HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Doctoral School of Plant Science

Ph.D. Dissertation

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PHYLAZONIT) IN TOMATO (*Solanum Lycopersicon L*) PRODUCTION UNDER DIFFERENT IRRIGATION LEVELS.

DOI: 10.54598/004050

By

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Gödöllő, Hungary

2023

Title: Effect of Pant Growth Promoting Rhizobacteria (Phylazonit) in tomato (*Solanum Lycopersicon L*) production under different irrigation levels.

Name: MATE Plant Science Doctoral (PhD) School

Major Ph.D. program field: Crop Production and Horticultural Sciences

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1. INTRODUCTION

1.1. Importance and background

Nowadays, tomato is the most popular and important vegetable crop grown all over the world. European countries produce about 18 million tonnes of tomatoes two-thirds of the total was produced in Italy and Spain (11.3 million tonnes) in 2021 (Eurostat, 2022). Almost all tomatoes are processed into multi-food products except a small part consumed directly or traded as raw commodities. In 2022, processing tomato production was 39.7 Mt worldwide and 16.9 Mt in Europe (WPTC, 2022). Tomato production is influenced by the consumption demand. Tomato is available year-round and provides significant health benefits. Quality is the most characteristic of fresh or processed tomatoes. It is influenced by a variety of interactions, environmental factors such as light, temperature, and irrigation supply, as well as nutrient component solution and crop management. (Dorais 2007). The irrigation or water supply has a strong consequence on the yield as well as the quality of processing tomatoes (Helyes et al. 2014b).

In 2015, FAO reported that the water supply for agriculture accounted for 70% of the freshwater used in the world, mostly through irrigation. This has been essential for food production since irrigation reduces drought risk and increases crop diversification, therefore it also improves rural incomes. About a decade ago irrigated land in agriculture was about 20 percent but it contributed to 40 percent of global food production (FAO 2015). Processing tomatoes requires 400-800 mm of water from transplanting to harvest (Steduto et al. 2012).

Drip irrigation is very efficient in saving water, but it can be increased by applying deficit irrigation (DI) in the field (Selim et al. 2012). This irrigation method causes water stress to plants, but if the yield reduction is lower than the benefit we get from the water saving or quality improvement then the lower yield becomes less important (Johnstone et al. 2005; Pék et al. 2017). Effects of DI vary year by year and it affects crops differently, moreover, soil also influences it. The most common water deficit applied is 50% of evapotranspiration (Bakr et al. 2017), but other rates can be used as well (Patanè and Cosentino 2010). Other techniques include the application of different DI rates in different vegetative stages (Nangare et al. 2016) or simply terminating irrigation for the duration of different phonological stages (Kuscu et al. 2014; Lei et al. 2009).

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but contributed to 40 percent of global food production (FAO 2015). Processing tomatoes requires 400–800 mm of water from transplanting to harvest (Steduto et al. 2012).

Drip irrigation is very efficient in saving water by itself, but its efficiency can be increased by applying deficit irrigation (DI) in the field (Selim et al. 2012). This irrigation method causes water stress to plants, but if the yield reduction is lower than the benefit we get from the water saving or quality improvement then the lower yield becomes less important (Johnstone et al. 2005; Pék et al. 2017). The effects of DI vary year by year, and it affects crops differently; moreover, soil also influences them. The most common water deficit applied is 50% of evapotranspiration (Bakr et al. 2017), but other rates can be used as well (Patanè and Cosentino 2010). Other techniques include the application of different DI rates in different vegetative stages (Nangare et al. 2016) or simply terminating irrigation for the duration of different phonological stages (Kuscu et al. 2014; Lei et al. 2009).

PGPRs have many benefits in the soil environment; they enrich all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate, and potassium solubilization or mineralization (Adesemoye et al. 2008). They involve various biotic activities of the soil ecosystem to make it dynamic for nutrient turnover and sustainable for crop production (Bhardwaj et al. 2014). Singh and co-workers (2011) reported the application of biofertilizers as seed or soil inoculants, where the microorganisms multiplied and participated in nutrient cycling, which benefited crop productivity. In other research, PGPR has increased marketable yield significantly while reducing the fertilizer demand for tomato (Adesemoye et al. 2009). Other researchers found that PGPR is useful for enhancing tomato phytochemicals (Sabin et al. 2017), especially under stressful conditions (Ruzzi and Aroca 2015).

1.2. Objectives

The purpose of this research was to determine the effects of different regimes of irrigation combined with the PGPR application on the yield and quality of industrial tomatoes.

1. The effect of PGPR application and DI strategy on soil moisture and root microbial activities
2. The effect of PGPR application and irrigation levels on the physiological response of processing tomatoes
3. The effects of PGPR application and irrigation levels on the yield of processing tomatoes

4. The effect of PGPR application and irrigation levels on the fruit qualitative parameters of processing tomatoes

2. LITERATURE REVIEW

2.1. Production of tomatoes

The tomato belongs to the genus *Lycopersicon* whose Latin name is *Solanum lycopersicum L.* It is the second-most consumed and most valuable vegetable in the world after potatoes. The fruits are consumed fresh in salads or processed in the industry (Costa and Heuvelink 2005). Fruits provide an important source of antioxidant components, vitamins, and minerals (Fernández-Ruiz et al. 2011; Gulcin 2012; Helyes et al. 2012; Helyes et al., 2015). Tomato plants have their origins in the tropical South America region; the plant has grown perennial herbaceous plant, but it is often known as an annual crop.

Currently, the total tomato production in the world is around 118.7 million tons in 2021, and it shows an increasing trend over years. The first country growing tomato is China, which accounted for 34.7% of the total production at 64.768 million metric tons (mT). China devoted 1,107,485 hectares to tomato production in 2020, with a yield of 58.5 mT/ha. India stood in second place, with a production of about 20.573 million mT on 812,000 hectares in 2020 and a yield average of 25.3 mT/ha. Turkey came in third with 13.204 million mT planted on 181,879 hectares and a 72.6 mT/ha yield. The United States came in fourth place with 12.227 million mT, 110,439 hectares, and a yield of 110.7 mT/ha (https://www.tomatonews.com/).

According to data from the European Commission, total tomato production in the EU will fall by 3% year on year in 2022, to 6.2 million metric tons, due to a lack of irrigation water and increased substitution to alternative, more competitive crops with lower financial risk. EU fresh tomato imports increased by 14% in 2021 compared to 2020, which is expected to increase further in 2022. Morocco is the EU's largest supplier, accounting for 70% of total imports, followed by Turkey. It was expected that the EU would import more tomatoes from Morocco in 2022 as European tomato production is expected to fall due to rising input costs. (https://www.mintecglobal.com/).

In 2011 and 2013, the quantities of processed tomatoes were approximately 34 to 35 million tons, of which 24 to 25 million tons have been shipped each year to the final consumption (FAO 2015). The highest per capita consumption levels have been recorded for consumers in Australia and New Zealand, North America (NAFTA region), and Europe. In these regions, the annual

quantities consumed accounted for about 22 kg/person/year. About 14.5 kg of raw tomato in processed form have been recorded for a Group of countries including Iraq, Turkey, Iran, and the Arab Peninsula. The regions of Mediterranean Africa, the EU, and non-EC Europe consumed on average the equivalent of 11.5 kg of raw tomato in processed form. Per capita consumption in Andean America, West Africa, Yemen, Central America, and Ukraine can be described as "low" (Dossier 2014).

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2.2. Processing of tomatoes

In 2016–17, the EU produced more than 18 million metric tons of tomatoes, of which 60% was used in the processing industry. Spain, Italy, and Portugal accounted for 94 % of production for processing. In the period between 2014 and 2016, EU production of fresh tomatoes remained stable at 15kg per capita. It is expected to decline slightly to 14.4kg by 2030. In contrast, processed tomatoes are expected to increase slightly by +0.4% per year until 2030. The consumption is expected to increase from 20.5 kg per capita in 2014–2016 to above 21kg in 2030 (European Commission Report 2017). The production of processed tomatoes is most often classified as one of four major sub-categories: tomato paste, tomato sauces, ketchup, and other products which mainly consist of puree, whole canned tomatoes, and juices. (Boriss 2005).

In Turkey, about 8.9 million tons of fresh tomatoes are processed every year. The processed products are paste (80%), sliced tomatoes (15%), and the rest as ketchup, juice, and other products. The farmers growing tomatoes got a net profit of 1,804 and 2,513 USD/ha on the research area of 5.55 and 2.48 ha, respectively (Engindeniz 2007). In the US, the number of processed tomatoes was 5–6 times larger than the number of fresh tomatoes. The fruits are harvested by machinery and sold under contract. The average industrial tomato growing acreage was 263,000 acres in 1980, increasing by 15 percent in 2004 to 301,600 acres. The production value increased by nearly 100 percent over the same period; however, regarding dollars per pound, the processing tomatoes had a lower market value compared to the fresh market tomatoes due to a larger share of total crop value (Boriss 2005). In the 2020/21 season, Australia's production of industrial tomatoes was about 232,562 tons with an average yield of 55.4 mT per hectare, where 2,215 hectares were planted. Higher soluble solids achieved in that season averaged 5.01%, which continues the trend in recent years. (http://www.aptrc.asn.au)

The characteristic of processing tomato varieties is that a special type of tomato has different growth habits compared to fresh-market tomatoes. The processing tomatoes are dwarf habit, concentrated and uniform fruits setting and ripening, tough skins, and a high soluble solids content. The varieties are also evaluated according to their production potential, the size of the cluster, canopy, quality, and firmness to allow bulk transport and mechanized harvesting (Boriss 2005; Costa and Heuvelink 2005). Growing in an open-field system and are harvested by the machinery often applied to processing tomatoes. Fruits are required to have a thick pericarp, a small stem end, an absence of defects, and resistance to diseases. The report in the US showed the harvested tonnage of industrial tomatoes is 5–6 times larger than that of the fresh market. Fruits harvested in the field are transported to the manufacturing plant and transformed into paste within 6 hours after harvest (Boriss 2005, Maria et al. 2017).

Processing tomato plants are grown either directly sown or transplanted, with the transplanting method achieving more uniform plant emergence, density, and development. The sowing period is carried on from the end of February until May in the Northern Hemisphere or from August to mid-December in the Southern Hemisphere when the temperature of topsoil is 100C. The optimal sowing depth is 2–4 cm, and the quantity is about 100 000 seeds per hectare (1–1.5 kg/ha). Transplanting is carried out in a nursery in plug trays (25–35 mm diameter per seedling) at a density of between 750 and 1 000 seedlings per m2, and the plants are transplanted at a rate of 25 000–33 000 plants per ha. Seedlings are commonly transplanted at the 4–5 true-leaf stage, 4–7 weeks after sowing. Seedlings should be short,150-200 mm, including the root clod, and less if transplanting is done by machine, and have a thick stem base (diameter ≥ 4 mm). Density ranges from 2.5 to 3.3 plants/m2, and row spacing ranges from 0.75 m to 1.6 m. Depending on temperature, tomatoes start to flower 25-40 days after transplanting or 35–50 days after emergence. The life cycle of processing a tomato varies from 95 to 115 days. Tomato flowers develop from buds situated on the axis of the angle between the leaf and stem. Flowers form in sequence as the number of leaves increases on the stem, and flower and fruit initiation overlap vegetative growth for the whole period (Steduto et al. 2012).

The yield has increased by more than 50 percent in California and the Mediterranean countries over the past 30 years. A yield ranges from 60 to 120 tonne/ha for processing tomatoes. The soluble solids content of the juice of the most widely used cultivars can vary between 4.2 and 5.5 percent. The factories require a minimum quality for processing tomatoes: a juice acidity range

between 0.34 and 0.40 g/100 ml, reducing sugars between 2.5 and 3.0 g/100 ml, and Bostwick consistency between 8 and 12 cm/30 s. Dry matter content of fresh fruit ranges from 4.0 to 7.0 percent. Harvest Index (HI, the ratio of yield measured as dry matter to total above-ground biomass) normally ranges from 0.5 to 0.65 (Gary and Tchamitchian 2002; Steduto et al. 2012).
Considering the deficit irrigation turns Rinaldi et al. (2015). research on the AQACROP system on the processing of tomatoes. The results show that net income oscillated between 1,280 € ha -1 and 3,420 € ha-1 for seasonal irrigation water amounts equal to 170 and 570 mm/s, with the highest income at 370 mm (4,011 € ha-1). Applying the deficit water treatment allows adequate fresh fruit yield, water saving, and a net income compared with that obtained with irrigation at fixed times, and stable profitability over the years (Rinaldi et al. 2011).

2.3. Situation of tomato production in Vietnam.

Tomato was first cultivated in Vietnam 100 years ago. Recently the government of Vietnam established a project for manufacturing and processing of tomatoes for domestic and international markets. The objective was to produce 33,000 tons of concentrated tomatoes. The factory was built in Hai Phong, and the tomato planting area is planned in an area of 1,200–1,500 ha in the Red River Delta (Binh et al. 2014). Nowadays, the Government has announced the target for vegetable export, for this aim tomato is one of the object vegetables for export together with other kinds of vegetables such as cucumber, corn, and bean. For this reason, the tomato area has been increasingly expanded in recent years from 15,000-17,000 ha annually with the production of 15 – 17 tons/ha and more than 30 tons/ha in some intensive farming areas (Institute of Vegetables and Fruits, 2000).
Due to dietary habits, people almost consumed tomatoes fresh. Therefore, a potential market and the need for input in the processing industry have promoted the development of tomato cultivation. In 2010, the Government established a project for manufacturing and processing tomato using domestic and export. The objective was to produce 33,000 tons of concentrated tomatoes. The factory was built in Hai Phong and the tomato planting area is planned in an area of 1,200 – 1,500 ha in the Red River Delta (Binh et al. 2014).
In Vietnam, tomato is highly sensitive to several biotic and non-biotic stresses. Fungal diseases (especially early blight, late blight, and Fusarium wilt), bacterial infections (bacterial wilt, bacterial spot, etc.), and virus diseases (tobacco mosaic virus, leaf curl, spotted wilt, etc.) are serious problems. Abiotic stress like extreme temperature, salinity, drought, excessive moisture, and environmental pollution, also affect tomato yield. The yield can decline during the summer

due to the hot and humid climate. In North Vietnam in winter, the temperature is about 100 C or less, so the plants suffer chilling injury when exposed. Tomato fields might suffer from flooding due to frequent heavy rains from April to October (https://openjicareport.jica.go.jp).
The current processing cultivar, Hong Loan, is commonly used due to its wide adaptability. VL2000, an American F1 crossbreed, is being popularly grown in North Vietnam. MV1 was recognized in 1996 as a good check for heat tolerance in the spring and summer of the year. VL2200, an American crossbred, is a favourite variety in tomato-growing areas (Vien 2003)

2.4. Plant characteristics and vegetative growth.

2.4.1. Tomato plant characteristics

Tomatoes can be grown under different conditions, but the most suitable are high altitudes, with low humidity and high luminosity. In different altitudes and regions, the crop can be cultivated differently. Tomato plants may be grown for the whole year, with heights ranging from 500 to 900 m. At an altitude less than 300 m, it is preferably cultivated in the winter, and at an altitude above 1200 m, it is best cultivated in the summer. Tomatoes can be grown under different conditions, but the most suitable are high altitudes, with low humidity and high luminosity. In different altitude regions, the crop can be cultivated differently; a tomato plant may be grown for the whole year at an altitude of 500–900 m (Da Silva et al. 2008). In tropical and temperate climates, the cultivation of tomatoes takes place in open fields or greenhouses. During the growth period, the temperature had a great impact on the growth rate. In the germination phase, the ideal temperature is 16–29°C; from 21–240°C is the ideal average temperature for plant development, and for fruit set, the optimum temperature is 24°C during the day and 14–17°C at night. Temperatures between 20 and 24°C during the day and around 18°C at night are ideal for the formation of lycopene. The necessary time from germination to anthesis is around 45 days in a warm climate with the right light intensity and 90–100 days to reach the beginning of fruit ripeness (Helyes et al. 2014a).
There are three types of cultivated tomatoes: indeterminate, semi-determinate, and determinate. Indeterminate plants are tall, frequently more than 2 m high, with vegetative growth continuing much longer after the start of flowering than in the other two types. Fruit ripens gradually, starting from the basal fruit clusters (Costa and Heuvelink. 2005; Da Silva
et al. 2008).

Semi-determinate plants are less tall than the former, reaching a maximum height of 0.9–1.5 m. Their characteristic is that the main fruit clusters ripen together, but the plant will also continue to produce additional fruit. Indeterminate and semi-determinate tomatoes need to be staked or trellised, are grown for the fresh market, and are harvested by hand. Determinate types, like the so-called bush tomato, mostly rest on the ground and have a relatively concentrated flowering and fruit setting lasting only about three weeks. In this period, vegetative growth continues. Most fruit of determinate cultivars matures in a relatively short period, and for this reason, they are suitable for mechanical harvesting. Processing tomato cultivars are bred for firmness and strong skin and are of the determinate type.

Tomato plants are dicots and grow as a series of branching stems, with a terminal bud at the tip that does the actual growing. The plant varies and may reach up to 3 meters in height depending on the growth habit, and the primary root may grow several meters in length. The stem is angular and covered by hairy and glandular trichomes that confer a characteristic smell. Almost tomato plants have compound leaves, which are typically comprised of five to nine leaflets. Leaflets are petiole and dentate. All leaves are covered by glandular, hairy trichomes (Costa and Heuvelink. 2005).

The tomato fruit is classified botanically as a berry. Size varies from small cherry types with only two divisions of the ovary (locules) to large multilocular beefsteak types. The fruit shapes range from round to oblate (flat-round) with fresh market tomatoes while processing tomatoes are more elongated (oblong) or pear-shaped. Fruit colour can be yellow, orange, pink, red, or even white. The red colour comes from the pigment lycopene, while the orange and yellow colour comes from the beta-carotene pigment (Costa and Heuvelink. 2005). The fruits can be either bilocular or multilocular, inside the locular cavities are located 50 to 200 seeds and are enclosed in gelatinous membranes. On average, the diameter of seeds is small (5 x 4 x 2 mm) and lentil shaped. The outer skin is a thin and fleshy tissue comprising the remainder of the fruit wall and the placenta. (OECD 2017).

2.4.2. *Nutritional value of tomatoes*

Fresh and processed tomato products make a significant contribution to human nutrition owing to the concentration and availability of several nutrients in such products and to their widespread consumption. Composition tables show that ripe tomatoes (Lycopersicon esculentum, Mill.) contain 93–95% water and low levels of solid matter. Tomatoes usually contain from 5.5 to 9.5% total solids, of which about 1% are in the skins and seeds (Canene-Adams et al., 2005). The percentage of soluble solids in tomatoes varies over wide limits for some reasons, such as variety, the character of the soil, and especially the amount of irrigation and rainfall during the growing and harvesting seasons. In as much as tomato products, such as pulp and paste, are evaporated to a specific percentage of solids, their yield per ton of tomatoes varies with the composition of the raw tomatoes used in their manufacture. In tomato juice, the fraction of insoluble solids (cellulose, lignin, pectin substances) varies from 15 to 20% of total solids (Costa and Heuvelink. 2005; Da Silva et al. 2008; Martí et al. 2016).

Oxidative processes have been linked to numerous human protections against certain pathologies, with many substances derived from vegetable products, including tomatoes. These substances have differing functions, such as free radical scavengers, singlet oxygen quenchers, metal chelates, and inhibitors of enzymes involved in the formation of the active species of oxygen. Research has demonstrated that the antioxidant activity of some tomato components provides a protective effect against some types of cancer and ischaemic heart diseases (Martí et al. 2016).

Fresh tomatoes and other processed tomato products contribute significantly to human nutrition owing to the concentration and availability of several nutrients in these products and their widespread consumption. Ripe tomato fruit contains 93–95% water and 5.5–9.5% total solids (Table 1). Free sugar is the main part of the soluble solids; they are predominantly reducing sugars. The reducing sugars are mainly glucose and fructose. The total sugar content of fresh tomatoes is found to vary from 2.19 to 3.55%. Tomato juice contains eight organic acids: malic acid, citric acid, tartaric acid, succinic acid, acetic acid, and oxalic acid. Malic acid was found to be the major organic acid in fresh juice whereas pyrrolidone carboxylic acid was found to be the major organic acid in processed juice.

Table 1. Composition of mature-green and ripe tomato per 100g.

Component	Mature - green	Ripe
Water (%)	93.0	93.5
Calories	24.0	22.0
Protein (g)	1.2	1.1
Fat (g)	0.2	0.2
Carbohydrates		
Total (g)	5.1	4.7
Fibre (g)	0.5	0.5
Ash (g)	0.5	0.5
Calcium (mg)	13.0	13.0
Phosphorus (mg)	27.0	27.0
Iron (mg)	0.5	0.5
Sodium (mg)	3.0	3.0
Potassium (mg)	244.0	244.0

Source: Jones, 1999.

Ripe tomatoes are relatively rich in antioxidants: vitamin C (160–240 mg/kg-1), lycopene (30–200 mg/kg-1), provitamin A carotenes (6–9 mg/kg-1), and phenolic compounds; flavonoids (5–50 mg/kg-1), and phenolic acids (10–50 mg/kg-1). Also present in small quantities are vitamin E (5–20 mg/kg) and trace elements such as copper (0.1–0.9 mg.kg-1), manganese (1–1.5 mg/kg), and zinc (1–2.4 mg/kg), which are present in several antioxidant enzymes (Canene-Adams et al. 2005). Whole, red-ripe tomatoes contain nearly all the vitamin C activity in the reduced ascorbic acid form. Dehydro-ascorbic acid has been reported to constitute 1–5% of the total ascorbic acid in tomatoes. The ascorbic acid concentration in fresh, ripe tomatoes is about 25mg per 100g (Daood et al. 2013; Canene-Adams et al. 2005).

Many tomato products contain a high amount, and there are good sources of potassium, folate, and vitamins A, C, and E. Tomato products also consist of many beneficial micronutrients,

including carotenoids and polyphenols. Carotenoids, such as the red pigmented lycopene, β-carotene, a pro-vitamin A compound; phytoene, and phytofluene are all found in abundance in raw tomatoes and tomato products. Tomatoes also provide small amounts of the B vitamin complex: thiamine, niacin, and riboflavin (table 2). A study by Adame et al. (2001) also reported the connection between increased tomato consumption and reduced risk for both cardiovascular disease and prostate cancer. (Canene-Adams et al. 2005).

