DOI: 10.5965/223811712142022428

Revista de Ciências Agroveterinárias 21 (4): 2022
Universidade do Estado de Santa Catarina

The application of 28-homocatasterone brassinosteroid reduces blossom end rot in 'BRS Montese' tomatoes

A aplicação de brassinosteroide 28-homocatasterona reduz a podridão estilar em tomates 'BRS Montese'

Paulo Sérgio Gularte[1] (ORCID 0000-0003-2399-3546), Aquidauana Miqueloto Zanardi[2] (ORCID 0000-0001-6051-2882), Tiago Miqueloto[1] (ORCID 0000-0002-1846-4466), Odimar Zanuzo Zanardi[2] (ORCID 0000-0002-1872-0749), Cristiano André Steffens[1] (ORCID 0000-0003-0936-8656), Cassandro Vidal Talamini do Amarante[1](ORCID 0000-0003-0563-2093)

[1]Santa Catarina State University, Lages, SC, Brazil. * Author for correspondence: p.gularte7@gamil.com
[2]Federal Institute of Santa Catarina, São Miguel do Oeste, SC, Brazil.

Submission: 22/Mar/2022 | Acceptance: 09/May/2022

ABSTRACT

We assessed the effect of 28-homocatasterone on xylem functionality and its relationship with blossom end rot (BER) occurrence and the quality of 'BRS Montese' tomatoes. Tomato plants were cultivated in a protected environment, with a semi-hydroponic system. During full flowering, open flowers were selected, marked, and pollinated. After two days, the flowers received the application of 28-homocatasterone at a concentration of 10^{-6} M or deionized water (control). Treatments were reapplied weekly up to 24 days after the first application (DAFA). At 24 DAFA, fruits were harvested and evaluated for fresh mass, texture (skin rupture and pulp penetration forces), skin color, xylem functionality, membrane permeability, apoplastic calcium concentration, and BER incidence. The application of 28-homocatasterone at 10^{-6} M did not increase the fresh mass nor change the fruits' texture attributes. However, 28-homocatasterone increased or kept xylem functionality, which was assicated with increased apoplastic calcium concentration and reduced BER occurrence in 'BRS Montese' tomatoes. Thus, the application of 10^{-6} M catasterone could be an alternative for BER control in tomatoes.

KEYWORDS: *Solanum lycopersicum;* calcium; xylem vessels; physiological disorder.

RESUMO

Avaliamos o efeito da 28-homocatasterona na funcionalidade do xilema e sua relação com a ocorrência de podridão estilar (PE) e a qualidade de tomates 'BRS Montese'. Os tomateiros foram cultivados em ambiente protegido, com sistema semi-hidropônico. Durante a plena floração, as flores abertas foram selecionadas, marcadas e polinizadas. Após dois dias, as flores receberam a aplicação de 28-homocatasterona na concentração de 10^{-6} M ou água deionizada (controle). Os tratamentos foram reaplicados semanalmente até 24 dias após a primeira aplicação (DAPF). Aos 24 DAPF, os frutos foram colhidos e avaliados quanto à massa fresca, textura (força de ruptura da epiderme e resistência a penetração da polpa), cor da casca, funcionalidade do xilema, permeabilidade da membrana, concentração de cálcio apoplástico e incidência de PE. A aplicação de 28-homocatasterona a 10^{-6} M não aumentou a massa fresca nem alterou os atributos de textura dos frutos. No entanto, a 28-homocatasterona aumentou ou manteve a funcionalidade do xilema, o que foi associado ao aumento da concentração de cálcio apoplástico e redução da ocorrência de PE em tomates 'BRS Montese'. Assim, a aplicação de catasterona 10^{-6} M pode ser uma alternativa para o controle de PE em tomates.

PALAVRAS-CHAVE: *Solanum lycopersicum*; cálcio; vasos xilema; desordens fisiológicas.

INTRODUCTION

Tomato fruits (*Solanum lycopersicum* Mill.) are consumed worldwide, possibly because of their wide culinary versatility as well as the presence of lycopene and other antioxidant compounds valuable for human health (RAIOLA et al. 2014). In addition, tomato cropping is an important alternative to generate income for small and medium-sized farmers due to its easy handling and the high commercial value added to the fruits (SOLDATELI et al. 2020). However, the frequent occurrence of physiological disorders, such as the blossom end rot (BER) and tomatoes cracking, reduces crop yield (OLLE & WILLIAMS 2017).

