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A novel plant-based biostimulant improves plant performances under drought stress in tomato

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Abstract: Abiotic stress adversely affects crop production, causing yield reductions in important crops, including tomato (*Solanum lycopersicum*). Among different abiotic stresses, drought is considered to be the most critical one since limited water availability negatively impacts plants growth and development, especially in arid or semi-arid areas. The aim of this study was to understand how biostimulants may interact with critical physiological response mechanisms in tomato under limited water availability and to define strategies to improve tomato performances under drought stress. We investigated physiological responses of the tomato genotype 'E42' grown in open field under control condition (100% irrigation) and limited water availability (50% irrigation) and treated or not with a novel plant-based biostimulant named CycoFlow (Agriges, BN, Italia). Plants treated with the biostimulant showed an increase in stomatal conductance. The highest yield *per* plant was registered under the 100% water regimens in biostimulant-treated plants. Also, biostimulant-treated plants had higher pollen viability (+50.94% under water deficit) and higher fruit weight (+56.13% under water deficit) compared to non-treated plants. The treatment with the biostimulant had also an effect on antioxidants and pigments content in leaves and fruits. Altogether, these results indicate that the application of the biostimulant CycoFlow to tomato plants improved plant performances under limited water availability.

Keywords: bioassay; limited water availability; tomato yield; glycine betaine

1. Introduction

Transient or extended drought periods are common in agriculture of arid and semi-arid environments and will become more frequent with climate change [1; 2]. Generally, plants respond to drought with a series of physiological mechanisms including stomatal closure, repression of cell growth and photosynthesis, activation of stress hormones and antioxidant mechanisms, which overall lead to a reduction in plant biomass. The effects of transient water deficit are different from those caused by severe drought [3;4]. Lack of water and increase competition for water resources between agriculture and other sectors require exploring alternative and sustainable crop management strategies that can allow saving water for irrigation and still maintain satisfactory levels of crop production. One of the strategies that can be used to improve the responses of plants to stress conditions could be the use of biostimulants. The application of biostimulants has a positive impact on plant nutrition and growth, and also provides anti-stress effects [5]. This crucial role of biostimulants highlights the importance of increasing our knowledge of their physiological functions, which are still unclear. The aim of this study was to link physiological responses and agronomic performance of tomato plants exposed to water deficit and treated with CycoFlow, a novel plant-based biostimulant supplied by Agriges (Benevento, Italy).

2. Experiments

Experiments were carried out at the agronomy farm of the University of Naples "Torre Lama" located in Bellizzi, (Salerno), Italy (latitude 40°31′N; longitude14°58′E) on a clay-loam soil. Four weeks after seeding, after the third true leaf was fully expanded, tomato plants (genotype E42, available at the University of Naples, Department of Agricultural Sciences) were transplanted into open field on June 19th 2019. The experimental design was a randomized block design with three replicates for water treatment (well watered 100% *vs*- water deficit 50%) and biostimulant treatment (treated *vs*- non treated). The biostimulant treatment was combined with two irrigation levels: irrigation with 50% and 100% of evaporation determined using a Class A evaporation pan between two irrigations. The experimental field was irrigated every 10 days, using a drip irrigation system with 5 L/h (one emitter/plant). Water deficit was induced at 22 DAT (days after transplant), when the plants were fully formed and continued until the end of the experiment. The biostimulant was applied at the moment of transplanting and thereafter every 15 days, until the end of the cultivation cycle for a total of four applications, by fertigation with a 3 g *per* liter solution. CycoFlow is a plant extracts-based biostimulant produced by the Agriges company (Benevento, Italy) rich in glutamic acid (including glutamine) and glycine betaine, peptides, nucleotides, B vitamins, trace elements and other growth factors. Its chemical composition contains total nitrogen of 4.5% and organic carbon of 19.5% The biostimulant has a pH of 5.0, a density of 1200 kg/m^3 and an EC value of 15 dS/m [6]. During the experiment, stomatal conductance was measured with a steady state porometer (AP-4, Delta-T Devices, Cambridge, UK) and the total leaf water potential (Ψt) was measured with a pressure chamber (PMS Instrument Company, Albany, USA). Leaf dry matter content (LDMC) was measured on five leaves from at least five different plants. The LDMC, a surrogate for leaf tissue density relates to the nutrient retention within the plant [7], was expressed as the ratio between leaf dry mass and saturated fresh mass. Pollen viability was analyzed using five flowers *per* plant sampled from three different plants per replicate with DAB test according to Dafni et al., 1992 [8]. Harvesting started on August 12th 2019, 54 days after transplanting (DAT). Six plants per treatment were collected for biomass determination. Shoot biomass was calculated as the sum of aerial vegetative plant parts (leaves + stems) and fruits were counted and weighted. Samples of freshly harvested fully ripened tomato fruits and leaves were collected from each plot to determine antioxidant and pigments content by a colorimetric assay on freeze dried and finely ground sub-samples. The evaluation of total carotenoids, chlorophylls, lycopene and β-carotene was carried out according to the method reported by Wellburn and by Zouari et al. as modified by Rigano et al. [9,10,11]. Measurements of the content of reduced ascorbic acid (AsA) and total ascorbic acid (AsA + dehydroascorbate − DHA), were carried out by using a colorimetric method [12], with modifications reported by Rigano et al. [13,14]. The antioxidant capacity was analyzed by the FRAP assay carried out by using the ferric reducing/antioxidant power method [15] with slight modifications. Data were analyzed by ANOVA and means were compared by the Tukey's test.

