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Application of biostimulants in tomato subjected to water deficit: Physiological, enzymatic and production responses¹

Aplicação de bioestimulantes em tomateiro submetido à deficiência hídrica: Respostas fisiológicas, enzimáticas e produtivas

Mariane Peripolli^{2*}, Sylvio H. B. Dornelles³, Sidinei J. Lopes⁴, Luciane A. Tabaldi³,
Vinícius S. Trivisoli² & Jaíne Rubert⁵

¹ Research developed at Universidade Federal de Santa Maria, Departamento de Biologia, Santa Maria, RS, Brasil

² Universidade Federal de Santa Maria/Programa de Pós-Graduação em Agronomia, Santa Maria, RS, Brasil

³ Universidade Federal de Santa Maria/Departamento de Biologia, Santa Maria, RS, Brasil

⁴ Universidade Federal de Santa Maria/Departamento de Fitotecnia, Santa Maria, RS, Brasil

⁵ Universidade Federal de Santa Maria/Programa de Pós-Graduação em Agrobiologia, Santa Maria, RS, Brasil

HIGHLIGHTS:

The application of Seed⁺ and Crop⁺ biostimulants reduced the damage caused by water stress in tomato plants.

The mean productivity of tomato fruits increased with the use of biostimulants regardless of the soil water condition.

The application of biostimulants significantly influenced the activity of the enzymes SOD and POD.

ABSTRACT: The objective of this study was to evaluate the effect of the application of the biostimulants Seed⁺ and Crop⁺ on physiological and production variables and on the activity of antioxidant enzymes (superoxide dismutase - SOD and guaiacol peroxidase - POD) in tomato plants subjected to two soil water conditions. The experiment was carried out in a greenhouse, in a 2 x 2 x 6 factorial scheme, with two times of application of the biostimulants (flowering and fruiting), two soil water conditions (50 and 100% of soil water holding capacity) and six biostimulants (control treatment; Seed⁺; Seed⁺ + Crop⁺ 1x; Seed⁺ + Crop⁺ 2x; Crop⁺ 1x; + Crop⁺ 2x). The experimental design was completely randomized, with four repetitions. The biostimulants Seed⁺ and Crop⁺ increased the quantum yield of photosystem II (Fv/Fm), regardless of the time of application and water condition of the soil. The biostimulants Seed⁺ + Crop⁺ 2x and Crop⁺ 2x stood out in the pre-morning period, with an average Fv/Fm of 0.813, under the conditions tested. The highest SOD activity (372.12 U mg⁻¹ of protein) was obtained with Crop⁺ 2x biostimulant in fruiting and under water deficit. For POD, when under water deficit, the best results were obtained with the biostimulants Seed⁺ + Crop⁺ 2x, Crop⁺ 1x and Crop⁺ 2x in flowering (810.94; 691.19 and 921.59 U mg⁻¹ protein) and in fruiting (703.60; 800.00 and 972.62 U mg⁻¹ protein). Thus, the use of Seed⁺ and Crop⁺ biostimulants can be an alternative to help mitigate the damage caused by water deficit in tomato crop.

Key words: *Solanum lycopersicum*, water condition, oxidative stress

RESUMO: Objetivou-se neste estudo avaliar o efeito da aplicação dos bioestimulantes Seed⁺ e Crop⁺ nas variáveis fisiológicas e produtivas e na atividade de enzimas antioxidantes (superóxido dismutase - SOD e guaiacol peroxidase - POD) em plantas de tomateiro submetidas a duas condições hídricas do solo. O experimento foi conduzido em casa de vegetação, em esquema fatorial 2 x 2 x 6, sendo duas épocas de aplicação dos bioestimulantes (florescimento e frutificação), duas condições hídricas do solo (50 e 100% da capacidade de retenção de água no solo) e seis bioestimulantes (tratamento controle; Seed⁺; Seed⁺ + Crop⁺ 1x; Seed⁺ + Crop⁺ 2x; Crop⁺ 1x; + Crop⁺ 2x). O delineamento experimental foi inteiramente casualizado, com quatro repetições. Os bioestimulantes Seed⁺ e Crop⁺ aumentaram o rendimento quântico do fotossistema II (Fv/Fm), independente do momento de aplicação e da condição hídrica do solo. Os bioestimulantes Seed⁺ + Crop⁺ 2x e Crop⁺ 2x, destacaram-se no período de antemanhã, apresentando média de Fv/Fm de 0,813, nas condições testadas. A maior atividade de SOD (372,12 U mg⁻¹ de proteína) foi com bioestimulante Crop⁺ 2x na frutificação e sob déficit hídrico. Para POD, quando em deficiência hídrica, os melhores resultados ocorreram com os bioestimulantes Seed⁺ + Crop⁺ 2x; Crop⁺ 1x e Crop⁺ 2x na floração (810,94; 691,19 e 921,59 U mg⁻¹ de proteína) e na frutificação (703,60; 800,00 e 972,62 U mg⁻¹ de proteína). Assim, a utilização dos bioestimulantes Seed⁺ e Crop⁺ pode ser uma alternativa para auxiliar na mitigação dos danos causados pela deficiência hídrica na cultura do tomateiro.