The BER in tomatoes is characterized by the presence of dark stains that cause tissue softening and necrosis in the distal part of the fruits (ANDRADE et al. 2020). The occurrence of BER is directly related to the reduction of calcium (Ca) intake (FREITAS et al. 2011) as well as its redistribution in the xylem from the proximal to the distal regions of the fruits (FREITAS et al. 2010, FREITAS et al. 2014, REITZ et al. 2021).

The xylem vessels in fruits gradually lose their functionality with fruit growth (SONG et al. 2018). In tomatoes, the decrease in the density of the xylem vessels occurs predominantly in the distal region of the fruits, reducing Ca supply and favoring the BER incidence (SAURE 2005, PEET 2009). Techniques to increase Ca supply and its redistribution inside tomatoes may attenuate the occurrence of physiological disturbances linked to Ca deficiency in fruits. However, exogenous applications of Ca have shown limited effectiveness in reducing the incidence of BER in tomatoes (ANDRADE et al. 2020).

Brassinosteroids (BRs) are hormones produced by plants that play an important role in plant development (TAIZ & ZEIGER 2017). In plant tissues, BRs promote the organization of cellulose microfibrils and the regulation of cell division and expansion, besides increasing the contents of soluble solids and phenolic compounds in fruits (PLANAS-RIVEROLA et al. 2019, RAMOS et al. 2019). BRs also act in the formation of xylem vessels, regulating the procambial cell differentiation and tracheal elements (TAIZ & ZEIGER 2017). The only known BRs active forms are hitherto, brassinolide, 28-epibrassinolide, catasterone, and ponasterone (ALCANTARA-CORTES et al. 2019). Therefore, it is believed that exogenous applications of BRs in tomato flowers and fruits can stimulate the formation of new xylem vessels, reduce BER incidence, and improve fruit quality. This study evaluated the relationship between the application of 28-homocatasterone on the functionality of xylem vessels, on post-harvest quality, and BER occurrence in 'BRS Montese' tomatoes.

MATERIAL AND METHODS

For the experiment, tomato seedlings were produced from ‘BRS Montese’ tomato hybrid seeds in polystyrene trays containing *Sphagnum* spp*.* peat-based substrate expanded perlite, vermiculite, roasted rice husk, dolomitic limestone, agricultural plaster, and NPK mineral fertilizer (Carolina Soil® Ltda., Santa Cruz do Sul, RS). After emergence, the seedlings were treated with azoxystrobin + difenoconazole fungicide at a concentration of 30 mL of the commercial product to 100 L of water (Amistar Top®, Syngenta Proteção de Cultivos Ltda. São Paulo, Brazil) to avoid the incidence of phytopathogens. Seedlings with three fully expanded leaves were transplanted in polyethylene pots with a capacity of 7.0 L containing organic substrate with 83.4% of composted *Pinus* sp*.* bark, 10% of expanded vermiculite, 5% of roasted rice husk, 0.5% of acidity correctives, 0.5% of rock phosphate, and 0.6% of NPK mineral fertilizer (Maxfertil substratos® Ltda., Pouso Redondo, Santa Catarina, Brazil). The substrate had a pH of 6.0 ± 0.5, electrical conductivity of 0.5 ± 0.3 mS cm^{-1}, water retention capacity of 90%, and density of 310 kg m^{-3}. The pots were placed at a spacing of 1.2 m between rows and × 0.5 m between plants in a greenhouse at room temperature. The tomato plants were grown in a single stalk in polyvinyl chloride ribbons and wire (tutoring). The pruning in the apical meristem of the plants was carried out after the emission of the third inflorescence.