Giovannucci et al. (2002) reported on tomatoes and tomato products as contributing to a rich source of lycopene, neurosporene, gamma-carotene, phytoene, and phytofluene. Because of their potent antioxidant properties, tomato carotenoids have been reported to be positive against cancer (Giovannucci et al. 2002). Consumption of food containing carotenoids, lycopene probably protected against cancers of the mouth, pharynx, and larynx, and also lung cancer. Food containing β-carotene would most likely protect against esophageal cancer, while lycopene would protect against prostate cancer (Canene-Adams et al. 2005).

Table 2 Major vitamins and antioxidant compounds of tomato and tomato products

	Tomato products (per 100g)				
	Raw tomatoes	Catsup	Tomato juice	Tomato sauce	Tomato soup
Potassium, *mg*	237	382	229	331	181
α-tocopherol, *mg*	0.54	1.46	0.32	2.08	0.50
Vitamin A, *IU*	833	933	450	348	193
Vitamin C, *mg*	12.7	15.1	18.3	7.0	27.3
Total folate, μ*g*	15	15	20	9	7
β-carotene, μ*g*	449	560	270	290	75
α-carotene, μ*g*	101	0	0	0	0
Lycopene, μ*g*	2573	17007	9037	15152	5084
Lutein + zeaxanthin, μ*g*	123	0	60	0	1

Phytoene, µg	1860	3390	1900	2950	1720
Phytofluene, µg	820	1504	830	1270	720

Source: Canene-Adams et al 2005

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2.5. Impact of stress on tomatoes plant

Soil is a complex and dynamic system that supports plant growth. Plants grow and are influenced by a variety of stresses in the soil environment during development, which significantly reduces agricultural production. These stresses are biotic, such as those caused by plant pathogens and pests (viruses, bacteria, fungi, insects, nematodes, etc.), and abiotic, including salinity, drought, flooding, heavy metals, temperature, gases, and nutrient deficiency or excess. Abiotic stresses are considered to be the main source of yield reduction (Nadeem et al. 2014). Tomato is high water demand for both its vegetative and reproductive growth, especially during the flowering and fruit enlargement stages; therefore, water (drought stress) and temperature stress can have major negative impacts on canopy development, biomass production, and yield (Jangid and Dwivedi 2016).

2.5.1. Drought stresses

Stress is a disadvantageous condition in the plant that is influenced by external factors such as low and excess water supply, high and low temperature, and light, which lead to a contrary effect. Today, the global climate is changing in many regions, raising the temperature and atmospheric CO2 levels. Temperature changes are the main factor altering rainfall patterns; the lack of precipitation often triggers agricultural and hydrological droughts worldwide (Dai 2011; Osakabe et al. 2014). Many studies have been conducted on the effect of drought stress on plant biomass

and seed production. It could occur losing more than 50% of the potential production (Helyes et al. 2014a; Mafakheri et al. 2010; Ozbahce and Tari 2010; Santisopasri et al. 2001).

Water stress affects tomato plants in various ways, such as reduced growth and leaf surface area, flower shedding, lack of mineral absorption, fruit size reduction, fruit splitting, puffiness, and many physiological disorders related to calcium deficiency, such as blossom end rot, poor seed viability. The effect of drought stress and salinity level on tomato seeds reduces their germination percentage. Foolad and Lin (1997) experimented with tomato seed germination and found a reduction in seed weight due to osmotic stress (Foolad and Lin 1997; Singh et al. 2012).

Decrease in soil water status, plants have many anatomical and physiological changes to reduce the effect of stress. The root system increases the synthesis of abscisic acid (ABA)—the concentration rises up to 50 times more than in leaves. ABA has a significant role in signal transduction under water stress conditions; it regulates stomatal behaviour and reduces transpiration rate by closing stomata. Increased drought status promotes root growth and overshoot growth in plants. Under water stress conditions, ABA also regulates the root-to-shoot ratio of the tomato plant. The root: shoot ratio increases during a water deficit condition, it enhances the capacity of the plant to absorb more water and minerals (Osakabe et al. 2014).

Drought, when combined with other types of stress, has a complex effect on plant growth and development. The cell division and enlargement lead to a reduction in vegetative and reproductive growth. Leaf area and stem length get reduced due to the decrease in cell size. A decline in leaves' size, a lower aperture, a drop in the number of stomata, cell wall thickening, cutinization of the leaf surface, and a developed conductive system (increase in the number of large vessels) are some alterations that occur in plants exposed to drought. Optimal leaf area development and stomatal opening are essential factors for optimal photosynthesis in plants. The main effect of drought stress on plant morphology is size reduction. A low photosynthesis rate is one of the most important factors in the reduction of plant size and biomass production (Jangid and Dwivedi 2016; Osakabe et al. 2014).

Productivity and yield are strongly affected by drought stress. Crop yield is severely reduced under drought stress, and this might be attributed to drought-induced reduced stomatal conductance, reduction in CO2 assimilation rates, photosynthetic pigments, small leaf size, reduced stem extension, disturbed plant water relations, and reduced water use efficiency. Uptake of mineral nutrition is also reduced under low soil water conditions i.e., nitrogen, sodium, sulphur, potassium, magnesium, and calcium. Many scientific studies have revealed that under low water

conditions, tomato plants have reduced yield and fruit size (Bakr et al. 2017; Helyes et al. 2014a; Helyes et al. 2012; Helyes et al. 2015).

Lycopene is an antioxidant that plays an important role in the biosynthesis of carotenoids. It is responsible for the red color in tomato fruit and processed products (Helyes et al. 2014a). Experiments done by Liu et al. (2011) and Helyes et al. (2014) supported the finding that lycopene decreased between well irrigation and the control quality parameter in tomato genotypes under drought stress conditions and that there was a significant increase in lycopene content during water and salinity stress.

2.5.2. Salt stresses

Salt stress has enormous influences on the growth and yield of tomatoes. To cope with stress, the plant responds differently depending on the germplasm, lines, and cultivars. (Foolad and Lin 1997). An important indicator of how plants responded to salt stress was shoot length; there was a remarkable decrease in the fresh and dry weight of tomato shoots in response to salinity stress. The plants declined in the number of leaves, length of leaves, and dry matter accumulation along with increased NaCl concentration and free proline content. Salinity stress also caused an increase in cytotoxic ions (Na+ and Cl-) and Ca2+, with a corresponding reduction in K+ concentrations (Silva and Neto 2015).

2.5.3. Flood stress

Flooding is an environmental stress that is related to precipitation, especially in soils with poor drainage. The tomato is identified as a sensitive crop to flooding stress. Lower leaves of the stem, including yellowing and senescence, are indicators of flooding injury in plants and typically appear 4-6 days after flooding. Flooding in tomato plants results in reduced leaf elongation, leaf epinasty, and the formation of adventitious roots, and an increase in diffusive resistance consequently resulting in wilting of leaves. Kramer (1951) reported that the middle leaves of tomatoes showed epinasty curvature 24–48 h after flooding and tomato plants will recover from flood stress through the formation of new adventitious roots. In flooded tomato plants, stomatal closure is a protective mechanism where it is controlled by root-to-shoot signalling regulation. Stomatal closure is due to increased leaf water potential, less photosynthetic activity, and a shortage of oxygen in the roots. So this protective mechanism decreases the damage to the plant system by increasing the root resistance to water entry from the soil (Kramer 1951).

2.5.4. Heat stress

An increase in ABA level was observed when tomato plants were subjected to constant stressful temperatures (Daie 1980). Tomato plants develop a strong defense mechanism through the accumulation of phenolic compounds against stress at 350 °C, which is above the optimal temperature of 250 °C (Rivero et al. 2001). Heat stress decreases the sugar concentration of tomatoes (Rosales et al. 2007). Under heat stress, heat shock proteins in the cytoplasm protect protein biosynthesis (Miroshnichenko et al. 2005). Chloroplast heat shock protein has been reported to have a dual role in the tomato as it protects photosystem II from oxidative damage and is involved in the colour change of fruit stored at low temperatures (Neta-Sharir et al., 2005).

2.6. The importance of PGPR and Phylazonit fertilizers in plants

Bacteria are vital components of soils, and many biotic activities of the soil ecosystem involve bacterial synthesis to make it dynamic for nutrient turnover and sustainable for crop production (Ahemad and Kibret 2014). Bacteria also responded to plant growth by mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, and improving soil structure (Hayat et al.

2010; Ahemad and Kibret 2014). The PGPR (plant growth promoting rhizobacteria) bacteria presenting around/in the plant roots are mobilizing, transforming, and solubilizing the nutrients more than those from bulk soil, therefore, they are crucial for soil fertility (Ahemad and Kibret 2014).

The action mechanisms of PGPR depend on the location of the rhizosphere bacteria surrounding the root system. While bacteria are provided with mechanical support, facilitating water and nutrient uptake, plant roots also synthesize, accumulate, and secrete a diverse array of compounds. Plant roots also secrete chemical attractants for a vast number of heterogeneous, diverse, and actively metabolized soil microbial communities. There was a wide range of chemical compounds secreted by plant roots that modified the chemical and physical properties of the soil and thus, regulated the structure of the soil microbial community near the root surface (Dakora and Phillips 2002; Bhattacharyya and Jha 2012; Ahemad and Kibret 2014). These compositions depended on the physiological status and species of plants and microorganisms. It promoted the plant's symbiotic interactions and inhibited the growth of the competing plant species. Also, microbial activity in the rhizosphere affects rooting patterns and the supply of available nutrients to plants.

PGPR has been classified according to their functional activities as (i) biofertilizers (increasing the availability of nutrients to plants), (ii) phytostimulators (plant growth promotion, generally through phytohormones), (iii) rhizoremediators (degrading organic pollutants), and (iv) biopesticides (controlling diseases, mainly through the production of antibiotics and antifungal metabolites) (Somers and Vanderleyden 2004).

PGPR microorganisms are classified on their functional groups: nitrogen-fixing groups, that enhance the N uptake of plants, categorized as symbiotic N2 fixing bacteria, and non-symbiotic (free living, associative, and endophytic) nitrogen-fixing forms such as cyanobacteria (Anabaena, Nostoc), Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus, and Azocarus etc. Bacterial genera like Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium, and Serratia are reported as the most significant phosphate solubilizing bacteria group (contributing to plant phosphorus (P) uptake) (Bhattacharyya and Jha 2012; Bishnoi 2015).

A number of researchers have experimentally demonstrated the role of PGPR in agricultural production. Bashan et al (1995) suggested that the dose distribution in the field depends on the concentration of PGPR in the biofertilizer, the kind of microorganisms present in the product, and the crop. In the case of cereals, the biofertilizer made of N-fixing bacteria or

PGPR has a dose that varies from 1 to 3 kg ha1 and from 10 to 15 kg ha1 for vegetables with biofertilizers containing PGPR and AMF. For fruit crops, the applied dosage is 10 to 50 g· plant/day with solid products or 3 to 5 kg/ha/day with liquid products. The treatment frequency and times depend on vegetable and fruit crops.

In the case of PGPR, repeated applications occurred 3–4 times during the growing season, with an interval of 2–4 weeks.

The study on the germination of Asparagus officinalis L. revealed significant differences in percentage between fertilizer treatments and controls (Ge et al. 2016). The Nitroxin biofertilizer, comprising Azospirillum and Azotobacter, mixed with 30% vermicompost showed the greatest positive impact on the fresh and dry weight of root and shoot. Application of PGPR on seed germination of Crataegus pseudoheterophylla Pojark plants were also investigated. The PGPR treatments were Azotobacter chroococcum, Azospirillum lipoferum, Pseudomonas fluorescens, B. subtilis, and combinations.

Results showed a higher percentage of seed germination (18.33%) and speed of germination (4.82 numbers per day) for treatments containing all bacterial inoculants (Fatemeh et al. 2014).

Inoculation of Burkholderia sp. 7016 on tomato seedlings in a PDA plate after 7 days at 280 °C showed a significant increase in root length, root fresh weight, and dry weight of 38.79, 64.28, and 100%, respectively; while the stem diameter, plant height, shoot fresh weight, and dry weight were increased by 11.33 to 32.31%. The results of the field experiments showed that the treatment enhanced the tomato yield and significantly promoted the activities of soil urease, phosphatase, sucrose, and catalase (Gao et al. 2015).

Greenhouse tomatoes were treated with Bacillus licheniformis biofertilization and nitrogen fertilization. Treatments used nitrogen fertilization (NF) or nitrogen combined with biofertilization (BF) with nitrogen doses: 0, 25, 50, 75, and 100%. The results indicated increased lycopene content with the nitrogen dose in the BF treatments, and lower lycopene contents in the NF treatments. The contents of hydrophilic antioxidant compounds (vitamin C and total phenols), as well as antioxidant activity (DPPH• scavenging capacity and FRAP), increased as the nitrogen dose was reduced. In addition, B. licheniformis had a positive net effect on the synthesis of flavonoids by the plant at a 75% nitrogen dose (Ochoa-Velasco et al. 2016).

The bio-fertilizer Phylazonit from Phylazonit Ltd. (Nyíregyháza, Hungary) contains bacteria: Pseudomonas putida, Azotobacter chroococcum, Bacillus circulans, Bacillus megaterium with germ number: 109 pieces.cm-3, the suggestion of the producer for all arable and horticultural plant cultures in the amount of 10 to 20 l/ha, with 100 to 300 l/ha water quantity, applied on the whole land surface prior to seeding, planting and immediately worked into the soil,

or by means of a special device with 20 to 40 l/ha quantity of liquid applied on the whole surface then mixed into the seedbed, or injected in the bed simultaneously with the seeding ("Phylazonit Introduction" 2017). Previously, it was found that Phylazonit applied in wheat, corn, and cucumber in a climate chamber significantly increased the total root length, biomass production, and nutrient uptake.

Application of the bio-fertilizer Phylazonit MC resulted in significantly larger root length and the number of lateral roots per plant in comparison with those of the control plants (Gajdos et al. 2009).

According to other researchers, Phylazonit increased the extractable NO-3 in sandy soil, decreased the negative effect of wheat straw, helped in the decomposition of wheat straw, and caused a significantly higher amount of organic nitrogen (Kovács 2010). When Phylazonit was applied to maize, it led to a significant rise in the bacteria count compared with the control and improvements in soil properties (Makádi et al. 2007).

MATERIALS AND METHODS

3.1. Experimental fields and plant material

Open field experiments were conducted in 2015, and 2016 on two locations of the Institute of Horticulture's farm at the Hungarian University of Agricultural and Life Sciences, Gödöllő, Hungary; 47.594292, 19,359758 (Location 1) and 47.577380, 19.379573 (Location 2). The experimental design was laid out as a randomized block with three irrigation level blocks: Full irrigation (IR100), deficit irrigation (IR50), and no irrigation (IR0). The drip irrigation system supplied the amount of water required according to the crop's daily water demand. The experiment design was two ways factorial design with three levels of PGPR – Phylazonit inoculation and three levels of irrigation (Figures 1, 2, and 3). In both growing seasons 2015 and 2016, we used the hybrid processing tomato: Uno Rosso F1 (United Genetics Seeds Co. Hollister, CA, USA) for our plant materials. Seedling raising have been carried out in plastic trays (each seed each hole) in the greenhouse on the 15th of April using the Klasmann TS3 substrate (content white sphagnum peat 80%; frozen black sphagnum-peat 20%) for one month. Seedlings were transplanted into the field on the 17th of May in both years.

Figure 1. Experimental farm design and location of growing season 2015

Figure 2. Experimental farm design and location of growing season 2016

The tomato plants grew in tree blocks with four replications per treatment. Seedlings were arranged in double (twin) rows with a distance of 1.6 m between the bed center and 0.4 m between the twin rows and 0.2 m between the plants (Figure 3).

Experiment 1 in 2015: The experiment had done on the old farm of the Horticulture Institute at Szent István University (SIU), Gödöllö, Hungary (47.593609N, 19.354630E). The experimental field was on brown forest soil composed of sand, a sandy-clay mixture of sandy loam, in a texture consisting of 69% sand, 22% silt, and 9% clay, 1.57 g cm-3 bulk density, 19% field capacity, neutral in pH, free from salinity (0.16 dS m-1) and low in organic carbon, NO-3 N (5 g kg-1), P2O5 (15 g kg-1), K2O (35 g kg-1).

Experiment 2 in 2016: The experiment was carried out on the new farm of the Horticulture Institute at Szent István University (SIU), Szárítópuszta, Gödöllö, Hungary (47.577131N, 19.379739E). The soil was brown forest soil, loamy in texture (41% sand, 47.5 silt, and 11.5% clay) with a bulk density of 1.49g/cm3, 25% field capacity, free from salinity (0.212 dS m-1) and low in organic matter, containing NO-3 N (8.6 g kg-1), P2O5 (8 g kg-1), K2O (56.7 g kg-1).

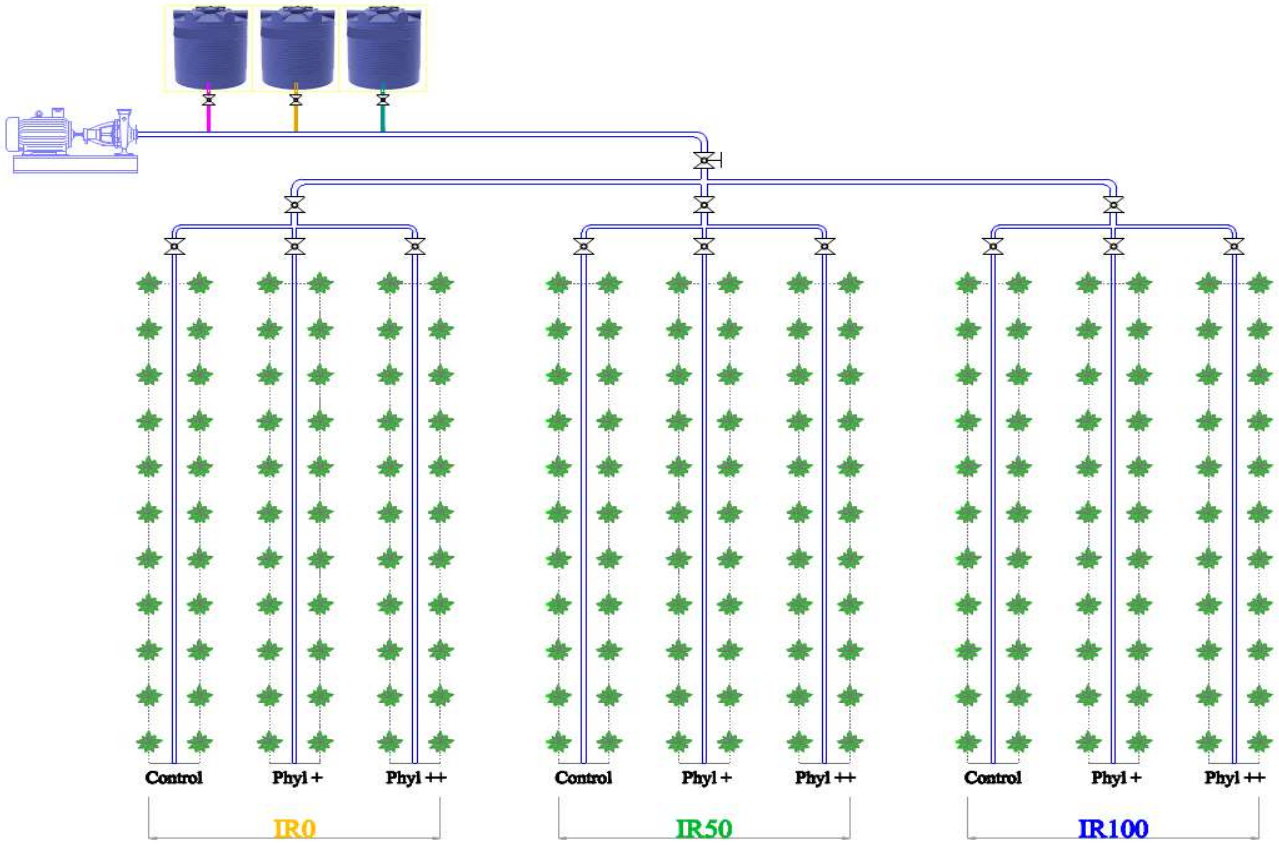

Figure 3. The diagram of Phylazonit inoculation and irrigation control in two years' experiment.

3.2. PGPR material and treatments

The commercial Phylazonit produced in Hungary (https://phylazonit.hu/) have been used in two years experiment. According to the manufacturer, Phylazonit is a mixture of Pseudomonas putida, Azotobacter chroococcum, Bacillus circulans, Bacillus megaterium produced by Phylazonit Ltd. Seedlings were inoculated with 1% Phylazonit MC® (Phyl+) or not (control). One-half of the inoculated seedlings has been inoculation again by adding 1% of the solution to the drip irrigation system (Phyl++). PGPR Phylazonit MC® contained living bacteria as follows: Bacillus megatherium var. Phosphaticum, as phosphorus mobilizes bacteria in *a concentration* of 1-2 x 108 cm-3, and Azotobacter chroococcum as free-living N2 fixing bacteria, in *a* concentration of 1-2 x 109 cm3.

Figure 4. Commercial Phylazonit product.
(*https://phylazonit.hu/termek/phylazonit-organic-tk/)*

3.3. Metrological data.

Temperature and precipitation forecasts were obtained from the National Metrological Institute (http://www.met.hu/en/idojaras). We used it to determine the daily water demand from plants according to the daily average air temperature and rain-fall. In the growing season 2015, we found a good distribution of the precipitations, and four heavy rains happened. In opposite to the 2015 growing season, tomatoes plants experienced more heavy precipitations and cooler temperature during 2016.

3.4. Irrigation supply.

There were two different irrigation regimes (IR), the calculation of the air temperature based on the weather forecast data from the National Metrological Institute.
According to Pék and co-workers (2014), the optimum irrigation supply (IR100) was estimated from *the* expected daily average temperature (in °C) divided by five expressed in *millimeters* (Equation 1); deficit irrigation (IR50) supply calculated by haft of optimum irrigation. Tomato plants were given *a* water supply three times a week through *a* drip system (Figure 3).