Palavras-chave: *Solanum lycopersicum*, condição hídrica, estresse oxidativo

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* Corresponding author - E-mail: mperipolli@gmail.com

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) has socioeconomic importance in the world, with global production of 174 million tons, and its main producers are: United States, Italy and Turkey (IBGE, 2019). In Brazil, the average production is approximately 4.5 million tons, with São Paulo, Goiás and Minas Gerais as the main producing states, accounting for 81% of the national production (IBGE, 2019). Crop yield is directly linked to abiotic and biotic factors in the field; however, the imbalance of these factors is a serious threat to agriculture (Ahmed et al., 2016). Damage to the plant can occur at the molecular, biochemical, morphological and physiological levels (Loreti et al., 2016).

Water deficit is one of the factors that most interfere in the morphophysiological processes of the plant, as it is responsible for several biochemical and metabolic processes (Mozdzen et al., 2015). Physiologically, water deficit causes oxidative stress in plant cells, increasing the production of reactive oxygen species (ROS), in addition to reducing plant growth and yield (Yasar et al., 2016; Alves et al., 2018). ROS detoxification mechanisms exist in all plants and encompasses the activation of various enzymes, including superoxide dismutase (SOD), catalase and guaiacol peroxidase (POD) (Meloni et al., 2003).

In order to mitigate the damage caused by water deficit, the development of biostimulants can contribute to improving the physical-chemical properties of the soil and the absorption, translocation and use of nutrients by plants, including increased resistance to various stresses (Calvo et al., 2014; Jardim, 2015). They contain in their formulation extracts of seaweed, *Ascophyllum nodosum*, which generates tolerance against biotic and abiotic stresses, favoring the signaling in plants for the production of elicitors or osmoprotective substances, for having high contents of glycine-betaine (GB) and proline (Bulgari et al., 2015).

Although the beneficial effects of biostimulant application have been proven for several crops, studies with tomatoes are scarce and may serve as a basis for further research. Thus, the objective of this study was to evaluate the effect of the application of biostimulants Seed⁺ and Crop⁺ on the physiological and production variables and on the activity of antioxidant enzymes (SOD and POD) in tomato subjected to two soil water conditions.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse in the municipality of Santa Maria, RS, Brazil (29° 38' 39.82" S and 53° 57' 44.26" W, and altitude of 113 m). The experimental design was completely randomized in a factorial scheme 2 x 2 x 6, corresponding to: factor A - two soil water conditions (50 and 100% of soil water holding capacity - WHC); factor B: two times of application of biostimulants (beginning of flowering and beginning of fruiting); and factor C: biostimulants (control treatment; Seed⁺; Seed⁺ + Crop⁺ 1 x commercial dose; Seed⁺ + Crop⁺ 2 x commercial dose; Crop⁺ 1 x commercial dose; Crop⁺ 2x commercial dose), with four repetitions.

Soil water holding capacity (WHC) was determined by drying until it reached constant weight (oven at 70 °C). Subsequently, the dry soil was irrigated until its reached saturation and then weighed. By subtraction between the mass of the pot with dry soil and the mass of the pot with moist soil, the amount of water required to reach 100% WHC was obtained.

Sowing was carried out in polystyrene trays with 200 cells filled with Mecplant[®] substrate, which were kept in a greenhouse, under floating irrigation. For treatments with Seed⁺, which contains in its formulation micro and macronutrients, in addition to 20% of fermented extract of *Ascophyllum nodosum* seaweed, the biostimulant was applied at dose of 100 mL 100 L⁻¹ of water, as indicated in the leaflet, in the floating and in the other treatment, using only water in the floating. The seedlings remained in this system until the moment of transplantation.