During the experiment, the plants were fertigated using drippers (semi-hydroponic system), without reusing the nutrient solution drained by the pots. For the vegetative stage, the nutrient solution used was composed of 800 g of calcium nitrate, 158 g of potassium nitrate, 55 g of monoammonium phosphate, 450 g of magnesium sulfate, 166 g of NKP (52% of phosphorus in P_2O_5 and 34% of potassium in K_2O water-soluble), 413 g of potassium chloride, 10 g of ConMicros Light, and 44 g of chelated iron (6%). For the reproductive stage, the plants were fertigated with a solution composed of 1.100 g of calcium nitrate, 250 g of potassium nitrate, 61 g of monoammonium phosphate, 450 g of magnesium sulfate, 159 g de NKP, 522 g of potassium chloride, 10 g of ConMicros Light, and 44 g of chelated iron (6%). In both phases, the solutions were pre-balanced and diluted to 1,000 L of solution suitable for growing tomatoes. The electrical conductivity (EC) of the solutions leached by the vessels was measured every 24 h. The CE adjustment was performed whenever values were below or above 2.5 mS cm^{-1}. The need for irrigation was determined using the crop evapotranspiration method, calculated by the sum of crop evapotranspiration (ETc), water application intensity, and system efficiency (SOUSA et al. 2011).

For phytosanitary management, sprays of azoxystrobin + difenoconazole fungicides were carried out (Amistar Top®, Syngenta Proteção de Cultivos Ltda., São Paulo, Brazil) at a concentration of 40 mL of product for 100 L of water and mancozeb + metalaxyl-M (Ridomil®, Syngenta Proteção de Cultivos Ltda., São Paulo, Brazil) at a concentration of 300 g of commercial product for 100 L of water. For the control of pest insects, lambda-cyhalothrin insecticide was applied (Karate Zeon 50 CS®, Syngenta Proteção de Cultivos Ltda., São Paulo, Brazil) at a concentration of 45 mL of commercial product for 100 L of water.

Twenty two days after transplanting (when 60% of the plants had their first inflorescence open), the open flowers were selected, marked, and manually pollinated. Two days after pollination, each inflorescence was sprayed with 0.002 mL of a 28-homocatasterone solution at a concentration of 10^{-6} M or deionized water (control). Treatments were reapplied weekly up to 24 days after the first application (DAFA). At 24 DAFA, fruits were harvested and assessed for fresh mass, texture (skin rupture and pulp penetration resistance forces), skin color, xylem functionality, membrane permeability, apoplastic Ca concentration, and BER incidence. The fruits were harvested 24 DAFA due to this is the period that started the occurrence of BER (ANDRADE et al. 2020).

The fresh mass of the fruits was measured right after harvest with the aid of a precision analytical balance (0.0001 g). Texture attributes (peel strength and pulp breaking force) were measured on opposite sides of the equatorial region of the fruits using an electronic texture analyzer (TA.XT-plus®, Stable Micro Systems Ltda., UK). For that, we used a probe with a diameter of 2 mm, introduced at a depth of 10 mm with pre-test, test, and post-test speeds of 6, 3, and 10-mm s^{-1}, respectively, and the results were expressed in Newton (N).

The skin coloration was assessed using a 450 G Delta Vista colorimeter (Delta Color Indústria e Comércio de Equipamentos Eletrônicos Ltda., São Leopoldo, Rio Grande do Sul, Brazil), in which the parameters L (*lightness*), C (*chromaticity*), and *h°* (*hue angle*) were analyzed. The color of the epidermis was determined by two readings taken in the equatorial region and on opposite sides of the fruits (more and less exposed to solar radiation).

The xylem functionality was assessed using the infusion technique with basic fuchsin dye at 1%, according to the procedure described by DRAŽETA et al. (2004). The fruits were harvested, and the peduncle was immediately submerged in ultrapure water to avoid xylem embolism. In the laboratory, we removed a section of approximately 0.2 cm of the peduncle and the remainder of the peduncle was immersed in basic fuchsin dye (1%). To remove the boundary layer of air, a fan was used close to the fruits. The number of xylem vessels in each fruit was visually quantified 24 h after immersion of the peduncles in the dye.

The soluble Ca content in the apoplast was quantified in 12 discs (4 discs/fruit), with 1 cm in diameter × 0.3 cm thickness. The discs were removed below the skin and in the proximal, median, and distal regions of the fruits. Afterward, the discs were washed in deionized water for 10 sec to reduce the contamination with extravasated Ca from tissues present on the surface. Each disc was placed in a 5 mm diameter × 115 mm height glass funnel adapted on a kitasato and subjected to vacuum. On each disc, 600 µL of an isotonic mannitol solution (0.2 M) was pipetted and the eluate was collected in the kitasato. The soluble Ca contents in the apoplast were quantified in plasma-induced emission spectroscopy and the results were expressed in µg g^{-1} of FW (fresh weight).

