

Doctoral School of Plant Science

Ph.D. Thesis

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

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1. INTRODUCTION

1.1. Importance and background

Nowadays, tomato is the most popular and important vegetable crops 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 the tomatoes are processed into multi-food products besides 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 tomato. It is influenced by variety 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 tomato (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 tomato requires 400-800 mm of water from transplanting to harvest (Steduto et al. 2012).

Drip irrigation is very efficient in saving water 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). 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 terminate irrigation for the duration of different phonological stages (Kuscu et al. 2014; Lei et al. 2009).

PGPRs have many benefits in the soil environment, it enriches all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate, and potassium solubilisation or mineralization (Adesemoye et al. 2008). They involve various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production (Bhardwaj et al. 2014). Singh and co-workers (2011) reported the application of bio-fertilizers as seed or soil inoculants, where the microorganism multiplied and participated in nutrient cycling and benefited crop productivity. In other research PGPR has increased marketable yield significantly, while reducing the fertilizer demand in tomato (Adesemoye et al. 2009). Other researchers found, that PGPR is a useful tool for enhancing phytochemicals in tomato (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 processing 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 physiological response of processing tomatoes
- 3. The effect 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. MATERIALS AND METHODS

2.1. Experimental fields and plant material

Open field experiments were conducted in 2015, 2016 on two locations of the Institute of Horticulture's farm at Szent István University, 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). In both growing season 2015 and 2016, we used the hybrid processing tomato: Uno Rosso F_1 (United Genetics Seeds Co. Hollister, CA, USA) for our plant materials.

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 bed center and 0.4 m in between the twin rows and 0.2 m between the plants.

2.2. PGPR material and treatments

The commercial Phylazonit produced in Hungary(https://phylazonit.hu/) have been used in two years experiment.

2.3. Metrological data.

Temperature and precipitation forecasts were obtained from the National Metrological Institute (<u>http://www.met.hu/en/idojaras</u>).

2.4. 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 expected daily average temperature (in °C) divided by five expressed in millimetre (Equation 1); deficit irrigation (IR50) supply calculated by haft of optimum irrigation. Tomato plants have received water supply three times per week through drip system.

2.5. Plant nutrition

During 2 years experiments, the plants fertilization demand and plant protection were conducted following Helyes and Varga (1994). Every week, 5 grams of the Ferticare (14-11-25) per square meter have been added to the cultivated area through the drip irrigation system to provide the plant nutrition. Ferticare 14-11-25 is a complex granulate chlorine-free fertilizer manufacture by YARA Company.

2.6. Harvesting

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

2.7. Field measurements

Field measurements depended on the weather condition, in two years growing, all of the measurements have been taken the same process. However, the first crop the period of water stress was longer, therefore, the measurements were taken frequently than in the second crop.

2.7.1. Soil water content

Soil water content was measured by digital soil moisture meter PT1 (Kapacitiv Kft., Budapest, Hungary) at six different soil depths (5, 10, 15, 20, 25, and 30 cm) before watering. Soil water content were taken continuously

during the plant development and prior to irrigation.

2.7.2. Stomatal conductance

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

2.7.3. Relative chlorophyll index

Relative chlorophyll index of the tomato leaves was measured by "Konica Minolta SPAD-502"; it is a rapid and non-destructive method to determine chlorophyll content in the field.

2.7.4. Chlorophyll fluorescence.

Chlorophyll fluorescence of the plants was determined by the PAM 2500 portable fluorimeter (Walz-Mess und Regeltechnik, Germany).

2.7.5. Leaf water potential (ψ leaf)

Leaf water potential (ψ leaf) was measured by pressure bomb (PMS Instruments Co., Corvallis, OR, USA).

2.7.6. Canopy surface temperature measurement (°C)

Canopy surface temperature determined by infrared thermometer technique (Raytek Raynger MX4, Santa Cruz, CA, USA).

2.7.7. Water use efficiency

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

2.8. Laboratorial measurements

2.8.1. *Proline:* The determination of proline was conducted as described by Bates et al., 1973.

2.8.2. Soil microbial activity

Total microbial activity was measured according to fluorescein diacetate hydrolysis (FDA) method (Green, Stott, and Diack 2006). The protocols to assess fluorescein diacetate hydrolysis activity was reported by Adam and Duncan (2001).

