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Abscisic acid and 24-epibrassinolide regulate blossom-end rot (BER) development in tomato fruit under Ca^{2+} deficiency

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Abstract

Calcium ($Ca^{2+)}$ is an essential macronutrient in plants, and low concentrations of this nutrient may result in development of a physiological disorder known as blossom-end rot (BER) in tomato. Hormones can regulate the accumulation of Ca^{2+} and, consequently, fruit susceptibilities to BER. The objective of this study was to evaluate the effect of gibberellin (GA), abscisic acid (ABA), and 24-epibrassinolide (EBL) on Ca^{2+} accumulation and BER incidence in tomato. The 'Tyna c.v' tomato plants were sprayed biweekly, during anthesis, with water (control), GA (28.9 µmol L^{-1}), ABA (90.8 µmol $L^{-1}$and 136.2 µmol L^{-1}), EBL (0.01 µmol L^{-1} and 0.1 µmol L^{-1}). Treatments were applied until the physiological maturity of fruits of the first raceme in a completely randomized design and then, the following evaluations were performed: percentage of BER, growth evaluations, Ca^{2+} contents, physical-chemical parameters in fruits, stomatal conductance and transpiration. ABA and EBL treatments reduced BER incidence in a range of 6.6 to 9.0 %. The bioregulators used had no effect on plant growth, fruit diameter, length, or color. However, application of GA and EBL reduced titratable acidity and, the first also reduced soluble solids content in the fruit. All treatments, except GA treatment, increased Ca^{2+} contents in the fruits, when compared with the control. The highest fruit Ca^{2+} content was observed in plants treated with 0.01 µmol L^{-1} of EBL. According to our results, ABA and EBL increased Ca^{2+} concentrations and decreased BER incidence and thus there is a great potential for their use in agriculture in tomato fruit.

Keywords: Bioregulators; Calcium Balance; Stomatal conductance; Transpiration; Titratable acidity.
Abbreviations: ABA_abscisic acid; BER_blossom-end rot; BR_brassinosteroids; Ca^{2+}_calcium; EBL_24-epibrassinolide; GA_gibberellins; ROS_reactive oxygen species; SS_soluble solids; TA_titratable acidity.

Introduction

Calcium (Ca^{2+}) is an essential macronutrient in plants, actively playing a role in growth and development (White and Broadley, 2003). This nutrient plays an important role in the cell wall structure and also in the maintenance of the integrity and functioning of cell membranes (Hepler, 2005; Marschner, 1995; White and Broadley, 2003).

Translocation of Ca^{2+} occurs almost entirely via xylem, moving from roots to aerial part of plants. Therefore, absorption is highly dependent on transpiration rate and plant growth (Ho et al., 1993). Uptake through the roots occurs by apoplastic and simplastic pathways (Karley and White, 2009); however, as Ca^{2+} is maintained at low concentrations in the cytoplasm, the apoplastic pathway is believed to be predominant (White and Broadley, 2003). After entering the root, Ca^{2+} movement upward is regulated by means of sap flow in the xylem in response to the water potential gradient between the roots and the aerial parts of the plant (leaves and fruits).

Ca^{2+} is not commonly limiting under field conditions, but there are a number of disorders generally associated with low levels of this ion in plant tissue such as small root development, blossom-end rot, leaf necrosis, apple bitter pit, aqueous spots, among others (White and Broadley, 2003). Visual symptoms of Ca^{2+} deficiency in fruit tissue begin with cellular plasmolysis and soaked appearance of tissues, which progress to cell death and necrosis, resulting in dark staining of tissues (De Freitas et al., 2011). In tomato, watermelon, melon, and pepper, Ca^{2+} deficiency in distal parts of fruits is considered the main cause of the onset of symptoms of a physiological disorder known as blossom-end rot (BER) (Ho and White, 2005).

Studies suggest that a genetic selection process aiming to increase fruit size and weight may have favored the selection of mechanisms that inhibit accumulation of Ca^{2+} and/or encourage storage of this ion in cellular organelles, reducing Ca^{2+} bound to the cell wall and allowing fruit growth (De Freitas et al., 2012b). These metabolic modifications may have led to increased fruit susceptibility to BER as large and long tomatoes are more susceptible to BER than small, round tomatoes (Ho and White, 2005).