3. Results

The water regimen significantly increased the leaf water potential compared to plants under full irrigation, while the treatment with the biostimulant did not affect this parameter (**Table 1**). The treatment with the biostimulant CycoFlow had a significant effect on stomatal conductance, which under full irrigation increased by 84.01% after treatment (**Fig. 1a**). Leaf dry matter content (LDMC) was significantly affected by the separate effect of water regimen and biostimulant treatments.The treatment with the biostimulant caused an increase in leaf dry matter content under water deficit. Only the water regimen had an effect on shoot biomass. Pollen viability decreased by 23.39% under water deficit (**Table 1**). On the contrary, plants treated with Cycoflow and subjected to water deficit showed an increase in pollen viability of 50.94% compared to non-treated plants (**Fig. 1b**). Interestingly, the treatment with the biostimulant increased fruit weights (up to 56.13% under water deficit) (**Table 1**). Water deficit had a significant effect on the number of fruits, that strongly decreased under stress. Althogheter, both the water regimen and the biostimulant treatments had an effect on final yields. The highest yield *per* plant was registered under the 100% water regimen in biostimulant treated plants (**Table 1)**.

Table 1. Leaf water potential, stomatal conductance, pollen viability, leaf dry matter content and biometric parameters of E42 treated with the biostimulant CycoFlow under two irrigation regimens. Asterisks indicate significant differences according to ANOVA (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). Different letters indicate significant differences according to Tukey's post-hoc test ($p < 0.05$).

	100%		**50%**		**Significance**		
	Non-treated	**Treated**	**Non-treated**	**Treated**	**W**	**B**	**WxB**
Leaf water potential (Mpa)	8.67 ± 2.08 a	7.5 ± 0.87 a	13.33 ± 2.02 b	10.33 ± 1.53 ab	**	ns	ns
Stomatal conductance (cm/s)	174.17± 42.79 a	320.5± 79.35 b	162.17± 30.67 a	199 ± 51.27 a	***	**	*
Leaf dry matter content (g/g)	0.072 ± 0.008 bc	0.103 ± 0.015 c	0.019 ± 0.012 a	0.055 ± 0.008 b	***	**	ns
Shoot FW (kg)	2.55 ± 0.79 a	5.07 ±1.85 b	0.50 ± 0.11 a	2 ± 0.48 a	**	ns	ns
Pollen viability (%)	0.73 ± 0.12 b	0.77 ± 0.1 b	0.53 ± 0.08 a	0.8 ± 0.08 b	***	**	***
Fruit weight (g)	7.13 ± 2.16 ab	8.30 ± 1.16 b	5.38 ± 1.38 a	8.40 ± 1.57 b	ns	**	ns
Number of fruit	123.17 ± 67.14 b	177 ± 59.58 b	36.33 ± 38.66 a	35.17 ± 22.18 a	***	ns	ns
Yield (kg/pt)	1.25 ± 0.27 b	1.76 ± 0.60 b	0.07 ± 0.02 a	0.44 ± 0.19 a	***	*	ns

