



**Hungarian University of Agriculture and Life Sciences**

**Doctoral School of Plant Science**

**Ph.D. Dissertation**

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

**By**

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## ACRONYMS

PGPR - plant growth promoting rhizobacteria  
WUE - water use efficiency  
SDD - stress degree day  
Fv/Fm – chlorophyll fluorescence  
SPAD – chlorophyll content  
T<sub>leaf</sub> – leaf surface temperature  
T<sub>air</sub> – air temperature  
HPLC - high performance liquid chromatograph  
Brix – soluble solid content  
ASC - ascorbate  
DHA – di-hydro-ascorbate  
ROS - reactive oxygen species  
ET<sub>c</sub> - crop evapotranspiration  
ET<sub>0</sub> - the reference of evapotranspiration  
K<sub>c</sub> - crop coefficient  
RI - optimum water supply treatment  
DI - deficit irrigation treatment  
NI - non-irrigated treatment  
RH - relative humidity  
T<sub>min</sub> - minimum temperature  
T<sub>max</sub> - maximum temperature

# 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,  $\beta$ -carotene, lutein, and ascorbic acid were determined in fruits.

## 2. LITERATURE REVIEW

### 2.1. The experimental plant

#### 2.1.1. *Lycopersicon esculentum* M.

The tomato is one of the species of *Solanaceae*. The two geographical locations in Mexico, Vera Cruz and Puebla are considered to be a center of domestication. As can be seen from the distribution of wild species, the progenitor of the tomato is mainly found in a narrow, dry, tropical, coastal region of Ecuador and Peru and some other regions of Northern Chile. The wild species, *L. Lycopersicum* var. *cerasiforme*, is assumed to be an immediate ancestor of the tomato. A wide stretch of Ecuador and Peru abounds with this form of tomato.

Mexico, not the Andean regions, was a pioneer in introduction of tomato to Europe. The name ‘tomato’ was derived from the Nahuatl language of Mexico. The early introduction of tomatoes into Europe was hampered by the dangerous food reputation attributed to the relationship to poisonous *Solanaceae* species such as belladonna and mandrake.

In Italy, therefore, it was initially used only as an ornamental. In France, the fruit was called “pomme d’amour” or love apple. In Italy, they were called “pomi d’oro” or golden apple, suggesting those first introduced were yellow fruited.

Rubatzsky & Yamaguchi (1997) reported that the cultivation of tomato had gained momentum in Europe during the 20<sup>th</sup> Century.

Some benefits pertaining to the cultivation of tomato are a shorter life cycle and flexible horticultural technique that also comprise grafting or cutting (Wien, 1997). Two main tomato types are currently grown:

First type: the determinate or “bushy” tomato is mainly used for processed food and is the most important outdoor commercial type in the USA. It has a time-limited flowering stage followed by a stage of fruit development.

Second type: the indeterminate or “vine” tomato, largely used for the production of fresh fruits in greenhouses and home gardens, produces inflorescences and flowers continuously throughout the plant’s life.

#### 2.1.2. A botanical characteristic of tomato

Tomatoes are usually annuals in temperate regions or short-lived perennials in the tropics. Plants grow from 0.5-2.0 m tall, with solid and thick stems. Growth habit can vary from erect to semi prostrate and some also exhibit substantial vining. Taproots usually are strong and deep, some occasionally reach depths of 3 m. Small glandular hairs that appear on stems, leaves, and peduncles



have a noticeable odour. Leaves are compound pinnate, coarsely toothed and often curled, but also can be smooth. The inflorescence is borne opposite and between leaves. Usually, 4-12 flowers develop on a broad, flat raceme. Flowers are perfect, about 2 cm in diameter and often pendent with a yellow star-shaped corolla, yellow anthers are united to form a tube. Self-pollination is commonly observed. Flowers are not unable to nectar, however, the process of cross-pollination is carried out by bees at varying frequencies (Rubatzky and Yamaguchi, 1997).

**2.1.3. The economic importance of tomato**

The tomato (*Lycopersicon esculentum* Mill.) is a member of the *Solanaceae*. The plant species are native to South America (Mexico) and Central America, but today people across the world plant it especially in temperate climates and in greenhouses only. Tomato is one of the most popular vegetables and one of the most important fruit crops. Tomato is the second largest cultivated plant in the world (Rubatzky and Yamaguchi, 1997).

Global tomato processing in 2019 was 37.38 million tons (mT), 2.4 million tons above the 2018 volume (Figure 1).

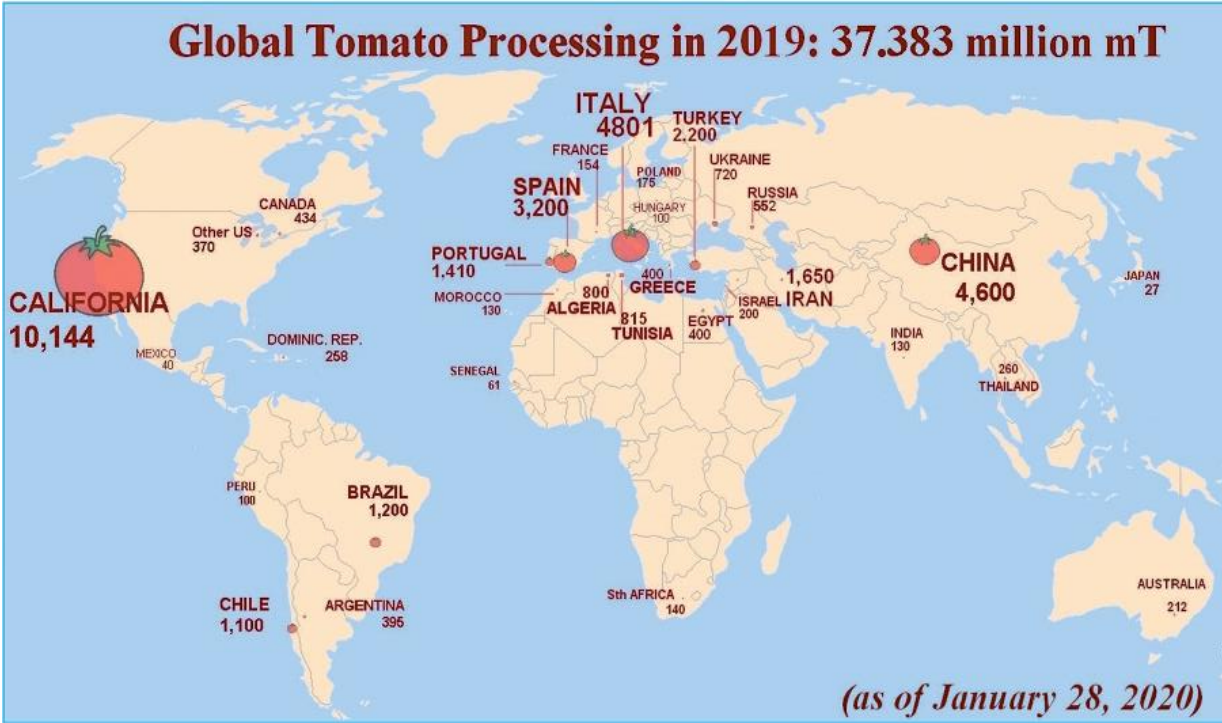


Figure 1. Global tomato processing in 2019. Sources: Tomato news 2020 yearbook

The largest tomato producers were California, USA, where 10.1 million tons, were processed, followed by Italy 4.8 million tons and China 4.6 million tons. Spain was the fourth largest producer in the world, with 3.2 million tons, then Turkey 2.2 million tons, Iran 1.6 million tons, Portugal

1.4 million tons, Brazil 1.2 million tons, Chile 1.1 million tons and Tunisia 0.8 million tons, in 2019. Hungary’s production was only 0.1 million tons (Tomato news 2020).

The United States is a leader in yield and production of processing tomatoes. The volume of tomato produced in wet conditions is limited because of fungi and some diseases. Production volume between developed and developing countries is fairly close. With continuing strong growth in worldwide demand for fresh and processed tomatoes, many countries have steadily increased production to satisfy domestic and international markets.

Last 10 years average of processing tomato shows production process increasing year by year. If we compare 1989 and 2019, it was increased by 15.224 million tons (Figure 2).

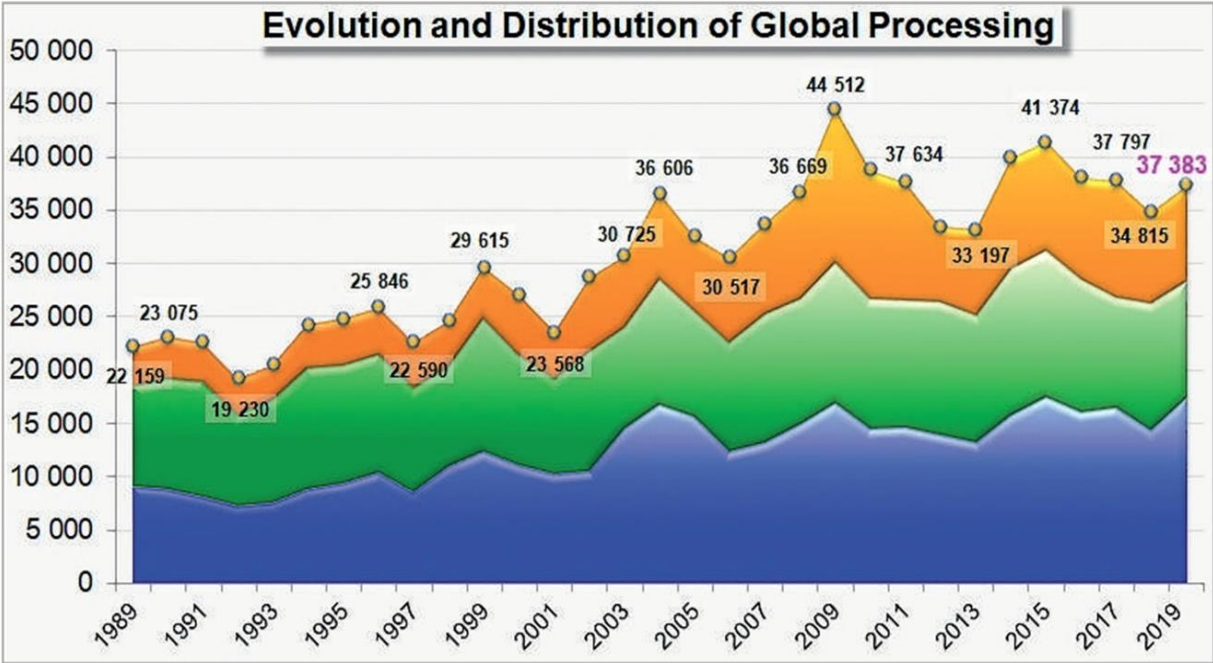


Figure 2. Tomato global production in 1989-2019  
 Resource: <http://www.tomatonews.com/pdf/yearbook/2020/>

Tomato products expressed a clear rise in the world exports in this sector, consumption of tomato-based products reportedly increased, according to estimates gathered during the latest study commissioned by the World Processing Tomato Council, by around 4% in 2018/2019 compared to the average level of the three previous marketing years. The slight upturn recorded worldwide has been expressed by a moderate rise in the level of overall individual consumption. Global tomato consumption was 38.3 million tons, in 2018/2019. Average consumer consumed the equivalent of 5 kg of fresh tomato in processed form, 2% more than on average during the previous three marketing years (Tomato news 2020). World tomato production volume is continuing strong growth, but Hungarian production volume little decreased and last few years increasing slowly,

since 2008 (Table 1). World tomato average production was 166470.7 tons and 168.3 tons in Hungary. The world highest yield was 382.7 tons in 2018. And highest yield of Hungary was 90.4 tons, in 2008 (Table 1).

Table 1. World and Hungarian tomato production (2008-2018)

Year	World			Hungary		
	Area (ha x 10 <sup>3</sup> )	Yield t ha <sup>-1</sup>	Production (t x 10 <sup>3</sup> )	Area (ha x 10 <sup>3</sup> )	Yield t ha <sup>-1</sup>	Production (t x 10 <sup>3</sup> )
2008	4223.0	335.4	141648	2.28	90.4	205
2009	4419.0	351.4	155308	2.34	82.3	192
2010	4430.0	346.0	153314	1.87	71.7	134
2011	4582.0	348.0	159489	1.98	82.7	163
2012	4803.0	339.8	163211	1.28	85.0	108
2013	4848.0	340.8	165239	1.74	78.0	135
2014	4903.0	356.5	174787	1.88	81.5	153
2015	4799.0	368.4	176823	2.26	88.5	200
2016	5013.0	355.4	178158	2.08	83.2	173
2017	4846.0	373.3	180945	2.19	84.2	184
2018	4762.0	382.7	182256	2.50	81.8	204
Average	4693.5	354.3	166470.7	2.0	82.7	168.3
Max	5013.0	382.7	182256.0	2.5	90.4	205.0
Min	4223.0	335.4	141648.0	1.3	71.7	108.0

Source: FAOSTAT. <http://www.fao.org/faostat/en/#data/QC>

## 2.2. Nutritional value of tomato

The tomato (*Lycopersicon esculentum Mill.*) contains 93-95% water and low levels of solid matter. Tomatoes are relatively rich in antioxidants: vitamin C (160-240 mg kg<sup>-1</sup>), provitamin A carotenes (6-9 mg kg<sup>-1</sup>) (Table 2). Also present in small quantities are vitamin E (5-20 mg kg<sup>-1</sup>), flavonoids (5-50 mg kg<sup>-1</sup>) and trace elements such as copper (0.1-0.9 mg kg<sup>-1</sup>), manganese (1-1.5 mg kg<sup>-1</sup>) and zinc (1-2.4 mg kg<sup>-1</sup>) which are present in several antioxidant enzymes (Bilton et al. 2001).

Fruits and vegetables are good sources of natural antioxidants for the human diet, containing many different antioxidant components which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline in addition to other health benefits (Cao et al., 1996; Cohen et al., 2000; Knekt et al., 2002; Liu et al., 2000; Sweeney et al., 2002; Velioglu et al., 1998; Wang et al., 1996). These antioxidants include carotenoids, vitamins, flavonoids, other phenolic compounds, dietary glutathione, and endogenous metabolites (Larson, 1988). Total carotenoid contents and antioxidant activities related to the colour of tomato which was the highest in the red tomatoes followed by purple, orange, pink and yellow ones (Li et al. 2013).

Table 2. Analytical data of tomato fruit

Composition	Database			
	1	2	3	4
	g kg <sup>-1</sup> fresh matter			
Water	931	942	933	ni
Protein	7.0	9.5	9.0	9.6
Fat	3.0	2.1	2.0	ni
Carbohydrate	31.0	34.5	32.0	ni
	mg kg <sup>-1</sup> fresh matter			
Fe	5.0	5.0	4.0	6.0
Cu	0.1	0.9	0.6	0.9
Zn	1.00	2.4	1.4	1.5
Mn	1.00	1.4	1.1	0.9
Vitamin C	170	242	180	160
Vitamin E	12.2	8.00	10.00	ni
Carotene	6.4	8.2	6*	7.6
Folates	0.17	0.39	0.23	ni

ni - not indicated; \* - beta-carotene equivalent (Holland et al., 1992; Scherz and Senser, 1989; Feinberg et al., 1991; Price, 1976)

### 2.2.1. Carotenoids

Early researchers (Kuhn and Grundmann, 1932) found that tomato carotenoids consisted predominantly of carotenes. Trombly and Porter (1953) listed 19 carotenes obtained from tomato extracts with lycopene and  $\beta$ -carotene usually constituting the major proportion of the carotene fraction. The two polyenes (colorless carotenoids), phytoene and phytofluene have been isolated frequently from tomato fruit (Porter and Zscheile, 1946; Rabourn and Quackenbush, 1953; Tomes, 1963).

Carotenoids are pigments which play a major role in the protection of plants against photooxidative processes. Carotenoids are among the most common natural pigments, and more than 600 different compounds have been characterized until now, with  $\beta$ -carotene as the most prominent (Olson and Krinsky, 1995). Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. Only plants, bacteria, fungi, and algae can synthesize carotenoids, but many animals incorporate them from their diet. Carotenoids serve as antioxidants in animals, and the so called provitamin A carotenoids are used as a source for vitamin A. Most carotenoids can be derived from a 40-carbon basal structure, which includes a system of conjugated double bonds.