Studies have shown that BER does not occur exclusively due to insufficient availability of Ca^{2+}, but rather due to poor supply of Ca^{2+} caused by reduced transport of Ca^{2+} to the distal tissue of the fast growing fruit and also by an increased demand of the distal tissue for Ca^{2+} by accelerating fruit expansion (Ho, 1998; Saure, 2005). In addition, a number of substances such as gibberellins (GA) (De Freitas et al., 2012a), abscisic acid (ABA) (De Freitas et al., 2014), ammoniacal fertilizers (Bar-Tal et al., 2001; Castro and Malavolta, 1976), together with environmental conditions such as water stress (Guichard et al., 2005), can modify this translocation, preventing Ca^{2+} from reaching the fruit. Other factors affect the partition of Ca^{2+} in plants, such as excessive rate of transpiration or even the lack of it, induced by high temperatures and/or low relative humidity. Leaves have higher transpiration rates than fruits, thus resulting in a higher Ca^{2+} concentration in leaves (De Freitas et al., 2011).

Plant hormone ABA is known to reduce stomatal opening and to decrease transpiration of leaves, which may restrict the flow of sap and Ca^{2+} from the xylem to leaves and increase it to fruits (De Freitas et al., 2014; Guichard et al., 2005). Gibberellins are plant hormones responsible for the growth of plant tissue, being a trigger for cellular expansion, together with auxins (Ho and White, 2005). Studies have shown that, they inhibit tissue differentiation (Aloni, 2001) and may affect development and functionality of xylem vessels responsible for Ca^{2+} transport in the plant, from root to fruit (Saure, 2005). Studies in which ABA was applied in tomato plants showed increased Ca^{2+} in the pericarp tissue at the end of the peduncle. This occurred due to an increased xylem flow and decreased phloem flow towards fruits. In addition, a higher apoplastic Ca^{2+} concentration was observed in fruits, decreasing the permeability of membranes and reducing the incidence of BER (De Freitas et al., 2013).

Effects of brassinosteroids (BR) on the development of BER are being studied, but researches are not conclusive how it works. Some studies showed that they induce stress tolerance (Schnabel et al., 2001) and increase cell viability, strengthening their capacity to eliminate ROS (Liu et al., 2009).

Studies using 24-epibrassinolide (EBL) have shown an increased activity of antioxidant enzymes, as well as a synthesis of antioxidant substances such as ascorbic acid (Liu et al., 2009). In addition, BR is known for its role in the development of xylem vessels, and in its absence, there is a predominance of phloem vessel formation (Nagata et al., 2001). Accordingly, its presence can stimulate the accumulation of Ca^{2+} and reduce the levels of reactive oxygen species (ROS) in tissues, reducing fruit susceptibility to BER.

If this bioregulators can cause modifications in plants water relations, plant and fruit development, adaptation to the environment and nutrients partition, then they might control or minimize the effects and incidence of blossom-end rot.

Thus, the objective of this study was to evaluate the effect of bioregulators gibberellin, abscisic acid and 24-epibrassinolide, in the calcium partition and development of BER in tomatoes.

Results

Blossom-end rot incidence and physiological evaluations

ABA_1 and ABA_2 (90.8 µmol L^{-1} and 136.2 µmol L^{-1}, respectively) and EBL_1 (0.01 µmol L^{-1}) treatments showed a 6.6 - 9.0% reduction in BER incidence compared with control treatment (Fig. 1). GA and EBL_2 (0.1 µmol L^{-1}) treated plants had similar BER incidence to control plants. The BER incidence was higher in the first racemes and lower in the second, third and fourth racemes (Table 1). Application of bioregulators had no effect on plant growth (Table 2). The average height of all plants was 154.7 cm. No significant differences were observed between diameter and length of fruits among treatments (Table 2). Color of epidermis was statistically the same for all treatments, which presented a mean value for the 30° color angle (°h) at the red ripe stage (López Camelo and Gómez, 2004).

The titratable acidity (TA) was lower in fruits treated with GA, EBL_1 and EBL_2 than in control fruit (Table 3). There was no significant variation of fruit pH among treatments, with a mean of 3.7. Only GA treated fruit showed lower soluble solids content, compared with control fruit (Table 3). The observed SS/AT ratio (SS, Soluble solids) was around 6 and treatments using EBL promoted a higher SS/AT ratio, compared with control and ABA treatments (Table 3).

In leaves, a reduced Ca^{2+} content was observed in treatments with ABA_1 and EBL_2, when compared with control (Table 4). This reduction was more pronounced in ABA_1 treatments. In fruits, the lowest Ca^{2+} levels were observed in the control and GA treatments (Table 4). The highest levels of Ca^{2+} were observed in treatments with EBL_1 (Table 4).

