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BLOSSOM-END ROT CONTROL IN TOMATO (*Solanum lycopersicum* L.) FRUIT USING PLANT GROWTH REGULATORS

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ABSTRACT

The tomato (*Solanum lycopersicum* L.) is the second most produced vegetable worldwide. Mexico ranks eighth as a producer and is the main exporter. This crop adapts to diverse climates and soil types, although it is susceptible to diseases and physiological disorders. Blossom-end rot (BER) is the most relevant, with damage that can reach up to 50 % of greenhouse production. This study aimed to evaluate the effect of foliar application of abscisic acid (ABA) combined with 24-epibrassinolide (EBL) to assess BER of tomato fruit in plants fed with low calcium (Ca) concentration (45 mg L^{-1}) in the supplied nutrient solution. Likewise, the physiological and metabolic changes that these growth regulators generate *in planta* were determined. A 4 × 4 factorial experiment was established in a completely randomized design. Four concentrations of ABA (0, 60, 100, and 140 mg L^{-1}) and four of EBL (0, 0.0024, 0.0048, and 0.0096 mg L^{-1}) were evaluated as factors, with four replications. The percentage of BER and fruit set, photosynthesis, stomatal conductance, intercellular CO_2, transpiration, Ca concentration in leaves and fruits, and the concentration of amino acids and proteins were determined. In the first evaluation (20 d of treatment), treatments ABA3EBL2 and ABA3EBL0 showed a lower incidence of BER (80 and 66 %). In the second evaluation, EBL decreased transpiration by 50.6 %. ABA1EBL1 (35.7 %) and ABA3EBL0 (30.5 %) increased fruit Ca, while ABA2EBL1 increased leaf Ca by 62.2 %. ABA0EBL1, ABA1EBL1, ABA3EBL0, and ABA1EBL3 increased total amino acids more than twofold, and ABA2EBL2 increased protein by up to 13.6 %. These results indicate that the early application of ABA and EBL reduces BER and improves physiological and metabolic parameters in tomato plants.

Keywords: abscisic acid, calcium, physiological disorder, environmental factors, 24-epibrassinolide.

INTRODUCTION

The tomato (*Solanum lycopersicum* L., Solanaceae family) is one of the most cultivated plant species in the world (Caruso *et al*., 2022). It is the second most important horticultural crop worldwide. In 2023, Mexico ranked as the eighth largest producer

and exporter of tomatoes. The main producing states are Sinaloa, San Luis Potosi, Michoacan, Jalisco, Morelos, Baja California Sur, Sonora, Puebla, and Zacatecas (SIAP, 2023). Tomatoes are the most important vegetables exported from Mexico and provide a significant economic benefit to farmers and marketers. In 2023, the production volume in Mexico was approximately 3.6 million Mg (SIAP, 2023).

Climate change and extreme weather events are causing significant negative impacts on crop production. Tomatoes are adaptable to almost all climatic regions; however, environmental stresses are the main limitations on yield and potential quality (Gerszberg and Hnatuszko-Konka, 2017). Abiotic stress can lead to the alteration of multiple processes in the plant, such as increased transpiration, decreased photosynthesis and photosynthetic pigments, decreased stomatal conductance, decreased turgor, and the production of reactive oxygen species (Sharma *et al*., 2020).

Blossom-end rot is a physiological disorder that causes significant losses in many horticultural crops, including tomato, watermelon, and pepper. It occurs in all tomato-producing areas worldwide and results in unmarketable fruits and decreased supply, resulting in significant economic losses (Hagassou *et al*., 2019). On the other hand, calcium (Ca) deficiency in tomato fruits can cause cell membrane rupture, followed by solute extravasation and cellular plasmolysis, resulting in the formation of blossom-end rot symptoms (Saure, 2014). Phytohormones play an important role in the onset of Ca deficiency disorders (de Freitas *et al*., 2018). Growth regulators such as abscisic acid (ABA) and 24-epibrassinolide (EBL) help plants tolerate different types of biotic and abiotic stresses.

The objective of this study was to evaluate the effect of foliar applications of ABA and EBL with a limited Ca content in the supplied nutrient solution on blossom-end rot in tomato fruit, as well as to determine the physiological and metabolic changes these compounds cause in tomato plants. The hypothesis was that tomato plants fed with a low dose of Ca and treated with foliar applications of ABA and EBL would show a significant decrease in blossom end rot while also causing beneficial physiological and metabolic changes in the plant.

MATERIALS AND METHODS

Location of the experiment

This study was conducted at the Postgraduate College Campus Montecillo, located at 19° 27′ 51″ N and 98° 54′ 15″ W, at an altitude of 2250 m. The experiment was carried out under greenhouse conditions in an area of 96 m^2. Two side curtains and a zenithal curtain protected with anti-aphid mesh at 25 threads per inch were used for ventilation. The average minimum temperature was 5.7 °C and the maximum was 41.9 °C, with a relative humidity of 33 %. The greenhouse and tutoring material (rings and hooks) were disinfested with quaternary ammonium salts (Aniba Plus, Mexico) at a dose of 0.2 mL L^{-1} of water.