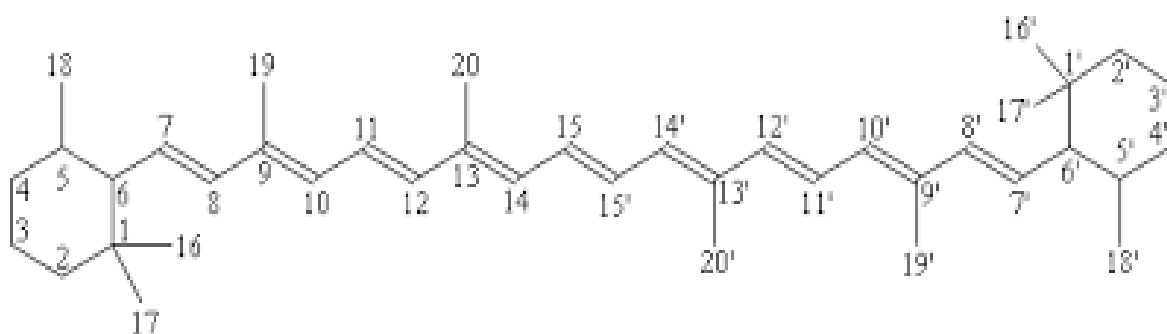


Figure 3. Structure and nomenclature of carotenoid

The central chain may carry cyclic end-groups which can be substituted with oxygen-containing functional groups (Figure 3). Based on their composition, carotenoids are divided in two classes, carotenes containing only carbon and hydrogen atoms, and oxocarotenoids (xanthophylls) which carry at least one oxygen atom.

Carotenoids are isoprenoid molecules that are common to all photosynthetic tissues. They are divided into the hydrocarbon carotenes, such as lycopene and  $\beta$ -carotene (Figure 4) or xanthophylls, typified by lutein (Figure 4).

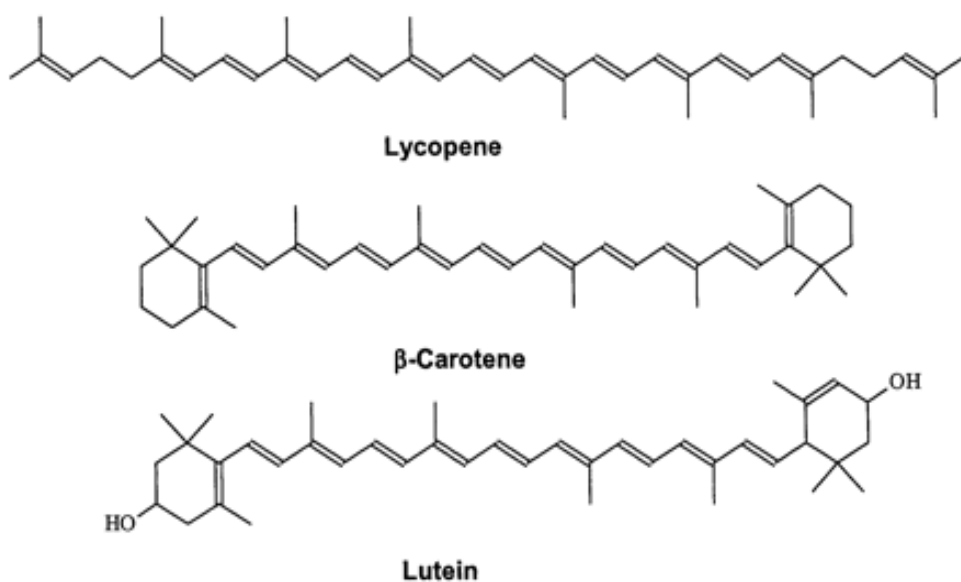


Figure 4. Structures of typical carotenoids.

The antioxidant defense system of the organism is a complex network and comprises several enzymatic and non-enzymatic antioxidants (Sies, 1993). It has been suggested that interactions between structurally different compounds with variable antioxidant activity provides additional protection against increased oxidative stress. Vitamin C, for instance, the most powerful water-soluble antioxidant in human blood plasma, acts as a regenerator for vitamin E in lipid systems (Niki et al., 1995).  $\beta$ -Carotene might also play a role in such radical transfer chains (Truscott, 1990; Bohm et al., 1997). There is evidence from in vitro studies, that  $\beta$ -carotene regenerates tocopherol from the tocopheroxyl radical. The resulting carotenoid radical cation may subsequently be repaired by vitamin C. Synergistic interactions against UVA-induced photooxidative stress have been observed in cultured human fibroblasts when combinations of antioxidants were applied with  $\beta$ -carotene as main component (Böhm et al., 1998a, Böhm et al., 1998b).

In biological systems, light exposure leads to the formation of reactive oxygen species which are damaging to biomolecules and affect the integrity and stability of subcellular structures, cells and tissues (Stahl and Sies, 2001; Krutmann, 2000). Photooxidative processes play a role in the pathobiochemistry of several diseases of light-exposed tissues, the eye and the skin.

Carotenoids are essential components of human diets, providing precursors for biosynthesis of vitamin A, which is a well-known carotenoid derivative with widespread biological functions (Krinsky and Johnson, 2005). Lycopene, the most abundant carotenoid in ripe tomato, is regarded as a bioactive component with regard to treating chronic diseases and lowering risk of cancer and cardiovascular disease (Sandmann et al., 2006, Ford and Erdman, 2012).

According to Tonucci et al. (1995) the carotenoids were detected and quantified lycopene, lycopene-5, 6-diol, lutein,  $\alpha$ ,  $\beta$ ,  $\gamma$ - and  $\zeta$ -carotenes, neurosporene, phytoene, and phytofluene. As expected, lycopene was the most abundant carotenoid, ranging in concentration from 0.3 mg 100 g<sup>-1</sup> in vegetable to 55 mg 100 g<sup>-1</sup> in tomato paste. The concentration of  $\beta$ -carotene ranged from 0.23 mg 100 g<sup>-1</sup> in tomato soup to 1.51 mg 100 g<sup>-1</sup> in vegetables. Lutein was found at very low concentrations in all products analyzed except tomato paste, which contained 0.34 g 100 g<sup>-1</sup> (Tonucci et al., 1995). In addition, Moretti et al. (1998) have shown that fruit bruising at the breaker stage could significantly decrease (-37%) the total carotenoids content in the locular tissue of tomato fruit at the ripe stage.

### 2.2.2. Ascorbic acid (Vitamin C)

L-ascorbic acid (Figure 5) is a  $\gamma$ -keto-lactone used in cells as an electron donor. The first product of its oxidation is the free radical monodehydroascorbate. This transforms spontaneously to ascorbate (ASC) and dihydroascorbate (DHA). ASC is the principal biologically active form, but DHA, the main oxidation product, also exhibits biological activity. Since DHA can be easily transformed into ASC in the human body, it is important to measure both ASC and DHA in fruit and vegetables for vitamin C activity (Lee and Kader, 2000).

Fresh fruit and vegetables, such as tomatoes, are the principal source of ascorbic acid (vitamin C) for humans, primates, and a few other mammals and passerines that are unable to synthesize this vitamin. Fruit ascorbic acid content is also valuable from an agronomic point of view, as well as documented evidence exists that the molecule, a prevalent antioxidant, can contribute to both biotic and abiotic stress tolerance in crops (Davey et al., 2000; Muckenschnabel et al., 2002; Kuzniak and Sklodowska, 2005), and also to post-harvest fruit quality (Davey and Keulemans, 2004; Malacrida et al., 2006).

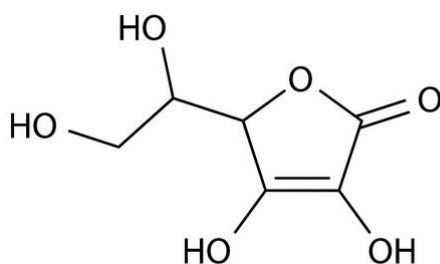


Figure 5. Structure of Ascorbic acid

The mean value of ascorbic acid content of 41 tomato varieties was 15.0 mg 100 g<sup>-1</sup>, and individual varieties ranged from 10.7 to 20.9 mg 100 g<sup>-1</sup>, wet weight basis. Tomatoes are a good dietary source of ascorbic acid (Vitamin C), however the ascorbic acid content varies greatly. Many factors contribute to this variation, and environmental growing conditions have been reported as having major effects on the ascorbic acid composition (Hamner et al. 1945, Mustard, 1946). Most researchers have found less than 100 percent variation in ascorbic acid content between different varieties for a single season and growing location (Maclinn and Fellers, 1938).

Tomatoes have remarkable concentrations of folate, vitamin C, and vitamin E (Gahler et al., 2003). Total vitamin C levels range from about 8 to 40 mg 100 g<sup>-1</sup> between species and varieties (Bertin and Génard, 2018).

### **2.2.3. Soluble solid content**

Fruit dry matter content, both soluble solids content and total solids content, constitutes one of the most important quality components in processing tomatoes (*Lycopersicon esculentum* Mill). Soluble solids content which accounts for about 80% of total solids content and consists primarily of sugars and organic acids, is directly related to the case yield of product per ton of tomato, while level and quality of insoluble solids is related to consistency of tomato products (Stevens and Scott 1990). Stevens and Rick (1986) reported susceptibility to diseases which affect water uptake of the plants and can have much larger effect on solids than genotypic variation for fruit solids content. Often selection in segregating populations is not effective because variation in irrigation, soil texture, disease resistance etc. may have large effects on solids content.

Soluble solid content (SSC) is one of the most important factors determining the quality and price of fresh fruits. Tomato fruits are rich in polyphenols, which constitute the largest part of the antioxidant content of the soluble solids (Proteggente et al., 2002). George et al. (2004) observed a huge variance (104-400 mg kg<sup>-1</sup>) in the polyphenol content of different tomato cultivars. According to Poysa (1993) and other researchers they found that soluble solid content (Brix°) can be very high without irrigation, although there is limited variability in commercial tomato cultivars (4.5-6.25%) (Patanè and Cosentino, 2010; Le et al., 2018).

### **2.3. Abiotic stress**

Environmental factors influence development, growth and biological yield of tomato. The ecological environment, in terms of tomato production, the habitat is the result of combined effects of climate, soil and biotic factors. Each of these environmental factors consists of many elements



which may affect plants by different properties and intensities. Two environmental factors belong to the abiotic factors: climate and soil which include as follows:

Climate: radiation, temperature, water, wind, CO<sub>2</sub> concentration and air pollution

Soil: structure, chemical components, nutrients, soil water and air content (Wien, 1997).

### **2.3.1. Water efficiency**

Vegetable crops have high water needs so it is necessary to develop growing strategies to optimize the efficiency of water use and maintain the yield and its quality (Nagare et al. 2016). Specially, processing tomato requires good agricultural conditions during all development stages to harvest. Water management (irrigation management) plays a major role in quality and quantity of tomato yields. Stevens and Rick (1986) consider the difficulty of water management to be the most important deterrent to high yields of high quality tomatoes. Processing tomato requires 400-800 mm of water during the whole development to harvest (Battilani et al. 2012).

Drip irrigation is a highly efficient method of water application, which is also ideally suited for processing tomato in the open field (Battilani et al., 2009; Helyes and Varga, 1994).

The sustainable use of water in agriculture has become a priority and the adoption of irrigation strategies which may allow saving irrigation water and maintaining satisfactory yields, thus improving water use efficiency, may contribute to the preservation of this even more restricted resource (Parry et al., 2005; Topcu et al., 2007). A recent positive approach to attain the goal of improving water use efficiency in agriculture is conventional deficit irrigation. The goal of deficit irrigation is to increase crop water use efficiency by reducing the amount of water applied with watering or by reducing the number of irrigation (Kirda, 2002). Water saving irrigation strategies such as deficit irrigation may allow to optimize water productivity in hot and dry climate areas, stabilizing yield and improving quality (Costa et al., 2007). Some studies have shown that water deficit during certain stages of growing season improves fruit quality, although water limitations may also determine fruit yield losses (Patanè and Cosentino, 2010). Deficit irrigation has been shown to reduce the production costs, preserve the water consumption, and it also has a positive effect on the processing quality of tomatoes (Favati et al., 2009; Patanè et al., 2011).

Drip irrigation system and deficit irrigation are both the most effective for tomato production (Selim et al., 2012, Pék et al., 2014). Although this irrigation method can cause water stress to plants, if the yield reduction is lower than the benefit derived from the water savings or from quality improvement (Johnstone et al., 2005; Pék et al., 2017). The effects of deficit irrigation vary depending on seasonal weather conditions and affect crops differently; it is also influenced by soil

(Helyes and Varga, 1994). The most common water deficit applied is 50% of evapotranspiration (Bakr et al., 2017), that can be used in different vegetative stages (Kuşçu et al., 2014; Nagare et al., 2016), or simply terminating irrigation for the duration of various phenological stages (Johnstone et al., 2005; Lei et al., 2009; Kuşçu et al., 2014). Water use efficiency is expressed by the ratio of the yield to amount of water in processing tomato (Battilani et al. 2009) and it can be considered a relative constant for a given crop under a given climate (Patanè et al. 2011). WUE expresses the efficiency of water in plant production (Patanè and Saita, 2015), which may contribute to saving irrigation water (Parry et al., 2005).

The knowledge of the plant response to water stress is important in order to determine the timing of irrigation, the applied water amount (Feres and Evans 2006). The period from fruit setting to the end of fruit development is the most sensitive to water deficiency (Helyes and Varga, 1994), when the degree of water stress tolerance of cultivars can be determined by the measurement of the physiological traits. According to Helyes (1990) and Cselotei and Helyes (1988) irrigation had affected the foliage temperature and yield of tomato. Decrease in soil water content induces the stomatal closure to reduce the water loss of plants. Nevertheless, the long-term stomatal closure results not only the reduction in transpiration but photosynthesis inhibition (Sing and Reddy 2011). Researchers found reduction in chlorophyll content under moisture deficit could be attributed to the fact that water stress damages the photosynthetic apparatus by causing changes in the chlorophyll contents and components (Kenneth et al., 2017). Reduction in moisture led to a decrease in the leaf relative water content, stomatal conductance, and fruit yield.

### **2.3.2. Temperature and radiation**

Temperature is not a growth factor supplying energy or constituents but primarily controls the rates of chemical reactions. Temperature controls plant development including morphogenesis and plant quality.

Plants can only survive and grow within their temperature limits and if during the seasonal cycle sufficient time is supplied and growth is sufficiently efficient. However, species-specific temperature limits, the minima and maxima, which define the ranges of survival are difficult to specify. Factors that affect these include the tremendous plasticity caused by adaptation, the duration of cold stress, the developmental stage, the level of activity or dormancy and cultivar differences (Wien, 1997). According to Dumas et al. (2003) the formation of lycopene depends on the temperature range and seems to occur between 12 and 32 °C. This process was found to be at an optimum between 16–18 and 26 °C in both cherry type variety VFNT cell suspension cultures and fresh tomato fruit harvested at the pink-ripe stage and left to ripen for several

weeks. The production of lycopene is inhibited by excessive sunlight and the best conditions are sufficiently high temperatures along with sufficiently dense foliage to protect the fruit from direct exposure to the sun. However, in tomatoes harvested for processing, the lycopene levels were enhanced by 5% at incubation temperatures of 30 and 34 °C and by 33% at 37 °C, and decreased in salad tomatoes incubated at temperatures above 30 °C (Dumas et al. 2003).

McCollum (1954) had observed that tomato fruits exposed to direct sunlight in the field often developed poor color due primarily to a low content of lycopene, because of the high temperatures of the exposed fruits. Lycopene synthesis in excised fruits from cultivars with various strains of pigments was drastically inhibited at 32°C in every strain which produced this pigment (Tomes, 1963). From the breaker point, Koskitalo and Ormrod (1972) distributed greenhouse-grown tomato plants in controlled environment chambers at 4 different temperature regimes respectively. Twenty-one days after, the fruit carotenoids contents were decreased from 64.8 to 24.2 mg kg<sup>-1</sup> for lower regimes, except for β-carotene. Baqar and Lee (1978) showed that 30°C temperature drastically reduced carotene synthesis in cultivar Rouge de Marmande, except for β-carotene and Grierson and Kader (1986) confirmed that the synthesis of lycopene, but not β-carotene, was inhibited by temperatures within the range 30-35°C.

Relatively high temperature or low light intensity of ripening fruit, may probably lead to reduction in ascorbic acid content due to oxidation (Murneek et al. 1954). However, in greenhouse, Liptay et al. (1986) observed seasonal variations of cv. Jumbo fruit vitamin C content from 70 to 230 mg kg<sup>-1</sup> fresh matter at the mature-green stage, in direct relation with temperature variations.

Light is effective until chlorophyll disappears. Carotene is increased by illumination of tomato fruits during ripening. If exposed to direct sunlight during development, the fruits will be higher in carotene when ripe than shaded fruits (McCollum, 1954).

Lipton (1970) found that the incidence of defective coloration of the shoulders or sides of fruits was higher in fruit exposed to the sun than in fruit shaded by foliage and seemed to be influenced by infrared and short-wave radiation. Thomas and Jen (1975) established that red light and the intensity of red light had a positive effect on carotenoid synthesis of detached mature-green tomato fruit and this effect was not a temperature effect. The far-red light suppressed carotenoid production, as compared to dark control.