Equation 1: Optimum water supply (IR100)

$$Io = \left(\frac{Tmax + Tmin}{2}\right)/5$$

Io: optimum water supply (mm).

T max: daily maximum temperature (°C)

T min: daily minimum temperature (°C)

In 2015, the field got 186.3 mm of water due to the rainfall. For this reason, the control block (IR0) - no irrigation block – received only 186.3mm of water supply from rain. The deficit irrigation block (IR50) had got 50% of the required water demand, and a total of 316.3 mm, which included the rainfall, and *the* optimum irrigation supply (IR100) received a total of 436.3mm with the rain amounts (Figure 5).

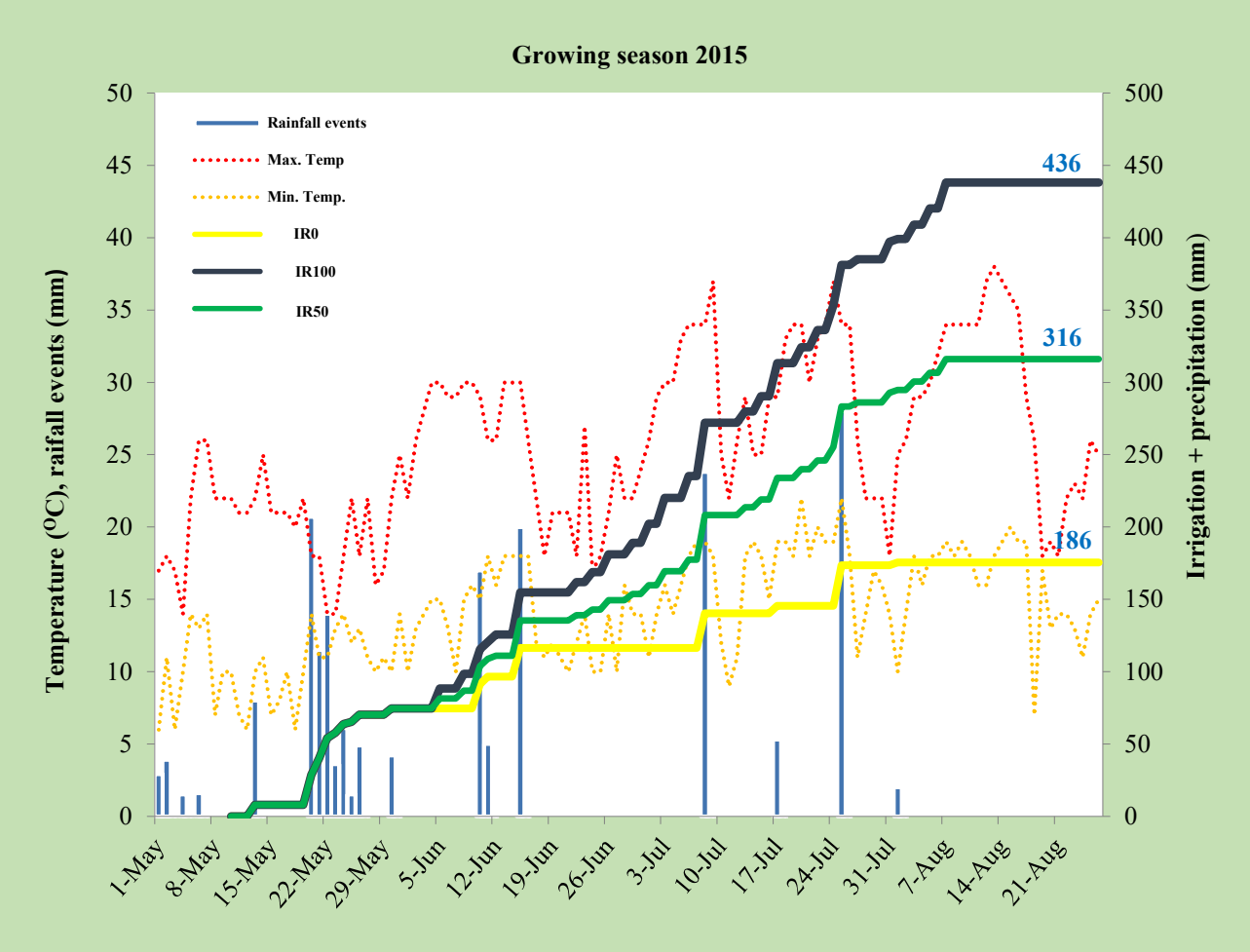

Figure 5. Meteorological and irrigation data during tomato vegetation period in 2015.

In the growing season of 2016, we have done the same procedure for the irrigation regulation. The amount of water was determined based on the air temperature and implemented through the drip irrigation system. We applied three levels of water supply in the experiment: no irrigation block (IR0) that received 296mm of rain; deficit irrigation (IR50) block got 50% of the calculated water demand with a total of 388mm that included the rainfall; and optimal irrigation (IR100) block got a total of 480mm including the precipitation (Figure 6).

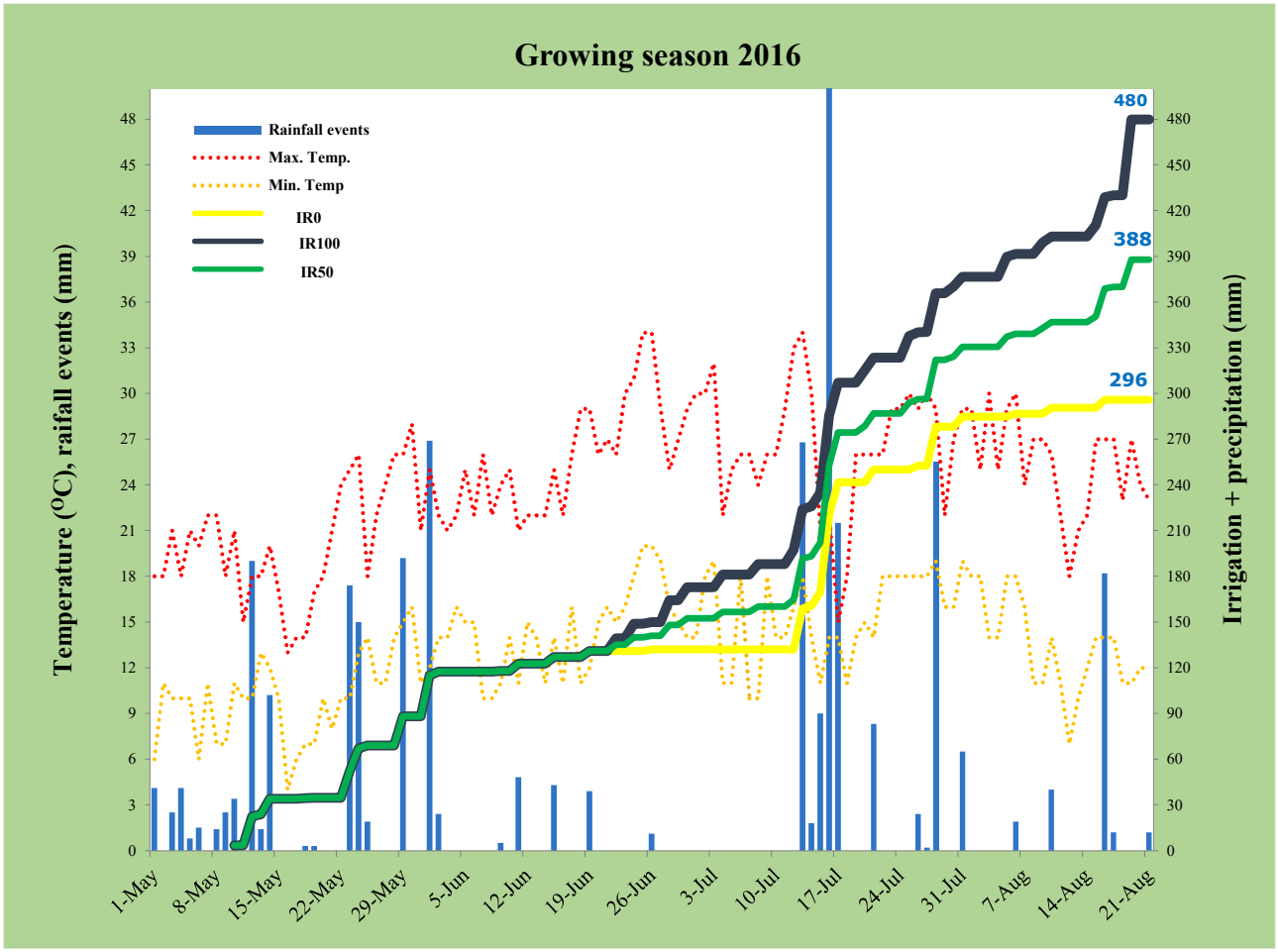

Figure 6. Meteorological data during tomato vegetation in 2016 from May to August.

3.5. Plant nutrition

During a 2 -years experiment, the plants' fertilization demand and plant protection were conducted following Helyes and Varga (1994). Every week, 5 grams of Ferticare (14-11-25) per square meter have been added to the cultivated area through the drip irrigation system to provide plant nutrition. Ferticare 14-11-25 is a complex granulate chlorine-free fertilizer manufactured by YARA Company. Ferticare fertilizer was added 7 times for each growing season. Table 3 shows the number and amount of nutrients per plant, doses, or calculation (Bakr, 2018.) The total amount of different macronutrients per square meter *follows*: 791 mg of total N, 616 mg of P2O5, 1407 mg of K2O, 238 mg of SO3-, and 126 of MgO and six different micronutrients: 5642 µg of Fe, 5642 µg of Mn, 1127 µg of B, 564 µg of Cu, 564 µg of Zn, and 112 µg of Mo through the drip irrigation system. We applied such a nutrition program to *fulfill* the fertilizer demand of the crop. In 2015, we added 25 grams of NovaTech® premium 15+3+20 (+3+10) before transplanting the field. NovaTech® manufactured by COMPO EXPERT from Germany, it is supported with DMPP

(3,4–dimethylpyrazolphosphate) that reduces the N-leaching and nitrification, *the* active phase of DMPP to 4-10 weeks (depending on temperature and humidity of soil), and the transformation of ammonium to nitrate.(http://agro.house/en-GB/Products/Details/1192)

Table 3 Amounts of macro and micro – nutrients (µg plant^{-1}) provided during the experiment.

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

Source: Bakr, 2018

In 2015, we also added 25 grams of NovaTech® premium 15+3+20 (+3+10) previously before transplanting the field. NovaTech® manufactured by COMPO EXPERT from Germany; it supported with DMPP (3,4–dimethylpyrazolphosphate), which can reduces N-leaching and nitrification, active phase of DMPP about 4 to 10 weeks, (depending on temperature and humidity of soil) the transformation of ammonium to nitrate.(http://agro.house/en-GB/Products/Details/1192)

3.6. Harvesting

In 2015, harvest was done two weeks earlier in the no irrigation block (IR0-block); because the tomato plants faced severe water deficit stress; and *therefore,* shortened their growth period. The first harvest *of* IR0-tomato plants *was* on *11th August* and then *on* 25th August by IR50 and IR100. In the 2016 season, unlike the previous season, we harvested all the samples after 100 days of growing.

Ten tomato plants for each replicate were cut off at the soil surface. *Their* total fresh weight of them *was* measured immediately. Then, the fruits were removed from the shoots, *and* the shoot weight of each sample from all replicates and treatments was recorded. The fruits *were* classified into marketable, green, and rotten fruits*,* and recorded the weight and number of total, marketable, and rotten fruits.

3.7. Field measurements

Field measurements depended on the weather conditions. In a 2-year growing, all the measurements were taken *through* the same process. However, for the first crop, the period of water stress was longer, and the measurements were taken *more* frequently than in the second crop.

- ✓ Soil water content was measured continuously during the growth period and before irrigation.
- ✓ Stomata conductance was recorded during the fruit setting on *the* 19th, 26th of June, and 13th of July in 2015, and on *the* 13th of July in 2016. Four readings per plant and four plants in each subplot with 4 replications in each treatment (4 leaves * 4 plants * 4 replications).
- ✓ Single-Photon Avalanche Diode (SPAD) readings were taken once *on the* 3rd of July in 2015, and *the* 13th of July in 2016. Four readings per plant and four plants in each subplot with 4 replications in each treatment (4 leaves * 4 plants * 4 replication).
- ✓ Chlorophyll fluorescence measurements were done every week on *the* 19th, 26th of *June* 3rd, 30th of July, and 6th of August in 2015, and *the* 29th of June; 6th*,* and 13th of July in 2016. One reading per plant for one plant in each subplot with 4 replications in each treatment. (1 leaf * 1 plant * 4 replications).
- ✓ Leaf water potential (ψL) *was* evaluated in 3 consecutive weeks in 2015 (on *the* 19th, 26th of June, and 3rd of July), and once (on *the* 13th of July) in 2016. One reading per plant and four plants in each subplot with 4 replications in each treatment (1 leaf * 4 plants * 4 replications).

- ✓ Canopy temperature was measured every week on *the* 12th, 19th, 26th of June 3rd, 30th of July, and 6th of August in 2015, and *the* 22nd, 29th of June, 6th, and 13th of July. Four readings per plant and four plants in each subplot with 4 replications in each treatment (4 leaves * 4 plants * 4 replications)

3.7.1. Soil water content

We measured the soil water content by *a* digital soil moisture meter PT1 (Kapacitiv Kft., Budapest, Hungary) (Figure 7) at six different soil depths (5, 10, 15, 20, 25, and 30 cm) before watering. Soil water content *was* taken continuously during the plant development and prior to *irrigation.*

Figure 7. Digital soil moisture meter PT1

3.7.2. Stomatal conductance

The water loss from the plant's leaves was detected using a porometer Delta-T, type AP4 from the UK. The equipment can measure the stomata conductance or stomata resistance account to the diffusion conductance. It compares the humidification within the chamber to readings from the calibration plate. (Figure 8).

Figure 8. Porometer Delta-T, type AP4.

3.7.3. Relative chlorophyll index

The relative chlorophyll index of the tomato leaves was measured by "Konica Minolta SPAD-502" (Figure 9); it is a rapid and non-destructive method to determine chlorophyll content in the field. A significant correlation between chlorophyll content and Single-Photon Avalanche Diode (SPAD), so SPAD values could be used for Nitrogen content in leaves (Martínez et al., 2015). The SPAD meter evaluates the difference between the transmittance of a red (650 nm) and an infrared (940 nm) light through the leaf, generating a three-digit SPAD value (Uddling et al., 2007).

Figure 9. Single – Photon Avalanche Diode (SPAD) device.

3.7.4. Chlorophyll fluorescence.

The chlorophyll fluorescence of the plants was determined by the PAM 2500 portable fluorimeter (Walz-Mess und Regeltechnik, Germany) (Figure 10). During the development stage, the measurement had done at midday on fully developed top leaves of a single plant in each replicate. The leaf clips were placed on the leaves for 35 minutes for dark adaption before performing measurements. The maximum photochemical quantum yield of PSII was characterized by the Fv/Fm ratio using the fast kinetics method in the Pam Win 3.0 software (Van Goethem et al. 2013).

Figure 10. PAM 2500 device. (*Source: Internet)*

3.7.5. Leaf water potential (ψ leaf)

Leaf water potential (ψ leaf) was measured by a pressure bomb (PMS Instruments Co., Corvallis, OR USA) (Figure 11). Four replications per treatment were sampled in both years, all samples were selected in newly matured leaves at midday for three consecutive weeks (Gonzalez, 2001).

Figure 11. Pressure bomb device.

3.7.6. Canopy surface temperature measurement (°C)

Canopy surface temperature was determined by the infrared thermometer technique (Raytek Raynger MX4, Santa Cruz, CA, USA) (Figure 12) which allows faster and more precise measurement without touching the plant (Bőcs et al. 2009). The device can read from -30 to 9000C with accurate (± 1% in reading) (https://www.farnell.com/datasheets/44260.pdf). The measurements have done weekly 6 times in 2015 and 4 times in 2016.

Figure 12. Infrared thermometer device. (*Source: Internet)*

3.7.7. Water use efficiency.

Water use efficiency (WUE, kg m-3) was calculated as the ratio of marketable yield on a fresh weight basis at harvest (FW, t ha-1) and total water used (ET, m3 ha-1), as measured by water balance as it is shown in Equation 2.

Equation 2: Water Use Efficiency (WUE)

WUE=(Total Yield (ton per hectare)/(Total water *consumed* (cubic per hectare)

$$WUE = \frac{Total\ Yield\ \ (ton\ per\ hectare)}{Total\ water\ cosumed\ (cubic\ per\ hectare)}$$

3.8. Laboratorial measurements

3.8.1. Proline

The determination of proline was conducted as described by Bates et al., 1973. Proline concentration was determined according to the acid-ninhydrin method - mature leaves (at the fruit setting stage) were chosen from 4 marked plants in one subplot and four subplots per treatment. Proline content was measured during the water stress period on 3rd July 2015 and 15th July 2016. A 0.5g of the leaf (a mixture of 4 leaves in each subplot) was ground in a mortal with the presence of a small spoon of quartz sand and 5 ml of 3% *sulfosalicylic* acid. The crude extract was centrifuged at 3000 rpm for 10 min, and the supernatant was added 2ml of acid-ninhydrin, 2ml of ortho-phosphoric acid (6 Molar), and 2ml of glacial acetic acid. After that, it was incubated for one hour in the underwater bath at 1000C. Incubated tubes were left for 5min at room temperature to finish the reaction. Spectrophotometer (Shimadzu U-2900, Tokyo, Japan) was used to record the absorbance of the extracts at 520nm. Proline concentration in the extracts was estimated using the calibration curve for proline standards (Figure 13) based on the fresh weight (Claussen, 2005).

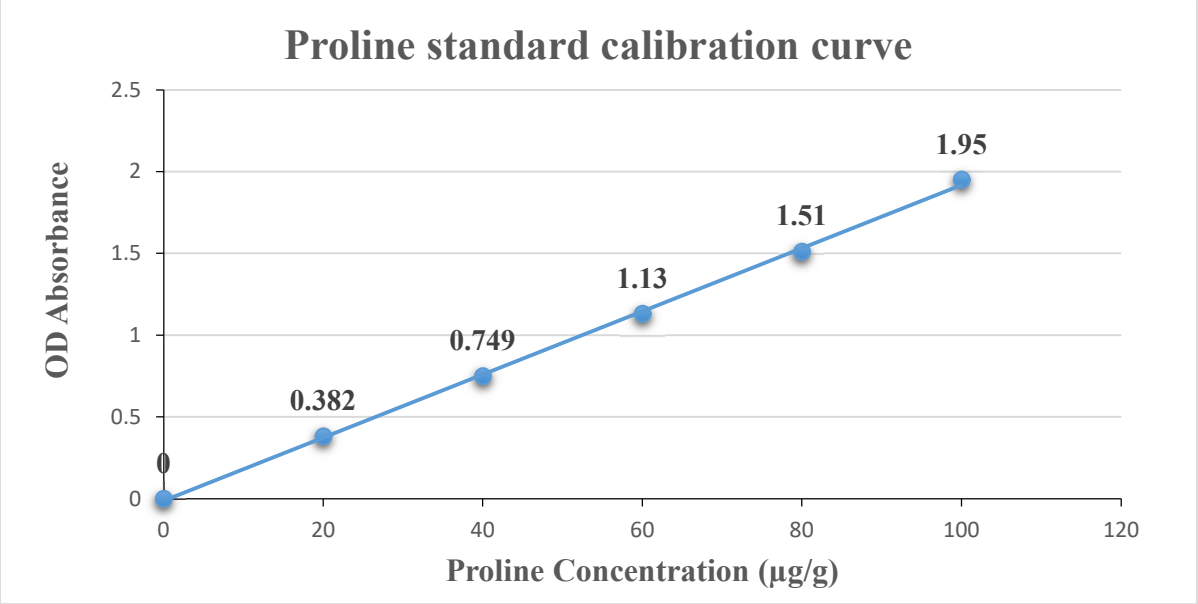

Figure 13. Standardization of proline curve.

3.8.2. Soil microbial activity

Total microbial activity was measured according to the fluorescein diacetate hydrolysis (FDA) method (Green, Stott, and Diack 2006). It is an accurate and simple method to estimate soil microorganisms. The protocols to assess fluorescein diacetate hydrolysis activity was reported by Adam and Duncan (2001).

3.8.3. Root colonization determination

We selected four plants randomly from subplots in the same treatment with soil core 25x25x25 cm and dug them out for investigation of root colonization. Then, the representative subsample of the roots regarding different treatments was cut into 10 mm pieces, and five randomly chosen pieces in each sample were stained by Trypan Blue. The stereomicroscope at x100 magnification was used to estimate the internal fungal structure and the percentage of root length colonized was determined by the gridline intersect method (Phillips and Hayman, 1970; Giovannetti and Mosse, 1980).

3.8.4. Analysis of carotenoid components and vitamin C.

Extraction of carotenoids

Carotenoid extraction was done according to the method of Daood et al (2013). Five grams of well-homogenized tomato has been taken in triplicate followed by disintegration in a crucible mortar in the presence of quartz sand. The water was then removed by adding 25ml of methanol along with the repeat disintegration of the aggregating bulk. After the addition of 60 ml of a 1:5 Methanol: dichloroethane solution, the mixture was transferred quantitatively into a 100 ml conical flask. The mixture was shaken up for 15 min by a mechanical shaker. A few drops of distilled water were added to separate the two phases. The pigment-containing lower layer was separated in a separating funnel, dried over anhydrous sodium *sulphate*, and passed into a round-bottom flask. The organic solvent was evaporated with vacuum by rotary evaporator (IKA® RV10, Sigma-Aldrich Ltd., Budapest, Hungary) at a maximum of 40C and the residues were re-dissolved in 5ml of HPLC grade acetone.