Genetic material

Tomato (*Solanum lycopersicum* L.) hybrid 'El Cid' seeds were sown in a 200-well styrofoam tray filled with peat (Kekkilä, Finland) as a substrate. The irrigation water used to prepare the Steiner nutrient solution (SNS) recorded an electrical conductivity (EC) of 0.44 dS m^{-1} throughout the crop cycle. In addition, it contained the following ions, expressed in milligrams per liter (mg L^{-1}): K = 3.519, Ca = 15.831, Mg = 16.659, Na = 60.49, PO_4 = 1.267, SO_4 = 0.912, HCO_3 = 306.22, Cl = 0.01, B = 0.08, Cu = 0.022, and Zn = 0.004. The EC indicated for the different SNS concentrations refers only to the fertilizer input.

The seedlings were irrigated with SNS at 0.69 dS m^{-1} EC, with the pH adjusted to 5.5. The complete SNS contained the following macronutrients, expressed in milliequivalents per liter (meq L^{-1}): NO_3^- = 12, $H_2PO_4^-$ = 1, SO_4^{-2} = 7, K^+ = 7, Ca^{2+} = 9, and Mg^{2+} = 4. The concentrations of SNS applied during the different phenological stages of the crop were adjusted based on this complete solution. The fertilizers used for SNS preparation at the various concentrations included calcium nitrate ($Ca(NO_3)_2.4H_2O$) (YaraTera, CALCINIT; Madrid, Spain), potassium sulfate (K_2SO_4) (Ultrasol NKS 46, SQM, Chile), monopotassium phosphate (KH_2PO_4) (Ultrasol MKP, SQM, Chile), magnesium sulfate ($MgSO_4.7H_2O$) (Sulmag Sal Epsom, Peñoles, Mexico), and a microelements mixture (Tradecorp AZ, Tradecorp, Spain).

Transplanting was carried out 40 d after planting. The irrigation regime was adjusted according to the phenological stage of the plant: during early growth, irrigation was applied for 1 min every hour; during the vegetative stage, for 2 min every 2 h; during flowering and fruiting, for 3 min every 2 h; and during ripening, for 4 min every 2 h. The average water output per dripper was 23.8 mL per minute.

Experimental design and treatments

The experiment had a 4 × 4 augmented factorial arrangement in a completely randomized experimental design. The assessed factors were abscisic acid (ABA) from a commercial product (PC) (5 %, Nutrigota; Guanajuato, Mexico) and 24-epibrassinolide (EBL) (85 %, Sigma-Aldrich; MO, USA), each evaluated at four levels. The ABA concentrations used were 0 (ABA0, 0 mg L^{-1} PC), 60 (ABA1, 1200 mg L^{-1} PC), 100 (ABA2, 2000 mg L^{-1} PC), and 140 mg L^{-1} (ABA3, 2800 mg L^{-1} PC). In the case of EBL, the concentrations were 0 mg L^{-1} (EBL0), 0.0024 mg L^{-1} (EBL1), 0.0048 mg L^{-1} (EBL2), and 0.0096 mg L^{-1} (EBL3).

The experimental unit consisted of a single tomato plant in a 13 L black polyethylene bag. Sixteen treatments, corresponding to the combinations of ABA and EBL levels, were applied to plants receiving a low Ca concentration in the nutrient solution (45 mg L^{-1}) plus Ca from irrigation water (15.8 mg L^{-1}) at 99, 106, and 113 d after transplant (dat). An augmented control was included, consisting of plants supplied with an adequate Ca concentration in the nutrient solution (180 mg L^{-1}) in addition to the Ca from water (15.8 mg L^{-1}), without foliar application of growth regulators. Each treatment was replicated four times, for a total of 68 plants.

Experiment development

From transplantation onwards, plants were irrigated with 25 % SNS (EC 0.5 dS m^{-1} and pH 5.5). Past 23 dat, the SNS concentration increased to 50 % (EC 1 dS m^{-1}). At 43 dat, a nearly complete SNS was prepared (except for Ca concentration). Half of the nutrients required for a complete SNS was supplied with fertilizers (90 mg L^{-1}) plus Ca supplied from water (15.8 mg L^{-1}). The remaining macro- and micronutrients were supplied according to the requirements of a complete SNS.

Three months after transplanting, the amount of Ca supplied through fertilizers was reduced (45 mg L^{-1}). The complete SNS had an EC of 2 dS m^{-1}; however, when modifying the amount of Ca in the solution, the EC varied. With Ca at 50 % of the requirement, the EC decreased to 1.9 dS m^{-1}, and with Ca at 25 % of the requirement, the EC decreased to 1.8 dS m^{-1}.

After 10 d of irrigating the plants with 45 mg L^{-1} of Ca, that is, at 99 dat, when the fifth fruit cluster was in anthesis, the treatments were applied with a spray bottle, using 125 mL of the solution per plant. The liquid was sprayed onto the stem, leaves, and opening flowers. To prevent the product from drifting to other plants during application, a plastic curtain was used to cover the target plant.

Variables evaluated

Twenty days after each application, the plant was evaluated for flower number, flower set percentage, blossom-end rot percentage, net photosynthetic rate, CO_2 assimilation, stomatal conductance, and transpiration. Total soluble protein, total free amino acids, and Ca concentration were determined in fruits from clusters 5, 6, and 7. Two fruits per cluster were considered for fresh-harvest analysis. Fruits were weighed and stored at -70 °C in an ultra-low temperature freezer (Thermo Scientific S/819442-162, USA). For dried fruit analysis, two fruits per cluster from each plant were dried at 72 °C for 72 h in a forced-air oven (Riossa HCF-125D, Mexico). Furthermore, a leaf was taken from the seventh cluster in each plant and dried for 48 h under the same conditions as the fruits. Before analyzing fresh fruits, they were ground in a porcelain mortar with a pestle using liquid nitrogen.