Somers et al. (1951) found that the ascorbic acid content of the fruits was associated with the degree to which they were shaded inside the plants: vitamin C content increased significantly from 298 mg kg<sup>-1</sup> fresh matter in “full shade” fruit to 344 mg kg<sup>-1</sup> fresh matter in “fully exposed” fruit. This was confirmed by Murneek et al. (1954) greenhouse-grown tomatoes (cvs. Marglobe and

Stokesdale) were usually lower in vitamin C than those grown outdoors, due chiefly to lower light intensity and shorter days during late fall, winter and early spring. There seemed to be a seasonal increase in concentration in vitamin C in field-grown fruit from early summer to late summer (250 to 350 mg kg<sup>-1</sup> fresh fruit). There was a strong positive correlation between vitamin C concentration and light intensity. In shade situation (by foliage) might reduce it by 15-20% compared to in light situation. The side of a tomato that was directly exposed to light was invariably higher in vitamin C than the shaded side. Brown (1954) also reported that fruits receiving direct sunlight were higher in ascorbic acid than fruits shaded by leaves or artificial cover, showing that, like many previous studies, light is the predominant factor in ascorbic acid production and accumulation in plant material. Venter (1977) demonstrated also that vitamin C content of tomato fruit (cv. Sieger) increased (from 250 to 400 mg kg<sup>-1</sup> fresh matter) with the length of the radiation period, with differences between shaded or unshaded fruits on the same plant or between shaded or unshaded fruit sections. López-Andréu et al. (1986) also found lower fruit vitamin C values in greenhouse with less direct sunlight than for field cultivated fruit. Another field study on the effects of shading with netting (0, 35, 51 or 63% shade) conducted in Egypt on two tomato cultivars showed that ascorbic acid content decreased with increasing shading while the best yield components were obtained from plants grown under 35% shading (El-Gizawy et al, 1993). Adegoroye and Jolliffe (1987) found that in tissues of fruit directly exposed to radiation, ascorbic acid content was decreased, although treated fruit exhibited some capacity for ascorbic acid accumulation.

Light exposure seems to be favorable to vitamin C accumulation in the tomato fruit, somewhat like for carotene synthesis in fruit. Thus, fruit vitamin C and  $\beta$ -carotene contents might be affected positively by not too close plant spacing to provide radiation and the use of cultivars naturally presenting a somewhat poor foliage as well as the use of moderate nitrogen rates to avoid excess of vegetative growth.

The temperature measurement of the plant canopy should be monitored for heat stress (Bates and Hall, 1981; Bócs et al., 2009; Helyes, 1990). The simplest is the stress degree day (SDD), which can be computed as a difference of leaf surface temperature and air temperature (Helyes et al., 2006; Idso et al., 1981; Jackson et al., 1977). According to Takacs et al. (2019) SSD is more feasible for heat stress monitoring in the case of using infrared thermometer for leaf temperature measurements.

## **2.4. Biotic stress**

### **2.4.1. Biofertilizer impact on plants**

Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria). PGPR is fixing N<sub>2</sub>, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant–microbe symbioses (Vessey, 2003). The most intensively researched use of PGPR has been in agriculture and horticulture. Several PGPR formulations are currently available as commercial products for agricultural production. Biofertilizers stimulate plant growth, increase fruit yield and reduce disease (nematode) of tomato population by antagonistic behavior. Highest number of fruits per plant was 10.66 and had significant differences compared with untreated plants. Treated plant had high yield and low nematode eggs in soil (Almaghrabi et al. 2013). Chinese researchers found that tomato's disease was reduced by 66.1-73.6% using biofertilizer, respectively, compared to the control. Yield increases with bacteria in this trial ranged from 49.5 to 70.8% (Jian-Hua et al. 2004).

During last decades, the role of PGPR in mitigation of abiotic stresses in plants has been studied (Dimpkpa et al. 2009, Nadeem et al. 2014). During salt stress these microbes activate the plant antioxidant defense machinery to regulate different enzyme activity that scavenges the reactive oxygen species (ROS) (Islam et al. 2015). Rhizobacteria has been shown to increase the drought tolerance by regulating the levels of proteins polysaccharides and important phytohormones. The field experiments are currently carried out to augment the PGPR mediated salt and drought tolerance in tomato (Singh et al. 2018).

## **2.5. Effect of water stress on physiological traits of tomato**

Under drought, plants reduce water loss by stomatal closure which results not only in a reduction of transpiration but the inhibition of photosynthesis coupled with reducing CO<sub>2</sub> uptake (Sing and Reddy, 2011). As a result of decreasing photosynthesis, the amount of available metabolites required for the development of plants decreases (Dorji et al. 2005, Kulkarni and Phalke, 2009), therefore the height and yield of plants decrease (Agbna et al. 2017). The photosynthetic activity of the crops is one of the important factors influencing the yield that can be monitored by the measurement of physiological traits such as chlorophyll content of leaves, net photosynthetic rate, and stomatal conductance (Song et al. 2012). Climatic effects can be mitigated not only by irrigation, but also by the cultivated varieties that use water efficiently and tolerate drought stress (Köksal et al. 2008; Sezen et al. 2008; Nemeskéri et al. 2010, 2015). Over-irrigation negatively

affects the generative growth due to the excess vegetative growth (Williams et al. 2010; Pires et al. 2011) and it reduces the yield processing quality (Çolak et al. 2015; Lahoz et al. 2016). Water stress occurring in the vegetative growth stage leads to short plants with small leaf area (Nielsen and Nelson 1998), while during generative stage, it causes flower drop and pod abortion (Boutraa and Sanders 2001; Young et al. 2004; Foolad 2005; Fang et al. 2010), resulting in a significant decrease in yield. Nemeskeri et al. (2019) reported during flowering period, under regularly irrigated conditions, the SPAD, Fv/Fm, and canopy temperature related to stomatal conductance. Stomatal conductance had significant influence on yield and quality under non-irrigated and well-irrigated conditions while the SPAD value and canopy temperature had significant influence under deficit irrigated conditions.

## **2.6. Effect of water stress on yield of tomato**

Modern agriculture is faced with two tasks: (1) to produce enough food for a growing global population, and (2) to ensure satisfactory crop quality while using water resources efficiently. Efficient water application is critical to the successful production of vegetables. One of the major benefits of drip irrigation is that it allows the grower to use less water and fertilizer than conventional irrigation methods, such as surface and sprinkler irrigation.

Yrisarry et al. (1993) conducted a study that consisted of three irrigation water levels (0.5, 0.9 and 1.3 crop evapotranspiration) for VF 6203 processing tomato cultivar. Results showed total yield increased when the amount of water was also increased. It was also determined that the increased volume of irrigation water and low fruit nutrient content.

Djurović et al. (2016) found the highest fresh tomato fruit yields were achieved under full irrigation, covering 100% of crop evapotranspiration. The full irrigation treatment also resulted in the greatest dry weight of the fruits ( $1.1 \text{ kg m}^{-2}$ ). The average fruit weight was rather uniform and ranged from 71.7 g to 75.4 g with deficit irrigation at 50% of crop evapotranspiration.

The marketable yield did not significantly differ among plots irrigated. Marketable yield was negatively affected by the early water shortage in no irrigation treatment, due to the high fruit losses (>44%). The effects of deficit irrigation on fruit quality were generally the converse of those on fruit yield. Water use efficiency was positively affected by deficit irrigation, suggesting that the crop does not benefit from the water when it supplied to fulfil total crop requirements for the whole season. Yield response factor, which indicates the level of tolerance of a crop to water stress, was 0.49 for total dry biomass and 0.76 for marketable yield, indicating that in both cases the reduction in crop productivity is proportionally less than the relative evapotranspiration deficit (Patane et al. 2011).

## 2.7. Effect of water stress on phytochemicals of tomato

Antioxidants are believed to be important in the prevention of diseases. Lycopene is one of the main antioxidants to be found in fresh tomatoes and processed tomato products. The lycopene content also accounts for the redness of the fruit, which is one of the main qualities for which industry and consumers now look. Other carotenes (such as  $\beta$ -carotene), vitamin C, vitamin E and various phenolic compounds are also thought to be health-promoting factors with antioxidant properties. Since the antioxidant content of tomatoes may depend on genetic factors, the choice of variety cultivated may affect the results at harvest. To be able to control the antioxidant content of tomatoes at the field level when growing a given variety, it is necessary to know the effects of both environmental factors, especially water supply regimes and the agricultural methodology (Dumas et al. 2003). Khachick et al. (1992) reported that tomatoes contain mainly lycopene and also  $\beta$ -carotene,  $\zeta$ -carotene, phytofluene and phytoene and traces of lutein,  $\alpha$ -carotene etc. The composition of carotenoids greatly depends on the variety. The wild varieties can contain up to twice the lycopene and vitamin C quantity of the cultivated varieties (Stevens and Rick 1986). Red fruit varieties total lycopene content was higher than yellow fruit varieties.

Water plays an important role in plant life. In many localities, it is the limiting factor for agricultural crops and hence increasing yield. Therefore, for adequate use of water, attempts should be made to obtain maximum yield with minimum water supply.

In the deficit irrigation treatment with lower water supply plant growth, and in particular the number of fruit settings were depressed and the sugar and vitamin C concentrations in the fruits were significantly increased, especially during fruit ripening. The higher levels of sugars, titratable acids, aroma volatiles and vitamin C are responsible for the higher fruit quality under conditions of lower water supply (Veit-Kohler et al., 2000; Nahar and Gretzmacher, 2002).

Several experiments have shown that increasing the water supply significantly increases the yield, reduces the Brix content of the fruit however the Brix yield ( $t\ ha^{-1}$ ) is significantly increased (Helyes et al., 2010). The content of glucose, fructose, sucrose, malic acid, ascorbic acid and citric acid increased significantly with water stress. Veit-Kohler et al. (2000) showed average sugar and lycopene content was quite uniform, while the irrigation regime had a significant effect on the average organic acid content and total antioxidant activity. Deficit irrigation treatments resulted in a higher organic acid content and higher total antioxidant activity than full irrigation (Djurović et al. 2016). Irrigation water has a positive effect on fruit yield but negative effect on soluble solids content (SSC) and antioxidant components (Pék et al. 2014, Helyes et al. 2014).

In a study of the effect of 4 irrigation regimes (40, 50, 60 and 70% depletion of available soil moisture) on tomato cultivars Pusa Ruby, Pusa Early Dwarf and Sioux, the lycopene content in tomato fruit was reduced by moisture stress (Naphade, 1993). If this were verified, there could be

an antagonism between improving some basic technological characteristics of the tomato fruit juice (dry matter content, °Brix, acidity) and improving lycopene content by means of water management at the field level. On the contrary, in red and pink cherry tomato cultivars, Matsuzoe et al. (1998) found that total carotene of fully ripe fruits and the amount of lycopene were increased by soil water deficit. In red and pink large-fruited tomatoes, soil water deficit also tended to increase the amount of lycopene per fresh matter in the outer pericarp region but it had no effect on the amount and distribution of  $\beta$ -carotene and xanthophylls (Zushi and Matsuzoe, 1998).



### 3. MATERIALS AND METHODS

#### 3.1. 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 6a, b).

The soil was brown forest soil, which was loamy in texture (consisting of 41% sand, 47.5% silt, and 11.5% clay). The soil characteristics of the field site are presented in Table 3.

Table 3. Chemical properties of the soil at the experimental site

Soil layer	Soil characteristic							
	Sand, %	Silt, %	Clay, %	Humus, %	pH <sub>H2O</sub>	P <sub>2</sub> O <sub>5</sub> , mg kg <sup>-1</sup>	K <sub>2</sub> O, mg kg <sup>-1</sup>	NH <sub>4</sub> , mg kg <sup>-1</sup>
0–60 cm	41	47.5	11.5	1.6	7.9	281	203	2.5

#### 3.2. 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. In the figure 7a, b compared tomato's characteristics of H1015 hybrid cultivar between the different irrigation treatments and growth stages, in dry year (2018) and wet year (2020). 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<sup>-2</sup>. 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 (10 l stock solution per 1 m<sup>3</sup> water) before planting out.

Three bacteria treatments and non 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 7a, b).

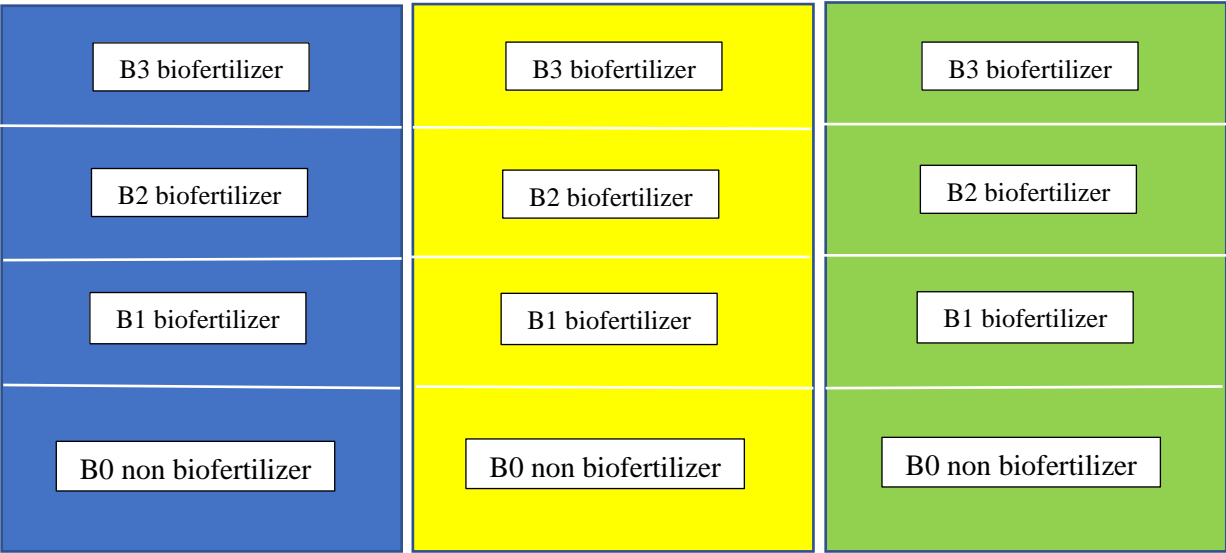


Figure 6a. Experimental field location and design in 2018

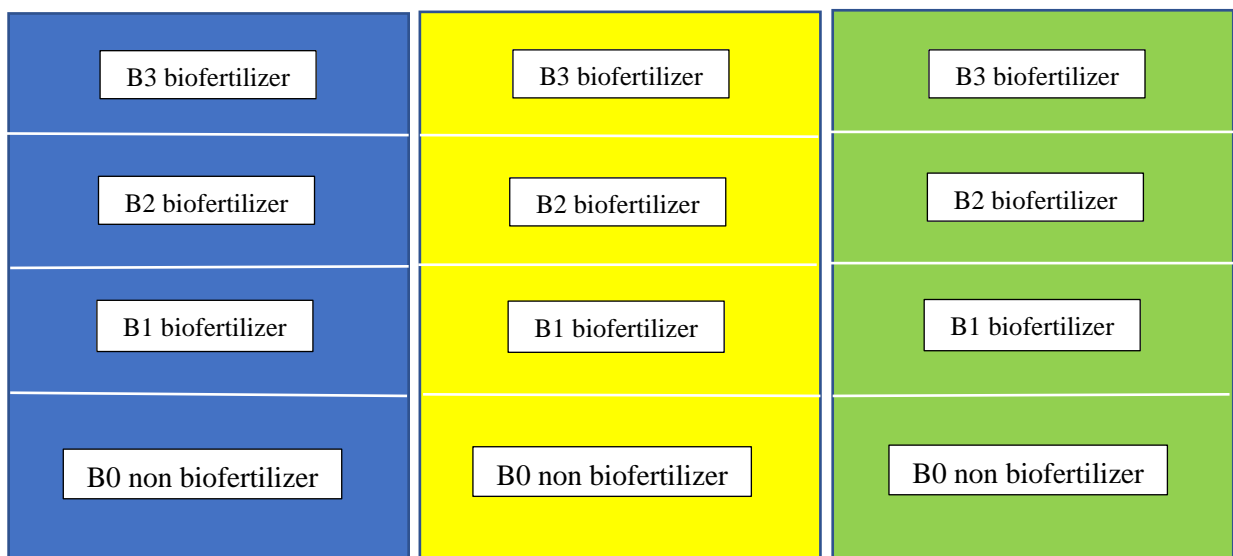


Figure 6b. Experimental field location and design in 2020



Vegetative stage  
in NI treatment



Flowering stage  
in NI treatment



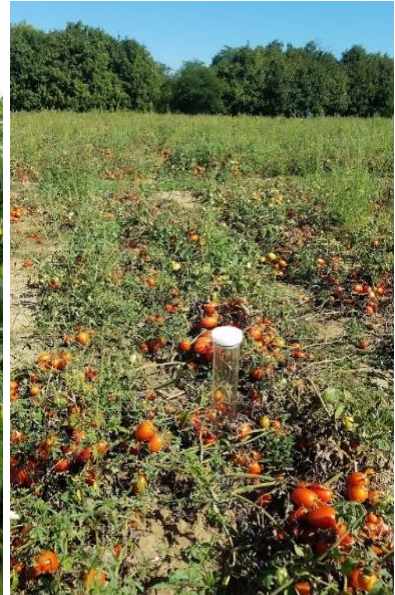
Fruit ripening stage  
in NI treatment



Vegetative stage  
in RI treatment



Flowering stage  
in RI treatment



Fruit ripening stage  
in RI treatment

Figure 7a. H1015 tomato's growth stages and comparison of different treatments in 2018



Vegetative stage  
in NI treatment



Flowering stage  
in NI treatment



Fruit ripening stage  
in NI treatment



Vegetative stage  
in RI treatment



Flowering stage  
in RI treatment



Fruit ripening stage  
in RI treatment

Figure 7b. H1015 tomato's growth stages and comparison of different treatments in 2020

### 3.3. Meteorological data

Weather forecasts from the National Meteorological Institute (<http://www.met.hu/en/idojaras/>) 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 (Figure 8). 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.