The lowest transpiration rates were observed in treatments with ABA_1 and ABA_2 (Table 4). Stomatal conductance was lower in ABA_1, ABA_2 and EBL_2-treated plants, when compared with control (Table 4).

Correlations and principal component analysis

There was a significant positive correlation among several factors; however, a highly significant correlation (R = 0.9) between BER and Ca^{2+} concentration in fruits was verified, being the highest among all variables observed (Table 5). Likewise, BER is positively correlated with leaf Ca^{2+} concentration, stomatal conductance and leaf transpiration (Table 5), as well as negatively correlated with Ca^{2+} concentration in fruits. Soluble solids content is negatively correlated with incidence of BER in fruits (Table 5).

Principal component analysis was used to reduce dimensionality of database (Fig. 2), and a total of four dimensions explained 88.51% of total database variability. First component explained 34.10% of database, and the main variables that contributed to this component were soluble solids, pH, height, fruit number leaf Ca^{2+} and stomatal conductance. Second component explained 28.62%, and the main variables were % BER, fruit Ca^{2+} fruit diameter, fruit length and color index. Third component explained 22.55%, and variables were titratable acidity, ratio and transpiration. Fourth component explained 8.73% of database, and its main variable was weight and finally the fifth component explained 5.99% of database and no variable was explained in this component.

Table 1. Average percentage of blossom-end rot incidence in fruits by raceme, in the first 4 racemes.

Treatments^{1}	1st raceme (%)	2nd raceme (%)^{ns2}	3rd raceme (%)	4th raceme (%)
C	20.62±1.09 ab^{3}	4±8.94	0	0
GA	21.6± 3.45 a	0	0	0
ABA_1	14.04±5.47 c	2.22±4.96	0	0
ABA_2	13.34±6.03 c	6.81±10.89	0	0
EBL_1	11.66±2.19 c	0	0	0
EBL_2	14.72±0.85 bc	0	3.33±7.45 ^{ns}	4±8.94 ^{ns}

^{1}C = control; GA = 28.9 µmol L^{-1}; ABA_1= 90.8 µmol L^{-1}; ABA_2 = 136.2 µmol L^{-1}; EBL_1 = 0.01 µmol L^{-1}; EBL_2 = 0.1 µmol L^{-1}. ^{2}ns= the values were not statically different (Tukey 5%). ^{3}The average followed by same letter were not statically different (Tukey 5%)

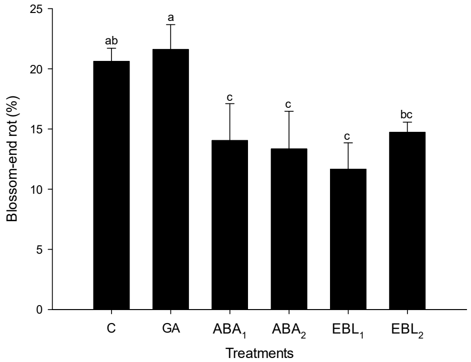

Fig 1. Percentage of blossom-end rot in the first raceme after 45 days of pollination of 'Tyna' tomatoes. Treatments: C = control; GA = 28.9 µmol L^{-1}; ABA_1= 90.8 µmol L^{-1}; ABA_2 = 136.2 µmol L^{-1}; EBL_1 = 0.01 µmol L^{-1}; EBL_2 = 0.1 µmol L^{-1}. Averages followed by the same letter are not statically different (Tukey 5%)

Table 2. Plant height, diameter, length, and average weight of fruits, number of fruits by raceme and epidermic color of fruits from 'Tyna' tomato

Treatments^{1}	Height (cm)^{ns2}	Diameter (mm)^{ns}	Length (mm)^{ns}	Weight (g)	Fruit number^{ns}	Color index^{ns}
C	151±14.8	44.6±4.0	54.8±5.4	556.5 ab^{3}	5.2±4.1	32.5± 1.7
GA	165±9.9	44.5±3.5	56.6±5.7	700.5 ab	6.9±3.2	32.1±1.2
ABA_1	154±10.0	44.1±3.0	55.3±4.6	673.8 ab	5.8±2.9	33.3±1.1
ABA_2	154±13.4	46.7±3.8	56.7±4.1	714.8 a	4.1±7.9	32.5±0.6
EBL_1	152±8.3	48.4±4.7	56.8±3.3	858.8 a	6.6±3.8	32.9±1.5
EBL_2	152±6.1	43.8±3.6	52.9±4.5	346.6 b	4.3±2.7	31.2±1.7
cv %	7.0	7.52	7.8	28.8	25.73	ns