Evaluation of physiological parameters

A portable meter (LI-6400, LiCOR; Lincoln, NE, USA) was used to measure CO_2 assimilation rates, stomatal conductance, transpiration, and net photosynthetic rate. Readings were taken from the first leaf immediately following the fifth and seventh clusters, 20 d after each ABA and EBL application.

Nutritional analysis, primary and secondary metabolites

Calcium was determined in fruits and leaves by wet digestion according to Alcántar-González and Sandoval-Villa (1999). The extracts were analyzed using an induction plasma optical emission spectroscopy (ICP-OES 725-ES, Agilent; Santa Barbara, CA, USA). Antioxidant activity, free amino acids, total protein, and hydrogen peroxide

were analyzed in fruits. Antioxidant activity was measured according to Castañeda-Castañeda *et al*. (2008). Extracts were read at a wavelength of 517 nm. Total free amino acids were determined using the ninhydrin method (Moore and Stein, 1954) and read at a wavelength of 570 nm. Total protein determination was based on the methodology of Bradford (1976) with some modifications, at an absorbance of 595 nm. For hydrogen peroxide quantification, the methodology of Sergiev *et al*. (1997) was used, at an absorbance of 390 nm. All readings were measured using a spectrophotometer (Synergy 2 Microplate Reader, Biotek, USA).

Statistical analysis

Data from the greenhouse and laboratory were analyzed using analysis of variance (ANOVA), logistic regression, or Poisson regression, depending on the data type. For ANOVA, when the null hypothesis was rejected, *post hoc* comparisons were performed with Tukey's test at a significance level of 0.05. When the null hypothesis was not rejected, pairwise comparisons were conducted using the Bonferroni adjustment. All analyses were performed using the R software v4.3.1 (R Core Team, 2023).

RESULTS AND DISCUSSION

Effect of growth regulators on flower number, flower set percentage, and blossom-end rot percentage

The results obtained from the statistical tests to compare the effect of the application of abscisic acid (ABA) and 24-epibrassinolide (EBL) or their combinations showed effects on the number of flowers, percentage of setting, and percentage of apical rot (Table 1).

Table 1. Results of statistical tests of the effect of the application of abscisic acid (ABA) and 24-epibrassinolide (EBL) on the variables number of flowers, percentage of setting, and percentage of blossom-end rot in tomato (*Solanum lycopersicum* L.).

Evaluation (days after starting treatment)	Variable/statistical test	*p* value
	Number of flowers	
2 (27)	Poisson regression	0.148
3 (34)	Poisson regression	0.126
	Fruit set percentage	
1 (20)	Kruskal-Wallis	0.546
2 (27)	ANOVA	0.604
3 (34)	ANOVA	0.691
	Blossom-end rot percentage	
1 (20)	ANOVA	0.019
2 (27)	Logistic regression	0.067
3 (34)	Logistic regression	0.684

For the number of flowers, tests were conducted 27 and 34 d after the start of treatment (dast); in both cases, no significant differences were recorded between the factor levels and between the factor combinations (p = 0.148 and 0.126). This may be due to factors such as temperature, since maximum temperatures of up to 41.9 °C were recorded. Temperatures above 27 °C negatively affect tomato flower formation. In greenhouse production, the optimal relative humidity ranges between 50 and 80 % (Castellanos, 2009; Shamshiri *et al*., 2018); however, in this experiment it averaged only 33 %. The mean number of flowers per plant was 7.8 at the second evaluation and 7 at the third. Similar results to those obtained in this study have been reported. Verma *et al*. (2014) applied three doses of gibberellic acid (GA; 20, 30, and 40 ppm) and naphthaleneacetic acid (NAA; 15, 30, and 45 mg L^{-1}) in tomato and obtained significant differences in the number of flowers. Kumar *et al*. (2018), when evaluating three doses of GA (20, 30, and 40 ppm), found that the application increased the number of flowers by 10, 12, and 15 %, respectively, compared to the control. Likewise, Tepkaew *et al*. (2022) indicated that the application of brassinosteroid type 7,8-dihydro-8α-20-hydroxyecdysone (DHECD, 0.1 μM L^{-1}) increased up to 225 % the number of flowers per inflorescence compared to the control in mango (*Mangifera indica* L.).

Similarly, growth regulators did not affect fruit set percentage across factor levels or factor combinations in the three evaluations conducted in this study. This could have been due to the temperature spikes and low relative humidity during the experiment, which interfered with pollination and fruit set, despite the fact that EBL promotes tolerance to environmental stress (Ahammed *et al*., 2015). Likewise, Riboldi *et al*. (2018) found no significant differences in fruit set after applying three growth regulators (GA, ABA, and EBL) to tomato plants without Ca supplementation. However, in *Annona squamosa* L., EBL application at a dose of 2 mg L^{-1} was found to promote fruit set, surpassing the control by up to 97 % (Aly *et al*., 2021).

On the other hand, the treatments evaluated had significant differences in blossom-end rot (BER) in the first evaluation carried out on fruits from the fifth cluster (Figure 1). In the case of ABA, the concentration of 140 mg L^{-1} (ABA3) significantly reduced BER by 45.2 % compared to ABA0 and 77.6 % compared to ABA1, which had a higher percentage of incidence. Furthermore, the ABA3EBL0 and ABA3EBL2 combinations induced a significant reduction in BER (66 and 80 %, respectively), compared to the combination without applying ABA0EBL0 regulators, and up to 88 and 93 %, in the same order, compared to ABA1EBL0, which presented the highest incidence of BER (90 %).