### 3.4. 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 4). 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).

$$\text{Equation 1. Evapotranspiration (} ET_c \text{)} = ET_0 \times K_c$$

Where  $ET_c$  is the crop evapotranspiration (mm),

$ET_0$  is the reference of evapotranspiration (mm),

$K_c$  is crop coefficient.

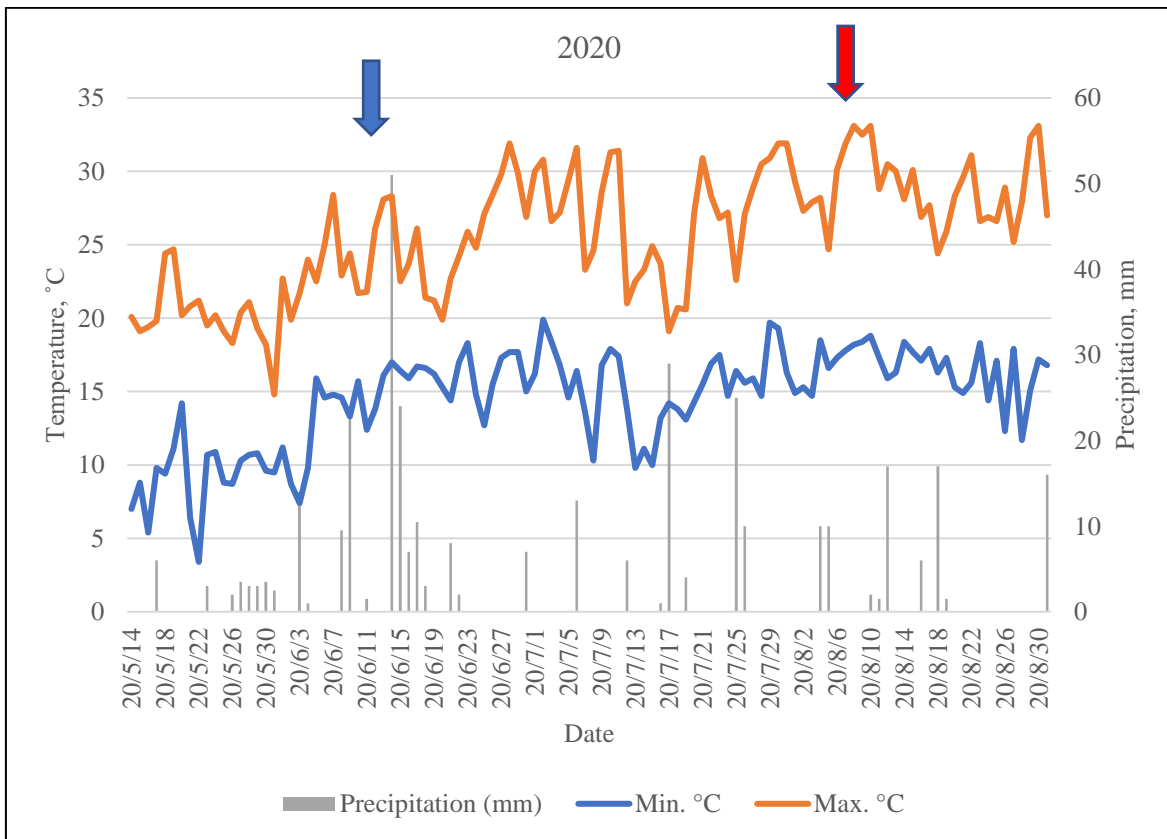
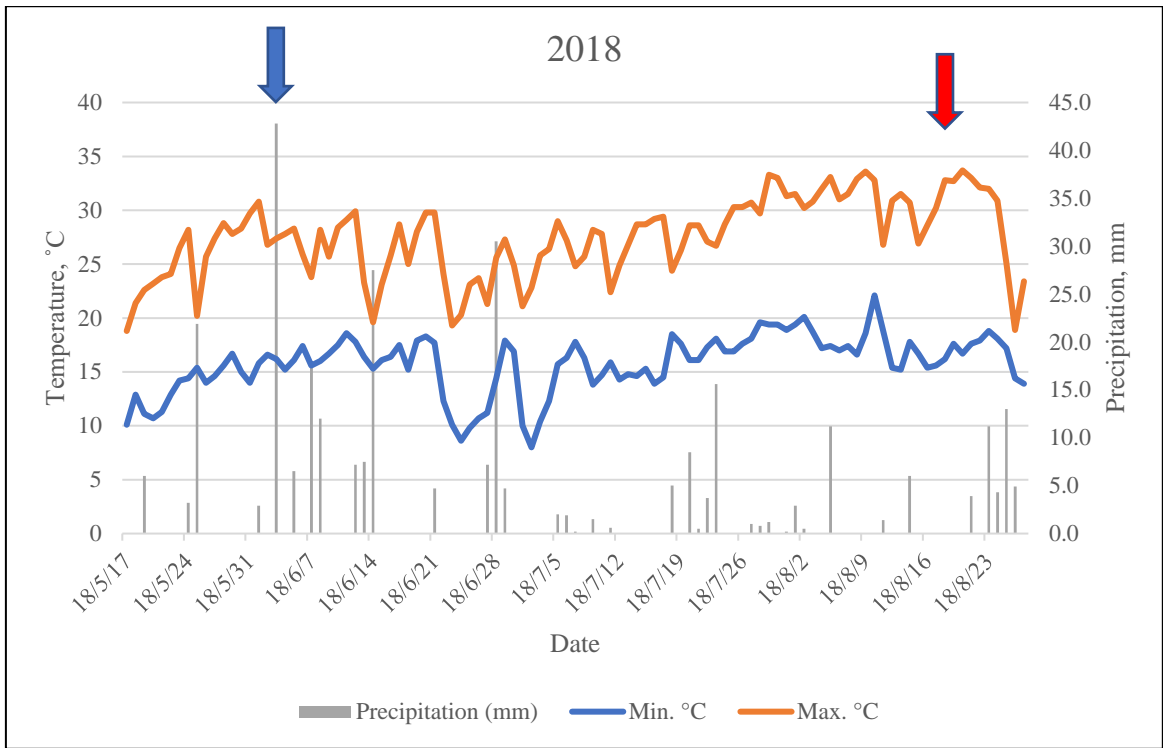


Figure 8. Meteorological data 2018 and 2020

Table 4. 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 <sub>c</sub> restoration	385	411.8	20	9	17.2	10.4
RI	100% ET <sub>c</sub> restoration	465	459.7	20	9	0	0

ET<sub>c</sub>-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 4).

### 3.4.1. 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<sup>-3</sup>) was calculated as the ratio between total marketable fruit weight (kg ha<sup>-1</sup>) and total water used (m<sup>-3</sup> ha<sup>-1</sup>).

### 3.5. Fertigation

Throughout both growing seasons plant nutrition requirements and plant protection were regulated after Helyes and Varga (1994). Macro and micro fertigation was done through the drip irrigation system by adding 0.01 kg of the NH<sub>4</sub>NO<sub>3</sub>, 0.95 kg of Ca (NO<sub>3</sub>)<sub>2</sub>, 0.02 kg Mg (NO<sub>3</sub>)<sub>2</sub>, 0.05 kg of Polyfeed, 0.07 kg of KNO<sub>3</sub>, and 0.02 kg of KCl to hectare of the cultivated area. Fertilizer was added 5 times throughout the growing season, in 2018.



In 2020, there was heavy rain and precipitation, therefore fertilization was little increased. During the growing season, main nutrient was applied 4 times and total N - 188 kg, P<sub>2</sub>O<sub>5</sub> – 89 kg, K<sub>2</sub>O – 317 kg to hectare of the cultivated area.

### **3.6. Harvesting**

The harvest date was 27 August 2018 and 1 September 2020. Plants were harvested at once after 103-110 days of growing (Figure 9). 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).



Figure 9. Heinz 1015 F1 hybrid cultivar

### **3.7. Experimental field measurements**

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

#### **3.7.1. Soil moisture**

The soil moisture was measured with PT-1 (Kapacitív Kkt., Hungary) (Figure 10). Measurements were taken with three (bottom, medium and head side of field) replications in each treatment.



Figure 10. PT-1 equipment for soil moisture

### 3.7.2. Chlorophyll content

Chlorophyll content of leaf was measured by SPAD 502 (Minolta, UK) portable chlorophyll meter and it was given as SPAD values (Figure 11). 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).



Figure 11. Chlorophyll meter SPAD 502

### 3.7.3. Chlorophyll fluorescence

Chlorophyll fluorescence was measured by portable fluorimeter PAM 2500 (Walz-Mess und Regeltechnik, Germany) (Figure 12). 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).



Figure 12. Fluorimeter PAM-2500

### 3.7.4. Canopy temperature

The infrared remote thermometer (Raytek Raynger MX4, Santa Cruz, CA, USA) was used to record the canopy temperature (Bócs et al., 2009) (Figure 13). 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  $900^{\circ}\text{C}$  (<http://www.farnell.com/datasheets/44260.pdf>). We measured ten leaf surface temperature in each treatment from flowering to fruit development stages of tomato.

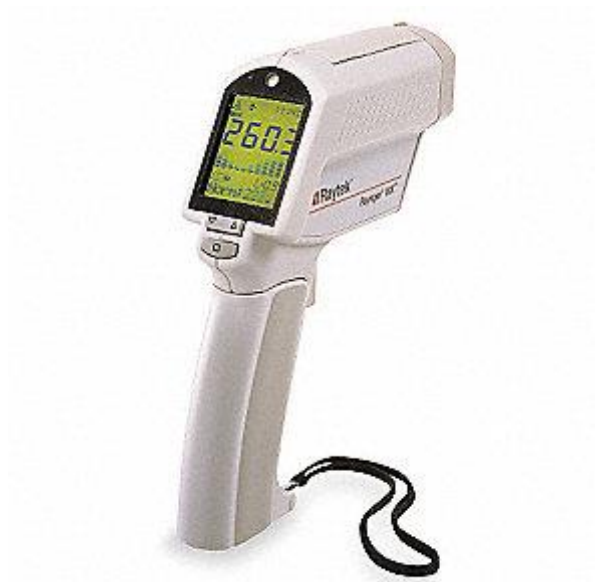


Figure 13. Thermometer Raytek MX4

### 3.7.5. Stress degree day (SDD)

The temperature measurement of the plant canopy was used to monitor heat stress. The heat stress was monitored from flowering to fruit development stages of tomato. Leaf temperature was measured every week during the warmest hours (12:00 pm). SDD was calculated as a difference of leaf surface temperature and air temperatures registered at the time of leaf surface temperature measurement ( $T_{\text{leaf}} - T_{\text{air}}$ ).

$$\text{Equation 3. Stress degree day (SDD)} = (T_{\text{leaf}} - T_{\text{air}})$$

Where  $T_{\text{leaf}}$  – leaf surface temperature and  $T_{\text{air}}$  – air temperature.

## 3.8. Phytochemical analysis

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

### 3.8.1. 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,  $\beta$ -carotene, 8- $\beta$ -apo-carotenal, ascorbic acid and tocopherols were purchased from Sigma-Aldrich (Budapest, Hungary).

### **3.8.2. 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  $-20^{\circ}\text{C}$  and used to determine the carotenoids.

### **3.8.3. 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  $40^{\circ}\text{C}$  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  $\mu\text{m}$  cellulose acetate syringe filter before injection on the HPLC column.

### 3.8.4. 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 (Figure 14). The separation and data processing were operated by EZChrom Elite software.



Figure 14. High performance liquid chromatograph (HPLC) instrument

Carotenoids were simultaneously separated on a core C-30, 2.6 $\mu$ , 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) (Figure 15). 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) (Figure 15).

Identification of carotenoids was based on comparison of retention time and spectral characteristics with those of available standards such as lutein,  $\beta$ -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  $\beta$ -8-apocarotenal as internal standard spiked with the samples. For quantification, the area of each compound was integrated at the maximum absorbance wavelength.

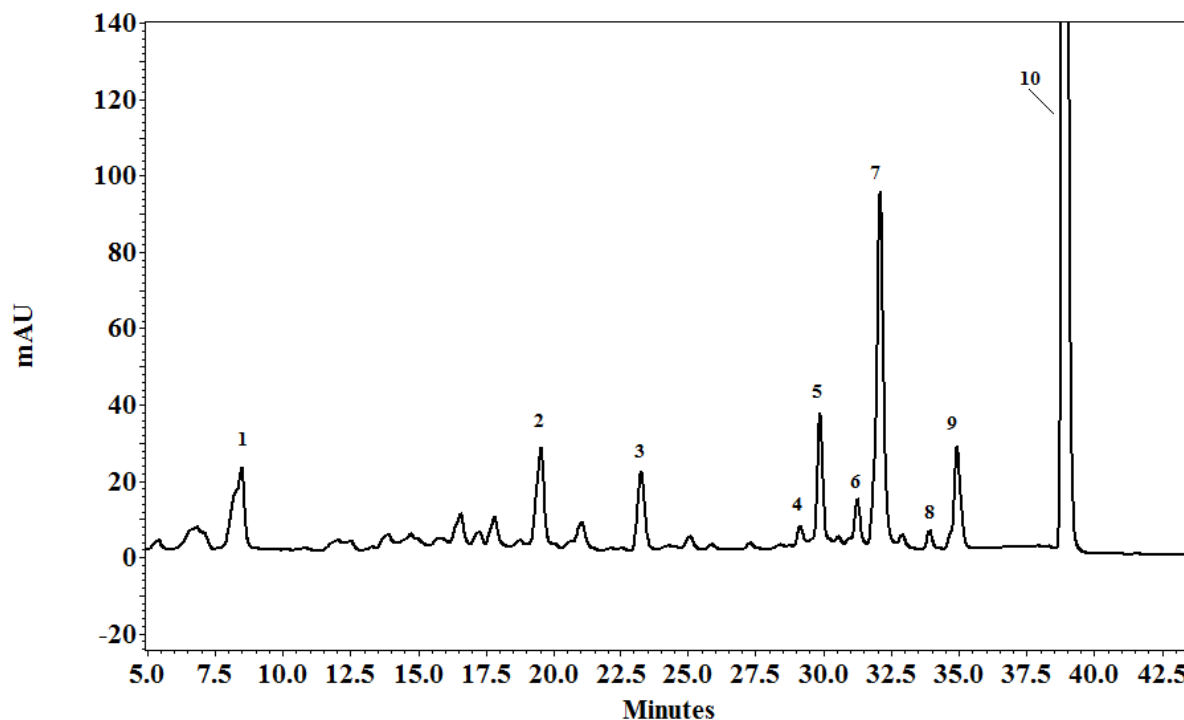


Figure 15. HPLC profile of tomato carotenoids separation.

Peaks are: 1-Lutein, 2- $\beta$ -carotene di-epoxide, 3-Lycopene epoxide, 4-Lycoxanthin, 5- $\beta$ -carotene, 6-15-cis-lycopene, 7-13-cis-lycopene, 8- $\gamma$ -carotene, 9-9-cis-lycopene, 10-all trans-lycopene.

Vitamin C (L-ascorbic acid) was separated on aqua Nautilus (Macherey Nagel, Düren, Germany),  $3\mu$ , 150 x 4.6 mm column with gradient elution of acetonitrile (A) in 0.01M  $\text{KH}_2\text{PO}_4$  (B) (Figure 16). 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 (Figure 16).

