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# The effect of plant growth-promoting rhizobacteria on yield, water use efficiency and Brix Degree of processing tomato

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**ABSTRACT**

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Open field experiments were conducted to investigate the effects of plant growth-promoting rhizobacteria (PGPR) (Phylazonit MC®) as a biofertilizer on processing tomato cultivar var. Uno Rosso $F_1$, grown under three different regimes of water supply. Field effectiveness of rhizobacteria inoculation on total biomass production, yield and water use efficiency, were examined in 2015 and 2016. Seedlings were inoculated with 1% liquid solution of Phylazonit MC® (*Pseudomonas putida*, *Azotobacter chroococcum*, *Bacillus circulans*, *B. megaterium*; colony-forming unit: $10^9$ CFU/mL) at sowing and planting out by irrigation. There were three different regimes of water supply: rain-fed control (RF); deficit water supply (WS50) and optimum water supply (WS100); the latter was supplied according to the daily evapotranspiration by drip irrigation. Total aboveground biomass (shoot and total yield) and red fruits yield were measured at harvest in August, in both years. Total biomass changed between 32.5 t/ha and 165.7 t/ha, the marketable yield from 14.7 t/ha to 119.8 t/ha and water use efficiency (WUE) between 18.5 kg/m$^3$ to 32.0 kg/m$^3$. The average soluble solids content of the treatment combinations ranged from 3.0 to 8.4°Brix. Seasonal effects were significant between the two years with different precipitation, which manifested in total biomass and marketable yield production. PGPR increased WUE only in WS50 in both years, while under drought stress and higher water supply, the effect was not clear. The effect of PGPR treatment on marketable yield, total biomass and WUE was positive in both years when deficit irrigation was applied and only in the drier season in the case of optimum water supply.

**Keywords**: vegetable crop; water stress; *Solanum lycopersicum* L.; microorganisms; rainfall

Tomato is one of the most popular and important vegetable crops grown all over the world. Processing tomato production was 38 Mt worldwide and 10.6 Mt in Europe in 2016 (World Processing Tomato Council 2017). Tomato quality is affected by the interactions between cultivars, environmental factors such as light, temperature and water supply, and the composition of the nutrient solution and crop management (Dorais 2007, Helyes et al. 2014).

Production of processing tomato requires 400–800 mm of water from transplanting to harvest. Drip irrigation is very efficient in saving water itself, but its efficiency can be increased by applying deficit irrigation (DI) in the field (Battilani et al. 2012). This irrigation method causes water stress to plants, but if the yield reduction is lower than the benefit it gets from the water saving or quality improvement, then the lower yield becomes less important (Johnstone et al. 2005, Pék et al. 2017).

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Effects of DI vary year by year and it affects crops differently, moreover soil also influences its impact (Helyes and Varga 1994). The most common water deficit applied is 50% of evapotranspiration (Bakr et al. 2017), but other rates can be used as well (Nguyen et al. 2012, Patanè et al. 2014, Al Mamun Hossain et al. 2017). Other techniques include the application of different DI rates in different vegetative stages (Kuşçu et al. 2014, Nangare et al. 2016), or simply terminated irrigation for the duration of different phenological stages (Johnstone et al. 2005, Lei et al. 2009). In processing tomato production, water use efficiency (WUE) is the ratio between the yield produced by the plant and the consumed water (Battilani et al. 2009). In this way, WUE can be considered as relatively constant for a given crop under a given climate (Patanè et al. 2014). WUE is a very useful index for demonstrating the efficiency of water use in plant production which may allow saving of irrigation water, contributing to the preservation of this restricted resource (Battilani et al. 2009).

Plant growth-promoting rhizobacteria (PGPR) have many benefits in the soil environment, they can improve the availability of micro- and macronutrients via nitrogen fixation, solubilisation of phosphate and potassium from sparingly soluble forms or mineralization of plant nutrients that are bound in organic matter (Adesemoye et al. 2008). PGPR have also increased marketable yield significantly, while reducing the fertilizer demand in tomato (Adesemoye et al. 2009). Other researchers found, that PGPR are a useful tool for enhancing phytochemicals in tomato (Sabin et al. 2017) especially under stressful conditions (Ruzzi and Aroca 2015).

Soluble solids content (SSC) is a refractometric index, which specifies the percentage of solids in tomato fruits, and it is measured in °Brix (Johnstone et al. 2005). For tomato processing, the lower yield per hectare and higher SSC of tomato fruits results in higher income, because of the high cost of transportation and volatilization (Grandillo et al. 1999, Hartz et al. 2005). Nowadays, profitable production is over 100 t/ha yield to stay in competition and reach high SSC, because of its strong negative correlation with the cost of processing (Barrios-Masias and Jackson 2014). The effects of yield levels are not so significant as the effect of SSC on cost (Rocco and Morabito 2016). The fulfilment of these two needs is a great challenge and demands professional research work related to the different production technology (Nichols 2006).