Based on the comparison of the effects of the combinations of growth regulators, considering the amount of Ca offered (45 mg L^{-1}), it is recommended to use the combination ABA3EBL0 since, although the BER is higher (10.7 %) than that obtained in ABA3EBL2 (6.3 %), the difference between both was not significant, which indicates that the difference was not due to the effect of the combinations; likewise, both treatments have significantly different effects than ABA1EBL0, while the rest of the combinations do not exceed the effect of any other combination (Figure 1).

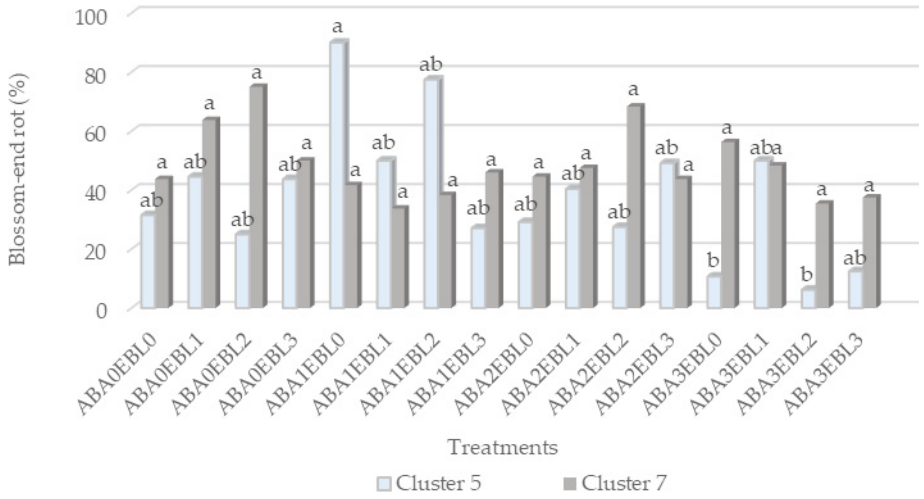

Figure 1. Effects of abscisic acid (ABA) levels and ABA × 24-epibrassinolide (EBL) combinations on blossom-end rot percentage (first and third evaluations 20 and 34 d after treatment initiation and 119 and 133 d after transplanting, respectively). ABA0: 0 mg L^{-1}; ABA1: 60 mg L^{-1}; ABA2: 100 mg L^{-1}; ABA3: 140 mg L^{-1}; EBL0: 0 mg L^{-1}; EBL1: 0.0024 mg L^{-1}; EBL2: 0.0048 mg L^{-1}; EBL3: 0.0096 mg L^{-1}. Values with the same letters are not statistically different (Tukey, $p \leq 0.05$).

When comparing BER across sampling times, incidence was higher in the third evaluation than in the first for 12 of the 17 treatments (Figure 1). The augmented control (180 mg Ca^{2+} L^{-1}, without ABA and EBL) showed no BER in the fifth cluster but reached 41.7 % in the seventh (data not shown). In contrast, the ABA0EBL0 treatment exhibited 31.5 % BER in the fifth cluster, increasing to 43.8 % in the seventh.

The decrease in BER in tomato with the use of ABA has been documented in various studies. Balate *et al*. (2018) reported an 86 % reduction compared to untreated plants. In contrast, de Freitas *et al*. (2014) indicated that 15 d after pollination, the application of ABA completely prevented BER, while the control presented an incidence of up to 19 %. Barickman *et al*. (2014) found that ABA treatments decreased BER incidence by 26.6 %. On the other hand, the use of EBL in tomato plants under abiotic stress with low Ca supply decreased the incidence of BER by 44.2 % (Riboldi *et al*., 2019).

The response of BER to ABA is attributed to its ability to activate specific mechanisms in both the plant and the fruit. These mechanisms may reduce xylem Ca transport to the leaves, enhancing Ca movement and uptake in the fruit. As a result, the apoplastic Ca concentration increases, leading to reduced membrane permeability and a lower incidence of blossom-end rot in fruit tissue (Barickman *et al*., 2014; de Freitas *et al*., 2014, 2011).

In the third evaluation (cluster 7), high percentages of BER were observed (Figure 1). This can be explained by the prolonged period of insufficient Ca supply, which extended beyond the requirements for commercial production (Steiner, 1984). At this

stage, the plants were also at an advanced phenological phase (3.5 months). As noted by Alcántar-González *et al*. (2016), during tomato ripening, premature root senescence and lignification of conductive tissues can restrict nutrient transport to developing fruits. In addition, during the second and third evaluations, greenhouse temperatures reached 40 °C with a relative humidity of 20 %, conditions that further limited nutrient uptake and transport, which intensified BER incidence.

According to Saure (2014), blossom-end rot occurs more frequently under conditions of low relative humidity, high light intensity, and elevated temperature, as these factors inhibit calcium transport to the rapidly growing distal fruit tissues. Moreover, insufficient Ca supply alone does not always induce BER symptoms; rather, the disorder is primarily driven by abiotic stress. In this experiment, unfavorable environmental conditions, including Ca deficiency, low relative humidity, and high temperature, were present, which contributed to the increased incidence of BER.