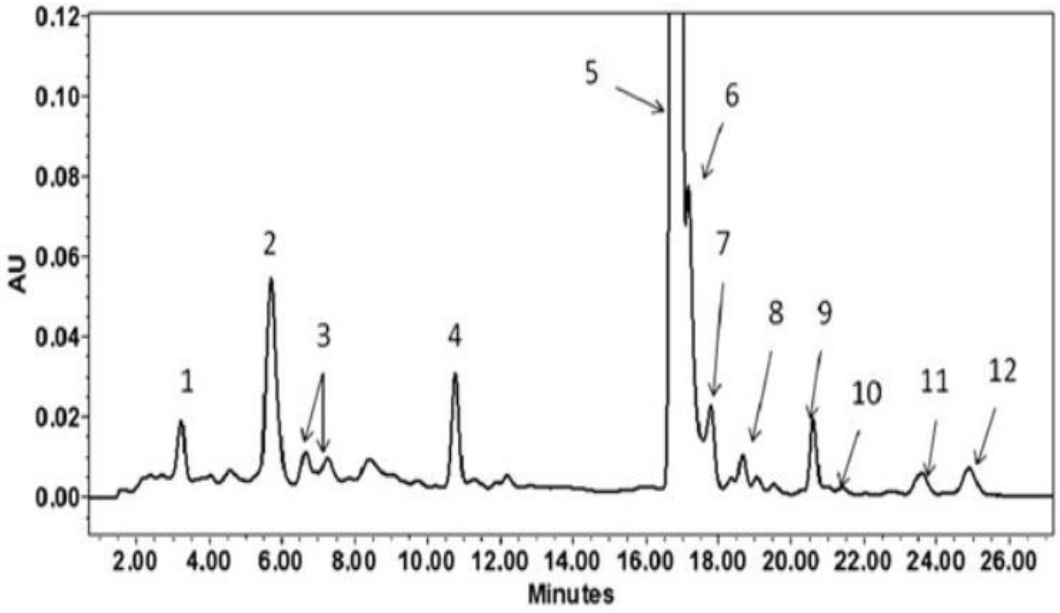

Figure 3. HPLC profile of tomato carotenoids separation on cross-linked ISIS column using gradient elution of water in acetone: 1: neoxanthin, 2: zeaxanthin, 3: lycopene epoxide, 4: lycoxanthin, 5: lycopene, 6: 9-cis-lycopene, 7: tetra-dehydrocarotenoid, 8: 13-cis-lycopene, 9: β-carotene, 10: cis- β -carotene, 11: antheraxanthin di-ester, 12: Lycophyll di-ester. (Source: H.G. Daood, 2013)

Extraction of ascorbic acid.

Ascorbic acid was extracted from 5 grams of well-homogenized tomato by crushing it in a crucible mortar and shaking it for 15 min with a 3% metaphosphoric acid solution. The mixture

was filtered through filter paper and purified by a 45-um nylon syringe filter before injection into the HPLC column.

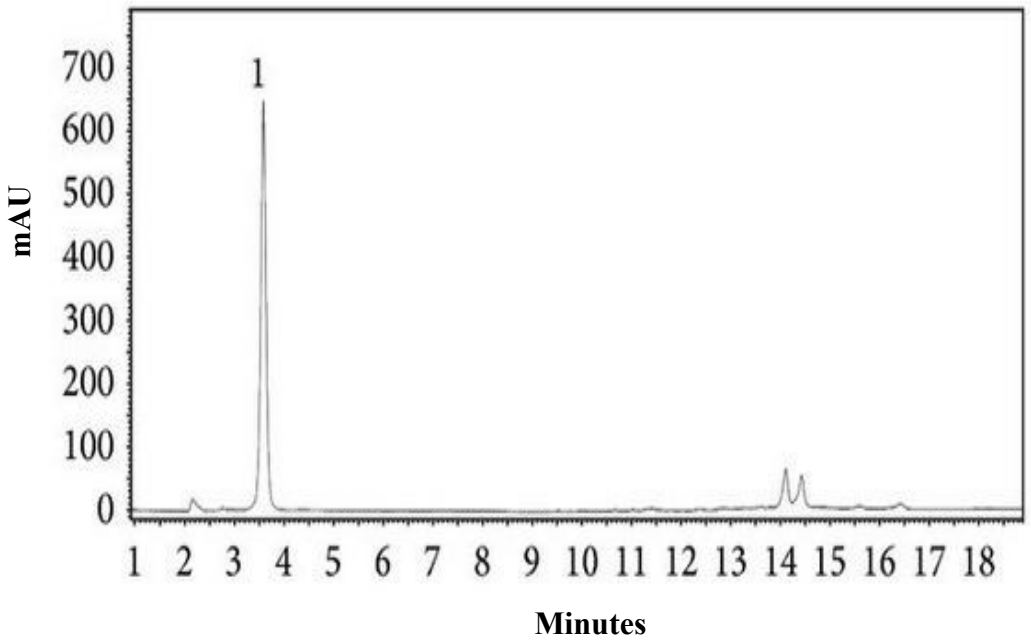

Figure 4. HPLC separation of Ascobic Acid on Nucleosil C18 aqua (Nautilus) column.

HPLC equipment and conditions.

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

The separation of carotenoids was done on cross-linked C-18, 3 µm, 150x4.6 mm column using a gradient elution of water in acetone as described by Daood et al., (2013). The column effluents were detected at their maximum absorption wavelength for identification and quantification. The retention properties and spectral characteristics of the detected peaks were compared with some available standard materials like lycopene, and β-carotene equivalent based on their spectral characteristics.

As for ascorbic acid, separation was performed on C-18, 240 x 4.6mm, 5 um column under ion-pair chromatographic conditions optimized and validated by Daood et al., (1994). Ascorbic acid was identified using standard material (Sigma-Alrrich, Budapest), from which stock and working solutions were prepared for getting the calibration curve.

Figure 5. HPLC separation devices.

3.8.5. Soluble solid content estimation.

The soluble solid content was determined by the Digital Refractometer Krüss DR 201-95 (Krüss Optronic, Hamburg, Germany), the values are reported as a percentage.

Figure 6. Digital refractometer devices.

3.9. Statistical analysis

Analysis of variances was conducted in two ways ANOVA, the software IBM SPSS Statistics for Windows, Version 22.0. (IBM Hungary, Budapest, Hungary) was used to run statistical analyses. The main effects were: Phylazonit inoculation (referred to as Phyl), Phyl with three levels (Control, Phyl+, Phyl++), and Irrigation supply (herein referred to as IR) with three variants (IR0, IR50, and IR100).

As a prerequisite for the statistical test, the assessment of the normality of the data was done by the Shapiro test. Due to our equal variances across groups, the Levene test was conducted to verify the homogeneity assumption.

The means of four replications were separated by the least significant difference (LSD, $P \leq 0.05$). In case of significant interaction between Phyl and IR, Tukey's HSD *post hoc* test was performed to determine significant differences among the treatments. Before data analysis percentage values for root colonization were arc-sine [square-root (X)] transformed. Pearson correlation coefficient is used to assess the direction and the strength of the linear relationships between (0Brix content, and marketable yield), (Soluble solid yield, and marketable yield), (proline content, and leaf water potential), (Canopy temperature, and Stomatal conductance)

4. RESULTS AND DISCUSSION

Our results had recorded in non-inoculated (Control), pre-transplant inoculation at sowing (Phyl+), and field inoculation at transplant (Phyl++), but only non-inoculated (Control), and field-inoculated at transplant (Phyl++) will be present in our work here because of the results in pre-transplant inoculation at sowing (Phyl+) (Appendices: Appendix 1 & 2: total biomass, WUE, SPAD, proline, canopy temperature. Appendices 4: the relationship between marketable yield and Brix°. Appendices 5 & 6: total carotenoid; lycopene, □-carotene, and Ascorbic acid did not give promising results compared to non-inoculated (Control) in two years.

Human activity (industry, transport, agriculture) has contributed to an increase in greenhouse gases emission which is the main cause of changes in climate conditions such as increasing temperature and reduction in precipitation in some areas, a rise in floods, and prevalence of hurricanes in other areas (Giannakoula and Ilias 2013; Garofalo and Rinaldi 2015; Peña-Gallardo et al. 2019.). PGPR (plant growth-promoting rhizobacteria) have informed by numerous authors on the positively affected plant tolerance to drought stress and plant-associated microorganism (Kloepper and Schroth 1981; Compant et al. 2010; Bhattacharyya and Jha 2012; Zivcak et al. 2016). In our two years field experiments, PGPR inoculation at transplant enhanced yield, growth, and water use efficiency under both deficit irrigation and full irrigation levels compared to control (non-inoculation) and pre-transplant at sowing (Appendices 1; 2; 3; 4; 5; 6) which is in agreement with previous studies of processing tomatoes (Helyes et al., 2012; Bakr et al., 2017 b).

In the case of the first experiment (season 2015), the farm has been used for many years for field studies. In opposite, in the case of the second experiment (season 2016) the field was left fallow for several years. There were differences in texture, field capacity, and water-holding capacity. The first experimental farm got lower holding water capacity and exhaust of nutrients, especially the microelements. Therefore, we have provided the first farm NovaTec® fertilizer (25 grams in each square meter) at the beginning of transplanting. Thus, plants received these elements: 605 mg of total N, 403 mg of P2O5, 806 mg of K2O, 323 mg of SO3-, and 97 mg of Mg O. In addition to three different micronutrients: 242 µg of Fe, 81 µg of B, and 40 µg of Zn.

4.1. Effect of irrigation on the water stress induction and soil water content

In the first eight weeks of seedling, tomato plants received the optimum with water supply in 2015. Different irrigation treatments started in the first week of June. A precipitation of 186.3 mm was measured, which did not cover the crop demand. The control block (IR0) got 186.3mm of water during the vegetative development. For this reason, the control tomato plants block (IR0) got stress by drought during the growing season. In the optimum (IR100) and deficit (IR50) irrigation treatments , the plants received 436.3 and 316.3 mm of water, respectively, including precipitation. The soil had water content range between 0.14-0.17, 0.11-0.14 and 0.07 – 0.10, corresponded to 73-89%, 58-73%, and 37-52% of field capacity in IR100, IR50 and IR0 blocks respectively. In the last three weeks, the average temperature was high (Figure 5), and it paired with low precipitation, which caused drought for processing tomato in 2015, which is usual in Hungary (Figure 18).

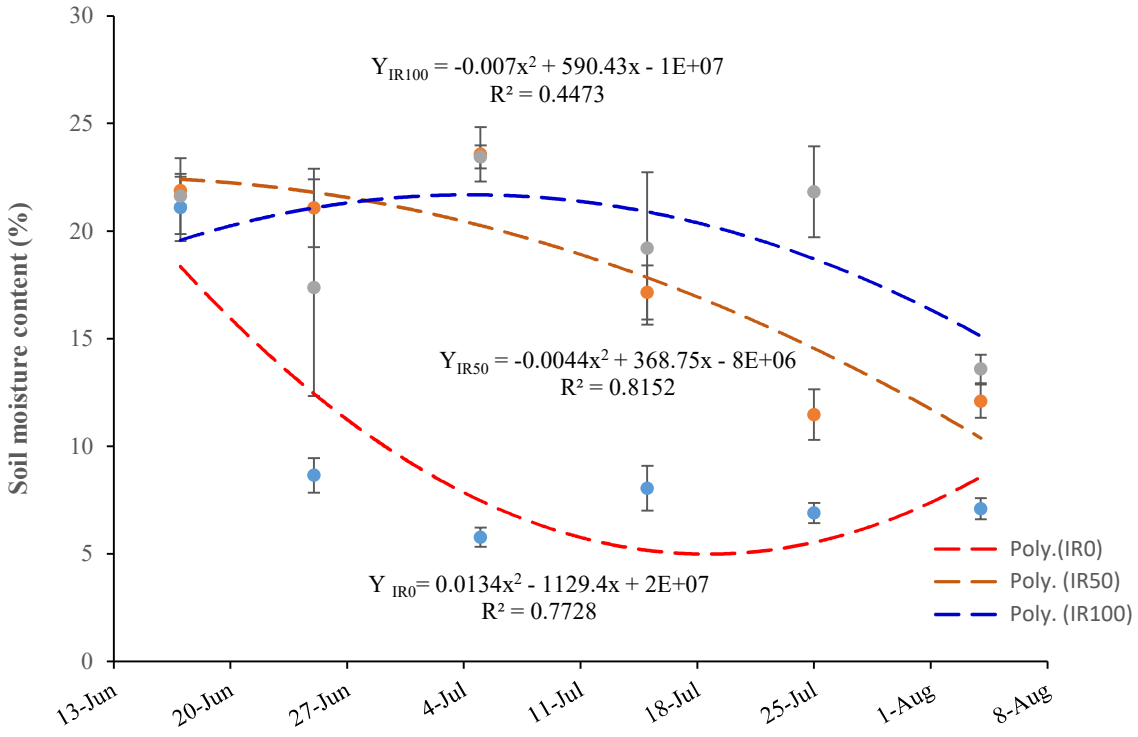

Figure 18: Soil moisture content during growing season 2015

In the second growing season of 2016 differed significantly from 2015, we started the irrigation after 5 weeks from transplanting because of the temporal distribution of precipitation. There was heavy rain in the middle of July and throughout the crop season, so the total precipitation amount

was 296 mm for plants in the rain-fed control. In the optimal (IR100) and deficit (IR50) irrigation, the plants received 480 mm and 388 mm of irrigation water, respectively. These values included 296 mm of rain (Figure 6). With the calculation of the volumetric water content, the field capacity ranged between 84-108%, 60 -76 %, and 52 – 68% corresponding to 21-27, 15- 19, and 13 – 17 % of volumetric water content in IR100, IR50, and IR0 blocks respectively. *The farm's* soil *was* loamy in texture (consisting of 41% sand, 47% silt, and 11.5% clay) with *a* bulk density of 1.49 g.cm3, and 25% of field capacity (Bakr, 2018). Throughout the growing season of 2016, there was a high level of precipitation *combined* with the high-water holding capacity of *the* soil, therefor the plants did not face the water stress in the control or deficit water supply (Figure 19).

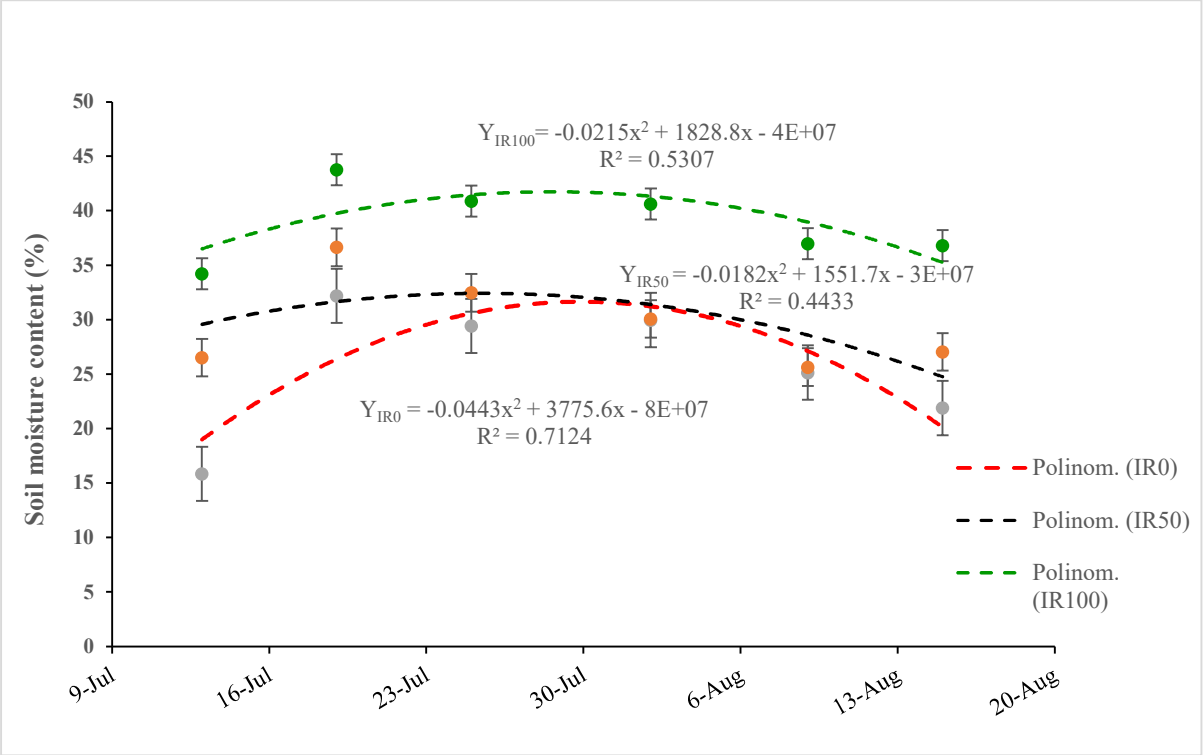

Figure 19: Soil moisture content during growing season 2015

4.2. Effect of irrigation on the root microbial activity

Some PGPR *is* free-living bacteria *that* occupy the tissues of living plants and cause unapparent and symptomatic infections. The bacteria play an important role in enhancing plant growth under stress and non-stress conditions (Nadeem et al. 2014; Zahir et al. 2004; Glick et al. 2007). Mechanisms of plant growth promotion include nitrogen fixation, phosphorus *solubilization*, siderophores production, plant grown regulators. (Berg 2009; Nadeem 2014). The dominant bacteria of PGPR are Pseudomonas, Enterobacter, Bacillus, Variovorax, Klebsiella, Burkholderia, Azospirillum, Serratia, and Azotobacter (Kaymak 2010; Nadeem, et al 2014). The colonization of roots by inoculated bacteria is an important step in the interaction between beneficial bacteria and the host plant.

We collected the samples randomly from above 20cm of the field experiments soils by using the wet sieving technique. In the first season, we estimated 3 to 4 spores in each gram soil and 4 to 5 spores in the second season. According to Maguro et al., 2015 and Bakr, 2018, the natural occurrence in agricultural soils in Hungary dominates by the Glomeracea family. Research work by Smith and Read (2008); Bakr (2018) showed that although the number of spores of root *bacteria* present in soil *was* not high, it plays an important role in newly cultivated *crops* even *though* the soil was left bore for several years.

PGPR root colonization found at harvest was similar to the colonization rate in Mycorrhizal inoculation (Bakr, 2018) or PGPR treatment (Cortivo et al. 2018). PGPR treatment slightly enhanced root colonization in inoculated plants even without irrigation treatment. Despite slightly higher colonization from Phyl++ plants from 65.2 to 68.3% in un-irrigation (IR0) to full irrigation (IR100) in 2015 and the highest colonization percent recorded in deficit irrigation (IR50) at 70.5% in 2016, however, there *was* no significant difference in the effect of PGPR combined with irrigation control (Table 4) in PGPR treatments. It may be related to the *increase* of efficient microbes, which displace the root–colonizing microorganism (Kloepper and Schroth, 1981). The importance of displaced microorganism is to percolate the water, increase the water uptake by the roots, or increases the water uptake by the whole plant (Lawrence et al. 1987).

In the first growing season, fluorescein diacetate (FDA) hydrolysis showed higher microbial activity in soil with no irrigation block. The deficit and optimal irrigation levels had no significant effect on fluorescein diacetate (FDA) hydrolysis although it had a slight increase. The increased microbial activity in deficit irrigation block (Table 4) may be related to the positive interaction between the PGPR and Mycorrhizal microorganisms (Bardi and Malusá, 2012; Malusá and

Ciesielska. 2015). Unlikely, in the 2016 season, the PGPR-Phyl++ inoculation did not enhance microbial activity in the root system (Table 4); the only explanation is a higher level of precipitation compared to results in the 2015 growing season.

Table 4. Effect of Irrigation level and PGPR application on Root colonization level (%) of processing tomato and soil microbial activity.

Water supply	Treatments	R. Col. (%)		FDA (µm p-nitrophenol g^{-1} hr^{-1})	
		2015	2016	2015	2016
IR0	Control	$54.5^{Aa} \pm 6$	$47.0^{Aa} \pm 18$	$0.78^{Ba} \pm 0.11$	$1.25^{Aa} \pm 0.2$
	Phyl ++	$65.2^{Aa} \pm 7$	$65.7^{Aa} \pm 14$	$0.79^{Ba} \pm 0.13$	$1.2^{Aa} \pm 0.3$
IR50	Control	$49.0^{Aa} \pm 3$	$53.0^{Aa} \pm 13$	$0.70^{Ba} \pm 0.07$	$1.14^{Aa} \pm 0.1$
	Phyl ++	$68.5^{Ba} \pm 9$	$70.5^{Ba} \pm 9$	$0.62^{Aa} \pm 0.03$	$1.1^{Aa} \pm 0.3$
IR100	Control	$54.8^{Aa} \pm 6$	$49.3^{Aa} \pm 8$	$0.62^{Aa} \pm 0.08$	$1.12^{Aa} \pm 0.2$
	Phyl ++	$68.3^{Ba} \pm 9$	$68.8^{Ba} \pm 8$	$0.62^{Aa} \pm 0.03$	$1.2^{Aa} \pm 0.3$
Phylazonit treatments (Phyl++)		**	**	**	ns
Irrigation levels (IR)		ns	ns	*	ns
(Phyl++) * (IR)		ns	ns	ns	ns

*Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001). Capital letters represent Phylazonit inoculation, small letters represent Irrigation levels effect.*

4.3. Effect of Phylazonit inoculation on physiological responses of tomato plants

4.3.1. Maximum photochemical yield and relative chlorophyll index

In 2015, the maximum photochemical yield (Fv/Fm) was recorded higher in the plants which received water supplied (IR50 and IR100) blocks than in non-inoculated or no water supply blocks (IR0). The maximum photochemical yield was the lowest value (0.69) in control plants with no irrigation (IR0), which means that during this stage of development, the plants were under heat stress or without water. However, Phyl++ inoculated treatment increased the photosynthetic efficiency at all irrigation levels (Table 5), improving plant growth and reducing *the* damage of photosynthetic apparatus in Phyl++ plants under drought stress (Delfine et al. 2000; Baker and Rosenqvist 2004; Thankappan et al. 2019). In the growing season of *2016, the* maximum efficiency (Fv/Fm) values of PSII *were* higher in deficit irrigation plants (IR50), and there were no *significant* differen*ces* from other treatments, which means no photo-oxidative damage neither in *fully* irrigated nor in unirrigated plants (Table 5).