^{1} C = control; GA = 28.9 µmol L^{-1}; ABA_1=90.8 µmol L^{-1}; ABA_2 = 136.2 µmol L^{-1}; EBL_1 = 0.01 µmol L^{-1}; EBL_2 = 0.1 µmol L^{-1}. ^{2}ns =The values were not statically different (Tukey 5%). ^{3}The average followed by same letter were not statically different (Tukey 5%)

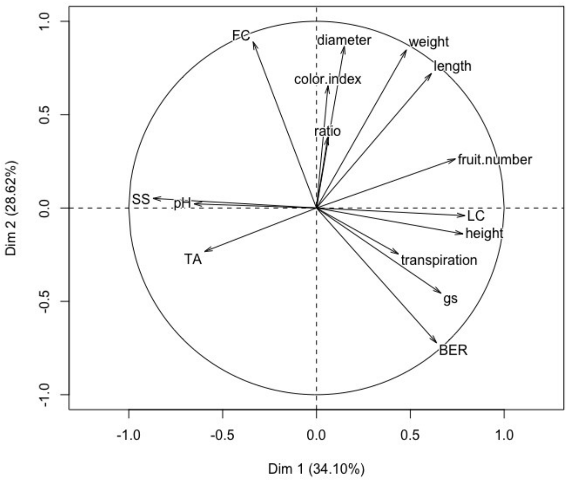

Fig 2. Relationship among variables (principal component analysis) shown by scatter plot of the first two principal components based on traits. BER = blossom end rot; Dim = dimension from principal component analysis database; FC = fruit Ca^{2+}; gs = stomatal conductance; LC = lead Ca^{2+}; SS = soluble solids; TA = tritratable acidity. First two dimensions represents 62,72% of the data.

Table 3. Tritatable acidity (TA), Soluble solids (SS), pH, and Ratio of ‘Tyna’ tomato fruits.

Treatments[1]	TA (% citric acid)	SS (°Brix)	pH[ns2]	Ratio
C	0.84 a[3]	5.04 a	3.62	6.0 c
GA	0.52 c	3.78 b	3.59	7.27 bc
ABA_1	0.85 a	5.58 a	3.69	6.56 bc
ABA_2	0.78 ab	4.62 ab	3.65	5.92 c
EBL_1	0.52 c	5.19 a	3.77	9.98 a
EBL_2	0.67 b	5.57 a	3.88	8.31 b
cv%	10.93	9.94	5.39	10.45

[1] C = control; GA = 28.9 µmol L^{-1}; ABA_1= 90.8 µmol L^{-1}; ABA_2 = 136.2 µmol L^{-1}; EBL_1 = 0.01 µmol L^{-1}; EBL_2 = 0.1 µmol L^{-1}. [2]ns = The values were not statically different (Tukey 5%). [3]The average followed. by same letter were not statically different (Tukey 5%)

Table 4. Stomatal conductance (g_s), transpiration, and Ca^{2+} content in leaf and fruit tissue of ‘Tyna’ tomato.

Treatments[1]	g_s (cm s^{-1})	Transpiration (µg cm^{-2} s^{-1})	Leaf Ca^{2+} g kg-1	Fruit Ca^{2+} g kg-1
C	0.99 ab[2]	7.82 a	24.68 ab	0.52 e
GA	1.02 a	8.28 a	25.87 a	0.62 d
ABA_1	0.83 d	7.21 b	17.75 d	0.82 b
ABA_2	0.74 e	6.64 c	23.80 ab	0.87 b
EBL_1	0.93 bc	8.33 a	23.24 b	0.95 a
EBL_2	0.90 cd	8.15 a	20.03 c	0.72 c
cv%	4.01	3.55	4.73	4.3

[1] C = control; GA = 28.9 µmol L^{-1}; ABA_1= 90.8 µmol L^{-1}; ABA_2 = 136.2 µmol L^{-1}; EBL_1 = 0.01 µmol L^{-1}; EBL_2 = 0.1 µmol L^{-1}. [2]The average followed by same letter were not statically different (Tukey 5%). DM= dry mass.