The seedlings were transplanted to 9-L black polypropylene pots, filled with 8.5 kg of soil, sieved, homogenized and with corrected acidity, according to the soil physical analyses report of the Soil Physics Laboratory – UFSM. The soil is considered type 2, belonging to sandy loam textural class (SBCS), with base saturation < 50, 60.6 of sand, 22.8% of silt and 16.6% of clay. Irrigation was carried out daily, leaving the soil close to its field capacity, in order to ensure a good establishment and initial development.

These amounts of water were supplied regularly through the weighing method, using an electronic scale (ACS System) with precision of 5 g, by adding water until reaching the total predetermined mass (pot + dry soil + water volume to reach 100 or 50% soil WHC). To determine soil water conditions (50 and 100% soil WHC), the following adapted formulas were used (Schwab, 2011):

$$MP50\% = (MPWHC - MPdry)0.5 + MPdry \quad (1)$$

$$MP100\% = (MPWHC - MPdry)1.0 + MPdry \quad (2)$$

where:

- MPn% - mass of pot for each treatment;
- MPWHC - mass of pot at water holding capacity; and,
- MPdry - mass of pot filled with dry soil.

The application of the biostimulant Crop⁺, composed of 10% extract of *Ascophyllum nodosum* and micro and macronutrients, was performed at doses of 100 and 200 mL 100 L⁻¹, applied in the flowering and fruiting development stages, considered to have the highest water demand for the crop. After the application of the biostimulant Crop⁺, water deficit was induced in the soil for a period of 15 days.

After the period of exposure to the treatments, evaluations of chlorophyll a fluorescence variables were performed in the middle third of the last fully expanded leaf of each plant. These evaluations were carried out in the pre-morning period (between 03 h 00 min and 06 h 00 min) and morning (between 07 h 00 min and 09 h 30 min) using the pulse-amplitude modulated fluorometer JUNIOR-PAM (Walz,

Germany). Initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence/maximum fluorescence ratio (maximum photochemical efficiency of PSII) (Fv/Fm), variable fluorescence/initial fluorescence ratio (Fv/Fo), effective quantum yield of PSII (YII125) and electron transport rate (ETR1500) were determined.

Enzymatic assays were conducted using fresh samples of roots and leaves. A portion of fresh tissue (0.5 g) of leaves from the middle third, macerated in liquid nitrogen, was homogenized in 3 mL of 0.05 M sodium phosphate buffer (with 1 mM EDTA, and 2% (w/v) polyvinylpyrrolidone - PVP), with pH 7.8. The homogenized mixture was centrifuged (13,000 x g for 20 min at 4 °C) and the supernatant was used to determine antioxidant enzymes. SOD activity was determined according to the spectrophotometric method, described by Giannopolitis & Ries (1977). The reaction mixture contained potassium 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 75 µM nitro blue tetrazolium (NBT), 0.1 mM EDTA, and 100 µL of enzymatic extract. The photochemical production of blue formazan from the NBT was monitored based on the increase in absorbance at 560 nm. The reaction was performed in test tubes at 25 °C, inside a reaction chamber under illumination of a 15-W fluorescent lamp. As a control, tubes with the reaction mixture were kept in the dark. The reaction began as the light was turned on and, after 15 min of illumination, the reaction was stopped, turning off the light. One SOD unit was defined as the amount of enzyme that inhibits NBT photoreduction by 50% (Beauchamp & Fridovich, 1971). In the assay, photochemically excited riboflavin is reduced by methionine to semiquinone, which gives an electron to oxygen, forming the superoxide radical, which in turn converts NBT into blue formazan. Superoxide dismutase catalyzes the reaction: $2O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2$.

Activity of the enzyme guaiacol peroxidase (POD) was determined according to Zeraik et al. (2008), using guaiacol as substrate. The reaction mixture contained 1.0 mL of potassium phosphate buffer (100 mM, pH 6.5), 1.0 mL of guaiacol (15 mM) and 1.0 mL of H₂O₂ (3 mM). After homogenization, 50 µL of the enzymatic extract of the plant was added to this solution. Enzyme activity was measured by oxidation of guaiacol to tetraguaiacol based on the increase in absorbance at 470 nm. The results were expressed in enzyme unit per mg of protein (U mg⁻¹ protein). For the calculation, the molar extinction coefficient of 26.6 mM⁻¹ cm⁻¹ was used.