Effect of growth regulators on physiological variables

In the first evaluation (119 dat, 20 dast) there was no effect of growth regulators on the physiological variables (Table 2), between the levels of the factors, or between their combinations. On the other hand, in the second evaluation (133 dat, 34 dast), the treatment with 0.0048 mg L^{-1} of EBL significantly reduced plant transpiration up to 50.6 % (Table 2) compared to the treatment without growth regulators (ABA0EBL0).

The effect observed with 0.0048 mg L^{-1} of EBL (EBL2) (Table 2) coincides with that reported by Riboldi *et al*. (2019), who indicate that when exogenous applications of EBL were made in tomato plants exposed to abiotic stress (low Ca supply), transpiration (7.6 %) and Ca concentration in the leaf (9 %) decreased. In turn, de Freitas *et al*. (2014) mention that the concentration of Ca in the fruit is not the primary cause of BER, but rather the combined action of several factors favors its appearance. Furthermore, reducing leaf transpiration can potentially decrease the movement of xylem Ca to the leaves and increase its movement to the fruit. Although in this study there was a decrease in transpiration and foliar Ca concentration after the application of EBL, the Ca concentration in the fruit (Table 3) was not affected.

Regarding transpiration (Table 2), the treatment that showed the greatest reduction over time was ABA2EBL2, with a decrease of 73.7 %, a net photosynthesis of 81.3 %, and stomatal conductance of up to 85.7 %. In contrast, the combination that showed the smallest decrease over time was ABA0EBL1, with 21 % in transpiration, 29.7 % net photosynthesis, and 8.3 % stomatal conductance. These results demonstrated a high correlation between net photosynthesis, stomatal conductance, and transpiration. On the other hand, intercellular carbon decreased by up to 49 %, with ABA2EBL3 showing the greatest reduction in this variable. However, several treatments had an increase in intercellular CO_2 concentration at the last measurement: ABA0EBL2 (30.6 %), Control A (22.5 %), ABA3EBL3 (19.1 %), ABA1EBL1 (17.4 %), ABA2EBL0 (15.5 %), ABA3EBL2 (9.5 %), and ABA0EBL1 (4.5 %).

Table 2. Statistical analysis of the effects of abscisic acid (ABA) and 24-epibrassinolide (EBL) on the physiological response of tomato leaves at 119 (evaluation 1) and 133 d (evaluation 2) after transplantation.

Variables		Evaluation 1				Evaluation 2			
		ABA	EBL	ABA*EBL	COMB	ABA	EBL	ABA*EBL	COMB
Np	Pr(>F)	0.130	0.934	0.890	0.753	0.468	0.054	0.121	0.086
Sc	Pr(>F)	0.113	0.401	0.493	0.318	0.276	0.186	0.166	0.147
Ci	Pr(>F)	0.398	0.213	0.550	0.436	0.512	0.770	0.948	0.950
T	Pr(>F)	0.158	0.730	0.843	0.692	0.453	0.035	0.252	0.122

Combination	Np^5	Np^7	Sc^5	Sc^7	CO_2i^5	CO_2i^7	T^5	T^7
	mmol CO_2 m^{-2} s^{-1}		mol H_2O m^{-2} s^{-1}		mmol CO_2 mol^{-1}		mmol H_2O m^{-2} s^{-1}	
ABA0EBL0	21.56	7.76	0.19	0.06	186.02	133.83	10.13	3.89
ABA0EBL1	17.33	12.19	0.12	0.11	132.80	138.74	5.75	4.54
ABA0EBL2	17.51	3.04	0.11	0.02	116.78	152.54	7.08	1.92
ABA0EBL3	16.25	10.33	0.13	0.07	172.29	117.29	6.11	3.10
ABA1EBL0	20.77	8.65	0.18	0.07	188.88	135.31	8.11	3.30
ABA1EBL1	19.50	6.11	0.12	0.04	118.19	138.78	7.54	3.04
ABA1EBL2	22.47	7.96	0.15	0.05	126.60	101.89	8.53	3.69
ABA1EBL3	22.01	6.33	0.23	0.04	210.21	111.98	10.43	2.80
ABA2EBL0	20.64	9.29	0.13	0.08	104.87	121.2	7.95	4.88
ABA2EBL1	20.21	6.99	0.15	0.05	136.98	128.96	7.69	2.92
ABA2EBL2	20.14	3.75	0.14	0.02	129.10	105.75	7.75	2.04
ABA2EBL3	19.04	8.61	0.15	0.06	164.00	83.68	7.26	3.24
ABA3EBL0	17.38	9.31	0.14	0.07	162.56	126.21	6.94	4.33
ABA3EBL1	19.39	8.71	0.13	0.07	139.39	127.05	6.86	3.78
ABA3EBL2	17.34	8.47	0.11	0.08	106.30	116.35	5.52	3.68
ABA3EBL3	18.70	9.15	0.12	0.08	120.01	142.99	6.96	3.48
Control A	19.22	6.76	0.14	0.07	119.00	145.73	6.65	3.53

Np: net photosynthesis; Sc: stomatal conductance; Ci: intercellular CO_2; T: transpiration; COMB: combination; [5]: readings from leaves located above the fifth cluster. [7]: Readings from leaves located above the seventh cluster. ABA0: 0 mg L^{-1}; ABA1: 60 mg L^{-1}; ABA2: 100 mg L^{-1}; ABA3: 140 mg L^{-1}; EBL0: 0 mg $L^{-1;}$ EBL1: 0.0024 mg L^{-1}; EBL2: 0.0048 mg L^{-1}; EBL3: 0.0096 mg L^{-1}; Control A: augmented control.