The PGPR bio-fertilizer Phylazonit MC® from Phylazonit Ltd. (Nyíregyháza, Hungary) can be used for many horticultural plant cultures (Gajdos et al. 2009, Balla-Kovács 2010).

The aim of this study was to investigate the effect of the PGPR product Phylazonit MC® on processing tomato cv. Uno Rosso $F_1$ under three different irrigation regimes. Plants treated at the time of sowing with PGPR were evaluated for the total biomass, yield, SSC and water use efficiency.

## MATERIAL AND METHODS

**Plant material**. Open field experiments were conducted with processing tomato cv. Uno Rosso $F_1$ (United Genetics Seeds Co. Hollister, USA), in 2015 (Location 1: 47.594292'N, 19.359758'E), 2016 (Location 2: 47.577380'N, 19.379573'E), Institute of Horticulture's farm at the Szent István University, Gödöllő, Hungary. Location 1 was classified as Calcaric Arenosol, sandy loam in texture, consisted 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) and low in organic carbon, $NO_3^-$-$N_{KCl}$ (5 g/kg), $P_{AL}$ (6.6 g/kg), $K_{AL}$ (29.1 g/kg). Soil type of location 2 was Calcaric Arenosol, loam soil in texture (41% sand, 47.5 silt and 11.5% clay) with bulk density of 1.49 g/cm$^3$, 25% field capacity, free from salinity (0.212 dS/m) and low in organic matter, consisted of $NO_3^-$-N (8.6 g/kg), $P_{AL}$ (3.49 g/kg), $K_{AL}$ (47.1 g/kg). The sowing was carried out on the 13$^{th}$ of April in a greenhouse using the Klasmann TS3 substrate in plastic trays (256 seeds/tray). The experimental design was a randomized complete block, 24 m$^2$ of plot size with four replicates. Seedlings were arranged in double (twin) rows with a distance of 1.6 m between bed centres and 0.4 m in between the twin rows and 20 cm between the plants. Seedlings were planted out 4 weeks after sowing at the development stage BBCH 104 (Hack et al. 1992). Weight of aboveground biomass and marketable yield were measured removing the total of 10 plants in each plot at harvest on August 18$^{th}$ and August 28$^{th}$ in 2015 and 2016, respectively.

**PGPR and irrigation treatments**. Immediately after sowing, plastic trays were inoculated with

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liquid solution of Phylazonit MC® (PGPR). Some trays were not inoculated (control). This solution is a mixture of *Pseudomonas putida*, *Azotobacter chroococcum*, *Bacillus circulans*, and *B. megaterium* produced by Phylazonit Ltd., located in Hungary (Gajdos et al. 2009, Balla-Kovács 2010). Seedlings were inoculated with 1% Phylazonit MC®(2 mL/plant) and again after planting out (41 667 plants/ha) the same solution (4170 L/ha) was given by the drip irrigation system.

Temperature and precipitation were recorded six times per hour using a Campbell 21X Datalogger meteorological station (Campbell Scientific Inc., Logan, USA). The daily amount of irrigation demand was calculated by potential evapotranspiration (ETc) and crop coefficient ($K_c$) using CROPWAT 8.0 software (Rome, Italy), where tomato was the reference crop (Surendran et al. 2017).

There were two different irrigation regimes, based on ETc: ETc × $K_c$, meaning optimum water supply (WS100), and half of this, 0.5 × ETc × $K_c$, DI (WS50), which were compared with unirrigated, rain-fed control (RF). The crop coefficient $K_c$, ranged between 0.4 and 0.7 from planting out (BBCH 104) to crop establishment (BBCH 109); between 0.7 and 1.1 from crop establishment to main flowering (BBCH 609); between 1.1 and 0.8 from the beginning of flowering to the beginning of fruit set (BBCH 701); between 0.8 and 0.6 from the beginning of fruit set to full maturity (BBCH 802) of the 1st and 2nd fruit truss. Harvest index (%) was calculated as the ratio of marketable yield (t/ha) and total biomass (t/ha).

Water use efficiency (kg/m$^3$) was calculated as the ratio of marketable yield on fresh weight basis (FW, kg/ha) at harvest and total water used (ET, m$^3$/ha), as measured by water balance (Patanè et al. 2014).