SPAD stands for Soil-Plant Analysis Development; SPAD value correlates with leaves' chlorophyll content. The high SPAD reading value *revealed* the low water and chlorophyll concentration simultaneously in the leaf (Wood et al., 1973; Nemeskéri and Helyes. 2019). In our experiment, SPAD reading reached a higher value in the Phyl++ inoculation samples than that in the control samples under no irrigation (IR0) and deficit irrigation (IR50) in 2015 (Table 5). These results are supported by Puangbut et al. (2017) and Adriano et al. (2018), *who* found higher chlorophyll content in inoculated plants under *drought-stress* conditions. The higher chlorophyll content is accompanied by photosynthetic efficiency improvement. Unlike in the growing season 2015, Phyl++ inoculation has been found not to affect the leaf chlorophyll content or SPAD values in *the* growing season 2016 (Table 5) in irrigation treatments (IR50 and IR100). This result is supported by the research on Arbuscular Mycorrhizal (AM) fungus inoculation in processing tomatoes (Bakr et al., 2018). Under the same conditions, AM fungus inoculation plants had no effect on chlorophyll content and SPAD value. It was approved that in two blocks (deficit and optimal water supplies) the irrigation treatments did not cause drought stress to the tomato plants grown in 2016.

Table 5. Effect of field Phylazonit inoculation and tree irrigation levels on maximum efficiency of PSII and SPAD of processing tomato leaves.

Irrigation levels	Treatments	Maximum efficiency of PSII (Fv/Fm)		SPAD	
		2015	2016	2015	2016
IR0	Control	$0.69^{Aa} \pm 0.08$	$0.75^{Aa} \pm 0.01$	$46.1^{Aa} \pm 0.8$	$53.8^{Ab} \pm 1.9$
	Phyl ++	$0.70^{Aa} \pm 0.04$	$0.76^{Aa} \pm 0.02$	$50.4^{Ba} \pm 2.5$	$54.5^{Ab} \pm 1.4$
IR50	Control	$0.76^{Ab} \pm 0.01$	$0.78^{Aa} \pm 0.01$	$47.7^{Aa} \pm 0.9$	$50.0^{Aa} \pm 1.4$
	Phyl ++	$0.79^{Bb} \pm 0.02$	$0.80^{Bb} \pm 0.02$	$49.5^{Aa} \pm 1.9$	$50.2^{Aa} \pm 2.7$
IR100	Control	$0.76^{Ab} \pm 0.03$	$0.77^{Aa} \pm 0.01$	$48.0^{Aa} \pm 1.7$	$47.1^{Aa} \pm 1.0$
	Phyl ++	$0.78^{Bb} \pm 0.05$	$0.77^{Aa} \pm 0.02$	$47.2^{Aa} \pm 1.5$	$49.2^{Aa} \pm 1.6$
Phylazonit (Phyl++)		**	**	*	ns
Irrigation levels (IR)		***	ns	ns	**
(Phyl++) * (IR)		ns	*	ns	ns

[1]*Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001). Capital letters represent PGPR inoculation; small letters represent Irrigation levels effect.*

4.3.2. Stomatal conductance and canopy temperature

Stomatal conductance is a measure of the degree of stomatal opening. It can be used as an indicator *of* the water status in *the* plant (Gimenez, et al. 2005). According to Ferreira and Katerji (1992), stomatal conductance can be measured on the first leaf above the terminal cluster, which well *characterizes* the water status of the whole plant. Stomatal conductance reflects also the water stress levels in plants caused by water deficits. In the control treatment with no Phyl++ inoculation, the average of stomatal conductance was 10.2 mmol m-2 s-1 in IR0, 18.7 mmol m-2 s-1 for the deficit irrigation (IR50), and 30.6 mmol m-2 s-1 for the *optimal* irrigated treatment. In 2015 and in 2016, the values were 31.1, 31.5, and 30.6 mmol m-2 s-1, respectively (Table 6). The data showed that in 2016 irrigation treatments had no effect on stomatal conductance. The explanation for this is that the heavy seasonal rain exceeded the *plants'* water requirements in some periods of the growing season of 2016.

In our research, we took the stomatal conductance measurements within three consecutive weeks when the daily temperature ranged between 25-300C and the light intensities were higher than 300 μmol m-2 s−1, which avoided any unexpected changes in the porometer

readings. Phyl++ treatments have significantly increased the stomatal conductance at deficit irrigation supply levels (IR50) from 18.7 to 23.9 mmol m-2 s−1 in 2015 and 31.5 to 33.6 mmol m-2 s−1 in 2016 (Table 6). It slightly enhanced the stomatal conductance in all irrigation (IR50 and IR100) treatments compared to the control block (IR0) in the two years of experiments. This result agreed with the research on processing tomatoes with Mycorrhizal treatments from Bakr, 2018 and Böcs et al. 2009.

Table 6. Effect of field Phyl++ inoculation and tree irrigation levels on Stomatal conductance (mmol $m^{-2}s^{-1}$) and Canopy temperature (^0C).

Irrigation levels	Treatments	Stomatal conductance (mmol $m^{-2}s^{-1}$)		Canopy temperature (^0C)	
		2015	2016	2015	2016
IR0	Control	$10.2^{Aa} \pm 1.5$	$31.1^{Aa} \pm 1.2$	$36.7^{Ac} \pm 2.4$	$25.1^{Aa} \pm 1.0$
	Phyl ++	$10.8^{Aa} \pm 1.6$	$30.2^{Aa} \pm 0.7$	$34.9^{Ac} \pm 1.2$	$25.1^{Aa} \pm 0.7$
IR50	Control	$18.7^{Ab} \pm 1.3$	$31.5^{Aa} \pm 1.0$	$31.6^{Ab} \pm 0.9$	$25.4^{Aa} \pm 0.5$
	Phyl ++	$23.9^{Bb} \pm 2.3$	$33.6^{Bb} \pm 0.6$	$30.4^{Bb} \pm 1.0$	$23.9^{Ab} \pm 0.8$
IR100	Control	$30.6^{Ac} \pm 1.8$	$30.6^{Aa} \pm 0.8$	$28.0^{Aa} \pm 0.6$	$25.2^{Aa} \pm 0.6$
	Phyl ++	$32.5^{Ac} \pm 1.2$	$31.0^{Aa} \pm 0.6$	$27.9^{Aa} \pm 0.5$	$25.8^{Aa} \pm 0.5$
Phylazonit (Phyl++)		***	*	**	ns
Irrigation levels (IR)		***	ns	***	ns
(Phyl++) * (IR)		***	ns	**	*

[1]*Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001). Capital letters represent PGPR inoculation; small letters represent Irrigation levels effect.*

Table 6 shows us the significant difference between irrigation treatments and unirrigated control. There was an upward tendency in the range of control, deficit, and fully irrigated plants, the more water supplies they had, the lower the canopy temperature became in the 2015 growing season. In control plants (IR0), the canopy temperature was 36.7 °C, for the deficit irrigated plants (IR50) it was 31.6 °C and for the fully irrigated plants (IR100) it was 28.0 °C. The reason that higher air temperature in 2015 led to higher canopy temperature could be the lack of water in plans. In 2016 the canopy temperature was lower than in 2015 and there were no significant differences between

the irrigation blocks. Phyl++ inoculated plants had a more efficient decrease (from 31.6 to 30.4 ºC) under IR50 (Table 6). The relationship between canopy temperature and stomatal conductance had reversed a trend in both seasons; canopy temperature correlated very strongly and negatively (r = 0.95) with the stomata conductance in the 2015 season and related to stomatal conductance in a weak negative (r = 0.28) relationship with stomatal conductance (Figure 20.) in 2016 growing season. Although under non-irrigated conditions (IR0) Phyl++ inoculation decreased the canopy temperature, which was associated with low stomatal resistance in 2015 under deficit irrigated conditions (IR50) e.g. 30.9ºC canopy temperature in control plants was associated with lower stomatal conductance (18,67 mmol m-2s-1) than the Phyl++ treated plants where the same temperature associated with higher stomatal conductance (24.28 mmol m-2s-1) (Figure 20). Vapor loss and CO2 assimilation were determined by stomatal conductance in plant leaves, and canopy temperature *has* a direct influence on photosynthesis and biomass production. Phylazonit inoculation plants had the same photosynthesis efficiency under deficit irrigation (IR50).

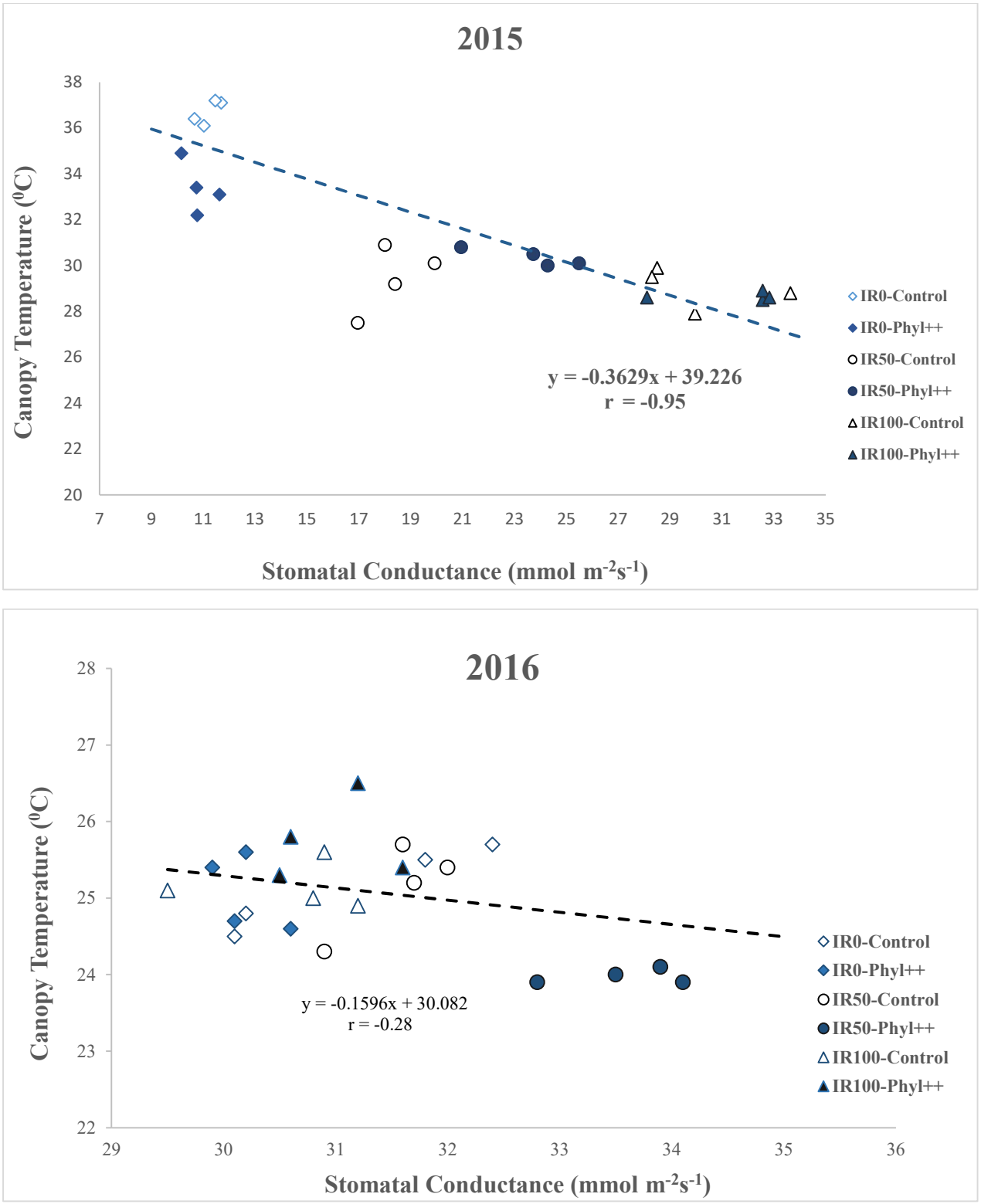

Figure 20. Relationship between stomatal conductance (mmol $m^{-2}s^{-1}$) and canopy temperature (^{0}C) in different irrigation levels and Phylazonit (Phyl++) treatment.

4.3.3. Leaf water potential

Plants manage drought stress by reducing water *vapor* and increasing water uptake, stomatal closure, smaller leaf area (leaf size and/or leaf number), and strengthened root system were strategies to avoid drought stress (Chaves et al. 2003). The leaf water potential (ΨL) is the most important index of water status in plants. It shows the potential to resist drought through water *uptake (*Bakr, 2018; Calcagno et al., 2011; Shinohara et al. 1995). Irrigation had *a* positive effect on the water potential (ΨL), decreased *the* irrigation levels, *and* the value of ΨL too (more negative) in *c*ontrol plant leaves from -0.9 MPa in IR100 to -1.1 MPa in IR50, and -1.6 MPa in IR0 in 2015 and from -1.02 MPa in IR100 to -1.05 MPa in IR50, and -1.12 MPa in IR0 in 2016 (Figure 21). These data also *show* the differences between the two growing years in *plant* water stress due to irrigation induction, when plants received much less water in 2015 and were just moderately stressed in 2016 in the no irrigation regime. Compared to Control plants, phylazonit inoculation (Phyl++) remarkably increased the ΨL in plant leaves by (15, 12, and 02%) in IR0, IR50, and IR100, respectively in the growing season of 2015 and by 06, 09, and 07% in IR0, IR50, and IR100, respectively in 2016 growing season (Figure 21.).

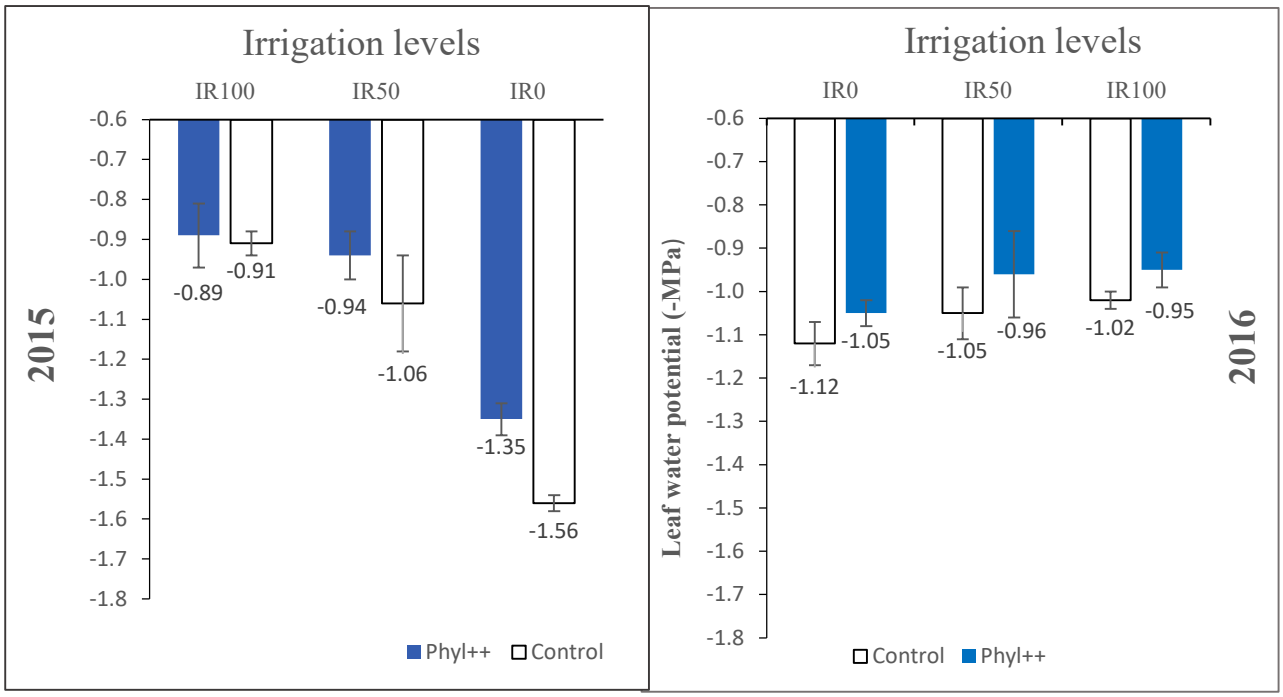

Figure 21. Leaf water potential (-MPa) of processing tomato in different irrigation levels and Phylazonit (Phyl++) treatment.

Our results are supported by the recent reports on maize (Sandhya et al. 2010), pennyroyal (Mentha pulegium L.) (Asghari et al., 2020), and tomatoes (Monica et al. 2016), which all illustrated that higher leaf water potential in host plants colonized by PGPR. PGPR

inoculation can affect the osmoregulation capacity by enhancing the soluble sugar, protein, and proline contents, leading to a higher water potential *gradient,* and thereby improving the water uptake and plant growth under stress *conditions* (Asghari et al., 2020).

4.3.4. Proline concentration

According to Hare et al. (1999), the most studied amino acid proline contributes to osmotic stress. Under two successive reductions *catalysed* by P5C synthetase (PC5S) and P5C reductase (PC5R), proline is *synthesized* in the cytosol and mitochondria from glutamate via Δ1-pyrroline-5- carboxylate (P5C). It has several major functions including mediating osmotic adjustment, protecting protein structures from denaturation, *stabilizing* cell membranes by interacting with phospholipids, scavenging ROS, and serving as energy and nitrogen sources (Claussen 2005). Other authors reported that there were higher proline concentrations *intolerant* plants under drought stress. Its biosynthesis and accumulation may be associated with the detoxification of ROS, a reduction in water potential, and a reduction in photosynthesis rates (Reddy et al., 2004; Claussen, 2005; Thapa et al., 2011)

Control and Phyl++ plants have increased proline accumulation more than two times in shoots as a response to irrigation stress (Figure 22) in two-year experiments with or without Phylazonit inoculation in non-irrigated blocks (IR0). In *full* irrigation block Phyl++ inoculation reduced the proline concentration compared to non-*inoculated but* reach significant levels in 2015. In Phyl++ plants shoot, the amounts of proline *were* reduced from 29.2 to 19.2 mg kg-1, and 25.0 to 15.6 mg kg-1 in two growing seasons, respectively. In 2015 and 2016, *the* IR50 block (Figure 22) *was* compared to control plants. Phyl++ inoculation increased the water status of host plants and lessened proline production. These *results* agree with the results conducted in mycorrhizal plants (Bakr et al. 2017) *who conducted the experiments with* processing tomatoes in open fields.

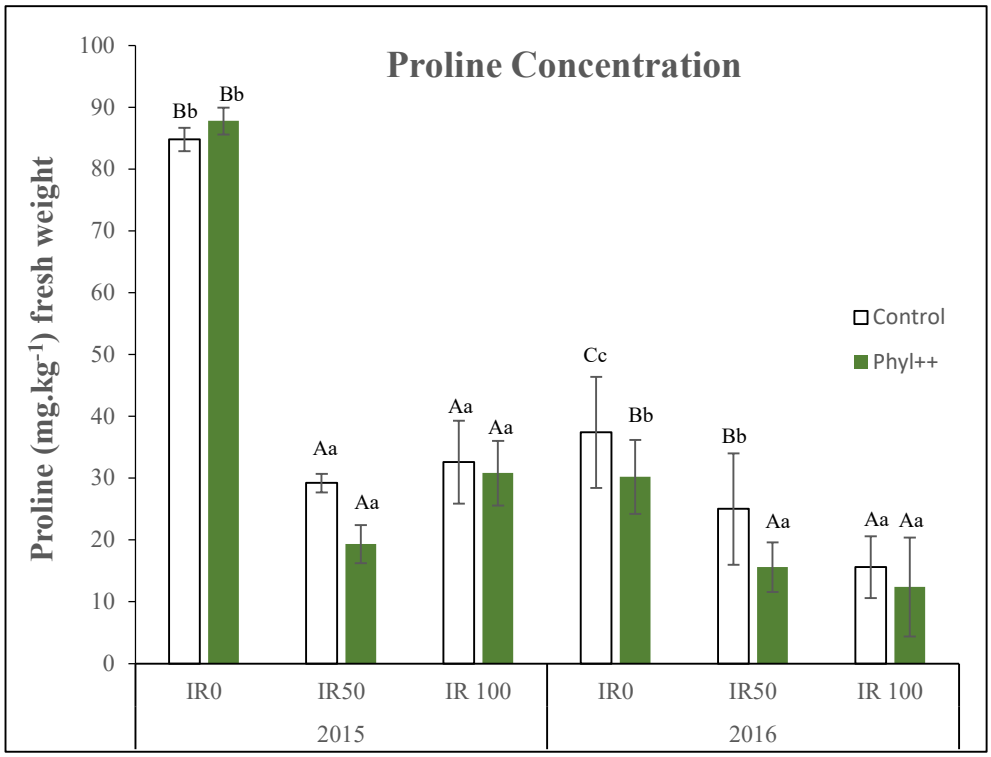

Figure 22. The concentration of proline (mg/kg) fresh weight in processing tomato leaves in different irrigation levels and Phylazonit (Phyl++) treatment combinations.

4.4. Effect of Phylazonit inoculation and irrigation on total biomass, harvest index and water use efficiency.

In both growing seasons, Phyl++ inoculation and irrigation regulation significantly increased the total biomass (fruits, stem, and leaves) (Figure 23) except for the control block (IR0). In the 2015 growing season, plants underwent two weeks of drought which caused decreased soil moisture and shortened the vegetative period (Figure 5). Compared to optimum irrigation treatment (IR100), in the control block (non-Phyl++ inoculation) decreasing irrigation reduced the total biomass by 64% in IR0, and 19% in IR50 in the first growing season, while in the growing season of 2016 by 8% in IR0, and 7% in IR50 compared to optimum irrigation level in the IR100 block. The effect of irrigation *significantly increased* the total biomass production by 228% and 284% in 2015, but only slightly in 2016 (1%, 10%), compared to the control (Figure 23). IR50 combined with Phyl++ inoculation increased total biomass by 32% (98.0 t/ha) and by 19% (165.7 t/ha) in 2015 and 2016, respectively. However, the Phyl++ application has increased total biomass significantly by 30% to 120.6 t/ha only in 2015 in the IR100 treatment, while it was not effective in 2016 (99%). Higher water supply resulted in a higher harvest index only in 2015 by 7% and

16% for IR50 and IR100, respectively, in agreement with Lei et al. (2009). Harvest index values increased from 0.63 to 0.63 in IR50 and from 0.59 to 0.66 in IR100 in 2016, but there was no significant difference between Phyl++ inoculation and control. The water demand for the processing tomatoes varied between 300 mm and 400 mm depending on the weather (Pék et al. 2017), which was covered by precipitation in 2016. Phyl++ inoculation increased the harvest index in all three irrigation regimes in 2015 and reached its maximum in deficit irrigation (Table 7).