The data obtained were tested for normality and homogeneity of variance (Shapiro-Wilk test and Bartlett test). Subsequently, analysis of variance and Scott-Knott test were performed to group the means at p ≤ 0.05, using the statistical program Sisvar® 5.3 (Ferreira, 2011).

RESULTS AND DISCUSSION

Water deficit can cause irreversible damage to leaf tissues, leading to important metabolic changes for plants such as stomatal restrictions on CO₂ supply and increased production of reactive oxygen species (ROS), which can degrade cellular components such as photosystem II and lipid membranes (Kim

et al., 2017). For fluorescence variables, significant differences by the Scott-Knott test (p ≤ 0.05) were only observed for the variables quantum yield coefficient (Fv/Fm) and apparent electron transport rate (ETR), which are used to detect disorder in the photosynthetic system caused by stresses, because the reduction in the values indicates inhibition of photochemical activity.

For the quantum yield of PSII (Fv/Fm) in the pre-morning period (Table 1), it can be observed that the application of biostimulants Seed⁺ + Crop⁺ 1 x, Seed⁺ + Crop⁺ 2 x, Crop⁺ 1 x and Crop⁺ 2 x favored the transport of electrons to photosystem II, when applied in flowering and under the condition of soil water deficit, with means of 0.72; 0.81; 0.75 and 0.80, respectively. Treatments with biostimulants differed statistically from the control treatment, under all tested conditions. In addition, there was no statistical difference regarding the time of application of biostimulants and soil water condition.

Despite not differing statistically (Table 1), the biostimulants Seed⁺ + Crop⁺ 2 x and Crop⁺ 2 x stood out from the other treatments, showing Fv/Fm means of 0.81, under the tested conditions. For the biostimulant Crop⁺ 2 x in flowering and fruiting (50 and 100% soil WHC), the values varied between 0.80 and 0.82 (Table 1). Photosynthetic metabolism is very sensitive to water availability, and plants with homeostatic metabolism have Fv/Fm values ranging from 0.75 to 0.85 (Wagner & Merotto Junior, 2014), where lower values indicate hampering of CO₂ fixation in leaf tissue, being an excellent indicator of plant stress. There were no differences in potential quantum yield of photosystem II (Fv/Fm) between the applications of biostimulant treatments in flowering or fruiting.

The results of fluorescence in the morning period are summarized in Table 2. The lowest values of maximum quantum yield of photosystem II (Fv/Fm) occurred in plants subjected to water stress and that did not receive biostimulant application, with values of 0.26 (flowering) and 0.24 (fruiting). These values indicate that the plants were in a situation of energy imbalance, causing losses in the photosynthetic capacity of plants under water deficit (Mishra et al., 2012). The increase in the Fv/Fm variable was proportional to the dose of the biostimulant Crop⁺, that is, the increase in biostimulant dose led to an increase in the Fv/Fm value, regardless of soil water condition and time of application.

Table 1. Potential quantum yield of photosystem II (Fv/Fm) in the pre-morning period in tomato leaves treated with biostimulants, in two times of application and under two soil water conditions

Biostimulant	50% WHC water condition		100% WHC water condition	
	Flowering	Fruiting	Flowering	Fruiting
Control treatment	0.25 a A	0.48 aB	0.54 aB	0.38 aA
Seed ⁺	0.60 b A	0.69 bA	0.72 bA	0.75 bA
Seed ⁺ + Crop ⁺ 1 x	0.72 cA	0.74 bA	0.75 bA	0.81 bA
Seed ⁺ + Crop ⁺ 2 x	0.81 cA	0.81 bA	0.81 bA	0.82 bA
Crop ⁺ 1 x	0.75 cA	0.77 bA	0.75 bA	0.79 bA
Crop ⁺ 2 x	0.80 cA	0.81 bA	0.82 bA	0.82 bA
Mean	0.66	0.72	0.73	0.72
CV (%)	12.50			

Means followed by the same letters, lowercase in the biostimulant factor column within the same soil water condition/time of application and uppercase in the soil water condition factor row within the same biostimulant/time of application, do not differ by Scott-Knott test at p ≤ 0.05; WHC - Soil water holding capacity