The membrane permeability was quantified through electrolyte leakage. For that, 400 mg discs were removed from the fruits healthy, washed three times in ultrapure water, and floated in vials containing 20 mL of deionized water. The electrical conductivity of the liquid suspension was analyzed in a conductivity meter (Lutron CD 4301, Lutron Electronic Enterprise Co. Ltd, Taipei, Taiwan) for 30, 60, and 120 min after incubation at room temperature, and the results were expressed as a percentage of total conductivity.

The BER incidence (%) was determined based on counting the number of fruits with visible BER symptoms and the total number of fruits collected at 24 DAFA.

The experiment was carried out following a randomized complete block design with two treatments and four replications and with each experimental unit composed of five plants. The data were initially submitted to the Bartlett (BARTLETT 1937) and Shapiro-Wilk (SHAPIRO & WILK 1965) tests to verify the homogeneity of variances and the residual normality, respectively. Once the assumptions of the normal model were met, the means were compared by Student *t*-test ($p<0.05$). All statistical analyses were performed using the statistical software SAS, version 9.1 (SAS INSTITUTE 2002).

RESULTS AND DISCUSSION

The application of 28-homocatasterone did not change the fresh mass and texture attributes (peel strength and pulp breaking force) of 'BRS Montese' tomatoes (Table 1).

In apples, the application of growth regulators can change the fresh mass of the fruits (MIQUELOTO et al. 2018), reducing cell division and expansion due to a smaller placental development, locule contraction, and absence of seeds (AYUB & REZENDE 2010). The results found in this study contradict those reported by RIBOLDI et al. (2018) in which the application of brassinosteroid in 'BRS Montese' tomatoes increased the diameter, length and fresh mass of the fruit. Fruit texture is a sensory attribute used by consumers as a criterion for their purchase choice. Reduced calcium concentrations may affect total and soluble pectin. Changes in cell wall components, especially cellulose, hemicellulose, and pectins affect texture modifications. Adequate Ca concentrations in fruit tissues promote the maintenance of texture, firmness, and resistance to damage as well as the reduction in the senescence process of the fruits (RAMOS et al. 2019).

Table 1. Fresh mass and texture of 'BRS Montese' tomatoes harvested 24 days after the application of 28-homocatasterone at a concentration of 10^{-6} M.

Treatment	Fresh mass[1] (g)	Texture[1] (N)	
		Peel strength	Pulp breaking force
Control	17.6 ± 1.0 a	11.31 ± 0.8 a	361.00 ± 9.1 a
28-homocatasterone	19.6 ± 3.1 a	10.44 ± 0.7 a	273.00 ± 10.3 a
t-test	21.91	3.46	3.50
P-value	0.6633	0.5507	0.439

[1]Data (mean ± SE) followed by the same letter in the column do not differ significantly by the *t*-test ($p<0.05$).

The application of 28-homocatasterone did not change the color parameters L, C, and *h*° of the fruits on the side that was less exposed to solar radiation (Table 2).

Table 2. Skin color of 'BRS Montese' tomatoes harvested 24 days after application of 28-homocatasterone at a concentration of 10^{-6}M.

Treatment	Skin color on the side more exposed to solar radiation[1]		
	L	*C*	*h*°
Control	48.1 ± 2.7 a	39.1 ± 0.9 a	42.8 ± 1.4 a
28-homocatasterone	51.4 ± 1.0 a	38.2 ± 0.7 a	34.0 ± 0.8 b
t-test	0.76	2.00	1.20
P-value	0.3473	0.7320	0.0028
	Skin color on the side less exposed to solar radiation[1]		
Control	75.8 ± 2.3 a	42.6 ± 0.8 a	106.6 ± 1.3 a
28-homocatasterone	73.9 ± 1.0 a	42.3 ± 0.4 a	105.7 ± 0.5 a
t-test	0.67	0.74	1.80
P-value	0.1172	0.8621	0.6487

[1]Data (mean ± SE) followed by the same letter in the column do not differ significantly by the *t*-test ($p<0.05$).