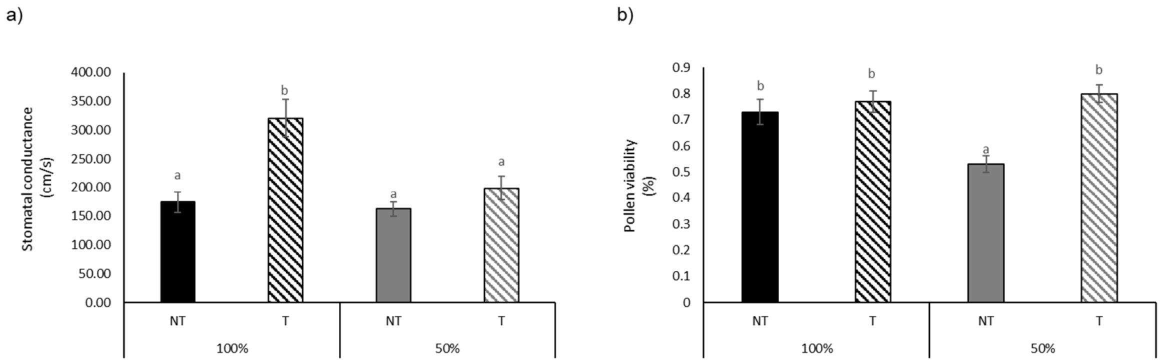

Figure 1. Effect of CycoFlow on (**a**) stomatal conductance, (**b**) pollen viability of 'E42'. Values are mean ± SE. Different letters indicate significant differences based on Tukey's test ($p \leq 0.05$).

3.3 Leaf antioxidants and pigments content

The treatment with the biostimulant had a significant effect on chlorophyll A content, that decresed in treated non-stressed plants (**Fig. 2a, Table 2**). A decrease in chlorophyll B content was also found (**Fig. 2b**). Interestingly, both the water regimen and the biostimulant treatments had an effect on ascorbic acid (AsA) content. The treatment with the biostimulant decreased the content of both reduced and total AsA under the 100% irrigation regimen (**Fig. 2c, d**). The antioxidant activity in the leaves increased by 98.09% after treatment with the biostimulant under limited water availability.

Table 2. Content of total AsA, reduced AsA, carotenoids, chlorophyll A and B (Chl A, B) and total antioxidant activity (Frap) in leaves of E42 treated with the biostimulant CycoFlow under two irrigation regimens. Asterisks indicate significant differences according to ANOVA (ns = not significant; * = p < 0.05; ** = p < 0.01; *** = p < 0.001). Different letters indicate significant differences according to Tukey's post-hoc test (p < 0.05).

	100%		**50%**		**Significance**		
	Non-treated	**Treated**	**Non-treated**	**Treated**	**W**	**B**	**WxB**
Total Asa (mg/100 g FW)	93.51±2.53 b	65.96±9.58 a	101.82±4.80 b	94.35±2.24 b	***	***	***
Reduced AsA (mg/100 g FW)	22.26±0.47 b	15.73±2.47 a	22.81±0.42 b	22.14±2.90 b	***	***	**
Carotenoids (mg/100 g FW)	25.16±3.59 ab	24.11±2.32 b	26.22±0.33 ab	27.43±0.45 b	**	ns	ns
Chl A (mg/100 g FW)	132.04±0.92 b	113.097±0.60 a	130.27±3.76 b	129.54±4.45 b	***	***	***
Chl B (mg/100 g FW)	51.02±2.50 b	43.95±4.86 a	51.05±4.67 b	52.67±3.53 b	**	ns	**
Frap (mmol TE/ 100 g FW)	179.48±18.14 a	202.48±65.77 a	174.38±18.50 a	345.44±66.35 b	**	***	**