In the first evaluation (Table 2), stomatal conductance remained between 0.11 and 0.15 mol H_2O m^{-2} s^{-1} in treatments where only EBL was applied (ABA0EBL1, ABA0EBL2, and ABA0EBL3), which represented between 21 and 42 % of the value observed in the treatment without growth regulators (ABA0EBL0). However, no statistically significant differences were detected either between factor levels or their interactions (Table 2), indicating that the observed variations were attributable to random variation rather than the effect of the growth regulators.

Table 3. Calcium concentration in fruits and leaves of tomato (*Solanum lycopersicum* L.) treated with abscisic acid (ABA) and 24-epibrassinolide (EBL).

Combination	Ca F1	Ca F2	Ca F3	Ca H
	(mg kg^{-1} dry biomass)			
ABA0EBL0	990.54 abc	804.11 cd	938.76 a	8075.57 efg
ABA0EBL1	1147.28 a	782.06 d	845.89 ab	6896.31 g
ABA0EBL2	997.38 abc	978.39 abc	775.76 ab	7750.75 fg
ABA0EBL3	1091.48 abc	830.79 cd	698.64 b	6969.1 g
ABA1EBL0	1143.6 a	863.43 bcd	784.84 ab	8958.40 def
ABA1EBL1	1060.54 abc	1091.26 a	762.52 ab	9339.46 cdef
ABA1EBL 2	1079.26 abc	937.48 bcd	764.23 ab	9131.32 def
ABA1EBL3	1116.98 ab	756.84 d	853.48 ab	11127.59 bc
ABA2EBL0	1052.10 abc	1019.83 ab	857.36 ab	11922.40 ab
ABA2EBL1	1035.30 abc	776.80 d	811.65 ab	13095.8 a
ABA2EBL2	885.60 bc	973.72 abc	764.90 ab	8835.2 def
ABA2EBL3	842.84 c	934.28 abcd	776.66 ab	9771.65 cde
ABA3EBL0	1051.19 abc	1049.62 a	810.60 ab	10324.95 bcd
ABA3EBL1	1029.19 abc	908.64 abcd	708.66 b	7938.13 efg
ABA3EBL2	1111.44 ab	856.52 bcd	668.46 b	9647.85 cde
ABA3EBL3	1034.91 abc	857.72 bcd	669.88 b	8712.93 defg

ABA0: 0 mg L^{-1}; ABA1: 60 mg L^{-1}; ABA2: 100 mg L^{-1}; ABA3: 140 mg L^{-1}; EBL0: 0 mg L^{-1}; EBL1: 0.0024 mg L^{-1}; EBL2: 0.0048 mg L^{-1}; EBL3: 0.0094 mg L^{-1}; Ca: calcium; F: fruit; 1: first evaluation (20 d after the start of treatments); 2: second evaluation (27 d after the start of treatments); 3: third evaluation (34 d after the start of treatments); H: leaf. Values with the same letters in each column are not statistically different (Tukey, $p \leq 0.05$).

The stomatal conductance values obtained in this study are consistent with those reported by Riboldi *et al*. (2019), who found no significant differences between EBL-treated tomato plants (0.15 mol H_2O m^{-2} s^{-1}) and the control (0.17 mol H_2O m^{-2} s^{-1}). Likewise, transpiration did not differ significantly between EBL treatment (2.32 mmol H_2O m^{-2} s^{-1}) and the control (2.51 mmol H_2O m^{-2} s^{-1}). In a previous study, Riboldi *et al*. (2018) also reported that the application of EBL in tomato plants did not produce significant differences in transpiration, although they observed a difference in stomatal conductance, with an increase of 6 to 9 %. In the second evaluation, both stomatal conductance and transpiration recorded lower values than those obtained in the first evaluation.

The decrease in photosynthesis across evaluations (Table 2) can be attributed to factors such as the use of growth regulators. ABA plays an important role in inducing stomatal closure (Engineer *et al*., 2016), which was observed in leaves located above the seventh cluster. Similarly, Hao *et al*. (2021) reported that foliar application of ABA in *Emmenopterys henryi* Oliv. under excess light stress reduced not only the

net photosynthetic rate but also stomatal conductance and transpiration, while simultaneously increasing intercellular CO_2 concentration.

In this study, photosynthesis, stomatal conductance, and transpiration showed similar behavior to that described above. While intercellular CO_2 concentration showed this trend in seven combinations, when growth regulators were used separately or at medium or high doses, intercellular CO_2 concentrations increased over time, with the exception of the ABA1EBL1 treatment. It was observed that different types of stress (biotic and abiotic) negatively affect gas exchange in the plant. Li *et al*. (2020) showed that tomato plants, when competing in high planting densities, tend to decrease stomatal conductance, transpiration, and net photosynthesis.

A second reason for the decrease in photosynthesis may be attributable to the plant's phenological phase (fruiting), combined with the climatic conditions (high temperature and low relative humidity), which caused a decrease in overall metabolism. Shamshiri *et al*. (2018) report that the optimal relative humidity range during all stages of tomato growth is between 50 and 70 %. Furthermore, under greenhouse conditions, when plant leaves are exposed to excessively hot air, transpiration is ineffective. These conditions were present during the experiment.