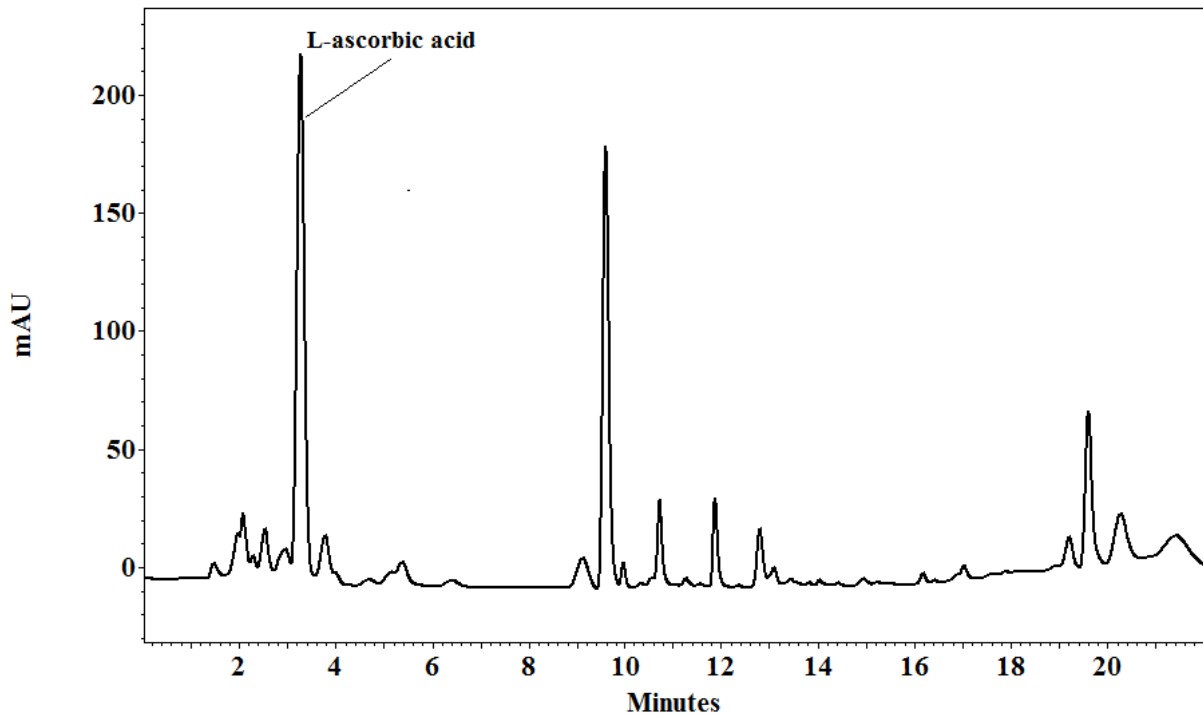


Figure 16. HPLC profile of L-Ascorbic acid separation.

### 3.8.5. 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 (Figure 17). 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.



Figure 17. Refractometer KRÜSS DR201-95



### **3.9. 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).

## **4. RESULTS AND DISCUSSIONS**

Worldwide, field crop production faces water stress that limits crops productivity, and biofertilizer 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). In our two years field-based trials, biofertilizer inoculation at transplant increased yield and enhanced growth and water use efficiency of plants under deficit water supply and full water supply levels compared to non-inoculated ones. Di Cesare et al. (2012) also found this result.

### **4.1. 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 (Table 5), 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 (Table 5). During fruit development stage there was high temperature (31.5 °C) and during beginning of flowering stage there was low temperature (13.6 °C).

Table 5. Meteorological data and accumulative water supply amount during the growth of tomato (2018)

Date	Stages	T <sub>min</sub> °C	T <sub>max</sub> °C	RH %	Soil moisture at 60 cm %	Precipitation (mm)	Irrigation mm (RI)	Σ DI (mm)	Σ RI (mm)
17.05-24.06	Growing	14.98	25.78	75.1	25.6	160.2	20.1	170.3	180.3
25.06-12.07	Beginning of flowering	13.69	25.11	72.2	26.2	48.6	35.1	66.2	83.7
13.07-20.07	Flowering	15.60	27.60	66.1	27.7	5.0	24.3	17.2	29.3
21.07-26.07	Flowering to fruit setting	16.90	28.33	74.4	27.8	28.3	19.5	38.0	47.8
27.07-09.08	Fruit development	18.34	31.52	70.3	27.2	17.8	44.0	39.8	61.8
10.08-23.08	Fruit ripening	17.36	31.22	65.8	24.8	22.5	17.2	31.1	39.7
27.08	Harvesting	15.90	24.60	79.0	-	22.2	0.0	22.2	22.2
	From planting to harvesting					<b>304.6</b>	<b>160.2</b>	<b>384.8</b>	<b>464.8</b>

T<sub>min</sub> = minimum temperature; T<sub>max</sub> = maximum temperature; RH-relative humidity, RI-regular irrigation, DI-deficit irrigation.

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 (Table 6). 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.

Table 6. Meteorological data and accumulative water supply amount during the growth of tomato (2020)

Date	Stages	T <sub>min</sub> °C	T <sub>max</sub> °C	RH %	Soil moisture at 60 cm %	Precipitation (mm)	Irrigation mm (RI)	Σ DI (mm)	Σ RI (mm)
14.05-24.06	Growing	3.4	28.4	74.1	23.7	181	19.4	193.3	200.4
25.06-12.07	Beginning of flowering	10.3	31.9	69.1	21.0	26	41.9	47.4	67.9
13.07-20.07	Flowering	9.8	27.2	76.5	26.4	34	22.6	45.5	56.6
21.07-26.07	Flowering to fruit setting	14.7	30.9	78.1	26.0	35	-	35	35
27.07-09.08	Fruit development	14.7	33.1	69.4	24.9	20	18.8	29.6	38.8
10.08-23.08	Fruit ripening	14.9	33.1	76.2	26.0	45	-	45	45
01.09	Harvesting	13.9	18.9	87.4	-	16	-	16	16
	From planting to harvesting					<b>357</b>	<b>102.7</b>	<b>411.8</b>	<b>459.7</b>

T<sub>min</sub> = minimum temperature; T<sub>max</sub> = maximum temperature; RH-relative humidity, RI-regular irrigation, DI-deficit irrigation.

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 (Table 6).

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 (Figure 7a, b).

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 (Figure 7a, b).

## **4.2. Photosynthetic efficiency and relative chlorophyll content**

### **4.2.1. Chlorophyll content (SPAD)**

Leaf chlorophyll content provides valuable information about physiological status of plants.

In 2018, chlorophyll content was highest in the full bloom stage and fluctuated widely in the next development stages. During full bloom stage, the level of chlorophyll was as follows: 52.6 SPAD in NI, 51.3 in DI and 52.4 in RI treatment. In the development period, SPAD value ranged from 45-52.6 in non-irrigated, 45.4-51.3 in deficit irrigated, and 44.8-52.4 in regular irrigated treatment. From flowering and fruit setting to fruit ripening period SPAD value was high under non-irrigated conditions in comparison with the well irrigated one in the drier 2018 (Figure 18a).

In the full bloom stage, leaf chlorophyll content (SPAD) was 55.8 in NI, 54.2 in DI and 52.4 in RI treatment, in 2020. This value was decreased slightly in the next development stages and decreased to 48.2 in NI, 41.9 in DI, 40.1 in RI treatment, in fruit ripening stage. Nevertheless, no significant difference in SPAD was detected between the non-irrigated and regular irrigated plants (Figure 18b). This sustained chlorophyll content was basis for a positive effect on photosynthesis and crop yield. Not only the water supply but the temperature influences the chlorophyll content of the leaves.

According to Wolken et al. (1955) temperature influences chlorophyll synthesis. During the experimental two years, optimum temperature positively influenced the chlorophyll synthesis in full bloom stage. The optimum temperature of general plant chlorophyll synthesis is about 30°C (Nagata et al., 2005).

### **4.2.2. Chlorophyll fluorescence**

The chlorophyll fluorescence value was 0.638-0.808 in NI, 0.689-0.805 in DI, 0.716-0.811 in RI treatment, in 2018. In the fruit development stage of each treatment, chlorophyll fluorescence was the highest (0.805-0.811) and it means during this stage plant was without water and heat stresses. But it reduced to 0.638-0.716 in fruit ripening stage (Figure 19a). This period indicates water and heat stress due to the cut off of irrigation 10-20 days before harvest. This is especially clear in the non-irrigated treatment, which is marked by red arrow (Figure 19a).

In 2020, chlorophyll fluorescence level ranged between 0.759-0.806 in NI, 0.753-0.800 in DI, and 0.733-0.810 in RI treatment. Under water deficiency chlorophyll fluorescence level was the highest at the fruit development stage, but it decreased in RI treatment (Figure 19b). The low chlorophyll fluorescence under regular irrigation (RI) is due to too much water caused by irrigation and heavy precipitation except for the first period of fruit ripening stage. After cut off of irrigation in the fruit ripening stage, water stress was affected in NI and DI treatment.

Mauro et al., (2020) reported the minimum chlorophyll fluorescence was negatively correlated to plant growth. This effect was also observed in our study.

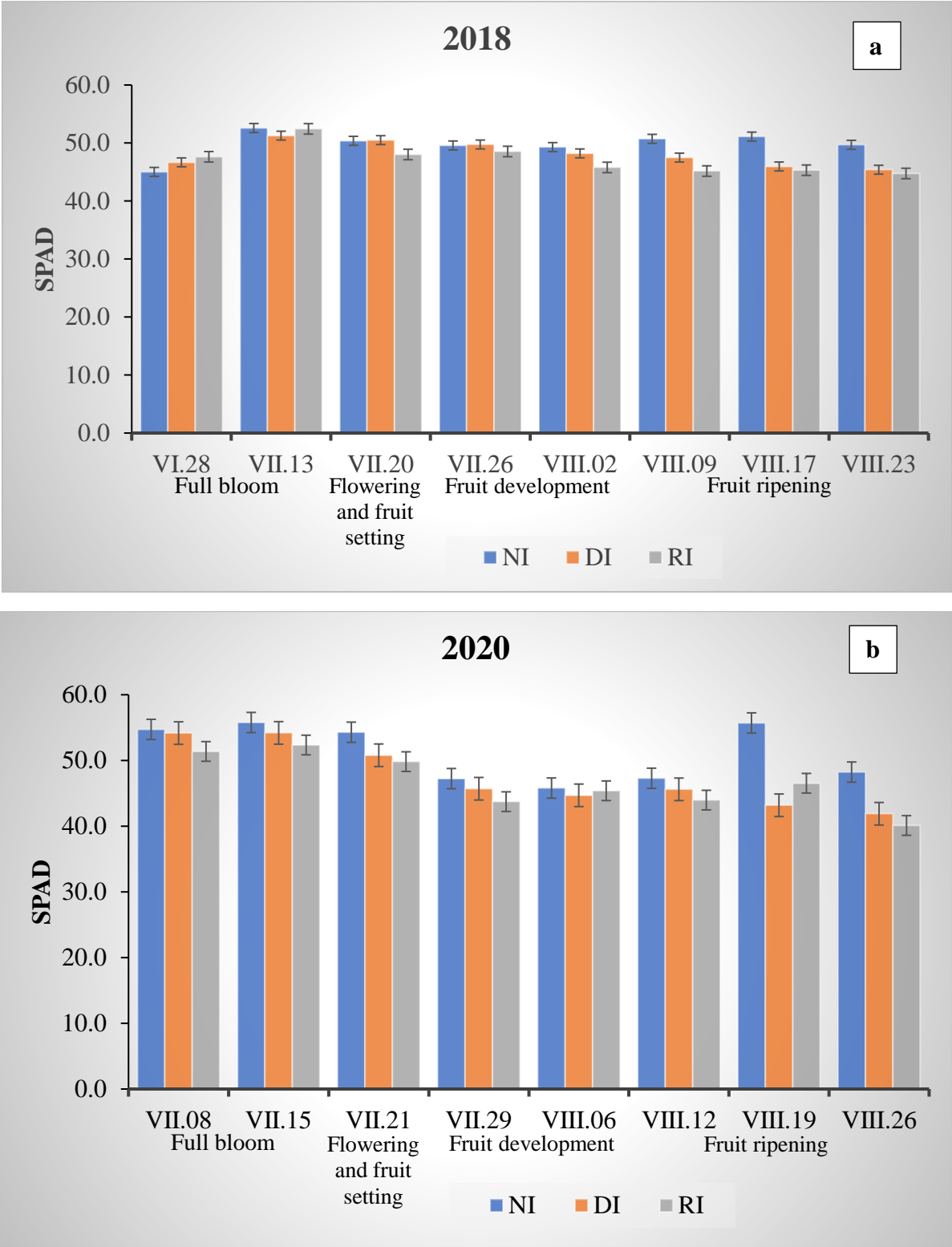


Figure 18. SPAD values under different water supply treatment

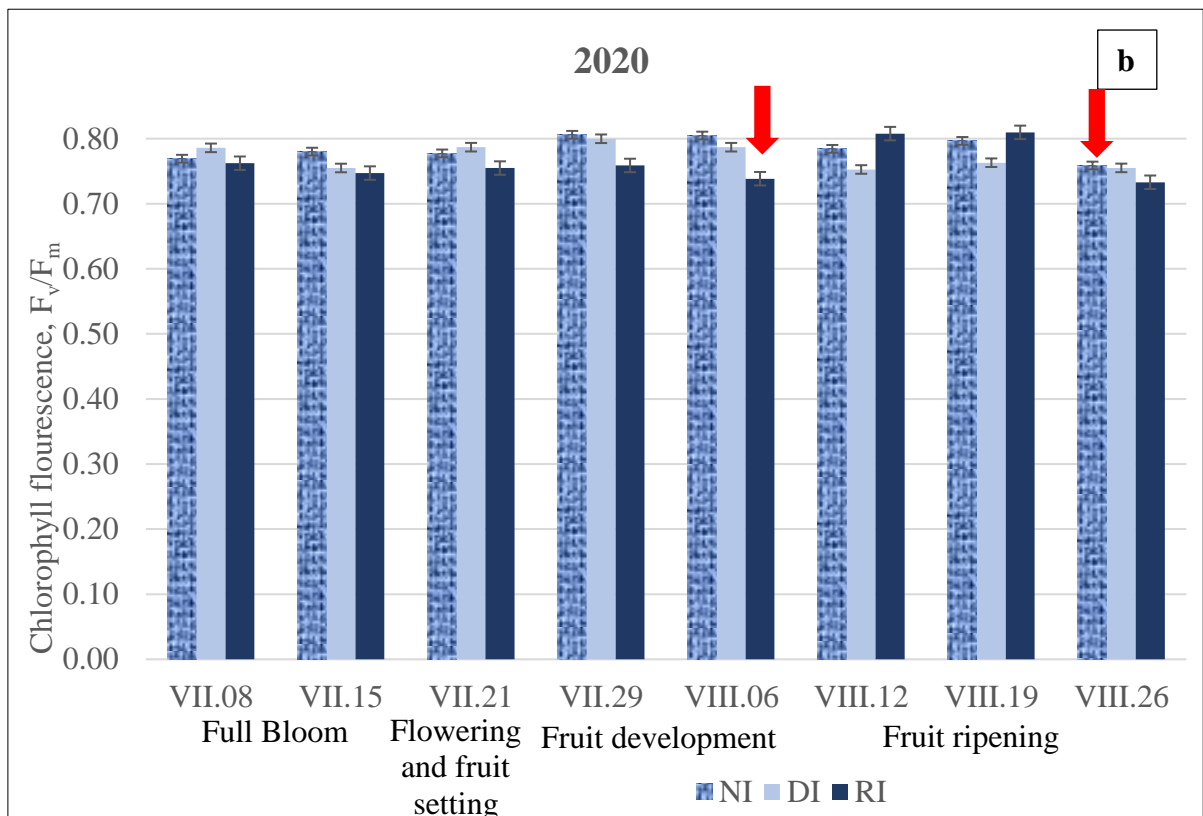
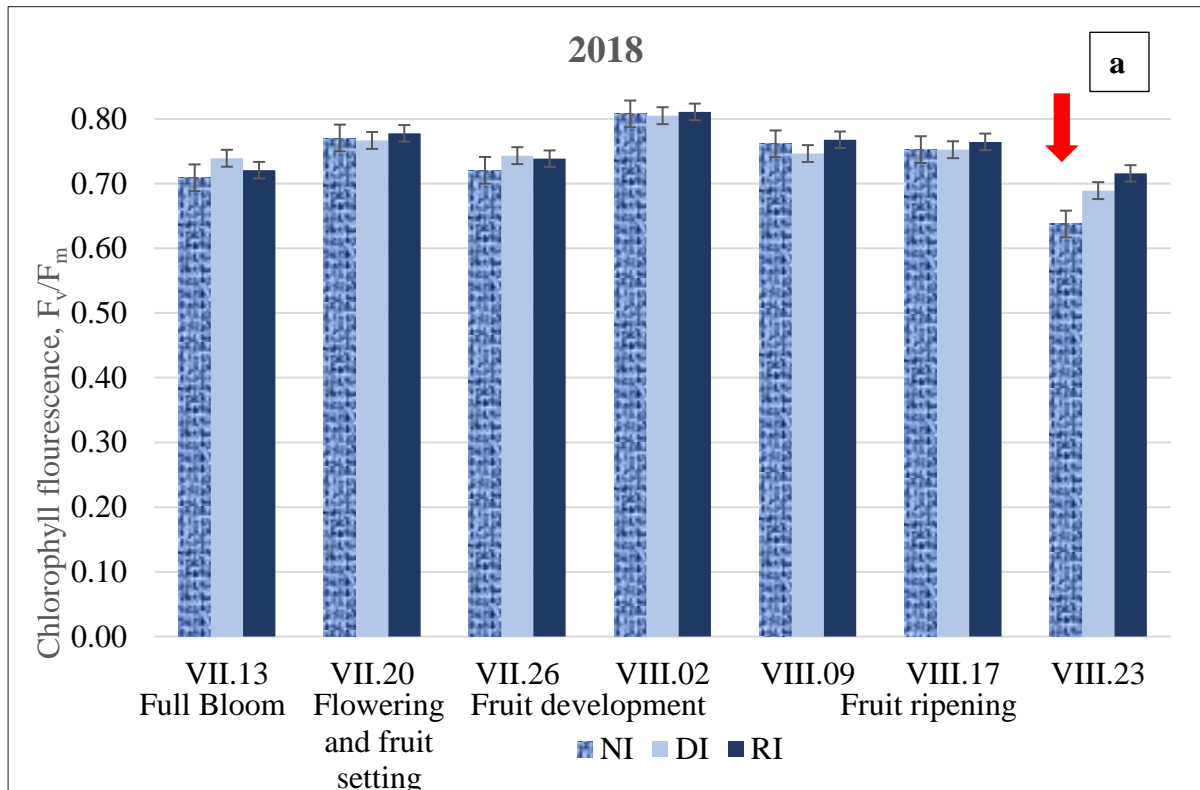