2.8.3. Root colonization determination

We determined by the gridline intersect method (Phillips and Hayman, 1970; Giovannetti and Mosse, 1980).

2.8.4. Analysis of carotenoid components and vitamin C.

Carotenoids and Vitamin C extraction was done according to the method of Daood et al (2013).

2.8.5. Soluble solid content estimation.

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

2.9. Statistical analysis

Analysis of variances was conducted by two ways ANOVA, the software IBM SPSS Statistics for Windows, Version 22.0. (IBM Hungary, Budapest, Hungary) was used to run statistical analyses.

3. 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: relationship between marketable yield and Brix. Appendices 5 & 6: total carotenoid; lycopene, β -carotene, Ascorbic acid did not give promising results compared to non-inoculated (Control) in two years.

In case of the first experiment (season 2015), the farm has been used for many years for field studies. In opposite, in 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 micro elements. Therefore, we have provided to 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 μ g of Fe, 81 μ g of B, 40 μ g of Zn.

3.1. 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. The different irrigation treatments started in the first week of June. The precipitation was recorded 186.3 mm,

which did not cover crop demand. Control block (IR0) only got 186.3mm of water during the vegetative development. Therefore, control tomato plants block (IR0) have got stress by drought during the growing season. Optimum (IR100) and deficit (IR50) irrigation supply have 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, and it paired with low precipitation, which caused drought for processing tomato in 2015, which is usual in Hungary.

In the second growing season of 2016 differed significantly from 2015, we started irrigation after 5 weeks from transplanting, because of the temporal distribution of precipitation. There was some 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. Optimal (IR100) and deficit (IR50) irrigation received 480 mm and 388 mm of irrigation water respectively, including 296 mm of rain. According to 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.

3.2 Effect of irrigation on the root microbial activity

PGPR root colonization were determined at harvest, which was similar to the colonization rate in Mycorrhizal inoculation (Bakr, 2018) or PGPR treatment (Cortivo et al. 2018). The PGPR treatment slightly enhanced the root colonization in inoculated plants even without present of irrigation treatment. Although a slightly higher in 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 were no significant different in effect of PGPR combine with irrigation control in PGPR treatments.

In the first growing season, fluorescein diacetate (FDA) hydrolysis showed higher in soil microbial activity in the no irrigation block. The deficit and full irrigation levels had non-significant effect on fluorescein diacetate (FDA) hydrolysis even it had a slight increase; microbial activity increasing in deficit irrigation block, may related to the positive interaction between the PGPR and Mycorrhizal microorganisms (Bardi and Malusá, 2012; Malusá and Ciesielska. 2015). Unlike in 2015, in the 2016 season the PGPR-Phyl++ inoculation did not enhance microbial activity in the root system; the only explanation is a higher level of precipitation compared to results in the 2015 growing season.

3.3 Effect of Phylazonit inoculation on physiological responses of tomato plants

3.3.1 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, improving plant growth and reducing damage of photosynthetic apparatus in Phyl++ plants under drought stress happened (Delfine et al. 2000; Baker and Rosenqvist 2004; Thankappan et al. 2019). In the growing season 2016, we recorded the values of PSII maximum efficiency (Fv/Fm) got higher in deficit irrigation plants (IR50) and there were no significant different from other treatments which means no photo-oxidative damage neither in full irrigated nor in unirrigated plants.

SPAD stands for Soil-Plant Analysis Development; SPAD value correlates with leaves' chlorophyll content. The high SPAD reading value indicates 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 in the Control under no irrigation (IR0) and deficit irrigation (IR50) in 2015. These results are supported by Puangbut et al. 2017; Adriano et al. 2018, higher chlorophyll content in inoculated plants under drought stress conditions. The higher chlorophyll content is accompanied by photosynthetic efficiency improvement. Unlike the growing season 2015, Phyl++ inoculation has been found no effect on leaf chlorophyll content or SPAD values in growing season 2016. in irrigation treatments (IR50 and IR100). It approved that in two blocks (deficit and full water supplies) or irrigation treatments, the tomato plants did not cause any drought stress in 2016.