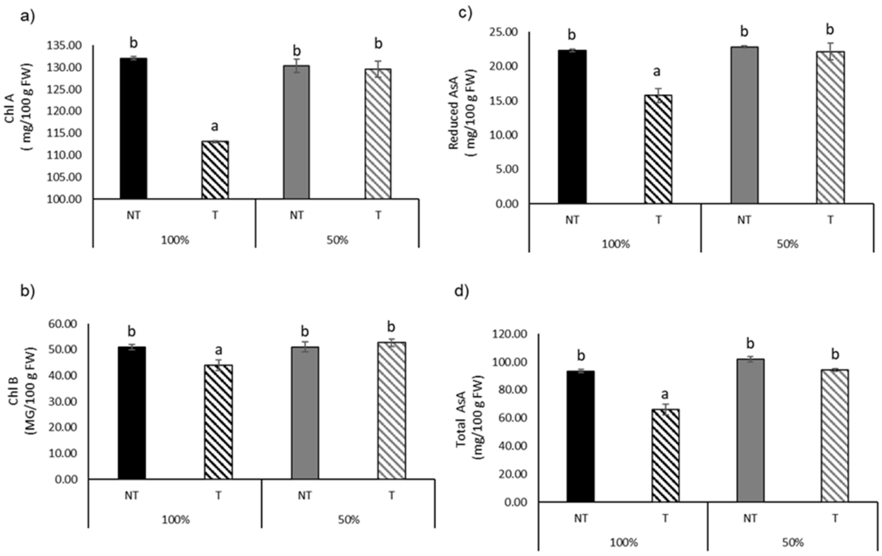

Figure 2. Effect of CycoFlow on the content of (**a**) chlorophyll A (Chl A), (**b**) chlorophyll B (Chl B), (**c**) reduced AsA, (**d**) total AsA in leaves of 'E42'. Values are mean ± SE. Different letters indicate significant differences based on Tukey's test (p ≤ 0.05).

3.3 Fruit antioxidants and pigments content

On fruits, water deficit increased the content of carotenoid by 42.80% compared to non stressed plants (**Fig. 3a, b; Table 3**). Reduced AsA, carotenoids and lycopene contents were significantly affected by the interaction between biostimulant treatments and water regimen (**Fig.3; Table 3**). The treatment with the biostimulant alone effected the content of Total Ascorbic Acid (**Table 3**).

Table 3. Content of total AsA, reduced AsA, carotenoids, β-carotene, lycopene and total antioxidant activity (Frap) in fruit of E42 treated with the biostimulant CycoFlow under two irrigation regimens. Asterisks indicate significant differences according to ANOVA (ns = not significant; * = p < 0.05; ** =

p < 0.01; *** = p < 0.001). Different letters indicate significant differences according to Tukey's post-hoc test (p < 0.05).

	100%		50%		Significance		
	Non-treated	Treated	Non-treated	Treated	W	B	WxB
Total Asa (mg/100 g FW)	115.40±11.41 b	100.99±6.68 a	111.50±7.69 ab	102.70±8.38 ab	ns	**	ns
Reduced AsA (mg/100 g FW)	94.20±4.90 b	84.65±7.15 a	91.11±5.03 ab	94.43±3.37 b	ns	ns	**
Carotenoids (mg/100 g FW)	11.61±0.51 a	15.47±0.95 c	16.58±0.32 d	13.31±0.41 b	***	ns	***
β-Carotene (mg/100 g FW)	0.34±0.05 a	0.33±0.03 a	0.40±0.02 b	0.37±0.07 ab	**	ns	ns
Lycopene (mg/100 g FW)	0.67±0.08 a	0.88±0.06 b	0.90±0.10 b	0.76±0.06 a	ns	ns	***
Frap (mmol TE/ 100 g FW)	413.55±48.20 a	426.52±58.38 a	845.10±79.03 b	882.24±73.71 b	***	ns	ns