Table 2. Potential quantum yield of photosystem II (Fv/Fm) in the morning in tomato leaves treated with biostimulants, in two times of application and under two soil water conditions

Biostimulant	50% WHC water condition		100% WHC water condition	
	Flowering	Fruiting	Flowering	Fruiting
Control treatment	0.26 aA	0.24 aA	0.39 aA	0.44 aA
Seed ⁺	0.50 bA	0.64 bA	0.40 aA	0.66 bB
Seed ⁺ + Crop ⁺ 1 x	0.61 bA	0.76 bB	0.63 bA	0.78 cA
Seed ⁺ + Crop ⁺ 2 x	0.79 cA	0.79 bA	0.74 bA	0.80 cA
Crop ⁺ 1 x	0.77 cA	0.78 bA	0.78 bA	0.78 cA
Crop ⁺ 2 x	0.84 cA	0.80 bA	0.81 bA	0.81 cA
Mean	0.64	0.69	0.60	0.68
CV (%)	10.88			

Means followed by the same letters, lowercase in the biostimulant factor column within the same soil water condition/time of application and uppercase in the soil water condition factor row within the same biostimulant/time of application, do not differ by Scott-Knott test at p ≤ 0.05; WHC - Soil water holding capacity

Significant reductions of Fv/Fm under water deficit were observed in *E. guineenses* (Chandrasekar et al., 2010), corroborating the control treatment of this study (Tables 1 and 2). In the present study, the application of biostimulants caused an increase in Fv/Fm values both under deficit and in the situation of adequate water supply, reaching values of 0.84 and 0.81 for the application of Crop⁺ 2 x under conditions of stress and adequate water supply, respectively (Table 2). The results show that the use of the biostimulant Crop⁺ alone or combined with Seed⁺ can be an alternative to mitigate the damage caused by water deficit to the photosynthetic structures of plants. In addition, the highest electron transport rate (ETR) was observed in plants that received biostimulants, indicating that they can enhance their photosynthetic activity.

ETR is useful to measure changes in the photosynthetic capacity of plants due to stress caused by water deficit, which can generate a super-reduction (Schöttler & Tóth, 2014). Statistical differences were observed when the treatments were applied in fruiting under 50% soil WHC (Table 3). Despite not differing statistically, in flowering under 50% soil WHC, the biostimulants Seed⁺ and Crop⁺ showed higher ETR values when compared to the control treatment.

Table 4 shows the activity of the SOD enzyme expressed in U SOD mg⁻¹ protein. For both biostimulants, Seed⁺ and Crop⁺, in general, when under water deficit, there was activation of SOD, compared with the control. Biostimulants have significantly influenced SOD activity, since water deficit generates oxidative stress through increased ROS, which can alter cell metabolism through damage to lipids, proteins and nucleic acids.

Table 3. Apparent electron transport rate (ETR) (μmol m⁻² s⁻¹) in the pre-morning period in tomato leaves treated with biostimulants, in two times of application and under two soil water conditions

Biostimulant	50% WHC water condition		100% WHC water condition	
	Flowering	Fruiting	Flowering	Fruiting
Control treatment	12.21 aA	14.47 aA	6.10 aA	14.23 aA
Seed ⁺	14.49 aA	31.33 aA	14.03 aA	50.20 bB
Seed ⁺ + Crop ⁺ 1 x	12.57 aA	48.33 bB	18.43 aA	31.30 aA
Seed ⁺ + Crop ⁺ 2 x	24.10 aA	43.70 bB	35.70 bA	38.77 bA
Crop ⁺ 1 x	24.97 aA	40.77 bA	31.70 bA	50.83 bA
Crop ⁺ 2 x	20.30 aA	57.23 bB	36.13 bA	51.87 bA
Mean	18.11	39.31	23.68	39.53
CV (%)	26.17			

Means followed by the same letters, lowercase in the biostimulant factor column within the same soil water condition/time of application and uppercase in the soil water condition factor row within the same biostimulant/time of application, do not differ by Scott-Knott test at p ≤ 0.05; WHC - Soil water holding capacity

Table 4. Specific activity of superoxide dismutase (SOD) (U mg⁻¹ protein) in tomato treated with biostimulants, in two times of application and under two soil water conditions