3.3.2 Stomatal conductance and canopy temperature

Various water stress levels in plants caused by water deficits also reflected in stomatal conductance. 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 only 30.6 mmol m⁻² s⁻¹ for the full irrigated treatment, in 2015 and in 2016, these were 31.1, 31.5 and 30.6 mmol m⁻² s⁻¹, respectively. The data showed that in 2016

irrigation treatments had no effect in stomatal conductance. The explanation for this is that heavy seasonal raining was exceeding plants water requirements in some periods of the growing season in 2016.

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. 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.

3.3.3 Leaf water potential

Irrigation had positive effect on the water potential (Ψ L), decrease in irrigation levels, decreased Ψ L too (more negative) in Control- 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. These data also shows the differences between the two growing years in plants 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 Ψ L in plant leaves by (15, 12, and 02%) in IR0, IR50, and IR100 respectively in the growing season 2015 and by (06, 09, and 07%) in IR0, IR50, and IR100 respectively in 2016 growing season.

3.3.4 Proline concentration

Control and Phyl++ plants have increased proline accumulation in shoots in response to irrigation stress by more than two times 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 it does not reach significant levels in 2015. In Phyl++ plants shoots, the amount of proline content reduced from 29.2 to 19.2 mg kg⁻¹, and 25.0 to 15.6 mg kg⁻¹ in two growing seasons respectively 2015 and 2016 from IR50 block (Figure 22) compared to Control plants. Phyl++ inoculation had increased the water status of host plants, and lessened proline production, theses result in agreement with the result conducted in mycorrhizal plants (Bakr et al. 2017) with the processing tomatoes in open field.

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

In both growing seasons, Phyl++ inoculation and irrigation regulation have significantly increased the total biomass (fruits, stem and leaves) (Figure 23) except for the control block (IR0). In the 2015 growing season, plants have undergone two week of drought which caused decreased soil moisture and shortened the vegetative period. Compared to optimum irrigation treatment (IR100), in the control block (non Phyl++ inoculation) decreasing irrigation have 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. IR50 combined with Phyl++ inoculation increased total biomass by 32% (98.0 t/ha) and by 19% (165.7 t/ha) in the 2015 and 2016, respectively. However, 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 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 of the three irrigation regimes in 2015, and reached its maximum in the deficit irrigation.

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 ($P \le 0.05$) higher than in IR100 and control (IR0) with 12% and 22% respectively in 2015. Phyl++ treatment resulted in significantly ($P \le 0.001$) 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 respective control without Phyl++ application. In a combination of treatments, Phyl++ could increase WUE only in irrigated plots in 2015.

3.5 Effect of Phylazonit inoculation and irrigation on yield parameters

3.5.1 Total yield and non-marketable fruits

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, the plants only received half of their water demand. The optimal irrigation supply (IR100) raised the yield by 57 tons per hectare. The same trend happened in 2016 but for less extend and not reaching significant levels.

The effect of PGPR (Phylazonit) inoculation on the field was positive at all the irrigation levels. In both years, experiments with the best interaction between irrigation and PGPR treatments under full irrigation in 2015 (102.7 tons per hectare) and in 2016 reached 160.9 tons per hectare. Total yield increased by about 34% in both 2015 and 2016 in Phyl ++ treatment compared to the Control plants. Beside 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. The higher yield loss in 2016 Phyl++ plants in IR50 can be explained by the highest total yield (160 tons per hectare). Higher percentage of the total yield was rotten due to heavy rains during the ripening period in 2016 comparing to the 2015 growing season. Phyl++ inoculation affected fruit quality positively including less rotten fruits in both seasons and at all irrigation levels.

3.5.2 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 amounting to 22% (IR50) and 51% (IR100) compared to the control. PGPR treatment combined with better water supply further increased the yield of tomato, but not in the 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.

3.5.3 Soluble solid content

In the two seasons, "Brix and marketable yield had an 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). In 2015, the highest Brix was recorded in the control treatment (IR0) both 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: very strong in 2015 season ($R^2 = 0.96$) and moderate in the 2016 growing season ($R^2 = 0.18$) 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 2015 growing season and in 2016 growing season (R from 0.70 to 0.72).