Figure 19. Chlorophyll fluorescence of tomato under different irrigation treatment in 2018 and 2020

### 4.2.3. Canopy temperature

In order to detect biotic and abiotic stress at leaf level thermal indices based on leaf temperature measurements have been commonly used (Helyes 1990; Böcs et al. 2009, Nemeskéri et al. 2018, Takacs et al., 2019). Under limited water supply condition, the plant need to decrease the transpiration with closing stomata (Nemeskéri et al. 2018) therefore the cooling of plants declined which lead to an increase in canopy temperature (Helyes et al. 2006). Measurement of canopy temperature of plants is suitable to monitor the water stress (Helyes 1990; Böcs et al. 2009).

In 2018, the canopy temperature also increased as the air temperature increased. Especially in the non-irrigated treatment, it was higher than the other two treatments. However, when the irrigation was stopped before the harvest, the temperature of the leaves increased in all three different irrigation treatments (Figure 20).

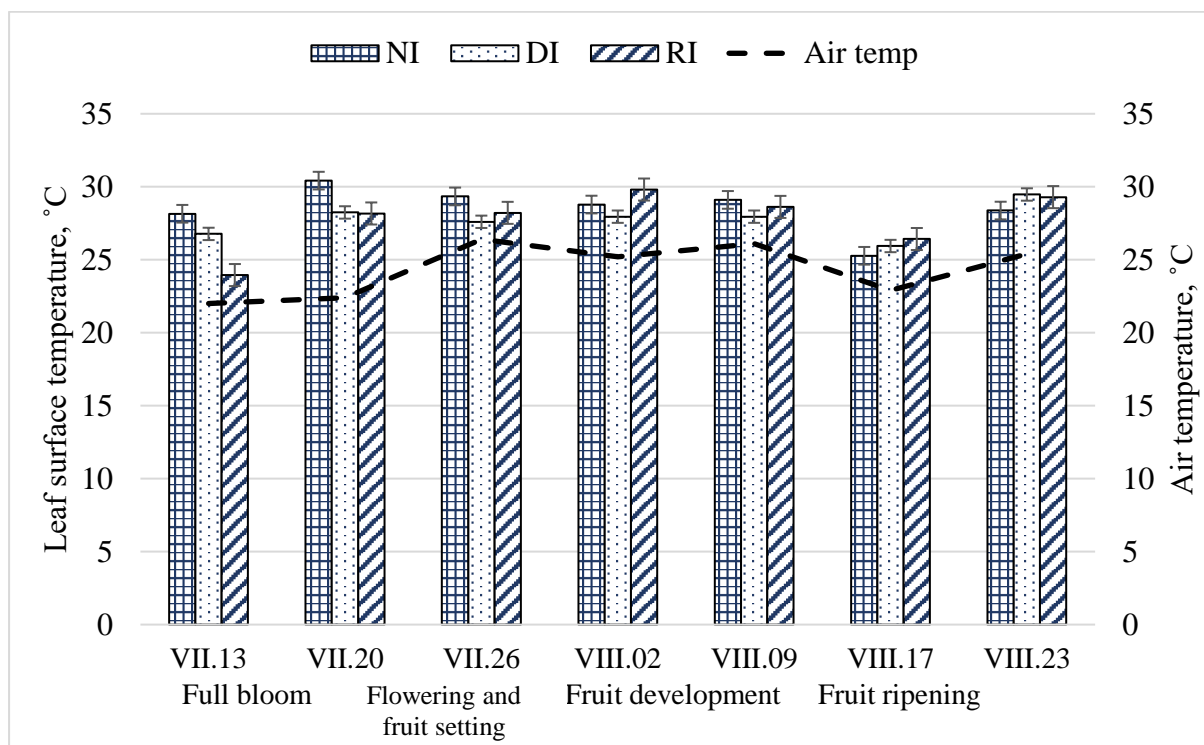


Figure 20. Leaf surface temperature and air temperature measurement in 2018

Regression analysis of relationship between leaf temperature under three different irrigation treatments and air temperature shows they have relationship between variables ( $R^2 = 0.51$ ) in the regular irrigation treatment. However, in the non-irrigation and deficit irrigation treatments there was no relationship between variables, in 2018 (Appendix 6, 7, 8).



The high canopy temperature shows the plants suffer from water stress. The highest difference in canopy temperature between the water supply conditions was detected during flowering stage which indicates that this is the most sensitive phase of plant to water stress (Figure 20).

The air temperature ranged between 17.4-25.3°C, leaf temperature fluctuated between 22.9-28.3°C in the non-irrigation treatment, 23.4-29.5°C in the deficit irrigation treatment, and 24.0-28.0°C in the regular irrigation treatment, in 2020. In the three different irrigation treatments, the leaf temperature was higher than the air temperature. Especially after the irrigation was stopped, the temperature of the leaves was the highest (Figure 21).

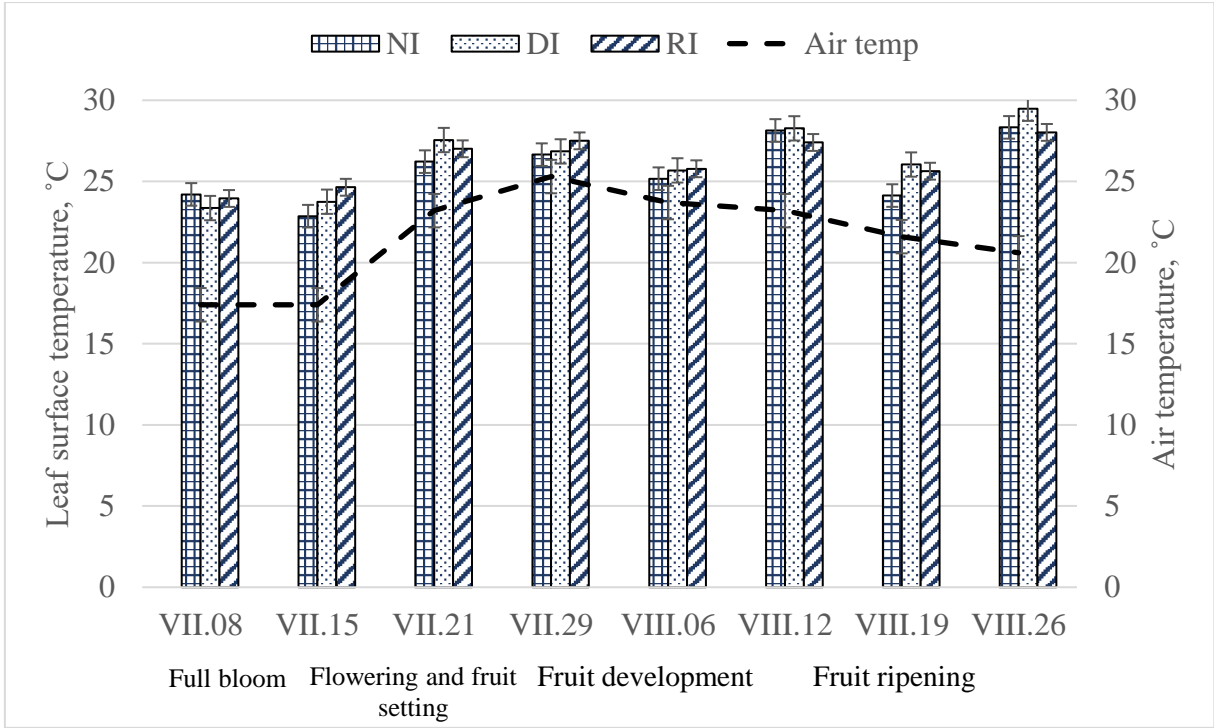


Figure 21. Leaf surface temperature and air temperature measurement in 2020

In 2020, regression analysis of relationship between leaf temperature under three different irrigation treatment and air temperature shows they have relationship between variables ( $R^2 = 0.50$ ) in the regular irrigation treatment. However, in the non-irrigation ( $R^2 = 0.32$ ) and deficit irrigation treatments ( $R^2 = 0.35$ ) there was no relationship between variables (Appendix 9, 10, 11).

The temperature measurement of the plant canopy should be monitored for heat stress (Bates and Hall, 1981; Böcs et al., 2009; Helyes, 1990). The heat stress was determined using the stress degree day method. Investigation of stress degree day of tomato showed tomato did not get heat stress in DI and RI treatments, in 2018. But in 2020, some days results showed there was stress but mean level of SDD shows there was no stress in the plant (Figure 22). Usually, plant stress was reported

above +40°C (Takacs et al., 2019; Helyes et al., 2006), but in our case meteorology showed air max temperature was +27.5-33.0°C during the two years in Godollo, Hungary.

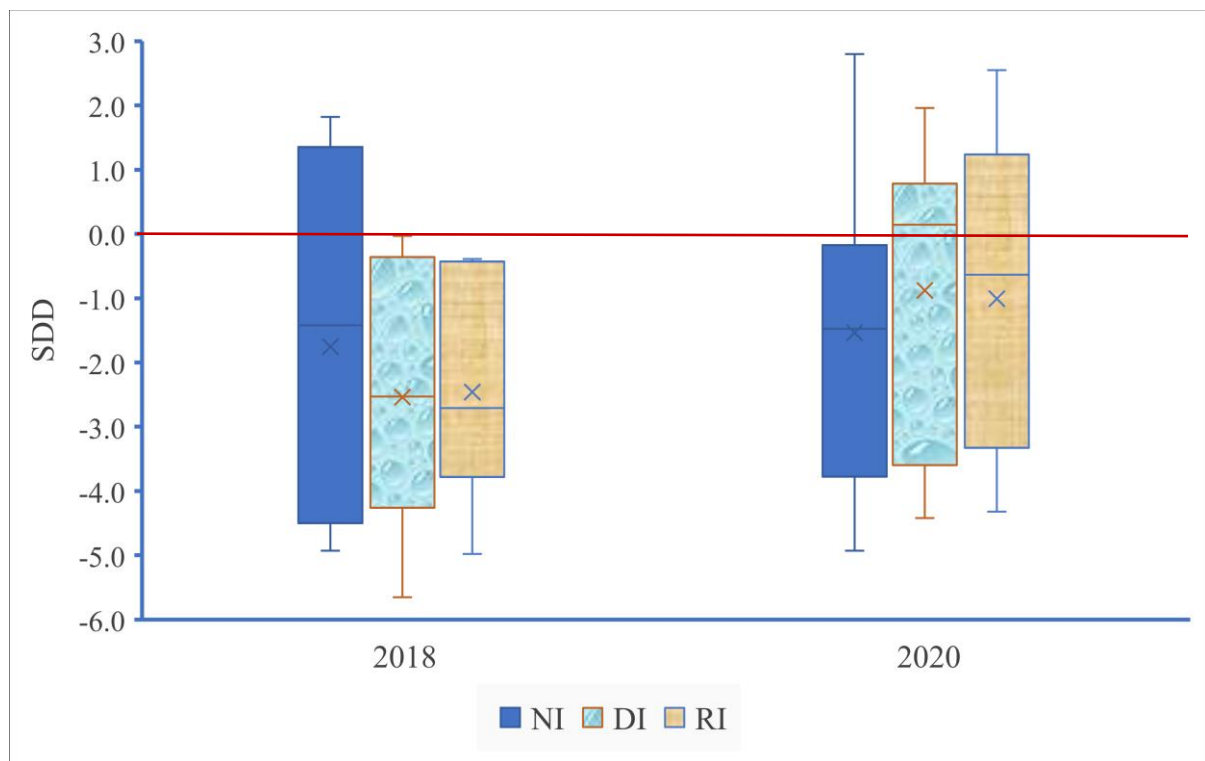


Figure 22. Comparison of stress degree day (SDD) in 2018 and 2020

After investigation of physiological traits, we calculated correlation between physiological traits and total yield. In the 2018 study a strong relationship between chlorophyll fluorescence and total yield ( $r = 0.82$ ) in the RI treatment was found. Also, between SPAD and total yield there was strong relationship ( $r = 0.70$ ) in the DI treatment. This indicates that SPAD and chlorophyll fluorescence have a strong effect on the yield in a drier year (Table 7). However, no correlation was observed between canopy temperature and total yield. Independently from the years chlorophyll fluorescence influenced significantly the yield under regular irrigation.

Table 7. 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 <sup>y</sup>	Total yield* t ha <sup>-1</sup>	Correlation
2018	NI	28.4 $\pm$ 1.6	50.0 $\pm$ 2.2	0.737 $\pm$ 0.05	50.6 $\pm$ 10.5	R <sup>2</sup> = 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 <sup>2</sup> = 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 <sup>2</sup> = 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 <sup>2</sup> = 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 <sup>2</sup> = 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 <sup>2</sup> = 0.88 Fv/Fm vs TY, R <sup>2</sup> = 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 7). 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.

#### 4.3. Total biomass and water use efficiency

Many researchers have noted that water use efficiency (WUE) has a significant effect on the fruit yields (Favati et al., 2009; Ozbahce and Tari, 2010, Patane et al., 2011). Nevertheless, the growth habit of tomato varieties and irrigation techniques influence the WUE. There was a higher WUE<sub>biomass</sub> in semi-determinate tomato plants in comparison with the determinate and indeterminate ones however an improvement in Brix yield and a higher WUE<sub>fruit</sub> has been shown in semi-determinate lines (Vicente et al. 2015). Luo and Lie (2018) found that conventional drip irrigation and alternate partial root-zone drip irrigation decreased tomato yield slightly but

increased the WUE by 7.8%. Agbna et al. (2017) using drip irrigation showed that deficit irrigation significantly increased the yield quality and irrigation water use efficiency compared to the full irrigation treatment.

Our study showed how the WUE affected the total biomass in the three different irrigation treatments. In 2018, strong relationship was found between WUE and total biomass in all irrigation treatments, especially in the DI and NI treatments, but Figure 23a shows that the relationship was positive ( $R^2 = 0.97$ ) in the DI treatment.