Table 5. Correlation analysis between physiological analysis and BER incidence to determine parameters potentially inhibiting ($-R^2$) or triggering ($+R^2$) BER in ‘Tyna’ tomato

Inhibiting BER	R^{2a}	Triggering BER	R^{2b}
Fruit Ca^{2+}	-0.93	Stomatal conductance	0.71
pH	-0.61	Height	0.55
Soluble solids	-0.59	Leaf Ca^{2+}	0.55
Fruit diameter	-0.55	Transpiration	0.30
Ratio	-0.43	Fruit number	0.26
Color index	-0.26		
Fruit total weight	-0.24		
Fruit length	-0.06		
Tritratable acidity	-0.01		

[a]Positive correlations mean a proportional correlation between two variables; [b] negative correlations mean inversely proportional correlation between the variables

Together, these results indicate that the ABA and EBL applications were able to alter the Ca^{2+} balance in the plants and reduce the incidence of BER in the fruits. The most pronounced effects were evidenced in EBL-treated plants, which did not show changes in fruit characteristics such as color, pH, and soluble solids, while increasing ratio values.

Discussion

In the present study, the incidence of BER was higher in first racemes, possibly due to rapid growth of the distal part of the fruits, which increases the demand for Ca^{2+}, compared with fruits of other racemes (Ho, 1998). During development of the first racemes, the plant presents a high vegetative growth, mainly in cultivars with indeterminate cycle, which have a continuous vegetative and reproductive growth, as is the case of 'Tyna'. This fact increases the competition for Ca^{2+} between leaves and fruits, and due to low Ca^{2+} mobility in the aerial part (Dražeta et al., 2004) associated to the higher leaf rates of transpiration and growth (De Freitas et al., 2011; Ho and White, 2005), the Ca^{2+} transport to the distal part is reduced, thus promoting the onset of the disorder (Ho, 1998).

A strategy that can be used to overcome this problem is to diminish Ca^{2+} transport to the leaves by reducing transpiration rates (Li et al., 2001). In the present work, high temperature peaks and low relative humidity, typical of the local winter, were observed, which may have contributed to the high transpiration rates observed (around 6 µg cm^{-2} s^{-1}) and, therefore, to the high content of Ca^{2+} in leaves. In addition, a decrease in relative air humidity, and consequently in the vapor pressure deficit, contributes to a greater occurrence of BER (De Kreij, 1996). In fact, through correlation analysis between evaluated parameters, it was possible to identify negative correlation between leaf transpiration and Ca^{2+} content in fruits.

Although the total Ca^{2+} content is important for the development of BER, metabolism of this nutrient at a cellular level may be even more relevant. Ca^{2+} may be bound to cell wall pectins, to phosphate groups of cell membranes, or even present within organelles and vacuole (De Freitas et al., 2012a). Thus, to minimize BER, more than raising Ca^{2+} levels in the soil, factors that modify nutrient partitioning between different drains should be considered.

In this regard, the use of bioregulators proves efficient as a way to alter physiological responses and thus minimizing damages caused by a high incidence of BER in fruits. The previous study reported an increased percentage of fruits with BER through exogenous applications of gibberellins, even without major changes in plant growth (De Freitas et al., 2011). However, in the present work, we verified that application of GA did not increase the incidence of BER in tomato fruits when compared with the control, although, in both cases, the percentage of BER were high when compared with other treatments. It is possible that high endogenous concentrations of GAs in control plants resulted in saturation of physiological responses to this hormone. In this regard, application of exogenous GA had no additional effect when compared with control plants.

The high percentage of BER observed in control plants, which are under the effect of endogenous GAs, and in plants treated with exogenous GA may be related to its effects on transpiration and stomatal conductance. In cases of high transpiration rates, the leaves become preferential drains to the detriment of the fruits (De Freitas et al., 2013). Although there was no significant reduction in Ca^{2+} content in the fruits, the presence of both endogenously and exogenously applied GAs leads to an accelerated growth of the fruits, increasing their demand for Ca^{2+} and favored the development of the disorder in fruits of control and GA-treated plants.