3.5.4 Carotenoids and ascorbic acid.

Regardless of yield, inoculation, and irrigation levels, total carotene production ranged from 0.8 to 12.1 kg ha⁻¹, which is almost a fifteen times difference (Table 10). The value of total carotene production depended on the marketable yield in the IR0, we found significant reduction in Phyl++ samples. However, irrigation regimes increased the carotenoids yield. In IR100 Phyl++ treatment enhanced slightly 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 carotene was recorded (12.1 kg ha⁻¹). Lycopene and β -carotene increased by 126 and 148%, respectively in Phyl++. 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 μ g g⁻¹) compared to the 2016 season range (330 to 418 μ g g⁻¹). According to Helyes et al, 2006, the ascorbic acid content in processing tomatoes were in the normal ranges from 286 to 446 mg kg⁻¹.

Increasing amount of irrigation has negatively affected and significantly reduced total carotenoid yeild 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. This negative trend was found more evident for lycopene from the IR0 to IR100. 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 10).

The effect of PGPR on total carotene and lycopene only emerged under irrigated conditions. However, positive effects were detected in the case of β -carotene in the rainfed control too. Phylazonit application did not affect ascorbic acid yields at all, but the effect of irrigation was expressive. Irrigation effect under IR100 irrigation regime was not significant for total carotene and β -carotene either, but it was expressional in the IR50 treatment to total carotene and the measured carotene components too. Total carotene, lycopene and ascorbic acid yields were affected by irrigation when additional water supply was not provided, but β -carotene wasn't.

4 CONCLUSIONS AND RECOMMENDATIONS

The two-year results in field-based experiment approved that commercially 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 effect than pre-transplant inoculation at sowing but increasing the cost. Colonization rate was higher than in control samples, Phylazonit inoculation improved plant development, yield, carotenoids, and lycopene as well as stomatal conductance, water use efficiency especially under deficit irrigation condition.

We found that the result on leaf water potential, stomatal conductance, canopy temperature, and water used efficiency in samples treated with Phylazonit (field -innoculation) 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 fieldtreatment enhanced the performances of tomato plants comparing to the Control samples. There were significant differences recorded in stomatal conductance, water use efficiency, canopy temperature, leaf water potential, Fv/Fm, SPAD content in the field-inoculation samples. Its partial reduced the water stress during the drought condition happened. These results supported

that Phylazonit symbiosis improve their host plants by increasing the water uptake through the regulation of the stomatal closure in plant.

Under deficit irrigation, Phylazonit inoculation at transplant enhanced the crop yields more efficiently than full irrigation. The results recorded higher in the number of carotenoids, lycopene, and β -Carotene and fruit set in 2016 growing season on loamy soil opposite 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 the better water holding and texture is accounted for the higher Phylazonit efficient performance on the tomato plant.

NEW SCIENTIFIC RESULTS

- 1. During 2 years of the experiments, I have found that, the time of treatment has a considerable impact on the efficiency of PGPR. The result from plants physiological responses, biochemical changes, plant production, and fruits 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 impact on the processing tomato plants could be reduced with Phylazonit application.
- 3. I supported that, PGPR-Phylazonit inoculation at transplant can enhance the water use efficiency, 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 impact by raising the water and nutrient uptake mechanism. Less organic and inorganic osmolytes in plants induced to moderate water deficit stress, supported by most important indices of plant water status (leaf water potential, stomatal conductance, and canopy temperature) are definite field-based proofs that the water and nutrient uptake meaningfully increased by the PGPR-Phylazonit inoculation. In another word PGPR-Phylazonit biofertilizer inoculation protected the plants from the water deficit
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instead of stimulating them to tolerate the stress. Also, it was found that, the positive effect of the PGPR-Phylazonit inoculation on stomatal regulation is partially contributed to the mediation of the water tress by sustaining plant soil water balance.

 I indicated that, PGPR-Phylazonit field-inoculation (Phyl++) could improve the fruit quality (higher Soluble solid-, Carotenoids-, βcarotene-, and lycopene- contents) accompanied by a meaningful increase of tomato yield particularly under deficit irrigation conditions.

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- 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. <u>https://doi.org/10.21273/HORTSCI13048-18</u>.
- 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.* <u>https://doi.org/10.17221/818/2017-PSE</u>.
- 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.

- 5. Le Anh Tuan, Helyes Lajos, Pék Zoltán, (2017). The simultaneous effect of water stress and biofertilizer on physiology and quality of processing tomato. Asian food conference.
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