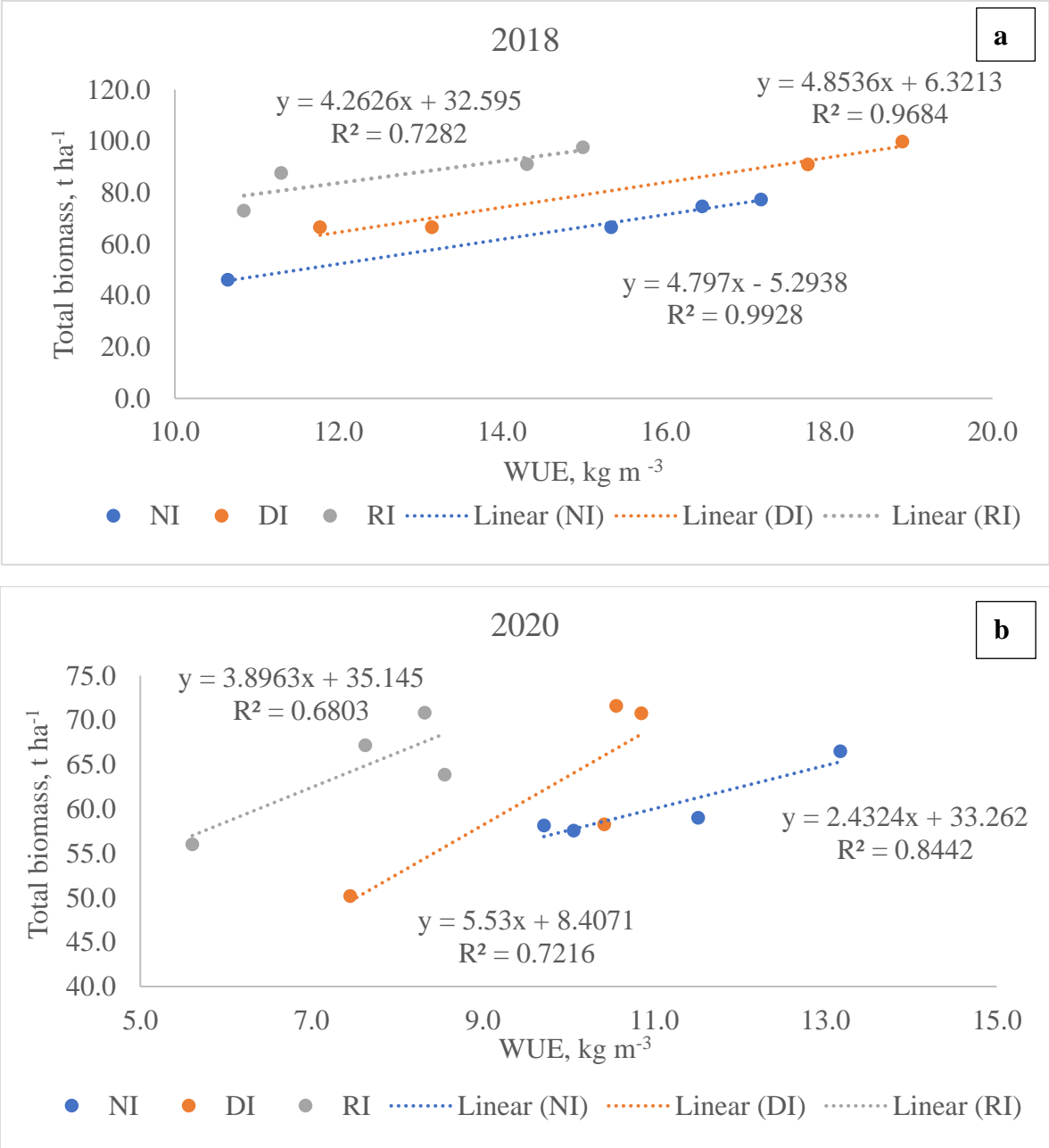


Figure 23. Regression analysis between total biomass and WUE in the different three irrigation treatments, 2018 and 2020

In 2020, WUE had a strong ( $R^2 = 0.84$ ) effect on the total biomass in the NI treatment. However, the highest biomass in the DI treatment indicated the advantages of the treatment (Figure 23b). During study years, the WUE has had a strong impact on total biomass, but there was a difference between the treatments in terms of the amount of biomass generated (Figure 23b). The total biomass and WUE result shows, the DI treatment not only saved water, but it also provided a higher yield than NI treatment (Figure 23a, b).

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.

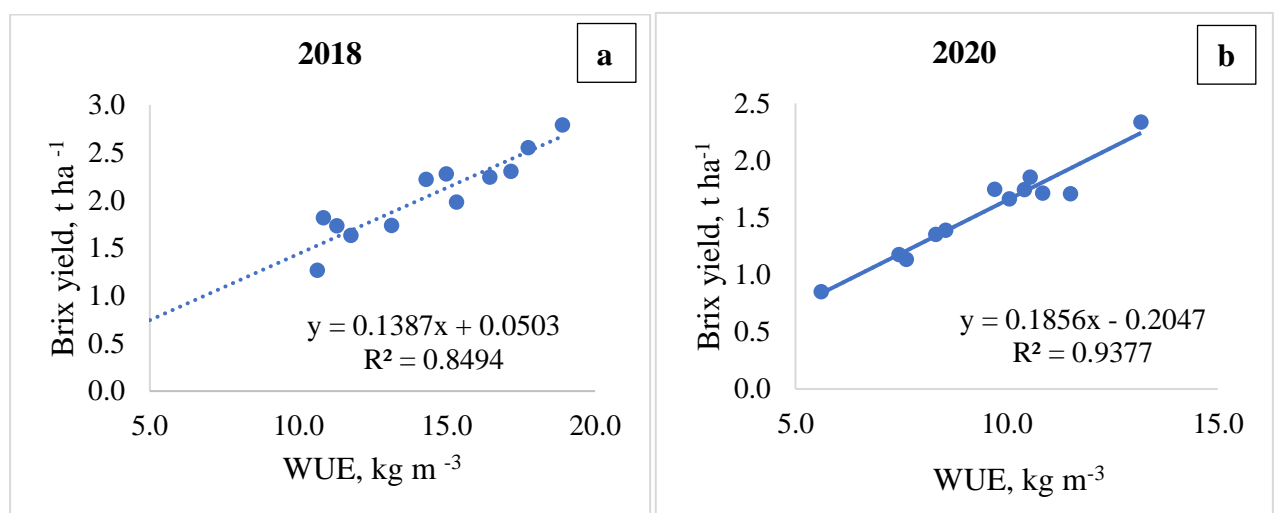


Figure 24. Relationship between Brix and WUE in 2018 and 2020

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 24a) in 2018 and  $R^2=0.93$  in 2020 (Figure 24b). This result also confirmed that of Böcs (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 ( $R^2=0.7547$ ).

#### 4.4. 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<sup>-1</sup> in the NI, 65 t ha<sup>-1</sup> in DI, and 72.3 t ha<sup>-1</sup> in the RI treatment, and in the marketable fruit category yield were ranged between 45.4-59.7 t ha<sup>-1</sup>, in green yield category 4.4-9.9 t ha<sup>-1</sup>, and in non-marketable yield category 0.9-2.7 t ha<sup>-1</sup>. 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 8).

According to researchers (Patanè and Cosentino, 2010; Battilani and Letterio, 2015, Helyes et al., 2019), the Brix value decreases with increasing irrigation. However, 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 8).

Table 8. Tomato yield in three different irrigation treatments in 2018 and 2020

Year	Treatment	Biomass, t ha <sup>-1</sup>	Total fruit yield, t ha <sup>-1</sup>	Marketable fruit yield, t ha <sup>-1</sup>	Green fruit yield, t ha <sup>-1</sup>	Non marketable fruit yield, t ha <sup>-1</sup>	Brix yield, t ha <sup>-1</sup>
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 (Appendix 1).

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<sup>-1</sup> in the three different irrigation treatments. In the marketable fruit category yields ranged between 34.6-40.4 t ha<sup>-1</sup>, in green yield category 1.7-2.5 t ha<sup>-1</sup>, and in non-marketable (rotten) yield category 7.1-12.2 t ha<sup>-1</sup>. 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 8).

As statistical analysis showed, Brix value of DI treatment was significantly different ( $P < 0.05$ ) from the RI treatment (Table 8). It can be established that deficit irrigation resulted in a remarkable increase in marketable yield but it decreased significantly the Brix value (Appendix 2).

Comparison of two years marketable yield, highest yield 59.2-59.7 t ha<sup>-1</sup> was observed in DI and RI treatments, in 2018 and 40.2-40.4 t ha<sup>-1</sup> was observed in DI and NI treatments, in 2020 (Figure 25). According to Bakr (2019) the optimum irrigation (RI) and deficit irrigation (DI) treatments increases the yield.

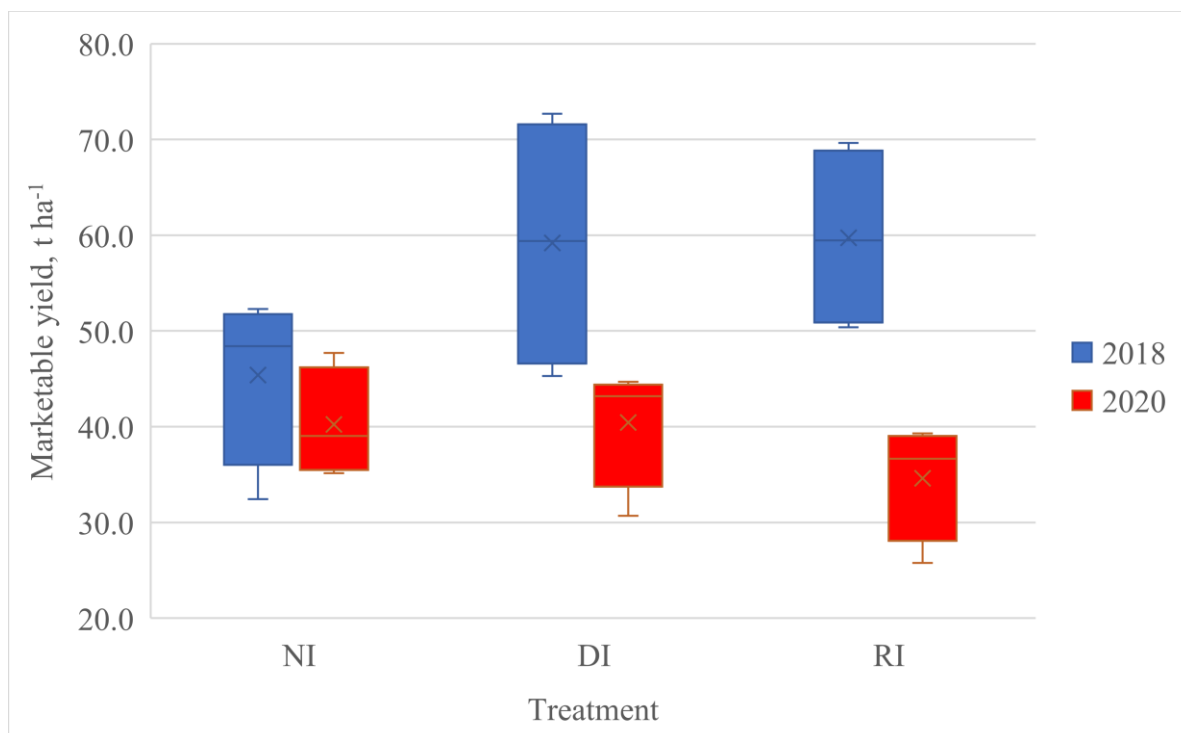


Figure 25. Marketable yield under different irrigation treatments in two years

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 25, Table 8).

#### 4.5. Effect of water deficiency on nutritive value of tomato fruit

Induction of abiotic stress in tomato plants has been proposed as a mechanism for improving the nutritional quality of fruits. Antioxidants are believed to be important in the prevention of diseases such as all kind of cancer and cardiovascular disease. Lycopene is one of the main antioxidants to be found in fresh tomato fruits. The lycopene content also accounts for the redness of the fruit,

which is one of the main qualities for which industry and consumers now look (Dumas et al., 2003).

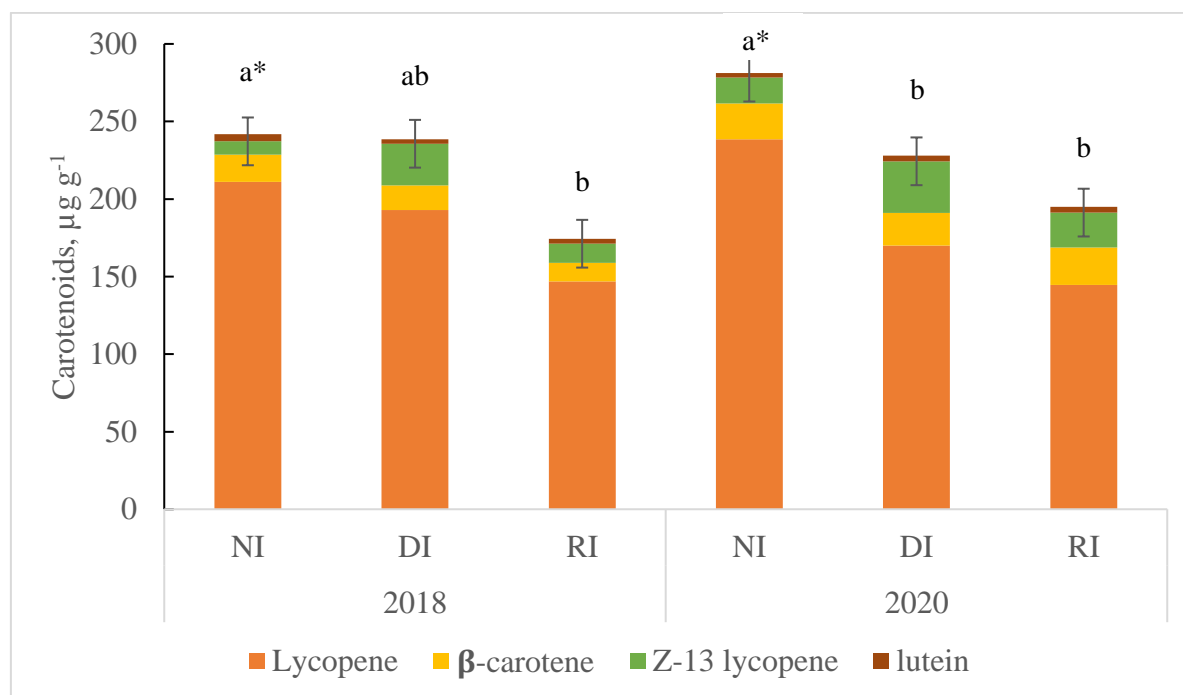


Figure 26. Effect of water supply on nutritional values of ripening fruit of tomato in 2018-2020. Different letters are significant different at  $P < 0.05$  level using Tukey's test.

In wet year (2020)  $\beta$  carotene and 13 cis lycopene content of tomato fruit was significantly higher than a drier (2018) year, however no change was detected in the others compared with the years (Appendix 3).

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 26). 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.

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 26). 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. Nevertheless, the other phytochemicals did not change significantly (Appendix 4).



Apart from the weather of years the results showed that lycopene,  $\alpha$  carotene and lycoxanthin content of fruits significantly decreased but 13-cis-lycopene content increased in the irrigated (DI, RI) conditions in comparison with that of non-irrigated ones (Appendix 5).

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 9).

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 9).

Table 9. 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 <sup>-1</sup>		BRIX		Vitamin C, $\mu\text{g g}^{-1}$	
		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.

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 9). 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 ( $79.8 \text{ t ha}^{-1}$ ), in 2018 and biofertilizer treatments under non-irrigated treatment ( $53.2 \text{ t ha}^{-1}$ ), 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 9). 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).

#### **4.6. 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).

Lycopene is the major carotenoid in tomatoes. Lycopene and  $\beta$ -carotene are the main pigments responsible for the characteristic colour of ripe fruits. Tomatoes contain a matrix of many bioactive components, including vitamin C, vitamin E, other carotenoids ( $\alpha$ -,  $\beta$ -,  $\gamma$ - carotene, lutein), and flavonoids (Bilton et al., 2001). Bilton et al. (2001) reported the major carotenoids were lycopene (90%),  $\beta$ -carotene (5–10%), and lutein (1–5%) with trace amounts ( $< 1\%$ ) of other carotenoids. In our studies observed same results of carotenoids that accumulate in ripe red tomato fruits. There was lycopene 81.8-83.4%,  $\beta$ -carotene 1.9-3.05%, and lutein 0.5%.

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 10). It means PGPRs have no significant effect on lycopene, but irrigation treatment has a significant effect on lycopene content.

$\beta$  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  $\beta$  carotene content (Table 10).

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 10).

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 10).

Matsuzoe et al. (1998) found that total carotene of fully ripe fruits and the amount of lycopene were increased by soil water deficit.

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 11). The irrigation treatments had significant effect on lycopene content.

$\beta$  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  $\beta$  carotene content (Table 11).

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 ( $P < 0.01$ ) effect on the lutein content, however biofertilizer did not significantly influence the lutein content, in 2020 (Table 11). 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 11).