In other way, researches conducting ABA application directly on fruits showed that this yielded a significant reduction in the percentage of BER incidence. This efficiency in maintaining adequate Ca^{2+} levels occurs mainly through control of leaf transpiration, modifying the Ca^{2+} partition between leaves and fruits (De Freitas et al., 2014). In PCA, it was possible to observe a positive correlation between BER, transpiration and leaf Ca^{2+} belonging to the same dimension. Since ABA regulates stomatal closure, its foliar application causes a greater part of Ca^{2+} that arrives at the aerial part of the plant to be directed to the fruits, when compared with plants not treated with ABA (De Freitas et al., 2014). In the present work, there was a significant reduction in the occurrence of BER by ABA treatments and reduction in transpiration rates and stomatal conductance as well as an increased Ca^{2+} levels in fruits when compared with the control.

Additionally, here we observed a beneficial effect of EBL_1 on the reduction of BER, associated with a decrease in the Ca^{2+} content in leaves and its increase in fruits, despite a significant reduction in transpiration rates in fruits of EBL-treated plants were not observed. Although some studies have shown a decrease in transpiration in response to BRs (Xu et al., 1994) and in tomato higher concentrations of EBL led to a total stomatal closure (Xia et al., 2014), the data obtained show that there is no clear relationship between transpiration and BER reduction in response to EBL treatments.

Under conditions of high temperature and low relative air humidity, it is possible that a situation of thermal stress in plants occur. Although during the course of the experiment extreme temperatures and low relative air humidity were observed, it is worth mentioning that EBL provides greater tolerance to environmental stresses (Ahammed et al., 2015). BRs act by increasing plant tolerance to stress (Schnabel et al., 2001), improving ability of cells to cope with ROS (Liu et al., 2009). It is possible that the reduction in the incidence of BER was directly related to this improvement in the antioxidant capacity of fruit cells.

In addition, studies have shown that BRs increase differentiation of transporter tissues, raising the concentration of xylem tissues (Nagata et al., 2001). Since Ca^{2+} moves through xylem, this higher amount of tissues may result in an improvement in Ca^{2+} rise, a fact observed in this work, in which there was a higher amount of Ca^{2+} in fruits in response to application of EBL. Thus, the lower percentage of BER in this study may still be explained by these two factors, acting together.

Therefore, it is speculated that these plants have become more adapted to this environmental condition, improving plants' physiological responses and directing more Ca^{2+} to fruits.

Although bioregulators may be able to reduce susceptibility to BER, it is desirable that they do not alter fruit quality. Treatments did not alter the color or number of fruits. pH values and soluble solids were also unaffected, except for GA, which reduced the soluble solids of fruits. Treatment with GA is widely used in fruits to increase their size, the size of peduncles, and to accelerate growth (De Freitas et al., 2012a). It is possible that, with faster growth of the fruits, accumulation of solids was lower. The pH has not been altered by treatments or in control fruits. Conversely, total acidity was reduced by treatments, mainly in EBL-treated plants, with exception to fruits that were treated with ABA.

Ratio is a relative value that measures the ratio of soluble solids to titratable acidity. It gives us information about the sensorial quality of fruits. We do not have data do support these ideas, but we believe that the growth conditions, nutrition, environment and climate could contribute to low pH, high tritratable content and then for ratio. Nevertheless, EBL treatment increased the values of the ratio, showing that the treatment, besides acting positively in reducing BER, contribute to improve their organoleptic characteristics

Materials and Methods

Plant material, growth conditions, and application of treatments

Elongated genotype of tomato, cultivar Tyna was cultivated in a greenhouse. Plants were seeded in trays with a ratio of commercial product (Plantmax HT, Eucatex Brazil) to expanded vermiculite, supplemented with 1 g NPK 10:10:10 L^{-1} and 4 g L^{-1} limestone, indicated for seed germination. Thirty days after planting, seedlings were transplanted into individual 30 L pots containing oxysol and organic substrate in the ratio 2:1.

The plants were fertilized every 20 days, during de growing and fructification time, with 10 g of slow release fertilizer containing N (16%), P_2O_5 (8%), K_2O (12%), MgO (2%), S (5%), Fe (0.4%), Cu (0.05%), Mn (0.06%), Zn (0.02%), B (0.02%), Mo (0.015%), but without Ca (Basacote Plus; Compo Expert; Soil fertilizer, Agricultural), to stimulate the incidence of BER, as well as soil with a low level of calcium (9 mmolc dm^{-3}) was used; added to pot for each plant. The plants were irrigated every other day until saturation of the substrate. Conditions of air temperature and relative humidity were monitored throughout the cycle, from planting to harvest, with a mean of 20°C and 71%, respectively.