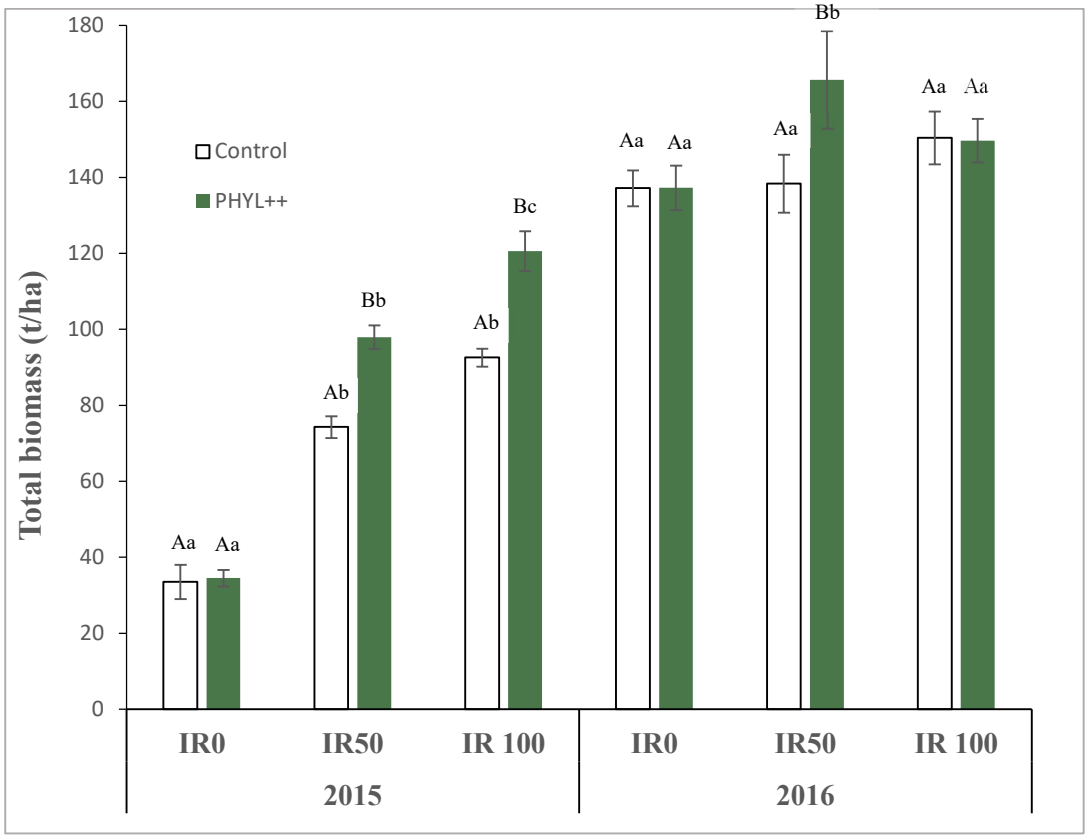

Figure 23. Total biomass (fresh weight) in different irrigation levels and Phylazonit (Phyl++) treatment.

Water use efficiency (WUE), as the main indicator of plant water status, is regulated by physiological processes (Lei et al. 2009). IR50 produced the best results of WUE (24.3 kg.m-3), significantly ($P \leq 0.05$) higher than in IR100 and control (IR0) with 12% and 22%, respectively in 2015. Phyl++ treatment resulted in significantly ($P \leq 0.001$) higher WUE in both IR50 (32%) and IR100 (30%). The maximum WUE was achieved at 32 kg.m-3 in IR50 with the Phyl++ treatment compared to the control without Phyl++ application (Table 7). In combination *with* treatments, Phyl++ could increase WUE only in irrigated plots in 2015. Deficit irrigation usually increases WUE (Patanè et al. 2010, 2014), but this effect was detected in this study only in combination with PGPR in 2015. With respect to all water supply regimes (Control (IR0), IR50,

IR100), no difference was found in *the* WUE of the control samples without PGPR in 2016, either. Better WUE was reached by PGPR treatments, in combination with *rainfall* (26.9 kg.m-3) and IR50 (30.9 kg.m-3) in 2016, which were mostly the same values as in the previous year (Table 7). WUE higher than 10 kg.m-3 is usual in the Mediterranean climate (Patanè et al. 2011, Kuşçu et al. 2014), and all results exceeded this value in both years.

Table 7. Effect of field Phyl++ inoculation and tree irrigation levels on Harvest index (%) and water use efficiency (WUE (kg.m-3)).

Irrigation levels	Treatments	Harvest index (%)		WUE ($kg.m^{-3}$)	
		2015	2016	2015	2016
IR0	Control	$0.44^{Aa} \pm 0.06$	$0.60^{Aa} \pm 0.07$	$19.8^{Aa} \pm 2.4$	$22.2^{Aa} \pm 1.9$
	Phyl ++	$0.68^{Bb} \pm 0.06$	$0.60^{Aa} \pm 0.05$	$18.5^{Aa} \pm 1.2$	$26.9^{Bb} \pm 1.7$
IR50	Control	$0.51^{Aa} \pm 0.04$	$0.60^{Aa} \pm 0.09$	$24.3^{Ab} \pm 0.9$	$21.3^{Aa} \pm 2.5$
	Phyl ++	$0.76^{Bc} \pm 0.03$	$0.63^{Aa} \pm 0.04$	$32.0^{Cc} \pm 1.0$	$30.9^{Bc} \pm 1.4$
IR100	Control	$0.60^{Aa} \pm 0.05$	$0.59^{Ba} \pm 0.07$	$21.7^{Ab} \pm 0.6$	$21.2^{Aa} \pm 1.5$
	Phyl ++	$0.75^{Bc} \pm 0.05$	$0.66^{Ba} \pm 0.04$	$28.3^{Bb} \pm 0.5$	$20.8^{Aa} \pm 1.9$
Phylazonit (Phyl++)		**	*	***	***
Irrigation levels (IR)		***	ns	***	***
(Phyl++) * (IR)		ns	ns	***	***

[1]*Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent PGPR inoculation; small letters represent Irrigation levels effect.* Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001).

4.5. Effect of Phylazonit inoculation and irrigation on yield parameters

4.5.1. Total yield and non-marketable fruits

Due to *the* soil structure (higher water holding capacity) of the second farm, the higher precipitation and to cool season in 2016 (Figure 6), and higher soil microbial activity (Table 4), fruit production was higher in *the* 2016 growing season compared to the 2015 growing season even without PGPR (Phylazonit) inoculation and irrigation supply.

In 2015, the irrigation supply strongly affected the total yield, even with or without the PGPR treatments. The total yield of the deficit irrigation (IR50) block increased by 43 tons compared to

the plants from the control block (IR0), in which block plants received only half of their water demand. The optimal irrigation supply (IR100) raised the yield by 57 tons per hectare. *A similar* trend happened in 2016 but for less extend and not reaching significant levels (Table 8)

The effect of PGPR (Phylazonit) inoculation on the yield was positive at all the irrigation levels. In 2015, the yield was 102.7 tons per hectare, while in 2016 a yield of 60.9 tons per hectare could be achieved as a result of the best interaction between irrigation and PGPR treatments under full irrigation. The total yield increased by 34% in both 2015 and 2016 in Phyl ++ treatment compared to the Control plants (Table 8). The enhancement in the total yield is most probably related to *the plant's* water status, nutrient uptake, and many physiological processes discussed in the next part. *Besides* the effectiveness in increasing the yield, Phyl ++ inoculation reduced the number of rotten fruits in both seasons and at all irrigation levels, except in deficit irrigation (IR50) in 2016 (Table 8). The high yield loss in 2016 Phyl++ plants in IR50 can be explained by the high increase in total yield (160 tons per hectare). *A higher* percentage of the total yield was rotten due to heavy rains during the ripening period in 2016 compared to the 2015 growing season (Table 8). Phyl++ inoculation positively affected the fruit quality including *fewer* rotten fruits in both seasons and at all irrigation supply levels. According to Bakr 2018, Mycorrhizal inoculation has helped tomato plants to better calcium uptake and led to better translocation of Ca+2 into the fruits, therefore, *lessening* blossom-end rot disorder (Figure 24) and minimizing losses due to fruit cracking.

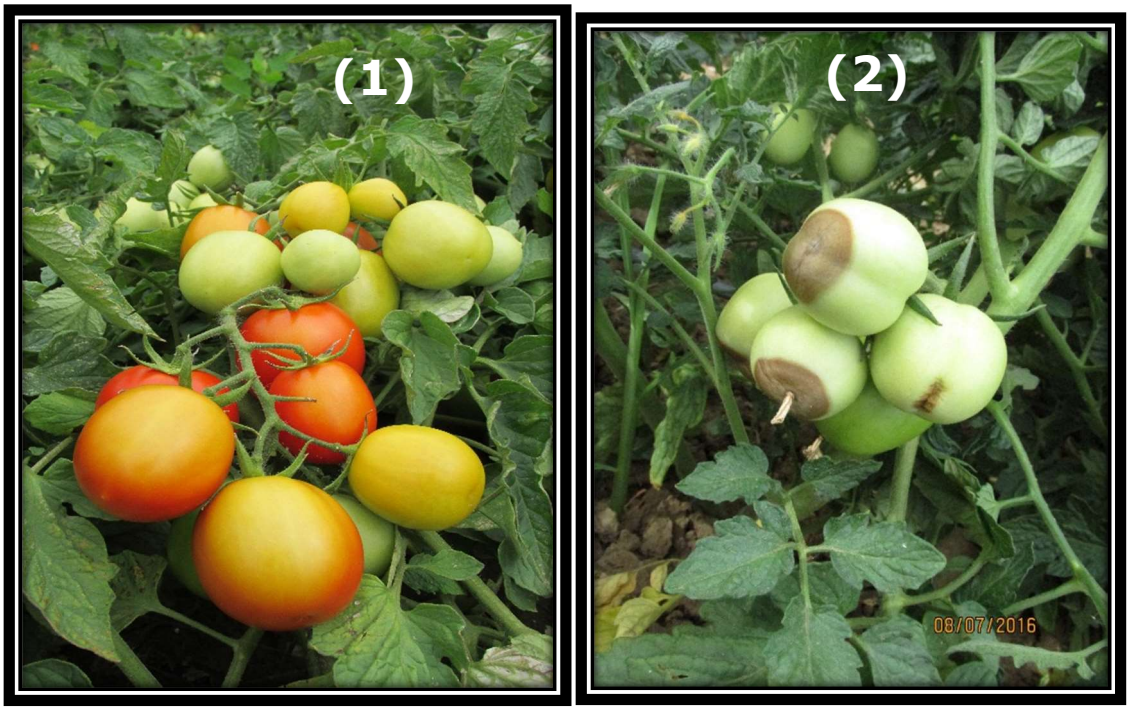

Figure 24. Uno Rosso fruits (1) and blossom-end rot disorder on the fruits (2)

Table 8. Effect of Irrigation level and PGPR application on total yield (t. ha^{-1}), rotten fruits (t. ha^{-1}), and rotten/total yield ratio (%)

Water supply	Treatments	Total Yield (t ha^{-1})		Rotten fruits (t ha^{-1})		Rotten / Total Yield (%)	
		2015	2016	2015	2016	2015	2016
IR 0	Control	$19.1^{Aa} \pm 4$	$108.6^{Aa} \pm 07$	$1.1^{Aa} \pm 0.4$	$43.4^{Ba} \pm 4.6$	$5.7^{Ba} \pm 2.8$	$40.0^{Ba} \pm 2.5$
	Phyl ++	$20.4^{Aa} \pm 2$	$115.4^{Ba} \pm 03$	$0.8^{Aa} \pm 0.5$	$35.5^{Aa} \pm 6.1$	$3.9^{Aa} \pm 3.0$	$31.5^{Aa} \pm 4.3$
IR 50	Control	$62.7^{Ab} \pm 2$	$119.2^{Aa} \pm 10$	$8.7^{Ab} \pm 1.8$	$40.6^{Aa} \pm 7.3$	$13.8^{Bb} \pm 3.1$	$36.0^{Ba} \pm 8.3$
	Phyl ++	$83.05^{Bb} \pm 5$	$160.9^{Bc} \pm 06$	$6.0^{Ab} \pm 3.0$	$46.6^{Ba} \pm 3.0$	$7.2^{Aa} \pm 4.1$	$30.5^{Aa} \pm 3.3$
IR100	Control	$76.3^{Ac} \pm 1$	$123.6^{Aa} \pm 12$	$15.7^{Ac} \pm 3.3$	$41.7^{Ba} \pm 13.8$	$20.5^{Bb} \pm 1.9$	$34.5^{Ba} \pm 7.2$
	Phyl ++	$102.7^{Bc} \pm 9$	$135.3^{Bb} \pm 05$	$10.4^{Ab} \pm 4.3$	$38.4^{Aa} \pm 8.0$	$10.1^{Aa} \pm 3.0$	$30.1^{Aa} \pm 4.6$
Phylazonit treatments (Phyl++)		***	**	*	**	**	***
Irrigation levels (IR)		***	***	**	***	***	***
(Phyl++) * (IR)		***	***	*	***	***	***

*Means with same letters are not significantly different at ($P<0.05$) as determined by Tukey's HSD test (Mean ± SD, n=4). Significant of source of variation (ns= not significant, * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$). Capital letters represent mycorrhizal inoculation, small letters represent Irrigation levels effect.*

4.5.2. Marketable fruits

In 2015, the marketable yield of IR50 and IR100 increased significantly by 384% and 465%, respectively, whereas in 2016, the respective yield increases were lower by 22% (IR50) and 51% (IR100) compared to the control (Figure 25). The PGPR treatment combined with *a* better water supply further increased the yield of *tomatoes*, but not in control (non-PGPR inoculated) and IR100 in 2016. IR50 combined with PGPR increased the marketable yield by 28% (to 72.6 t/ha) in 2015 and by 45% in 2016 reaching the highest value of 119.8 t/ha in that year (Figure 25). This finding agrees with previous studies *on* processing *tomatoes* (Helyes et al. 2014b, Pék et al. 2017). The vast difference in yield production between the 2015 and 2016 growing *years* due to the soil characteristics (brown forest soil - low holding water capacity) and the lack of precipitation, tomato plants had to face several stresses in 2015. The *higher* yield in 2016 is because of moderate water stress (higher precipitation) in no irrigation plots, and the loamy soil texture can hold higher amounts of *water (*Figure 12). For this reason, the PGPR inoculation in the plots without irrigation could give only 19% of the potential fruit biomass. Unlike in 2015, in 2016, the PGPR inoculation enhanced the marketable fruits by 10% *compared* to the control plants (67.3 t ha^{-1}).

Deficit irrigation strategies (IR50) is a method to save irrigation water and keep the yield in arid and Semi-arid area, where the rainfall from May to August are rare (Helyes et al. 2012; Rinaldi et al. 2015; Rinaldi et al. 2011; Delazari et al. 2019). However, processing *tomatoes* requires *a* high water supply through irrigation (Atherton and Rudicd. 1986). According to Hobson and Grierson (1993), PGPR is considered as *a* pre-harvest biotic factor affecting crop yield and quality. It is known that ripening can influence the quality attributes of the fruit (shape, size*, colour,* and texture) It has been proven that PGPR affects rice yield, increasing total biomass *by* about 20% (Chaintreuil et al. 2000; Baset – Mia and Shamsuddin, 2010), seed inoculation with PGPR enhanced seed germination and higher seed dry weight in maize (Gholami et al. 2008) and yield in tomatoes (Gagnb et al. 1993; Schober et al. 2007; Mena-Violante and Olalde-Portugal, 2007; Candido et al. 2015; Monica et al. 2016; Ochoa-velasco et al. 2016; Helyes et al. 2014a; Deák et al. 2015; Tripti et al. 2017; Nemeskéri and Helyes, 2019; Ahmed et al. 2020; Karthika et al.2020). Nemeskéri et al. (2022) also reported that tomato plants *treated* with PGPR resulted in 26% more ripe marketable fruits and 49% less unripe fruits under growing in a dry year, and deficit irrigation conditions.

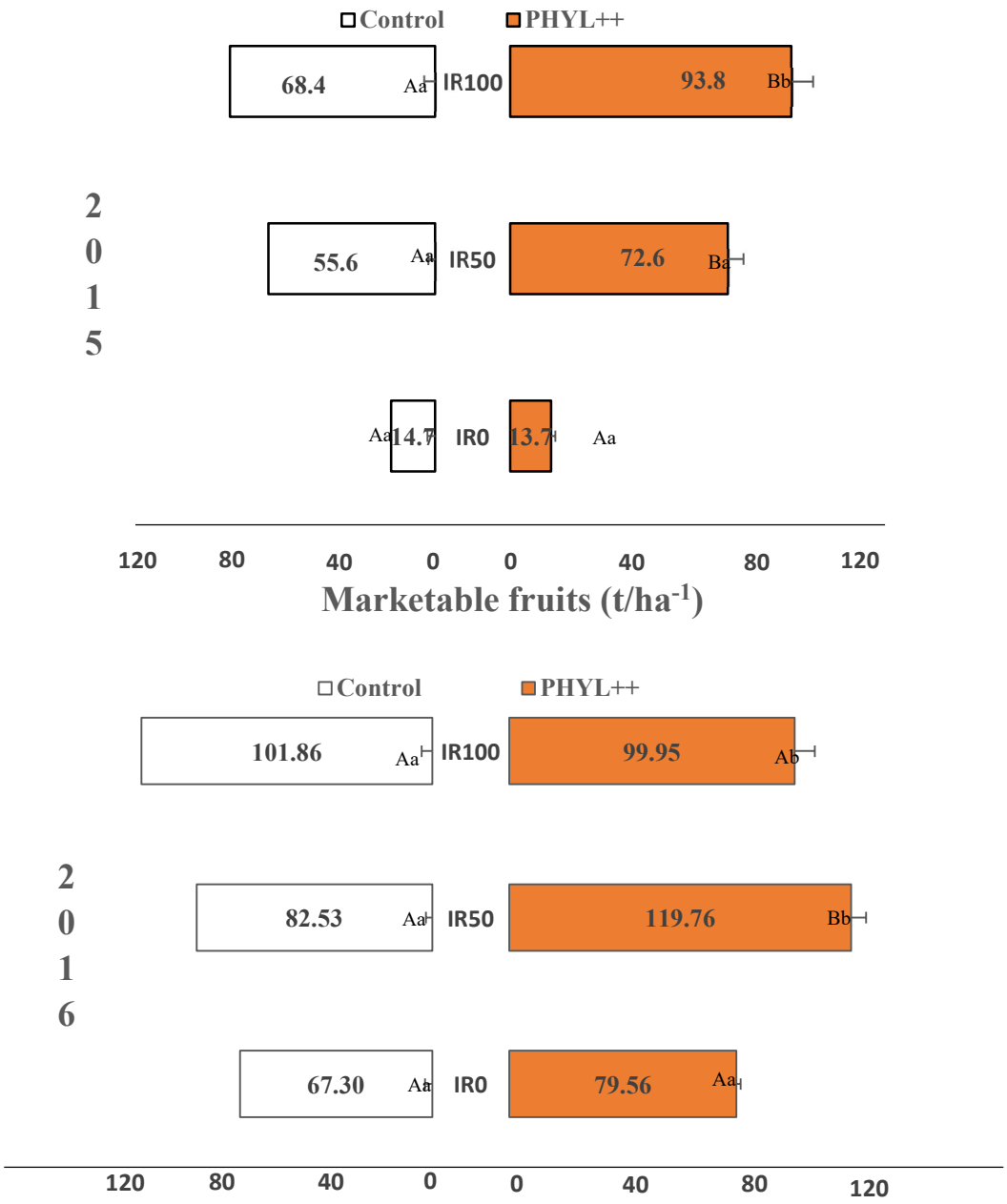

Figure 25. Effect of Irrigation level and PGPR application on marketable yield and control in three irrigations levels treatments.

4.5.3. Soluble solid content

In the two seasons, Brix and marketable yield had a negative relationship. The higher the yield production rose (more than 60 *t. ha*-1 in average), the lower the obtained °Brix was (below 5.5 in the irrigated samples) (Table 9). In 2015, the highest Brix was recorded in the control treatment (IR0) with and without irrigation levels (in control samples: 8.0 and Phyl++ samples 7.6, respectively). Linear regressions showed different levels of correlations between marketable yield and Brix affected by PGPR. It was strong in *the* 2015 season (R2 = 0.96) and moderate in the 2016 growing season (R2 = 0.18) for control plants. According to the slope of linear regressions, Phyl++ treatments slowed down the decrease of the soluble solid content along with the increased yield (R from 0.91 to 0.95) in *the* 2015 growing season and in *the* 2016 growing season (R from 0.70 to 0.72) (Figure 26). *These* results are supported by Bakr et al. *2017; Pék* et al. 2015; Helyes et al. 2012; Helyes et al. 2014a. Table 6 shows the highest loss in Brix from 8.03 to 3.73 (in 2015) and 3.65 to 3.20 (in 2016), is due to the fact that the higher levels of irrigation lead the higher water content in fruits and decreasing sugar content (Atherton & Rudich, 1986; Bark, 2017). Azotobacter chroococcum PGPR content as free living N2 fixing bacteria that improves nitrogen uptake on plants contributes to increased sugar content in fruits (Atherton & Rudich, 1986; Fan, 2017). Despite Brix content losses in non-inoculated and Phyl++ plants, yield increase led to an increase in the soluble solid content as the mass production per area increased in both the 2015 and 2016 growing seasons (Figure 27).