In contrast, Trouwborst *et al*. (2011) reported that in vertically trained crops such as tomato, the combined effects of leaf aging and reduced light intensity are associated with a decline in photosynthetic capacity. While leaf age alone significantly influences photosynthesis, the greatest impact is attributed to the reduction in photosynthetically active photon flux. As tomato plants grow under vertical training, lower leaves become progressively shaded, leading to reduced photosynthesis. The plants in this study were exposed to these conditions (vertical training and leaf aging), which was reflected in an overall decline in photosynthetic activity.

Effect of growth regulators on calcium concentration

In all treatments evaluated, fruit Ca concentration decreased as the number of harvested clusters increased (Table 3). Looking at the results of the first evaluation (20 d), treatments ABA0EBL1 and ABA1EBL0 had higher fruit Ca concentrations, exceeding the treatment without applying regulators (ABA0EBL0) by 15.8 and 15.5 %, respectively. However, no statistically significant differences were found between ABA1EBL0, ABA3EBL0, and ABA3EBL2. In the same first evaluation, the BER percentage of ABA1EBL0 was the highest (90 %), while in ABA3EBL0 and ABA3EBL2 it was significantly the lowest.

The differences in BER percentage cannot be explained by Ca deficiencies in the fruit. In the second evaluation, treatments ABA1EBL1 and ABA3EBL0 exceeded treatment ABA0EBL0 by 35.7 and 30.5 %, while in the second and third evaluations (27 and 34 d), Ca concentration in fruits of plants treated with ABA1EBL0 decreased by 24.5 and 31.4 %, respectively. This reduction is likely due to the increasing distance of the developing fruits from the nutrient supply points as the plant grows. These findings are consistent with those reported by Coolong *et al*. (2014), who state that as the number of harvested

clusters increases, not only does the concentration of Ca decrease (up to 18.8%), but so does the concentration of most nutrients.

In the third evaluation (34 dast), the combination without applied growth regulators (ABA0EBL0) recorded the highest Ca concentration in fruit, presenting significant differences with ABA0EBL3, ABA3EBL1, ABA3EBL2, and ABA3EBL3 (25.6, 24.5, 28.8, and 28.6 %, respectively). However, no significant differences were found for BER percentage. Although ABA0EBL0 had the highest concentration, it was lower than those recorded in the first evaluations. In the first evaluation (fifth cluster), the combinations of growth regulators ABA3EBL0 and ABA3EBL2 gave the best results. Riboldi *et al*. (2018) reported that Ca concentration in tomato fruit, compared to the control, increased from 57 to 67 % when ABA was applied, while with EBL it increased from 38 to 72 %. On the other hand, Riboldi *et al*. (2019) mentioned a Ca concentration in fruits treated with EBL and harvested 15 d after pollination (dap) had 3000 mg kg^{-1} of dry biomass, while the control presented 3760 mg kg^{-1} of dry biomass.

In this study, in fruits from the fifth cluster, the Ca concentration ranged from 840 to 1150 mg kg^{-1} of dry biomass in the different combinations. Meanwhile, for the sixth and seventh clusters, the Ca concentration was below 1090 and 940 mg kg^{-1} of dry biomass, respectively. Overall, the concentrations recorded in the three evaluations were lower than those reported by Riboldi *et al*. (2019), who found higher Ca levels even in fruits harvested at 15 dap. This discrepancy may be explained by the abiotic stress experienced by the plants in the present study. This result is similar to that reported by Maia *et al*. (2019), who found that nutrient concentration and accumulation were influenced by the imposed nutritional deficiencies.

Leaf Ca concentration was only measured in the third evaluation and showed significant differences between factor levels and factor combinations (Table 3). Treatment with ABA2EBL1 showed an increase of up to 62.2 % in leaf Ca concentration compared to the treatment without regulations (ABA0EBL0). Balate *et al*. (2018), working with tomato plants in soil, found a leaf Ca concentration of 30 620 mg kg^{-1} (control), while plants treated with 500 mg ABA L^{-1} had a Ca concentration of 37 000 mg kg^{-1} of dry biomass.

Riboldi *et al*. (2018), when evaluating the effect of growth regulators on Ca concentration in tomato leaves under hydroponic conditions, reported that foliar application of ABA (136.2 μM) increased Ca concentration to 23 800 mg kg^{-1} of dry matter. In contrast, a lower ABA dose resulted in 17 950 mg kg^{-1} of dry biomass, representing decreases of 28.1 and 3.6 % relative to the control, respectively. Regarding the use of brassinolides, Riboldi *et al*. (2019) reported that the application of EBL to leaves (0.01 μM) generated a concentration of Ca in leaves of 35 900 mg kg^{-1} of dry biomass, 9.1 % lower than in the control.

The lower Ca concentrations in leaves, compared with both the cited literature and the present maximum value of 13 095.8 mg kg^{-1} of dry biomass (ABA2EBL1) obtained in this study, can be explained by two main factors: 1) Riboldi *et al*. (2019) measured Ca in leaves located above the first cluster, whereas in this study, measurements were

taken from leaves above the sixth cluster; and 2) the experiment was conducted under conditions of reduced Ca availability. These results indicate that the limited Ca supply in the nutrient solution negatively affected all treatments.