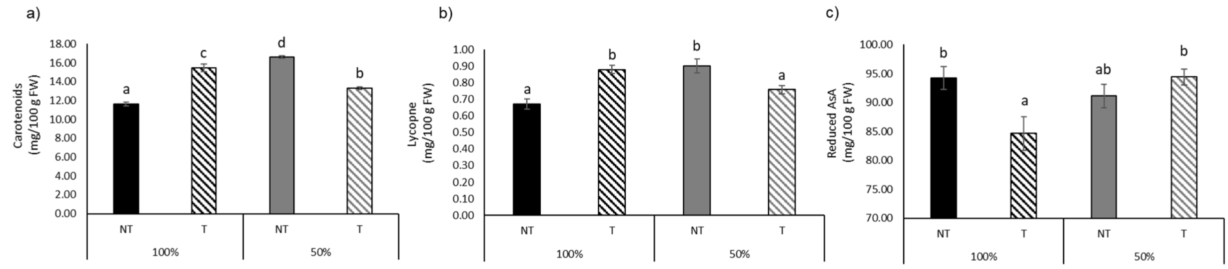

Figure 3. Effect of CycoFlow on the content of (**a**) carotenoids, (**b**) lycopene, (**c**) reduced AsA in fruit of 'E42'. Values are mean ± SE. Different letters indicate significant differences based on Tukey's test (p ≤ 0.05).

4. Discussion

In this study a tomato landrace was grown under water deficit in open field and was treated with a plant-based biostimulant named CycoFlow. Biostimulants have demonstrated to exert beneficial effects in alleviating stress in horticultural crops [16]. It has been reported that the positive effects of protein hydrolysates as stress protectant are increasingly important in the current global climate change scenario [17]. In this study, plant yield was reduced in water-stressed plants compared to well-irrigated ones, but after Cycoflow treatment both well-watered and water-stressed plants showed better performances in the field, in agreement with previous studies conducted on the same plant species and using the same biostimulant [6]. The presence of glycine betaine in CycoFlow may have enhanced the tolerance of tomato plants to water deficit. It has been previously demonstrated that glycine betaine applied exogenously by foliar application significantly increased stomatal conductance of tomato plants grown in well-watered, water-deficient or saline conditions [18]. Accordingly, a higher stomatal conductance was observed in tomato plants treated with the biostimulant. Moreover, the free amino acids present in the biostimulant may have acted as signaling molecules and may have promoted endogenous phytohormonal biosynthesis thus stimulating growth and also fruit setting [19]. Higher pollen viability was also observed after treatment with Cycoflow, which allowed water-stressed plants to have the same level of pollen viability as untreated well-watered plants. This result could be due to the high level of proline present in the biostimulant, an amino acid whose natural content in flower organs is 10 times higher compared to leaves. Moreover, it is known that the amino acid proline also favors the translocation of nutrients towards developing flowers (sink) [20]. The typical response to oxidative stress under drought is the reduction of chlorophyll content. Chlorophylls degradation and/or chlorophyll synthesis deficiency occur when plants are subjected to drought stress [21]. As reported also by Ma et al. [22], we did not observe any significant changes in chlorophyll content under different irrigation regimens in non-treated samples. The ability to maintain an optimal chlorophyll content during water deficit may be a key drought tolerance trait in this tomato line. Interestigly, the treatment with the biostimulant had a clear positive effect on the total antioxidant activities in the leaves of plant grown under limited water availability. These results are consistent with previous work that demonstrated that CycoFlow treatment induced the activation of the antioxidant defense system [6]. Fruit vegetables, in particular tomatoes, are considered good sources of lipophilic and hydrophilic antioxidant molecules such as lycopene and ascorbic acid, therefore the content of these compounds was here evaluated. In general, the content of carotenoids and lycopene were higher in the fruit treated with the biostimulant compared to the non-treated ones in well watered plants. The beneficial effects of Cycoflow on phytochemical compounds (i.e., lycopene) could be related to the activation of specific molecular and physiological mechanisms related to nitrogen metabolism [23]. The production and accumulation of lycopene with biostimulant application could be considered as an extra value to support human health [24].

5. Conclusions

In this paper we investigated the effects of the application of one plant-based biostimulant named CycoFlow on the nutritional quality and yield of tomatoes grown under limited water availability. The application of the CycoFlow biostimulant had a clear effect on plant growth and improved plant performances under stress conditions. Cycoflow application had also a clear effect on antioxidant activity and tomato fruit quality. It can be concluded that this plant-based biostimulant enhances defences mechanisms under water stress conditions, including the increase in antioxidants content. Additional research is needed to fully understand the mechanisms of action of this plant-based biostimulant.

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