Degree Brix (°Brix), as an indicator of the soluble solids concentration in tomato fruits, was estimated by the Digital Refractometer Krüss DR 201-95 from the homogenized fruit samples (Krüss Optronic, Hamburg, Germany).

**Statistical analysis**. The software IBM SPSS version 23.0 for Windows (IBM Hungary, Budapest, Hungary) was used for data analysis. The effects of Phylazonit MC®, irrigation regimes, and their interaction were determined by two-way ANOVA. Mean values ($n$ = 4) not sharing the same letters are significantly different at ($P \leq 0.05$) as determined by the Tukey's test.

## RESULTS AND DISCUSSION

**Yield and total biomass**. Irrigation for optimum (WS100) and deficit (WS50) water supply received in total (including rainfall) 426.3 mm and 306.3 mm, respectively. Seasonal temperature in both years was high. In 2015, heat was paired with low precipitation (186.3 mm) inducing drought stress for processing tomato, which is usual in Hungary (Pék et al. 2017). Season of 2016 differed significantly from 2015. The total precipitation amount was with 296 mm (RF) almost double compared to 2015. In addition, WS100 and WS50 received 480 mm and 388 mm of the total water, respectively, during the season of 2016.

In 2015, WS50 and WS100 increased significantly the marketable yield by 384% and 465% respectively, whereas in 2016, the respective yield increases were lower amounting to 22% (WS50) and 51% (WS100) compared to RF. PGPR treatment combined with better water supply further increased the yield of tomato, but not in RF and WS100 in 2016. WS50 combined with PGPR increased the marketable yield by 28% (72.6 t/ha) in 2015 and by 45% in 2016 reaching the highest value of 119.8 t/ha in that year (Figure 1). This finding is in agreement with previous studies of processing tomato (Helyes et al. 2014, Pék et al. 2017).

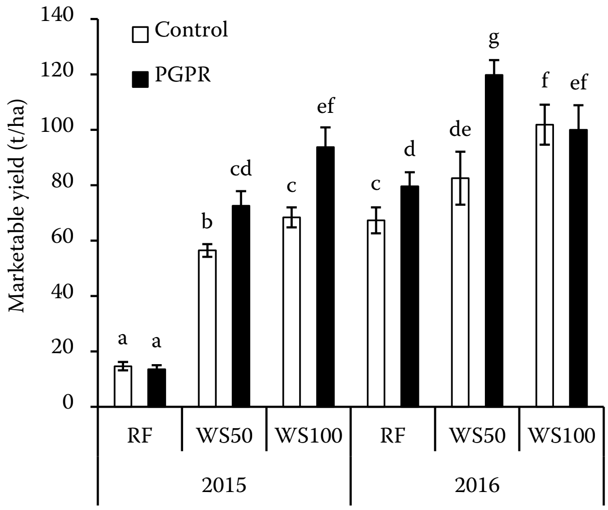


Figure 1. Mean values of marketable yield on fresh weight basis in different irrigation and plant growth-promoting rhizobacteria (PGPR) treatment combinations in 2015 and 2016. Columns bearing the same letter are not significantly different at $P \leq 0.05$ and vertical bars represent significant differences ($n$ = 4). RF – rain-fed control; WS50 – deficit water supply; WS100 – optimum water supply

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A positive effect of drip irrigation could be realized through the inhomogeneity of the soil moisture distribution concluded by Selim et al. (2012) and increased microbial activity mobilizing mineral nutrients by mineralization according to Wang et al. (2017).

SSC of tomato fruits was often very high without irrigation (Helyes et al. 2014). Irrigation decreased SSC significantly compared with control (8.03, 5.03, 3.73°Brix, respectively) in 2015, but had no significant effect (3.65, 4.45, 3.55°Brix) in 2016. Tendency in PGPR treatments combination with different water supplies was mostly the same (7.60, 4.13, 4.38°Brix) in 2015. As a result of better water conditions, decreasing SSC also appeared (4.10, 4.13, 3.05°Brix) in 2016. The greatest effect of increasing soil water deficit was the rise in soluble solids and a decrease in yield, which was expectable and agrees with the other researchers (Patanè and Cosentino 2010). However, a clear PGPR effect on SSC was not observed in this study, as found by other researchers (Selim et al. 2012).

Irrigation increased significantly total biomass production (228%, 284%) in 2015, but only slightly in 2016 (1%, 10%), compared to RF (Figure 2). WS50 combined with PGPR increased total biomass by 32% (98.0 t/ha) and by 19% (165.7 t/ha) in the two years, respectively. However, PGPR increased total biomass significantly by 30% (120.6 t/ha) only in 2015 in WS100 treatment, it was not effective in 2016 (99%).