Table 10. Effect of water supply (WS) and PGPR on phytonutrients of H-1015 F1 tomato (2018)

Water supply	PGPRs	Lycopene $\mu\text{g g}^{-1}$	$\beta$ carotene $\mu\text{g g}^{-1}$	Lutein $\mu\text{g g}^{-1}$	13 cis lycopene $\mu\text{g g}^{-1}$	Phytoene $\mu\text{g g}^{-1}$	Phyto- fluene $\mu\text{g g}^{-1}$
NI	B0	232.45 b	4.38 c	1.33 c	3.01 g	7.71c	4.22b
	B1	268.76 a	6.46 a	2.04 a	7.45 d	10.47b	5.42a
	B2	239.09 b	4.78 bc	1.47 c	6.23 e	9.62b	4.77b
	B3	248.38 ab	5.24 b	1.75 b	9.00 c	12.00a	5.44a
<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	6.66d	3.66c
	B1	208.29 b	4.49 c	1.08 d	6.83 e	7.70c	3.91c
	B2	228.04 b	5.21 b	1.38 c	5.64 f	10.81a	5.23a
	B3	188.81 cd	3.61 d	1.26 c	25.36 a	9.90a	5.03a
<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	5.81e	3.04c
	B1	136.87 d	3.63 d	0.77 e	3.48 g	5.31e	2.56d
	B2	161.81 d	4.73 bc	1.03 d	6.78 e	7.03c	3.40c
	B3	219.95 b	6.73 a	1.50 c	12.58 b	10.39a	4.96b
<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	**	***	***	**
	WSxPGPR	*	**	*	***	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 11. Effect of water supply (WS) and PGPR on phytonutrients of H-1015 F1 tomato (2020)

Water supply	PGPRs	Lycopene $\mu\text{g g}^{-1}$	$\beta$ carotene $\mu\text{g g}^{-1}$	Lutein $\mu\text{g g}^{-1}$	13 cis lycopene $\mu\text{g g}^{-1}$	Phytoene $\mu\text{g g}^{-1}$	Phytofluene $\mu\text{g g}^{-1}$
NI	B0	233.18	6.45	0.84	4.66 e	9.90b	4.75b
	B1	238.36	6.91	1.08	5.08 e	12.10a	5.71a
	B2	208.34	5.62	0.94	8.21 bc	10.24b	4.71b
	B3	196.62	5.83	1.11	5.56 e	10.20b	5.52a
<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	6.32d	2.75d
	B1	166.14	5.88	1.07	6.28 d	7.58c	3.60c
	B2	156.48	6.46	0.95	4.17 e	7.17c	3.48c
	B3	160.53	6.68	1.10	7.31 c	6.99d	3.54c
<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	6.41d	3.21c
	B1	168.29	8.09	1.42	15.77 a	9.66b	4.79b
	B2	143.68	6.45	1.31	3.66 f	7.99c	4.18b
	B3	172.22	7.95	1.40	3.93 f	9.19b	4.80b
<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,  $\beta$ -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  $\beta$ -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.

## SUMMARY

The global agricultural producers should provide healthy and high-quality vegetables products to the increasing population. 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. 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. This study determined the effects of some environmental stress on the growth of processing tomato under three different irrigation treatments and indicated which treatment had effects on tomato crop and fruit quality. The experiment was conducted under ecological conditions of Hungary, a temperate climate, in 2018 and 2020.

In this work, we used 3 irrigation and 4 biofertilizer treatments for processing of tomato in the experimental field. The cultivar H1015 hybrid of processing tomato (*Lycopersicon esculentum* Mill.) was used for the experiments. Changes in physiological traits were measured during reproductive stage of development in order to determine their effect on photosynthesis, growth and yield of tomato.

In our study, irrigation treatments had different effects on physiological traits. During fruit ripening stage in the non-irrigated treatment, SPAD and leaf temperatures were high and chlorophyll fluorescence levels were low. This was influenced by the temperature, water and drought stress.

The highest total biomass of tomato was observed in regular irrigation treatment (87.4/64.5 t ha<sup>-1</sup>) in 2018/2020. The lowest total biomass was observed in non-irrigation treatment (66.2/60.3 t ha<sup>-1</sup>) in 2018/2020. But most important is marketable fruit yield amount.

Although the biomass value of the RI treatment was high, the marketable fruit yield was low and the amount of rotten fruit was increased in both experimental years. However, the biomass and marketable fruit yields of the DI treatment were consistently high.

In the study years, fruit quality varied depending on the climate condition, irrigation and fertilization treatment. The vitamin C and carotenoids content, which determine fruit quality, were highest in the non-irrigation treatment. This shows that antioxidant compounds are increased in plants under the influence of water stress. The Brix value, vitamin C and carotenoids content were decreased with increasing irrigation. Nevertheless the weather of the years influenced the efficiency of irrigation that showed in the nutritional quality of the fruit.

Our study showed, the results of the 2018, brix value were higher in DI treatment than other treatments. The lycopene content in the DI treatment was close to that in the NI treatment, in the two experimental years.

The content of carotenoids decreased slightly even in the deficit irrigation treatment. Deficit irrigation supports to reduce water consumption and produce a quality fruit yield.

The combined treatment of biofertilizers and irrigation was affected on tomato fruit yield and quality. The 2018 and 2020 studies of Brix showed it was significantly different ( $P < 0.05$ ) between different biofertilizer treatments. Among the different biofertilizer treatments, the B1 bacterium had a positive effect on the Brix value, which was contained in the tomato fruit.

PGPRs had no significant effect on lycopene, but irrigation treatments significantly affected the lycopene content in 2018. Combined treatments had significantly ( $P < 0.001$ ) affected the lutein content ( $P < 0.05$ ) in 2018. Use of B1 treatment under NI and DI conditions increased the lycopene, beta carotene and lutein content in a drier year (2018).

In 2020, different irrigation treatment had significantly ( $P < 0.01$ ) affected the lutein content, however biofertilizer did not significantly influence the lutein content. B1 biofertilizer treatment increased the cis lycopene, phytoene and phytofluene content of tomato fruit in the wet year (2020) in comparison without biofertilizer treatment.

We recommended the use of the field-inoculation at transplant multi-species inoculum as an integrative method in the sustainable field production system. Our result confirmed the important role of combined treatment which are deficit irrigation and "B1" biofertilizer.

## RELATED PUBLICATIONS

1. Bulgan Andryei, Kitti Zsuzsanna Horváth, Zoltán Pék, Eszter Nemeskéri, Andras Nemenyi, Lajos Helyes (2018): Effects of irrigation and plant growth promoting rhizobacteria on processing tomato. In: 7th International Scientific Conference – Sustainable Development of Agriculture and Economy. Scientific Journal – Agricultural Economics. vol.09. Published by School of Economics and Business Mongolian University of Life Science. ISSN 2519-2000, 203-206 p.
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## APPENDICES

Appendix 1. Effect of years on yield of H-1015 F1 tomato

Years	Total biomass t ha <sup>-1</sup>	Green biomass t ha <sup>-1</sup>	Total yield t ha <sup>-1</sup>	Marketable yield t ha <sup>-1</sup>	Green yield t ha <sup>-1</sup>	Nonmarketable yield t ha <sup>-1</sup>	Brix°	Brix yield t ha <sup>-1</sup>	Vitamin C µg g <sup>-1</sup>
2018	83.33±17.1	15.17±2.8	68.16±16.0	58.64±13.2	7.74±3.9	1.78±1.4	3.69±0.4	2.14±0.4	45.11±7.5
2020	62.49±6.9	12.74±3.2	49.75±6.3	38.32±6.2	2.09±1.1	9.33±4.3	4.01±0.6	1.56±0.4	35.56±4.6
Average	72.91±16.6	13.95±3.2	58.96±15.2	48.48±14.5	4.92±3.9	5.56±4.9	3.85±0.5	1.85±0.5	40.33±7.8
Significance	**	*	***	***	***	***	ns	**	**

\*P≤0.05, \*\* P<0.01, \*\*\*P<0.001, ns=non significance.

Appendix 2. Effect of water supply on yield of H-1015 F1 tomato in average of years

Water supply	Total biomass t ha <sup>-1</sup>	Green biomass t ha <sup>-1</sup>	Total yield t ha <sup>-1</sup>	Marketable yield t ha <sup>-1</sup>	Green yield t ha <sup>-1</sup>	Nonmarketable yield t ha <sup>-1</sup>	Brix°	Brix yield t ha <sup>-1</sup>	Vitamin C µg g <sup>-1</sup>
NI	63.28	13.06	50.24	42.79	3.46	3.97	4.44 a	1.90	43.85
DI	78.31	14.06	64.26	53.91	5.24	5.10	3.76 b	1.99	39.33
RI	77.14	14.74	62.39	48.75	6.06	7.60	3.37 b	1.64	37.83
Average	72.91	13.95	58.96	48.48	4.92	5.56	3.86	1.85	40.33
Significance	ns	ns	ns	ns	ns	ns	***	ns	ns

\*\*\*P<0.001, ns=non significance. Mean values in the column having different letters are significantly different using Tukey's test.

Appendix 3. Effect of years on nutritional values of H-1015 F1 tomato

Years	Lycopene $\mu\text{g g}^{-1}$	$\beta$ carotene $\mu\text{g g}^{-1}$	$\gamma$ carotene $\mu\text{g g}^{-1}$	Lutein $\mu\text{g g}^{-1}$	9 cis- lycopene $\mu\text{g g}^{-1}$	13 cis- lycopene $\mu\text{g g}^{-1}$	Lycoxanthene $\mu\text{g g}^{-1}$	Phytoene $\mu\text{g g}^{-1}$	Phytofluene $\mu\text{g g}^{-1}$
2018	187.93±49.9	3.97±1.3	1.11±0.3	1.02±0.3	1.26±0.4	4.27±2.5	4.37±1.6	6.73±2.1	3.64±1.1
2020	177.16±51.8	6.20±1.6	1.13±0.3	0.98±0.2	0.94±0.3	6.76±3.2	5.23±1.3	7.54±2.5	3.57±1.4
Average	183.32±49.7	4.92±1.8	1.11±0.3	0.99±0.2	1.12±0.4	5.33±3.0	4.74±1.5	7.08±2.3	3.61±1.2
Significance	ns	**	ns	ns	*	*	ns	ns	ns

\* $P \leq 0.05$ , \*\*  $P < 0.01$ , ns=non significance.



Appendix 4. Effect of water supply on nutritive values of H-1015 F1 tomato

Years	Water	Lycopene $\mu\text{g g}^{-1}$	$\beta$ carotene $\mu\text{g g}^{-1}$	$\gamma$ carotene $\mu\text{g g}^{-1}$	Lutein $\mu\text{g g}^{-1}$	9 cis- lycopene $\mu\text{g g}^{-1}$	13 cis- lycopene $\mu\text{g g}^{-1}$	Lycoxanthene $\mu\text{g g}^{-1}$	Phytoene $\mu\text{g g}^{-1}$	Phytofluene $\mu\text{g g}^{-1}$	$\alpha$ carotene $\mu\text{g g}^{-1}$
2018	NI	232.45 a	4.38	1.30	1.33 a	1.24	3.01	5.13	7.71	4.22	
	DI	181.87 ab	3.87	1.04	0.82 b	1.21	6.22	4.44	6.66	3.66	
	RI	149.50 b	3.65	0.99	0.89 ab	1.34	3.60	3.54	5.81	3.04	
<i>Sign.</i>	*	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	
2020	NI	233.18 a	6.45	1.24	0.84	0.85	4.66	6.51	9.90	4.75	0.74 a
	DI	156.96 ab	5.43	1.08	1.04	1.17	9.31	4.91	6.32	2.74	0.29 b
	RI	141.38 b	6.72	1.07	1.04	0.78	6.31	4.28	6.41	3.20	0.49b
<i>Sign.</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>0.011</i>

\* $P \leq 0.05$ , ns=non significance. Mean values in the column having different letters are significantly different at  $P < 0.05$  level using Tukey's test.

Appendix 5. Effect of water supply on nutritional values of H-1015 F1 tomato in average of years

Water supply	Lycopene µg g <sup>-1</sup>	β carotene µg g <sup>-1</sup>	γ carotene µg g <sup>-1</sup>	Lutein µg g <sup>-1</sup>	9 cis- lycopene µg g <sup>-1</sup>	13 cis- lycopene µg g <sup>-1</sup>	Lycorhanthene µg g <sup>-1</sup>	Phytoene µg g <sup>-1</sup>	Phytofluene µg g <sup>-1</sup>	α carotene µg g <sup>-1</sup>
NI	232.75 a	5.26	1.27	1.12	1.07	3.71 b	5.72 a	8.65	4.45	0.74 a
DI	171.18 b	4.54	1.06	0.92	1.20	7.54 a	4.64 ab	6.51	3.27	0.29 b
RI	146.01 b	4.97	1.02	0.96	1.10	4.76 ab	3.86 b	6.07	3.11	0.49 ab
Average	183.32	4.92	1.11	0.99	1.12	5.34	4.74	7.08	3.61	0.51
Significance	**	ns	ns	ns	ns	*	*	ns	ns	**

\*P≤0.05, \*\* P<0.01 ns=non significance. Mean values in the column having different letters are significantly different using Tukey's test.

Appendix 6. Regression Statistics of leaf temperature (NI treatment) vs. air temperature in 2018

<i>Regression Statistics</i>	
Multiple R	0.26048
R Square	0.06785
Adjusted R Square	-0.1186
Standard Error	1.69428
Observations	7

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	1.04476	1.04476	0.36395	0.57264
Residual	5	14.353	2.8706		
Total	6	15.3978			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	22.9965	9.1279	2.51936	0.05322	-0.4675	46.4605	-0.4675	46.4605
Air temp	0.22566	0.37405	0.60329	0.57264	-0.7359	1.18718	-0.7359	1.18718

Appendix 7. Regression Statistics of leaf temperature (DI treatment) vs. air temperature in 2018

<i>Regression Statistics</i>	
Multiple R	0.48202
R Square	0.23235
Adjusted R Square	0.07882
Standard Error	1.07464
Observations	7

ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	1.7477	1.7477	1.51336	0.27334
Residual	5	5.77424	1.15485		
Total	6	7.52194			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	20.5981	5.78958	3.5578	0.01625	5.71555	35.4807	5.71555	35.4807
Air temp	0.29186	0.23725	1.23019	0.27334	-0.318	0.90173	-0.318	0.90173

Appendix 8. Regression Statistics of leaf temperature (RI treatment) vs. air temperature in 2018

<i>Regression Statistics</i>	
Multiple R	0.71615
R Square	0.51287
Adjusted R Square	0.41544
Standard Error	1.52799
Observations	7

ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	12.2905	12.2905	5.26414	0.07026
Residual	5	11.6738	2.33475		
Total	6	23.9642			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	8.94107	8.23199	1.08614	0.32698	-12.22	30.1021	-12.22	30.1021
Air temp	0.77397	0.33734	2.29437	0.07026	-0.0932	1.64112	-0.0932	1.64112

Appendix 9. Regression Statistics of leaf temperature (NI treatment) vs. air temperature in 2020

<i>Regression Statistics</i>	
Multiple R	0.566384017
R Square	0.320790855
Adjusted R Square	0.20758933
Standard Error	1.751617302
Observations	8

ANOVA				<i>Significance F</i>	
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	
Regression	1	8.694570965	8.694571	2.833803	0.143286703
Residual	6	18.40897903	3.068163		
Total	7	27.10355			

	<i>Standard Error</i>		<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	17.4732843	4.933441395	3.541804	0.012192	5.401588081	29.544981	5.401588081	29.54498051
Air temp	0.382330195	0.227119146	1.68339	0.143287	0.173410335	0.9380707	-0.17341033	0.938070725

Appendix 10. Regression Statistics of leaf temperature (DI treatment) vs. air temperature in 2020

<i>Regression Statistics</i>	
Multiple R	0.59667178
R Square	0.35601722
Adjusted R Square	0.24868675
Standard Error	1.8362235
Observations	8

ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	11.1840495	11.184	3.31702	0.11841202
Residual	6	20.2303005	3.37172		
Total	7	31.41435			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	17.0278867	5.1717353	3.29249	0.01656	4.37310628	29.68267	4.37310628	29.68266709
Air temp	0.43362475	0.2380894	1.82127	0.11841	-0.14895903	1.016209	-0.148959	1.016208524

Appendix 11. Regression Statistics of leaf temperature (RI treatment) vs. air temperature in 2020

<i>Regression Statistics</i>	
Multiple R	0.71256782
R Square	0.50775289
Adjusted R Square	0.42571171
Standard Error	1.11237077
Observations	8

ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	7.65807509	7.65808	6.189	0.04730484
Residual	6	7.42421241	1.23737		
Total	7	15.0822875			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
Intercept	18.5087202	3.13299943	5.90767	0.00105	10.8425467	26.17489
Air temp	0.35881809	0.14423282	2.48777	0.0473	0.0058931	0.711743



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