Table 9. Effect of PGPR inoculation and three irrigation levels on °Brix (g/100g) and °Brix yield ($t.ha^{-1}$) of processing tomato

Water supply	Treatments	^{0}Brix (g/100g)		^{0}Brix yield ($t.ha^{-1}$)	
		2015	2016	2015	2016
IR0	Control	$8.0^{Ac} \pm 0.1$	$4.5^{Aa} \pm 0.2$	$1.2^{Aa} \pm 0.1$	$2.4^{Aa} \pm 0.1$
	Phyl ++	$7.6^{Bb} \pm 0.2$	$4.1^{Aa} \pm 0.1$	$1.0^{Aa} \pm 0.1$	$3.3^{Aa} \pm 0.3$
IR50	Control	$5.0^{Bb} \pm 0.2$	$3.7^{Aa} \pm 0.1$	$2.8^{Aa} \pm 0.1$	$3.5^{Aa} \pm 0.3$
	Phyl ++	$4.1^{Aa} \pm 0.1$	$4.0^{Ba} \pm 0.2$	$3.0^{Ba} \pm 0.2$	$4.9^{Ba} \pm 0.3$
IR100	Control	$3.7^{Ba} \pm 0.2$	$3.6^{Aa} \pm 0.4$	$2.6^{Aa} \pm 0.1$	$3.1^{Aa} \pm 0.2$
	Phyl ++	$3.4^{Aa} \pm 0.2$	$3.5^{Aa} \pm 0.1$	$4.1^{Ba} \pm 0.4$	$3.7^{Ba} \pm 0.2$
Phylazonit treatment (Phyl++)		**	*	**	***
Irrigation levels (IR)		***	***	*	***
(Phyl++) * (IR)		**	**	*	*

*Means with same letters are not significantly different at ($P<0.05$) as determined by Tukey's HSD test (Mean ± SD, n=4). Significant of source of variation (ns= not significant, * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$). Capital letters represent PGPR inoculation, small letters represent Irrigation levels effect.*

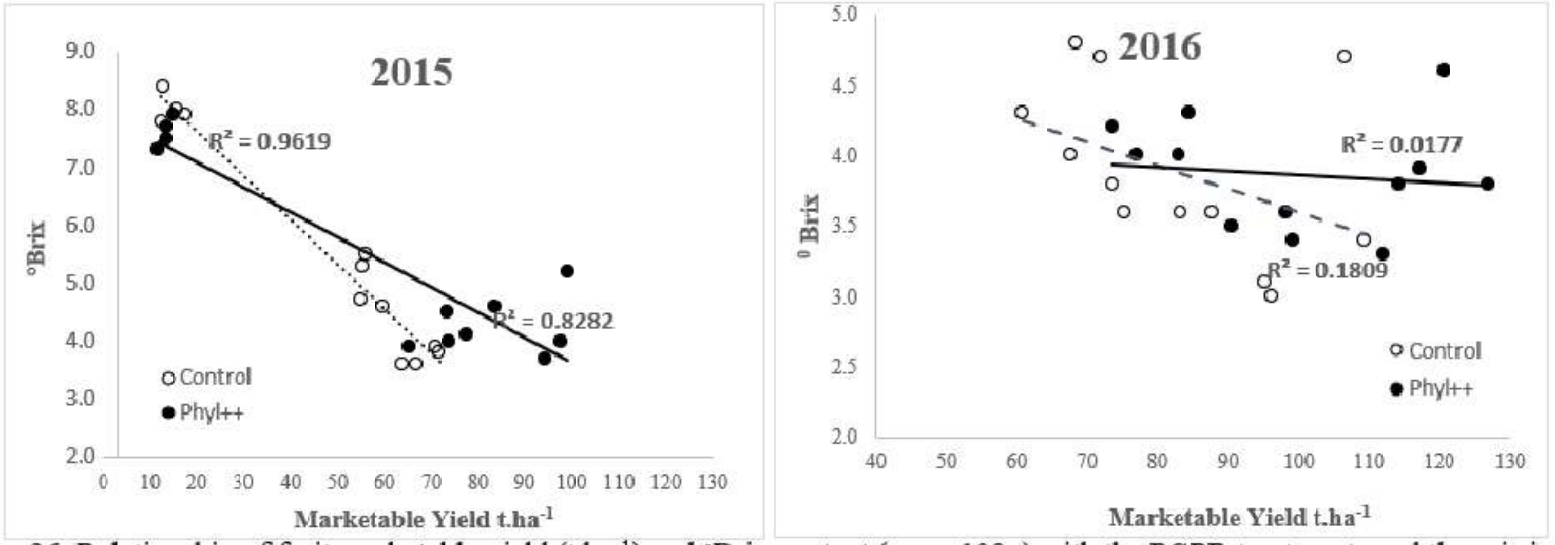

Figure 26. Relationship of fruit marketable yield (t.ha^{-1}) and °Brix content (g per 100g) with the PGPR treatments and three irrigation levels.

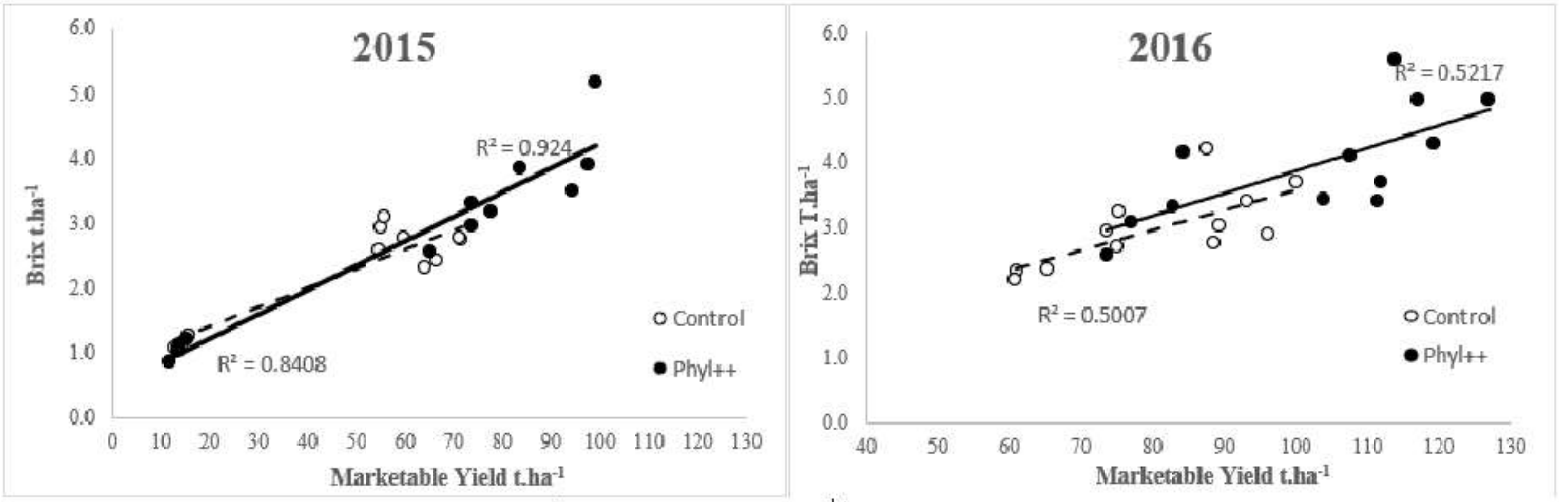

Figure 27. Relationship of marketable yield (t.ha^{-1}) and °Brix yield (t.ha^{-1}) and with the PGPR treatments and three irrigation levels.

4.5.4. Carotenoids and ascorbic acid.

Figure 14 shows the HPLC profile of tomato carotenoids as separated into several compounds such as neoxanthin, zeaxanthin, lycopene epoxide, lycoxanthin, lycopene, 9-cis-lycopene, tetra-dehydro-carotenoid, 13-cis-lycopene, β-carotene, cis-β-carotene, antheraxanthin di-ester, Lycophyll di-ester. The method was carried out in the Analytical Laboratory of the Hungarian Agricultural and Life Science University in Gődőllő, according to Daood et al. (2014). Because of *the* nutritional value, biological activities, and marketability of plant products, we concentrate on three main components: β-carotene, lycopene, and total carotenoids.

Table 10 displays the total carotenoids, lycopene, -carotene, and ascorbic acid levels in two growing seasons, 2015 and 2016. Increasing irrigation levels reduces total carotenoids, lycopene, and β-carotene content in fruits, but higher yields can compensate for this loss. According to Pék et al. (2014) and Helyes et al. (2006), abiotic factors such as water supply, temperature, and *sunlight* can affect the antioxidant composition and concentration of carotenoids in tomatoes. Lycopene is responsible for tomato reddening (Dumas et al 2003; Miguel et al 2006, Helyes et al 2014). Lycopene forms at lower temperatures ranging from 16 to 260 degrees Celsius; above 30 degrees Celsius, lycopene biosynthesis is inhibited (Tomes, 1963). This can explain the lower amount of lycopene in IR50 and IR 100 sample blocks of tomato fruits in *the* growing season *of* 2015 when the plants spent two weeks at high temperatures (Figure 8); however, except the controls and non-irrigated plants, which were harvested earlier.

Table 10. Effect of field Phyl++ inoculation and tree irrigation levels on total Carotene (µg.g^{-1}), lycopene (µg.g^{-1}), β-carotene (µg.g^{-1}), ascorbic acid (µg.g^{-1}) contents of processing tomatoes.

Water supply	Treatments	Total carotene (µg.g^{-1})		Lycopene (µg.g^{-1})		β-carotene (µg.g^{-1})		Ascorbic acid (µg.g^{-1})	
		2015	2016	2015	2016	2015	2016	2015	2016
IR0	Control	$136.3^{Bb} \pm 1.0$	$321.7^{Ab} \pm 9.4$	$100.6^{Bb} \pm 1.0$	$204.8^{Aa} \pm 4.8$	$2.63^{Aa} \pm 0.1$	$13.84^{Bb} \pm 1.0$	$329.9^{Ab} \pm 14.9$	$330.53^{Aa} \pm 11.2$
	Phyl ++	$62.0^{Aa} \pm 4.5$	$304.3^{Ab} \pm 9.4$	$45.4^{Aa} \pm 3.9$	$218^{Ab} \pm 11.4$	$1.51^{Aa} \pm 0.1$	$10.43^{Aa} \pm 1.6$	$324.5^{Ab} \pm 4.2$	$342.92^{Aa} \pm 27.8$
IR50	Control	$106.3^{Aa} \pm 3.8$	$128.6^{Aa} \pm 20.0$	$72.0^{Aa} \pm 2.9$	$233.3^{Ab} \pm 12.8$	$2.23^{Aa} \pm 0.2$	$10.58^{Aa} \pm 1.4$	$286.0^{Aa} \pm 12.8$	$418.24^{Ba} \pm 19.2$
	Phyl ++	$167.2^{Bb} \pm 12.4$	$160.0^{Aa} \pm 16.0$	$126.2^{Bb} \pm 9.7$	$270.0^{Bb} \pm 13.8$	$4.36^{Ba} \pm 0.7$	$9.59^{Aa} \pm 1.6$	$221.8^{Aa} \pm 9.2$	$383.16^{Ab} \pm 66.7$
IR100	Control	$94.3^{Aa} \pm 9.1$	$195.8^{Aa} \pm 11.3$	$66.1^{Aa} \pm 3.5$	$95.1^{Aa} \pm 9.3$	$2.41^{Aa} \pm 0.2$	$5.80^{Aa} \pm 0.8$	$272.9^{Aa} \pm 6.9$	$333.6^{Aa} \pm 36.5$
	Phyl ++	$76.7^{Aa} \pm 4$	$302.4^{Bb} \pm 6.5$	$50.4^{Aa} \pm 2.6$	$202.8^{Bb} \pm 31.4$	$2.42^{Aa} \pm 0.1$	$15.74^{Bb} \pm 1.58$	$249.6^{Aa} \pm 10.1$	$277.8^{Aa} \pm 42.7$
Phylazonit (Phyl++)		***	**	*	***	**	*	ns	*
Irrigation levels (IR)		***	**	***	ns	***	ns	**	*
(Phyl++) * (IR)		*	***	***	*	***	**	ns	ns

*Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001). Capital letters represent PGPR inoculation; small letters represent Irrigation levels effect.*

Regardless of yield, inoculation and irrigation levels, the total carotene production ranged from 0.8 to 12.1 kg ha-1, which is almost a fifteen- times difference (Table 11). In the IR0, depending on the marketable *yield significant* reduction in the value of total carotene production of Phyl++ samples. However, irrigation regimes increased the carotenoid content. In IR100, Phyl++ treatment slightly enhanced the lycopene (4.7 kg ha-1), β-carotene (227.3 g ha-1) and total carotene (7.2 kg ha-1) contents. In IR50, there was twofold difference in the total carotenoid yield between control and Phyl++ samples, in which the highest amount of total carotene was recorded (12.1 kg ha-1). Lycopene and β-carotene increased by 126 and 148%, respectively in Phyl++ (Table 11). In contrast, the amount of ascorbic acid in IR0 and IR50 had no significant difference between Phyl++ and control. There was a slight lower in Ascorbic acid content in the 2015 growing season (272 to 329 μg g-1) compared to the 2016 season range (330 to 418 μg g-1) (Table 10). According to Helyes et al, 2006, the ascorbic acid content in processing tomatoes were in the usual ranges (from 286 to 446 mg kg-1) for industrial tomotoes.

Increase of irrigation level has negatively affected and significantly reduced total carotenoid yield of marketable fruits from 18.8 kg ha-1 in IR0 and 19.1 kg ha-1 in IR50 to 13.5 kg ha-1 in IR100. Such trend was evident for lycopene from the IR0- to IR100-treated tomato fruits. Irrigation regimes had no effect on β-carotene yield, and the ascorbic acid levels did not have a clear trend without PGPR application. Moreover, beside the yield improvement of Phyl ++plants, PGPR treatment had doubled the total carotenoids and lycopene production in irrigated plots (Table 11).

The effect of PGPR on the measured components was unclear (Ruzzi and Aroca 2015). The positive impact of PGPR on the total carotenoid content was evident only in the deficit irrigation treatment. Lycopene and β-carotene content responded *in* the same way to the PGPR treatment. PGPR application has been reported to increase, *in* the same way, the concentration of carotene components slightly changes the ascorbic acid content under moderate water scarcity (Ordookhani et al. 2010). However, PGPR altered the lycopene and β-carotene yields negatively, along with the ascorbic acid yield. Response of the measured carotenoid components to PGPR treatment was not observable when transpiration was not limited (IR100). The mean values of β-carotene and ascorbic acid were 152.1 and 227.32 g ha-1 with the optimal irrigated treatment, respectively. On the contrary, more ascorbic acid was produced as a function of bio-fertilizer. *The effect* of *the* water supply was noticeable in many cases. Differences in the content of lycopene, β-carotene*,* and ascorbic *acid were* found between PGPR and its control at every water supply level. The effect of the PGPR treatment was not influenced by the irrigation when there was no water scarcity.

The effect of PGPR on total carotene and lycopene only emerged under irrigated conditions. However, *a* positive effect was observed in the case of β-carotene in the rainfed control. Phylazonit application did not affect ascorbic acid yield at all, but the effect of irrigation was expressive. *The effect* of irrigation on total carotene and β-carotene content under *the* IR100 irrigation regime was non-significant, but it was expressional in the IR50 treatment to total carotene and the measured carotene components. When additional water supply was not provided, the yield of total carotene, lycopene, and ascorbic acid was affected by irrigation, but that of β-carotene wasn't.

Table 11. Effect of field Phyl++ inoculation and tree irrigation levels on β-carotene ($\mu g.g^{-1}$) and Ascorbic acid ($\mu g.g^{-1}$) content of processing tomato.

Irrigation levels	Treatments	Total Carotene ($kg.ha^{-1}$)		Lycopene ($kg.ha^{-1}$)		β- Carotene ($g.ha^{-1}$)	
		2015	2016	2015	2016	2015	2016
IR0	Control	$2.01^{Ab} + 0.3$	$18.79^{Aa} + 1.4$	$1.48^{Aa} + 0.5$	$15.91^{Aa} + 1.2$	$39.8^{Ab} + 5.1$	$11.93^{Aa} + 1.4$
	Phyl ++	$0.83^{Aa} + 0.1$	$18.01^{Aa} + 1.5$	$0.62^{Aa} + 0.1$	$15.24^{Aa} + 1.2$	$20.4^{Aa} + 2.3$	$12.78^{Ab} + 1.1$
IR50	Control	$6.01^{Ab} \pm 0.7$	$19.11^{Aa} \pm 1.1$	$1.07^{Ab} \pm 0.5$	$10.75^{Aa} \pm 1.6$	$126.0^{Bc} \pm 21.6$	$12.67^{Ab} \pm 1.1$
	Phyl ++	$12.09^{Bb} + 1.7$	$40.39^{Bc} + 1.5$	$9.20^{Bb} + 0.8$	$34.11^{Bb} + 1.1$	$312.8^{Dd} + 79.2$	$20.78^{Bb} + 2.1$
IR100	Control	$6.45^{Ab} \pm 1.4$	$13.47^{Aa} \pm 2.3$	$4.55^{Ab} \pm 0.6$	$10.74^{Aa} \pm 2.0$	$152.1^{Bc} \pm 36.0$	$10.20^{Aa} \pm 1.4$
	Phyl ++	$7.21^{Ab} \pm 1.2$	$25.33^{Bb} \pm 2.6$	$4.72^{Ab} \pm 0.8$	$21.59^{Bb} \pm 2.3$	$227.3^{Cd} \pm 34.36$	$15.67^{Ab} \pm 3.4$
Phylazonit (Phyl++)		*	***	***	***	***	***
Irrigation levels (IR)		***	***	***	***	***	***
(Phyl++) * (IR)		**	***	***	***	***	***

*Means with same letters are not significantly different at ($p<0.05$) as determined by Tukey's HSD test (Mean+SD, n=4). Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001). Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001). Capital letters represent PGPR inoculation effect; small letters represent irrigation levels effect*

5. CONCLUSIONS AND RECOMMENDATIONS

The two-year results in a field-based experiment approved that commercial PGPR - Phylazonit strains can be used as an integrated application for processing tomato production, alleviated moderate water stress, and improved both production and fruit quality. The second treatment with the PGPR- Phylazonit (field-inoculation) at transplant can be a very successful strategy.

The results also approved that Phyllazonit in field inoculation is more effective than pre-transplant inoculation at *sowing but* increases the cost. The colonization rate was higher than in control samples, Phylazonit inoculation improved plant development, yield, carotenoids, and lycopene as well as stomatal conductance, and water use efficiency especially under deficit irrigation conditions.

We found that the result on leaf water potential, stomatal conductance, canopy temperature, and water used efficiency in samples treated with Phylazonit (field -inoculation) did not have the effect of reducing drought stress when the plants underwent water deficit conditions and did not have much effect to avoid the effects of drought (the results in water use efficiency, canopy temperature, SPAD and leaf water potential).

Under deficit irrigation or moderated drought stress, Phylazonit field treatment enhanced the performances of tomato plants compared to the Control samples. There were significant differences recorded in stomatal conductance, water use efficiency, canopy temperature, leaf water potential, Fv/Fm, and SPAD content in the field-inoculation samples. It partially reduced the water stress during the drought condition happened. These results supported that Phylazonit symbiosis improves their host plants by increasing the water uptake through the regulation of the stomatal closure in the plant.

Under deficit irrigation, Phylazonit inoculation at transplant enhanced the crop yields more efficiently than full irrigation. The results recorded a higher level of carotenoids, lycopene, and β-Carotene and fruit set in the 2016 growing season on loamy soil opposite the 2015 growing season.

For 2 years of experiments, the soil characteristics (texture and water holding) had an important role in the Phylazonit symbiosis effect. The loamy soil, in the 2016 season, had better water holding and texture *accounting* for the higher Phylazonit efficient performance on the tomato plant.

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NEW SCIENTIFIC RESULTS

1. During a 2-years experiment, I found that the time of the treatment has a considerable impact on the efficiency of PGPR. The result of the plant's physiological responses, biochemical changes, plant production, and fruit quality, I found that the field inoculation at transplanting with the commercial inoculum PGPR - Phylazonit is the efficient method in reducing drought stress in processing tomatoes.
2. I approved that the effect of drought stress on industrial tomato plants can be reduced by the Phylazonit application.
3. I supported that PGPR-Phylazonit inoculation at transplant can enhance the water use efficiency and total biomass and help host plants to assist the water stress impact, especially under deficit irrigation.
4. The results from water use efficiency in two years approved that PGPR-Phylazonit biofertilizer field-inoculation supported their host plant to overcome the drought stress impacts by raising the water and nutrient uptake mechanism. Less organic and inorganic osmolytes in plants induced to moderate water deficit stress, supported by the most important indices of plant water status (leaf water potential, stomatal conductance, and canopy temperature) are definite field-based proofs that the water and nutrient uptake meaningfully increased by the PGPR-Phylazonit inoculation. In other words, PGPR-Phylazonit biofertilizer inoculation protected the plants from the water deficit instead of stimulating them to tolerate the stress. It was also found that the positive effect of the PGPR-Phylazonit inoculation on stomatal regulation partially contributed to the mediation of the water tress by sustaining plant soil water balance.
5. I indicated that PGPR-Phylazonit field-inoculation (Phyl++) could improve the fruit quality (higher Soluble solid-, Carotenoids-, β-carotene-, and lycopene- contents) accompanied by a meaningful increase of tomato yield, particularly under deficit irrigation conditions.

SUMMARY

Climate change is currently having an impact on biosystems and humans. It reduces agricultural production and un-secure food production for the exponentially growing population. Rising temperatures and changing precipitation patterns make plant growth more difficult in many places and put pressure on the freshwater supply. Extreme weather events lead to desertification, and land degradation happens in many areas. Human activities include urbanization, increased living demand, population growth, and a scarcity of agricultural irrigation resources. Within the soil, the PGPR has been proven to promote plant growth directly or indirectly via the biocontrol of host plant disease, production of phytohormones, or improvement of plant nutritional status. The PGPR contents of Pseudomonas putida, Azotobacter chroococcum, Bacillus circulans, and Bacillus megaterium were found to play an important role in root morphology, plant growth, nutrient uptake, and drought tolerance.