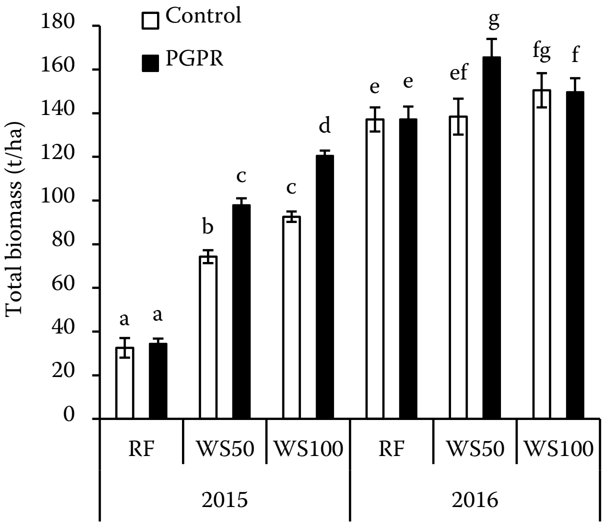


Figure 2. Mean values of total biomass (fresh weight) in different irrigation and plant growth-promoting rhizobacteria (PGPR) treatment combinations in 2015 and 2016. Columns bearing the same letter are not significantly different at $P \leq 0.05$ and vertical bars represent significant differences ($n$ = 4). RF – rain-fed control; WS50 – deficit water supply; WS100 – optimum water supply

Higher water supply resulted in harvest index higher by 7% and 16% WS50 and WS100, respectively only in 2015, in agreement with Lei et al. (2009), but it showed statistically no difference in 2016. 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. PGPR increased the harvest index in all of the three irrigation regimes in 2015, and reached its maximum in the deficit irrigation. Yet, there was no change in 2016 (Table 1).

**Water use efficiency**. WUE as the main indicator of plant water status is regulated by physiological processes (Lei et al. 2009). WS50 produced the best results of WUE (24.3 kg/m$^3$), significantly ($P \leq 0.05$) higher than in WS100 and RF with 12% and 22% respectively in 2015. PGPR treatment resulted in significantly ($P \leq 0.001$) higher WUE in both WS50 (32%) and WS100 (30%). The maximum WUE was achieved at 32 kg/m$^3$ in WS50 with the PGPR treatment compared to the respective control without PGPR application (Table 1). In a combination of treatments, PGPR could increase WUE only in irrigated plots in 2015. DI usually increases WUE (Patanè et al. 2011, 2014), but this effect was detected in this study only in combination with PGPR in 2015. With respect to all water supply regimes (RF, WS50, WS100), no difference was found in WUE of the control samples without PGPR in 2016, either. Better WUE was reached by PGPR treatments, in combination with RF (26.9 kg/m$^3$) and WS50 (30.9 kg/m$^3$) in 2016, which were mostly the same values as in the previous year (Table 1). Higher WUE than 10 kg/m$^3$ is usual in the Mediterranean climate (Patanè et al. 2011, Kuşçu et al. 2014, Giuliani et al. 2016), and all results exceeded this value in both years.

The most important quality factor of processing tomato is the °Brix, which can be very high without irrigation (Patanè and Cosentino 2010, Helyes et al. 2014, Kuşçu et al. 2014). °Brix was significantly higher in RF (7.3–8.4), and WS50 was also higher (3.6–5.5) than WS100 (3.0–4.9) without PGPR, while PGPR treatments showed higher variability in irrigated (3.0–5.2) and lower in RF (7.3–7.9) plots. WS50 and WS100 reached higher

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Table 1. Mean values of harvest index and water use efficiency (WUE) in different irrigation and plant growth-promoting rhizobacteria (PGPR) treatment combinations in two years

| | | Harvest index (%) | | WUE (kg/m$^3$) | |
|---|---|---|---|---|---|
| | | control | PGPR | control | PGPR |
| 2015 | RF | 0.44 ± 0.06$^{aA}$ | 0.68 ± 0.06$^{abB}$ | 19.8 ± 2.4$^{aA}$ | 18.5 ± 1.2$^{aA}$ |
| | WS50 | 0.51 ± 0.04$^{abA}$ | 0.76 ± 0.03$^{bB}$ | 24.3 ± 0.9$^{bA}$ | 32.0 ± 1.0$^{cB}$ |
| | WS100 | 0.60 ± 0.05$^{bA}$ | 0.75 ± 0.05$^{bB}$ | 21.7 ± 0.6$^{aA}$ | 28.3 ± 0.5$^{bB}$ |
| 2016 | RF | 0.60 ± 0.07$^{bA}$ | 0.60 ± 0.05$^{aA}$ | 22.2 ± 1.9$^{abA}$ | 26.9 ± 1.7$^{bB}$ |
| | WS50 | 0.60 ± 0.09$^{bA}$ | 0.63 ± 0.04$^{aA}$ | 21.3 ± 2.5$^{abA}$ | 30.9 ± 1.4$^{cB}$ |
| | WS100 | 0.59 ± 0.07$^{bA}$ | 0.66 ± 0.04$^{aA}$ | 21.2 ± 1.5$^{aA}$ | 20.8 ± 1.9$^{aA}$ |

