp. B8/1, *Acinetobacter* sp. PR7/2, *Aeromonas salmonicida* PR10, *Variovorax* sp. BAR04).
- I found that depending on seasonal variation, biofertilizer 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.
- It was found that the biofertilizer inoculation helped tomato plant to overcome the water stress impact through avoidance mechanism by increasing the water and nutrient uptake. In other words, biofertilizer inoculation protected the tomato plants from the water deficit instead of stimulating them to tolerate the stress.

CONCLUSION AND RECOMMENDATIONS

- The two years study examined how environmental factors such as precipitation, water supply and air temperature affect tomato growth and development stages, as well as yield and fruit quality, in tomato production.
- The result showed that water supply treatments and air temperature during vegetation period directly and indirectly effected photosynthesis and the antioxidant contents of fruits. As the air temperature rises, the canopy temperature increases and the chlorophyll content decreases, which negatively affected the fruit quantity. Especially, plant physiological traits have a strong effect on yield.
- Water stress conditions during plant growth increased the content of antioxidants in tomato plants. Water stress positively effected fruit nutrition, but negatively effected fruit yield. Use of regular irrigation resulted in a high yield with low nutritional quality particularly in the dry year but in the wet year it was a flooding stress producing a low yield and high non marketable yield. Moderate water stress using deficit irrigation resulted in sufficient yield and Brix yield and nonmarketable yield but nutritional quality of fruit is still low. Use of deficit irrigation improves the water use efficiency (WUE) which is more associated with biomass and Brix yield ($t\ ha^{-1}$) in dry (2018) year than in wet year (2020). Our results encourage the use of deficit irrigation in industrial agriculture systems, because it provides a fewer use of irrigation water without significant decrease in yield however the yield quality needs to be improved. Lycopene is one of the main antioxidants in fresh tomato fruits, and its amount was the highest under non irrigated condition independently of the weather conditions.

Lutein content was high under non-irrigated condition in a dry year but it was low in a wet year. These carotenoids of tomato were decreased by deficit irrigation. Plant growth promoting rhizobacteria (PGPR) promote the plant to endure the water scarcity but their effect on the Brix and vitamin C content of fruit differ due to the strong water stress. The results showed that use of B1 of PGPR treatments improved the Brix value of fruits independently of the weather conditions.

- In the field biofertilizer inoculant 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) occurred only in the year 2018. Use of B1 treatment under NI and DI conditions increases the lycopene, beta carotene and lutein content in a drier year.
- The better performance of deficit irrigation for total yield of tomato is accounted for the combined treatment, but more efficient performance of biofertilizer was recorded on fruit antioxidant content. However, B1 negatively influenced the vitamin C content, its effect on cis lycopene, phytoene and phytofluene content was significantly positive and a moderate increase in lycopene content could be detected in the wet year (2020).
- Our results encourage the use of B1 as “biofertilizer” 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 a sowing pre-transplant bio fertilizer inoculation, but economical aspects should be considered, since more inoculum is required.

RELATED PUBLICATIONS

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