Biostimulant	50% WHC water condition		100% WHC water condition	
	Flowering	Fruiting	Flowering	Fruiting
Control treatment	124.43 aA	144.44 aA	103.65 aA	104.38 aA
Seed ⁺	154.33 aA	156.56 aA	109.03 aA	105.13 aA
Seed ⁺ + Crop ⁺ 1 x	214.42 cA	215.06 bA	79.00 aA	86.90 aA
Seed ⁺ + Crop ⁺ 2 x	240.70 cA	244.92 bA	113.33 aA	92.18 aA
Crop ⁺ 1 x	192.63 bA	187.22 aA	86.30 aA	102.58 aA
Crop ⁺ 2 x	295.42 dA	372.12 cB	75.39 aA	91.36 aA
Mean	203.66	220.05	94.45	97.09
CV (%)	15.02			

Means followed by the same letters, lowercase in the biostimulant factor column within the same soil water condition/time of application and uppercase in the soil water condition factor row within the same biostimulant/time of application, do not differ by Scott-Knott test at p ≤ 0.05; WHC - Soil water holding capacity

The highest SOD activity was observed in plants under water deficit and application of the biostimulant Crop⁺ 2 x in fruiting, which reached a value of 372.12 U mg⁻¹ protein. Application of Crop⁺ increased SOD activity compared to the control and to the application of only Seed⁺, evidencing the role of this biostimulant in the defense against ROS. In plants without water deficit, no statistical difference was observed in relation to the application of biostimulants and the time of application.

During the evolutionary process, plants developed ROS removal systems, which can be of enzymatic antioxidant defense including enzymes SOD, POD and CAT, in addition to non-enzymatic antioxidants such as ascorbate, glutathione, proline, carotenoids and phenolic compounds. Changes in antioxidant mechanisms correlate the ability to defend against different stresses. Under water deficit conditions, important changes in the photosynthetic and antioxidant metabolism of *Coffea arabica* plants have been recorded (Deuner et al., 2011).

Tomato plants subjected to water deficit that did not receive application of treatments (T1) showed lower value of POD activity, differing statistically from plants treated with biostimulants (Table 5). There were higher POD values in the treatments Seed⁺ + Crop⁺ 2 x, Crop⁺ 1 x and Crop⁺ 2 x, that is, when using the biostimulant Crop⁺, alone or associated with Seed⁺, and under water deficit. Thus, it is observed that, regardless of soil water condition and application time, the biostimulants helped to enhance peroxidase enzymes in the plants.

In their study, Anjum et al. (2017) observed increased activity of CAT and SOD enzymes in maize plants under severe water

Table 5. Specific activity of guaiacol peroxidase (POD) (U mg⁻¹ protein) in tomato plants treated with biostimulants, in two times of application and under two soil water conditions

Biostimulant	50% WHC water condition		100% WHC water condition	
	Flowering	Fruiting	Flowering	Fruiting
Control treatment	247.88 aA	380.24 aB	129.61 aA	158.32 aA
Seed ⁺	375.54 bA	351.93 aA	136.26 aA	144.72 aA
Seed ⁺ + Crop ⁺ 1 x	415.20 bA	542.95 bB	138.83 aA	139.29 aA
Seed ⁺ + Crop ⁺ 2 x	810.97 dB	703.60 cA	134.26 aA	137.08 aA
Crop ⁺ 1 x	691.19 cA	800.00 dB	171.35 aA	104.02 aA
Crop ⁺ 2 x	921.59 eA	972.62 eA	143.10 aA	133.30 aA
Mean	577.06	625.22	142.24	136.12
CV (%)	12.07			

Means followed by the same letters, lowercase in the biostimulant factor column within the same soil water condition/time of application and uppercase in the soil water condition factor row within the same biostimulant/time of application, do not differ by Scott-Knott test at p ≤ 0.05; WHC - Soil water holding capacity

deficit. In the present study, there was increase in the activities of SOD and POD in plants exposed to water deficit conditions. Wang et al. (2010) reported that SOD and POD enzymes protect plants from oxidative damage under water stress conditions. In addition, the activity of these enzymes was higher with the presence of biostimulants, under the condition of water deficit, which may be related to the increase in tolerance to stress, with more efficient removal of reactive oxygen species.