Different letters in the same column and different capitals in the same row represent significant differences at $P \leq 0.05$ ($n$ = 4). RF – rain-fed control; WS50 – deficit water supply; WS100 – optimum water supply

marketable yield in the range of 65 to 126 t/ha with PGPR and only 55 to 109 t/ha without it. °Brix and marketable yield had an adverse relationship (Pék et al. 2017). The higher the yield production rose (more than 55 t/ha in average) the lower the °Brix obtained (below 5.5 in the irrigated samples). Regression analyses showed a different correlation between marketable yield and °Brix as affected by PGPR. According to the slope of power regressions, °Brix decreased to a lesser extent with increasing marketable yield when the plants were treated with PGPR than without PGPR, but this effect only prevailed under irrigated conditions (Figure 3).

Cultivar var. Uno Rosso was popular for processing tomato production near northern limit of cultivation in recent years, because of its good Brix yield per hectare (Andrejiová et al. 2016, Bakr et al. 2017, Helyes et al. 2017). Cut-off is a very useful DI method to increase °Brix under Mediterranean climate conditions (Mácua et al. 2003, Johnstone et al. 2005, Patanè et al. 2013), but not under Hungarian weather conditions (Helyes et al. 2012). Season-long deficit irrigation is more useful in Hungary, because of the expectable rainy period before harvest. Positive effects of irrigation on yield and of water deficit on °Brix agree with other researchers (Patanè et al. 2014, Pék et al. 2017). According to multiple correlation of determination, marketable yield had a great effect on °Brix with ($R^2$ = 0.75) or without ($R^2$ = 0.83) PGPR, which is due to better water supply (Figure 3).

Applied PGPR solution could increase tomato yield, and increase mineral nutrient availability by improved root growth, as previously published about *A. chroococcum* (Baba et al. 2018) and *B. circulans* (Mehta et al. 2015). Plant growth-promoting effects of *B. megaterium* and *P. putida* on tomato could be due to phytohormonal effects, like ethylene-related processes, which resulted in higher yield in rockwool (Hernández-Montiel et al. 2017) or in soil (Aslam et al. 2018).

Tomato yield, WUE and °Brix were significantly influenced by water supply and DI was a useful tool to enhance soluble solids content of fruit. The effect of PGPR treatment was not clearly positive under all treatment combinations, except for between 296–426 mm total water supply. A positive impact of PGPR treatment combined with DI (WS50) was realized in both years in terms of biomass production, marketable yield, harvest index and WUE. Additional studies are needed

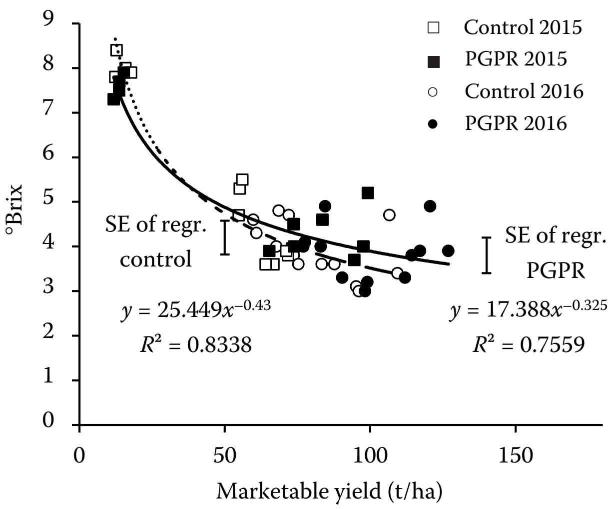


Figure 3. Effect of yield on soluble solids content (°Brix) with plant growth-promoting rhizobacteria (PGPR) and control in 2015 and 2016. Vertical bars represent standard error (SE) of regressions ($n$ = 24)

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to determine how the time of PGPR application and the amount of irrigation water can be further optimized.

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