Our main research goal was to investigate the relationship between commercial PGPR and the plant under field conditions and acquire the information for further practical usage. The efficiency of PGPR biofertilizer in the field is strongly influenced by temperature (hot or cold), precipitation, light, soil texture, and soil rhizosphere. In contrast to greenhouse or pot experiments, we can control many factors, such as microorganisms, macros - micros nutrition content, and light or water supply. The beneficial role of the PGPR symbiosis in the sustainable agriculture system and their economic importance in crop production have been published in many articles; however, Phylazonit biofertilizer is a new product necessary to optimize the field-inoculation at transplant to get the benefit from the symbiosis in a most efficient way.

We carried out our experiments in the Experimental Farm of the Institute of Horticulture at Szent István University, which is located in the Lower Park of Gödöllő in 2015 and in 2016 in Szárítópuszta. In this study, we inoculated the Phylazonit with the same magnitude regardless of inoculation timing, field area, or irrigation levels, resulting in efficient root colonization. Our results showed that the Phylazonit was more efficiently associated at transplant (Phyl++) than at pre-transplant inoculation (Phyl+) and control plants.

The results of open field and laboratory provide evidence that bacteria present in Phylazonit could not help their host plants to avoid or resist the drought stress under severe deficit water conditions that lasted for more than two weeks. In another way Phylazonit treatment is not the perfect method and cannot save the plants under severe drought stress conditions, therefore the combination between irrigation strategy and Phylazonit treatment is the most important key in establishing Phylazonit symbiotic association.

Deficit irrigation combined with Phyl++ inoculation increased total biomass by 32% and 19% in both seasons, resulting in higher WUE (24.3 kg/m3), which was significant ($P \leq 0.05$) in 2015 compared to IR100 and control (IR0), which increased total biomass by 12% and 22%, respectively; remarkably increased the ΨL in plant leaves by 12 %, and 09 %. It slightly enhanced the stomatal conductance in all irrigation (IR50 and IR100) treatments in the two years of experiments compare to the control block (IR0) from 18.7 to 23.9 mmol m-2 s-1 in 2015, and from 31.5 to 33.6 mmol m-2 s-1 in 2016 and lower canopy temperature in two years growing.

Phylazonit inoculation improved the water uptake in deficit irrigation treatment, it probably led to better nutrient uptake in plants, which in turn positively affected the fruit setting and the total yield compared to non-inoculated plants (from 62.7 to 83.05t ha-1 in 2015, and from 119.2 to 160.9 t ha-1 in 2016). Moreover, the probable enhancement of the mineral uptake in Phyl++ plants enhanced the fruit quality.

Besides the climate effect, the soil structure also had a great effect on the Phylazonit efficiency. In two experiment locations, there are two types of soil: loamy and sand loamy, our results showed higher yield on the loamy field, higher total carotenes (from 106 to 128 µg.g-1), lycopene (from 72 to 233 µg.g-1), and β- carotene (from 2 to 10 µg.g-1) contents compared to old sandy loam farm calculated as an average across all water levels. The higher microbial activity (1.1-1.25 m.g-1.hr-1 in the loamy rhizosphere) than (0.62-0.79 m.g-1.hr-1 in the sandy loamy rhizosphere) is that more soil moisture content resulted in better mycorhizosphere microbial interaction between plant roots and microbes, which enhanced nutrient absorptions.
The use of Phylazonit -inoculation at transplant multi-species inoculum as an integrative method in the sustainable field production system was recommended. As the research before, our results confirmed the key role importance of the irrigation strategy in the Phylazonit - crop symbiosis efficiency, therefore scheduling and regulating water amount based on soil characteristics and crop development stage are necessary to reach compromise results.

RELATED PUBLICATIONS

1. Tuan Anh Le, Zoltán Pék, Sándor Takács, András Neményi, Hussein G. Daood, and Lajos Helyes. (2018): The Effect of Plant Growth Promoting Rhizobacteria on the Water-yield Relationship and Carotenoid Production of Processing Tomatoes. HORTSCIENCE 53(6):816–822. https://doi.org/10.21273/HORTSCI13048-18.

2. Tuan Anh Le, Zoltán Pék, Sándor Takács and Lajos Helyes. (2018): The effect of plant growth-promoting rhizobacteria on yield, water use efficiency and Brix Degree of processing tomato. *Plant Soil Environ. Vol. 64, 2018, No. 11: 523–529.* https://doi.org/10.17221/818/2017-PSE.

3. Lajos Helyes., Le. Anh. Tuan, Jawdat Bakr and Zoltán Pék (2019). The simultaneous effect of water stress and biofertilizer on physiology and quality of processing tomato. Acta Hortic. 1233. ISHS 2019. DOI 10.17660/ActaHortic.2019.1233.9.

4. Zoltán Pék; Noémi Budavári ; Le Anh Tuan; Hussein Daood; Krisztián Halász; Gábor Gyulai; Peter Szuvandzsiev (2017): Amerikai örökségfajta (heirloom) paradicsomok talaj nélküli termeszthetőségének, morfometriai és beltartalmi értékeinek vizsgálata. KERTGAZDASÁG 49(1) 9-17.

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ACKNOWLEDGEMENTS

First, I would like to gratefully acknowledge my supervisor Professor Lajos Helyes for his scientific guidance, consistent support, motivation, and collaboration.

I would like to thank Dr. Andras Nemenyi and Dr. Zoltán Pék for the stimulating discussions and for providing much equipment for the measurements, Professor Eszter Nemeskéri and Dr. Attila Ombódi for the valuable reviews.

Special thanks to Professor Hussein G.Daood for his assistance in the HPLC analyses, and laboratory methodology.

Sincerely regards to my colleagues of the Department of Horticulture, all friends, and Ph.D. students for their patience and support during my work in the field and laboratory.

I acknowledge the permission from the Ministry of Education and Training, the Stipendium Hungaricum fund by Tempus Foundation, and the Vietnam Institute of Agriculture Engineering and Postharvest Technology.

Finally, I would like to express my special appreciation and thanks to my family for being a constant source of strength and inspiration.

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LIST OF ABBREVIATIONS:

PGPR	Plant growth – promoting rhizobacteria
IR	Irrigation regimes
IR 50	Deficit irrigation
IR100	Optimum irrigation supply
Phyl	Phylazonit inoculation
Phyl+	Phylazonit inoculation during the seedling raising period.
Phyl++	Phylazonit inoculation during the seedling rasing period and at the transplanting.
SPAD	Soil-Plant Analysis Development.
WUE	Water use efficiency
ψ leaf	Leaf water potential
FDA	fluorescein diacetate hydrolysis

APPENDICES

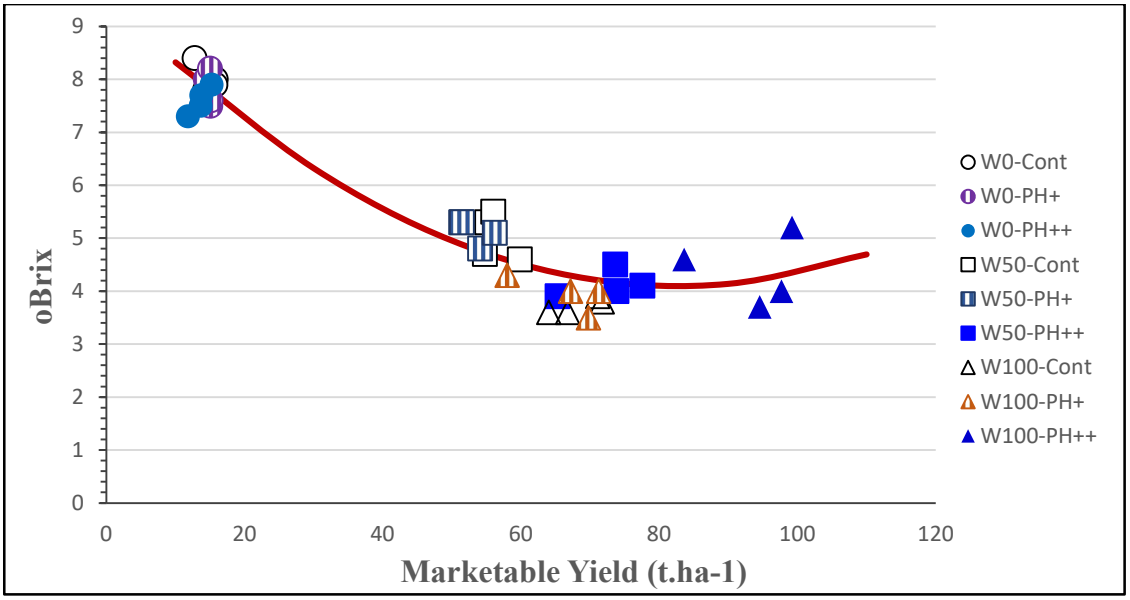
Appendices 1. *Season 2015: Total biomass, water use efficiency (WUE), SPAD and Canopy temperature of field PGPR contribution of non-inoculated (Control), pre-transplant inoculated (Phyl+), and field inoculated (Phyl++) plants in three irrigation supply treatments.*

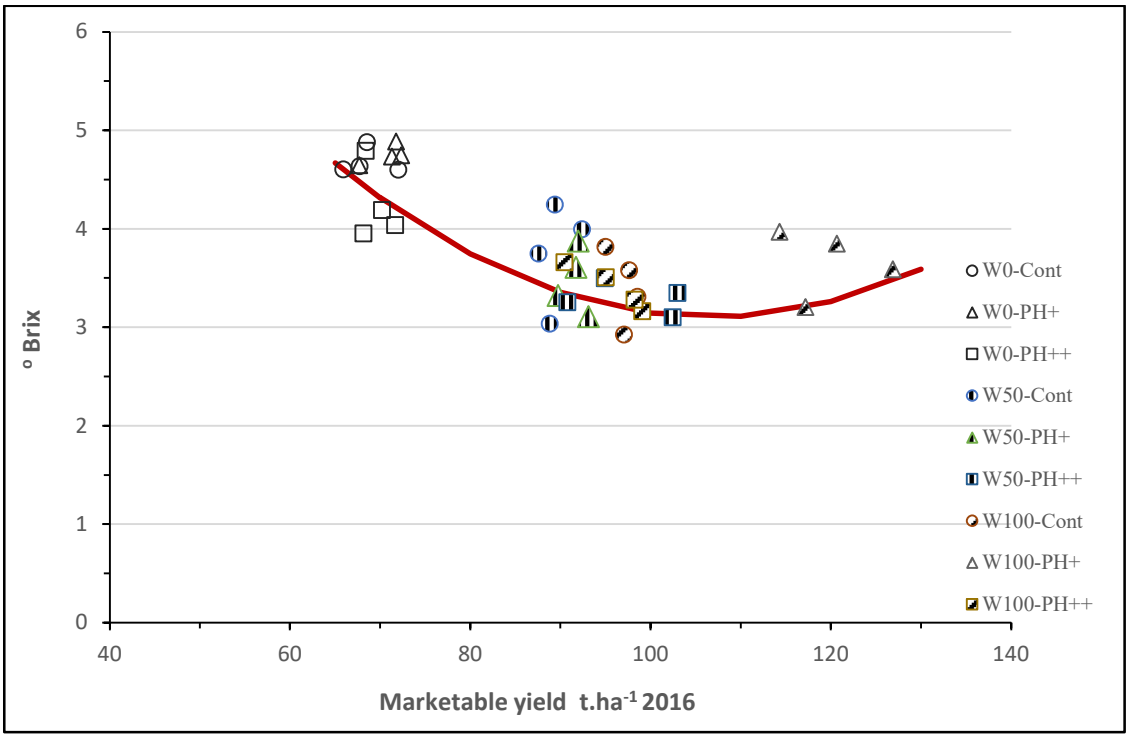
Water supply	Treatments	Total biomass $t.ha^{-1}$	WUE $kg.m^{-3}$	SPAD	Proline µg/g	T^0 Canopy ^0C
IR0	Control	$33.5^{Aa} \pm 4.5$	$18.0^{Aa} \pm 2.4$	$46.1^{Aa} \pm 0.8$	$84.8^{Bb} \pm 1.9$	$23.5^{Ac} \pm 1.5$
	Phyl +	$33.9^{Aa} \pm 2.1$	$18.2^{Aa} \pm 1.1$	$49.7^{Ba} \pm 2.3$	$79.9^{Ba} \pm 2.7$	$22.9^{Ab} \pm 1.3$
	Phyl ++	$34.5^{Aa} \pm 2.2$	$18.5^{Aa} \pm 1.2$	$50.4^{Ba} \pm 2.5$	$87.8^{Cb} \pm 5.4$	$21.5^{Ac} \pm 0.1$
IR50	Control	$74.3^{Ab} \pm 2.9$	$24.3^{Ab} \pm 0.9$	$47.7^{Aa} \pm 0.9$	$29,2^{Ab} \pm 1.5$	$21.1^{Bb} \pm 1.3$
	Phyl +	$82.6^{Bb} \pm 2.9$	$27.0^{Bb} \pm 1.0$	$50.1^{Aa} \pm 0.6$	$25.3^{Ab} \pm 1.2$	$19.0^{Aa} \pm 0.45$
	Phyl ++	$98.0^{Cb} \pm 3.1$	$32.0^{Cb} \pm 1.0$	$49.5^{Aa} \pm 1.9$	$19.3^{Aa} \pm 4.5$	$18.7^{Ab} \pm 0.53$
IR100	Control	$92.6^{Ac} \pm 2.4$	$21.7^{Ab} \pm 0.6$	$51.0^{Bb} \pm 1.7$	$32.5^{Aa} \pm 6.9$	$17.7^{Ba} \pm 0.46$
	Phyl +	$95.1^{Ac} \pm 1.1$	$22.3^{Ac} \pm 0.3$	$48.6^{Aa} \pm 0.6$	$29.7^{Ba} \pm 2.7$	$17.3^{Aa} \pm 0.42$
	Phyl ++	$120.6^{Bc} \pm 2.2$	$28.3^{Bc} \pm 0.5$	$47.2^{Aa} \pm 1.5$	$30.8^{Ba} \pm 2.0$	$16.9^{Aa} \pm 0.22$
Significant of source of variation		(ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001)				
Phylazonit (Phyl)		***	***	ns	*	***
Water supply (W)		***	***	ns	***	***
(Phyl) * (W)		***	***	***	**	ns

Appendices 2. *Season 2016: Total biomass, water use efficiency (WUE), SPAD and Canopy temperature relative field PGPR contribution of non-inoculated (Control), pre-transplant inoculated (Phyl+), and field inoculated (Phyl++) plants in three irrigation supply treatments.*

Water supply	Treatments	Total biomass $t.ha^{-1}$	WUE $kg.m^{-3}$	SPAD	T^0 Canopy ^0C
IR0	Control	$137.2^{Aa} \pm 5.5$	$22.2^{Aa} \pm 1.9$	$47.2^{Aa} \pm 2.2$	$26.1^{Ac} \pm 0.2$
	Phyl +	$133.6^{Aa} \pm 14.2$	$27.0^{Ab} \pm 3.5$	$49.7^{Aa} \pm 2.3$	$25.6^{Ab} \pm 0.8$
	Phyl ++	$137.3^{Aa} \pm 5.8$	$26.9^{Ab} \pm 1.7$	$50.4^{Aa} \pm 2.5$	$26.0^{Aa} \pm 0.7$
IR50	Control	$138.4^{Aa} \pm 8.2$	$21.3^{Aa} \pm 2.5$	$50.4^{Aa} \pm 3.3$	$25.0^{Ab} \pm 0.4$
	Phyl +	$155.4^{Bb} \pm 9.7$	$28.1^{Bb} \pm 2.2$	$50.1^{Aa} \pm 0.6$	$24.9^{Ab} \pm 1.2$
	Phyl ++	$165.7^{Bb} \pm 8.4$	$30.9^{Bc} \pm 1.4$	$48.7^{Aa} \pm 1.0$	$24.7^{Aa} \pm 0.3$
IR100	Control	$150.4^{Aa} \pm 2.2$	$21.2^{Aa} \pm 1.5$	$50.9^{Aa} \pm 1.7$	$23.2^{Ac} \pm 0.3$
	Phyl +	$156.6^{Ab} \pm 9.2$	$20.4^{Aa} \pm 1.2$	$48.6^{Aa} \pm 0.6$	$23.3^{Ab} \pm 0.3$
	Phyl ++	$149.7^{Aa} \pm 6.4$	$20.8^{Aa} \pm 1.9$	$47.2^{Ab} \pm 1.5$	$23.7^{Aa} \pm 0.2$
Significant of source of variation		(ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001)			
Phylazonit (Phyl)		*	***	ns	ns
Water supply (W)		***	***	ns	***
(Phyl) * (W)		**	**	*	ns

Appendices 3. *The relationship between marketable yield and Brix in two years growing season.*





Appendices 4. *Total carotenene, Lutein, Lycopene, β-carotene and Ascobic acid of non-inoculated (Control), pre-transplant inoculated (Phyl+), and field inoculated (Phyl++) plants in three water supply treatments in 2015 season.*

Water supply	Treatments	Total carotene kg.ha^{-1}	Lutein g.ha^{-1}	Lycopene kg.ha^{-1}	β-carotene g.ha^{-1}	Ascorbic Acid kg. ha^{-1}
IR 0	Control	2.0^{Ba} ± 0.3	29.03^{Ba} ± 5.3	1.5^{Ca} ± 0.2	39.8^{Ba} ± 5.2	4.9^{Aa}±1.3
	Phyl +	1.3^{Aa} ± 0.2	16.51^{Aa} ± 2.8	1.0^{Ba} ± 0.2	23.9^{Aa} ± 4.6	5.5^{Aa}±0.6
	Phyl ++	0.8^{Aa} ± 0.1	13.89^{Aa}± 1.4	0.6^{Aa} ± 0.1	20.4^{Aa} ±2.3	4.4^{Aa}±0.4
IR 50	Control	6.0^{Ab} ± 0.7	61.7^{Ab} ± 11.7	4.1^{Ab} ± 0.5	126.0^{Ab} ± 2.5	16.14^{Ab}±1.6
	Phyl +	8.8^{Bc} ± 0.9	64.41^{Ab} ± 8	7.0^{Bc} ± 0.7	194.6^{Ab} ± 25.5	17.2^{Ab}±1.4
	Phyl ++	12.1^{Cc} ± 1.7	119.63^{Bc} ± 21.4	9.1^{Cb} ± 0.8	312.8^{Bb} ± 79.2	16.2^{Ab}±2.3
IR 100	Control	6.5^{Ab} ± 1.4	47.76^{Ab} ± 8.3	4.5^{Ab} ± 0.6	152.1^{Ab} ± 36.0	18.7^{Ab}±1.6
	Phyl +	6.7^{Ab} ± 0.9	55.39^{Ab} ± 6.2	4.01^{Ab} ± 0.6	212.4^{Bb} ± 32.0	19.6^{Bc}±1.3
	Phyl ++	7.2^{Ab} ± 1.2	57.47^{Ab} ± 6.4	4.7^{Ac} ± 0.8	227.3^{Bb} ± 34.4	23.5^{Bc}±3.2
Significant of source of variation (ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001)						
Phylazonit (Phyl)		***	***	***	***	ns
Water supply (W)		***	***	***	***	**
(Phyl) * (W)		***	***	***	***	ns

Appendices 5. *Season 2016: Total carotenene, Lutein, Lycopene, β-carotene and Ascobic acid of non-inoculated (Control), pre-transplant inoculated (Phyl+), and field inoculated (Phyl++) plants in three water supply treatments.*

Water supply	Treatments	Total carotene kg.ha^{-1}	Lutein g.ha^{-1}	Lycopene kg.ha^{-1}	β-carotene g.ha^{-1}	Ascorbic Acid kg. ha^{-1}
No Water supply	Control	$21.1^{Ab} \pm 2.6$	$1.8^{Aa} \pm 0.8$	$13.4^{Ab} \pm 1.3$	$1.2^{Aa} \pm 0.2$	$22.7^{Aa} \pm 2.4$
	Phyl +	$24.9^{Aa} \pm 5.4$	$1.9^{Aa} \pm 0.4$	$15.4^{Ba} \pm 2$	$1.4^{Aa} \pm 0.2$	$27.2^{Ba} \pm 3.0$
	Phyl ++	$24.2^{Aa} \pm 3.6$	$2.0^{Aa} \pm 0.3$	$17.3^{Ba} \pm 1.8$	$1.3^{Aa} \pm 0.2$	$25.6^{Ba} \pm 1.0$
Half Water supply	Control	$10.7^{Aa} \pm 4.4$	$2.8^{Ab} \pm 0.4$	$19.1^{Ac} \pm 1.6$	$1.3^{Aa} \pm 0.1$	$34.8^{Ab} \pm 7.0$
	Phyl +	$19.5^{Aa} \pm 4.0$	$2.0^{Aa} \pm 0.7$	$14.2^{Aa} \pm 1.8$	$1.8^{Ba} \pm 0.4$	$45.4^{Ab} \pm 6.7$
	Phyl ++	$18.8^{Aa} \pm 5.0$	$4.1^{Ba} \pm 1.1$	$29.7^{Bb} \pm 2.4$	$2.1^{Bb} \pm 0.2$	$40.0^{Ab} \pm 6.0$
Full Water supply	Control	$18.3^{Ab} \pm 1.3$	$1.1^{Aa} \pm 0.4$	$8.9^{Aa} \pm 2.1$	$1.0^{Aa} \pm 0.1$	$34.3^{Ab} \pm 8.1$
	Phyl +	$18.8^{Aa} \pm 2.0$	$1.4^{Aa} \pm 0.8$	$11.9^{Aa} \pm 2.8$	$1.3^{Ba} \pm 0.3$	$29.9^{Aa} \pm 2.9$
	Phyl ++	$33.7^{Bb} \pm 1.6$	$3.6^{Ba} \pm 1.5$	$22.8^{Bb} \pm 7.9$	$1.6^{Ba} \pm 0.4$	$37.1^{Ab} \pm 3.6$
Significant of source of variation		(ns= not significant, * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001)				
Phylazonit (Phyl)		***	***	***	***	ns
Water supply (W)		***	*	***	***	***
(Phyl) * (W)		*	**	**	*	*