From pollination to physiological maturation of fruits of the first raceme (60 days after pollination), plants were sprayed biweekly with a 125-ml solution per plant containing water (control), GA (GA_3, PROGIBB, Sumitomo Chemical do Brasil, Sao Paulo, SP) (28.9 µmol L^{-1}), ABA_1 (Valent Biosciences, Libertyville, IL, USA) (90.8 µmol L^{-1}), ABA_2 (Valent Biosciences) (136.2 µmol L^{-1}), EBL_1 (Sigma-Aldrich, Saint Louis, MO, USA) (0.01 µmol L^{-1}) and EBL_2 (Sigma-Aldrich) (0.1

µmol L^{-1}). At the moment of the red ripe stage of fruits from the first raceme, evaluations of plants and fruits were carried out.

Growth evaluations

Evaluations were divided into plant and fruit analyses. The former consisted in measuring plants' final height. The latter evaluated the number of fruits, diameter and average length, and percentage of fruits with visual symptoms of BER. Only fruits of the first raceme were used for evaluations. Incidence of BER was also evaluated in subsequent racemes.

Determination of total leaf and fruit Ca^{2+} contents

Whole fruits and leaves, oven-dried at 65ºC until constant weight, were used. Samples of mature leaves were removed from the region close to the fruits of the first raceme. Five-hundred milligrams of dry material were taken, 6 mL of nitroperchloric acid (2:1) was added and the material digested in gypsum block at 240° C and completed to 15 g with distilled water. Total Ca^{2+} was determined by atomic absorption, according to Malavolta et al. (1997). Results were expressed in g of Ca^{2+} per 100 g of fruit dry matter.

Determination of physical-chemical parameters in fruits

Soluble solids (SS) were determined in fruit juice after trituration in a domestic centrifuge. A drop of juice was used, and readings were performed in an Atago digital refractometer, Palete 101 model (Atago, Ribeirao Preto, SP, Brazil), with two readings per repetition. Results were expressed as percentage. Titratable acidity (TA) was determined in fruit juice after crushing, where 10 g of juice was weighed and filled with distilled water to make 100 mL. Subsequently, it was titrated with NaOH (0.1 N) to the pH 8.1, under constant agitation, according to the methodology described by Carvalho et al. (1990). Results were expressed as % citric acid in the juice. Ratio: was expressed as a ratio between soluble solids/titratable acidity.
The color of fruit skin was determined using a Minolta colorimeter, CR-300 model (Konica Minolta Holdings Inc., Tokyo, Japan), with the following configuration: CIELab color system, D65 illuminator and standard observer 2º. Two readings per fruit were performed on opposite sides of its equatorial region, and the results were expressed in color angle (h).

Determination of stomatal conductance and transpiration

Leaf gas exchange was measured using A LI-COR-1600 machine (LI-COR Biosciences, Lincoln, NE, USA) model, according to manufacturer's recommendations. The analyses were performed between 9 and 11 am at 10, 25, 40, and 55 days after pollination of the flowers of the first raceme. Mature leaves close to first raceme were used. The values obtained for transpiration and stomatal conductance correspond to the average of the 4 evaluations performed on leaves during the period of experiment.

Experimental design

Experimental design was a completely randomized design, with 6 treatments, five replicates and two plants per replicate. Results were submitted to analysis of variance and, when significant, were submitted to the Tukey test at the 5% probability. Data were also submitted to principal component analysis (PCA).
For selection of factors to be analyzed, the Kaiser criterion (Kaiser, 1960) was used for factors with their own value greater than 1. Analysis was performed using the FactoMineR bookstore (Husson, 2014) in the R project.

Conclusion

Applied bioregulators influenced availability of Ca^{2+}, modifying contribution to fruits to the detriment of leaves. Application of ABA reduced the percentage of BER, keeping transpiration levels lower when compared with control and GA-treated plants. In addition, EBL treatments reduced the percentage of BER, but did not alter fruit characteristics such as color, pH, and soluble solids, while increasing ratio values, evidencing an improvement in the sensorial quality of fruits.
Despite the mechanisms presented here, more research should be carried out to understand mainly how BRs act in the control of BER in fruits and its relation with ABA. There is a great potential for their use in agriculture; however, the first factors for the consolidation of their use would be to study the ideal doses, periods and place of application, adverse effects they may cause in post-harvest storage, as well as market acceptance of these fruits. The commercial use of bioregulators can be recommended and their action spectra and efficiencies will be optimized in other varieties and experimental conditions through further research.

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