The antioxidant system can be enhanced through the application of products such as biostimulants. Studies have demonstrated an increase in the antioxidant system and a reduction in the incidence of lipid peroxidation in some crops subjected to water stress, compared to control plants, with the use of biostimulants (Elansary et al., 2016; Santaniello et al., 2017). The basis of biostimulants is the alga *Ascophyllum nodosum*, rich in glycine, betaine glycine and proline, which are related to the increase in the activity of antioxidant enzymes, promoting tolerance to biotic and abiotic stresses (Colla & Rouphael, 2015; Battacharyya et al., 2015).

For the transverse and longitudinal diameter of the fruit, there was no significant difference, while the mass can be observed in Table 6. It is possible to observe that the Crop⁺ 2 x treatments had the highest values (95.15; 92.50; 89.23 and 93.11 g), which did not differ from one another, demonstrating that the application of the biostimulant Crop⁺ 2 x led to higher values when treatment was not applied and under water stress. The control treatment produced fruits with lower average mass under both irrigation depths, regardless of the period evaluated, differing statistically from all other treatments.

The treatments did not differ regarding the time of application of biostimulants; however, it can be observed that their use, even under conditions of water deficit (50% WHC), remained equal or superior to treatments with 100% soil WHC. Thus, it is concluded that the use of biostimulants was beneficial, mitigating the damage caused by water deficit, thus maintaining yield levels.

The average yield of tomato fruits increased significantly with the use of biostimulants under both soil water conditions (Table 6), compared with the control treatment. These results may be a consequence of the effects of biostimulants on the potential quantum yield of photosystem II (F_v/F_m), electron transport rate and activity of antioxidant enzymes, which increased in the presence of biostimulants. Santos et al. (2012) found that the use of biostimulants promoted the translocation

Table 6. Mean values of total fruit production (g) of tomato treated with biostimulants, in two times of application and under two soil water conditions

Biostimulant	50% WHC water condition		100% WHC water condition	
	Flowering	Fruiting	Flowering	Fruiting
Control treatment	37.30 aA	28.01 aA	31.69 aA	48.24 aB
Seed ⁺	65.02 bA	68.67 bA	66.21 bA	66.52 bA
Seed ⁺ + Crop ⁺ 1 x	78.83 bA	78.23 cA	69.55 bA	67.90 bA
Seed ⁺ + Crop ⁺ 2 x	72.06 bA	65.70 bA	67.88 bA	68.71 bA
Crop ⁺ 1 x	72.98 bA	83.92 cA	83.23 cA	72.77 bA
Crop ⁺ 2 x	95.15 cA	92.50 cA	89.23 cA	93.11 cA
Mean	70.22	69.51	67.97	69.54
CV (%)	26.17			

Means followed by the same letters, lowercase in the biostimulant factor column within the same soil water condition/time of application and uppercase in the soil water condition factor row within the same biostimulant/time of application, do not differ by Scott-Knott test at $p \leq 0.05$; WHC - Soil water holding capacity

of a large amount of assimilates and metabolites involved in flowering. These data may be related to the use of macro and micronutrients, for example, calcium, which contributes to resistance to falls and to abortion of flowers and, consequently, increases the number of fruits produced (Oliveira et al., 2001).

The observations of the present study show that the application of biostimulants Seed⁺ and Crop⁺ was effective in triggering protection of tomato plants when subjected to water deficit, hence keeping the plant under physiologically balanced conditions and consequently ensuring productivity.

CONCLUSIONS

1. Application of biostimulants kept the potential quantum efficiency of photosystem II (F_v/F_m) close to normal. The biostimulants Seed⁺ + Crop⁺ 2 x and Crop⁺ 2 x, in the pre-morning period, led to an average of 0.81, under the tested conditions.

2. The highest SOD activity was obtained with application of the biostimulant Crop⁺ 2 x in fruiting (372.12 U mg⁻¹ protein). For POD, the highest activity was obtained with the biostimulants Seed⁺ + Crop⁺ 2 x, Crop⁺ 1 x and Crop⁺ 2 x, applied in flowering (810.94; 691.19 and 921.59 U mg⁻¹ protein, respectively) and fruiting (703.60; 800.00 and 972.62 U mg⁻¹ protein, respectively).

3. Tomato yield increased with increasing doses of the biostimulants Seed⁺ and Crop⁺, under the conditions of 50 and 100% soil WHC and regardless of the time of application.

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