Effect of growth regulators on amino acids and proteins

The concentration of total free amino acids in tomato fruits (Table 4) was influenced by most treatments. However, ABA0EBL1, ABA1EBL1, ABA3EBL0, and ABA1EBL3 showed statistically significant differences with the ABA0EBL0 treatment, surpassing it by 187.9, 151.5, 139.4, and 136.4 %, respectively. ABA0EBL1 had the highest amino acid concentration (0.95 mg g^{-1} of fresh biomass), followed by the augmented control (0.94 mg g^{-1} of fresh biomass) (data not shown). However, no significant differences were found between the aforementioned treatments with higher amino acid concentrations. Rouphael *et al.* (2021) found an amino acid concentration of 6.14 mg g^{-1} BF in tomato fruits (control), which increased by 11.4 % with the application of a biostimulant (Auxym, Rivoli Veronese, Italy). In contrast, the highest amino acid concentration

Table 4. Concentration of total free amino acids and total proteins in tomato (*Solanum lycopersicum* L.) fruits (seventh cluster) on the third evaluation (133 d after transplantation) from plants treated with abscisic acid (ABA) and 24-epibrassinolide (EBL).

Combination	Total free amino acids (mg g^{-1} BF)	Total soluble proteins (mg g^{-1} BF)
ABA0EBL0	0.33 b	0.22 ab
ABA0EBL1	0.95 a	0.09 de
ABA0EBL2	0.65 ab	0.16 bcd
ABA0EBL3	0.67 ab	0.07 e
ABA1EBL0	0.55 ab	0.17 abc
ABA1EBL1	0.83 a	0.15 bcde
ABA1EBL2	0.70 ab	0.12 cde
ABA1EBL3	0.78 a	0.17 bc
ABA2EBL0	0.36 b	0.21 ab
ABA2EBL1	0.69 ab	0.12 cde
ABA2EBL2	0.55 ab	0.25 a
ABA2EBL3	0.59 ab	0.19 abc
ABA3EBL0	0.79 a	0.14 bcde
ABA3EBL1	0.65 ab	0.11 cde
ABA3EBL2	0.76 ab	0.12 cde
ABA3EBL3	0.67 ab	0.18 abc

ABA0: 0 mg L^{-1}; ABA1: 60 mg L^{-1}; ABA2: 100 mg L^{-1}; ABA3: 140 mg L^{-1}; EBL0: 0 mg L^{-1}; EBL1: 0.0024 mg L^{-1}; EBL2: 0.0048 mg L^{-1}; EBL3: 0.0094 mg L^{-1}; BF: Fresh biomass. Values with equal letters are not statistically different (Tukey, $p \leq 0.05$).

recorded in this study, under the ABA0EBL1 treatment, was considerably lower, likely because the fruits were harvested at only 20 dap. This suggests that amino acid levels rise as fruits ripen. Moreover, the application of growth regulators, either individually or in the combinations ABA0EBL1, ABA1EBL1, ABA1EBL3, and ABA3EBL0, enhanced amino acid accumulation in tomato fruits grown under nutritional stress.

The treatments showed wide variability in total protein concentration (Table 4). Only ABA2EBL2 recorded higher protein concentrations than the treatment without the application of regulators (ABA0EBL0, 13.6 %). The ABA1EBL0, ABA2EBL0, ABA2EBL3, and ABA3EBL3 treatments did not show significant differences. The highest average concentrations of total soluble protein were observed either with ABA alone (ABA1EBL0 and ABA2EBL0) or with combined applications of ABA and EBL at medium to high doses (ABA2EBL2, ABA2EBL3, and ABA3EBL3), whereas EBL applied alone did not enhance protein concentration.

Dalyan (2023) mentions that foliar application of EBL in tomato plants under abiotic stress (fungicide) favored the increase of total proteins in the leaf up to 21.9 % compared to the control. Choi *et al*. (2011), when analyzing the protein concentration in 11 tomato varieties grown without abiotic stress under greenhouse conditions, found that the concentration remained in the range of 0.59 to 1.05 % in red fruit and 1.3 % in green fruit. Similarly, Rouphael *et al.* (2021) found a protein concentration of 2.4 % in ripe tomato fruits between 60 and 90 dat. The highest concentration in the present study was 0.025 % (0.25 mg g^{-1} of fresh biomass). This may be explained by the conditions under which the tomato plants were grown and harvested (abiotic stress due to low Ca supply, high temperatures, and low relative humidity).

The exogenous application of ABA enhances cell viability under stress by increasing the capacity of cells to scavenge reactive oxygen species (Liu *et al*., 2009). This effect is mediated by elevated antioxidant enzyme activity and the synthesis of antioxidant compounds, such as ascorbic acid, which also promotes the production of osmolytes, including amino acids like proline and glycine-betaine (Ahmad *et al*., 2017). Foliar application of ABA promotes antioxidant production (de Freitas *et al*., 2018). This explains why amino acid concentrations increased after the application of growth regulators.

CONCLUSIONS

The combined application of abscisic acid (ABA) and 24-epibrassinolide (EBL), particularly treatments ABA3EBL0 and ABA3EBL2 at the early phenological stage of the tomato plant, effectively mitigated the stress caused by low Ca supply, thereby significantly decreasing the incidence of blossom-end rot. The application of ABA and EBL did not affect stomatal conductance, net photosynthesis, and intercellular carbon, but it did affect transpiration. Treatments ABA0EBL1, ABA1EBL1, ABA1EBL3, and ABA3EBL0 favored the concentration of amino acids, while ABA2EBL increased the concentration of total proteins.

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