However, fruits treated with 28-homocatasterone had lower *h*° values in the region more exposed to solar radiation, indicating a greater intensity of the red color in fruits (Table 2). The application of 28-homocatasterone possibly induced greater expression of PSY-1 genes, responsible for carotenogenesis at the expense of PSY-2 (predominant in green tissues), increasing the activity of enzymes involved in the biosynthesis pathway of pigments, such as lycopene (RAMOS et al. 2019).

The application of 28-homocatasterone increases the number of functional xylem vessels and Ca concentration in the apoplast in the proximal, median, and distal regions of the fruits compared with the control treatment (Table 3).

In *Arabidopsis thaliana*, the application of BRs promoted the formation of new xylem vessels, since 28-homocatasterone enhanced the formation of BES1 (*bri1*-EMS-suppressor-1) and BZR1 (brassinazole-resistant-1) transcripts, transcription factors that promote the expression of several genes, including the factors that encode proteins to differentiate xylem vessel elements in the procambium (FUKUDA 2004, SAITO et al. 2018). Furthermore, the use of the BRs biosynthesis inhibitor in *Lepidium sativum* plants increased the number of phloem vessels and inhibited the development of secondary xylem, indicating that BRs influence the development of xylem vessels in plants (NAGATA et al. 2001). The xylem is a conductive tissue formed by lignin and cellulose, responsible for transporting water and mineral salts from the roots to shoot tissues (leaves, branches, flowers, fruits, and seeds) of plants (TAIZ & ZEIGER 2017). During fruit growth, some xylem vessels are compressed and lose their functionality due to the elongation process of the parenchyma cells, reducing the flow and redistribution of water and Ca in the fruits (DRAŽETA et al. 2004). Low Ca concentration in fruits reduces Ca availability, which can cause the loss of membrane integrity (MIQUELOTO et al. 2018). In our study, fruits treated with 28-homocatasterone had a higher concentration of apoplastic Ca than control fruits (only distilled water) (Table 3). The increase in Ca supply to the fruits was possibly due to the stimulation of xylogenesis promoted by the application of BRs (MIQUELOTO et al. 2018). Ca has low or no mobility in the phloem and is therefore transported to the fruits almost exclusively via the xylem (FAQUIN 2005). Other studies with the application of brassinosteroids in tomato also found an increase in apoplastic calcium and related to greater functionality and number of xylem vessels (FREITAS et al. 2017, RIBOLDI et al. 2018).

Table 3. The number of functional xylem vessels and apoplastic calcium in 'BRS Montese' tomatoes, harvested 24 days after application of 28-homocatasterone at a concentration of 10^{-6} M.

Treatment	Number of functional xylem vessels[1]			Apoplastic calcium[1]
	Proximal	Median	Distal	(µg g^{-1} FW)
Control	8.3 ± 2.0 b	7.3 ± 1.5 b	4.6 ± 0.8 b	1.5 ± 0.6 b
28-homocatasterone	28.0 ± 2.5 a	21.0 ± 2.0 a	19.0 ± 0.5 a	6.6 ± 1.0 a
t-test	4.28	5.38	2.19	3.12
P-value	0.0233	0.0079	0.0087	0.0047

[1]Data (mean ± SE) followed by the same letter in the column do not differ significantly by the *t*-test (p<0.05).

The application of 28-homocatasterone did not change the electrolyte leakage after 30 and 60 min of fruit incubation (Table 4).

Table 4. Electrolyte leakage after 30, 60, and 120 minutes of incubation of 'BRS Montese' tomatoes harvested 24 days after application of 28-homocatasterone at a concentration of 10^{-6} M.

Treatment	Electrolyte leakage[1]		
	30 min	60 min	120 min
Control	9.8 ± 1.2 a	39.3 ± 2.3 a	40.0 ± 2.5 b
28-homocatasterone	17.1 ± 2.0 a	39.9 ± 1.9 a	50.5 ± 2.2 a
t-test	1.80	1.90	2.70
P-value	0.1322	0.8512	0.0317

[1]Data (mean ± SE) followed by the same letter in the column do not differ significantly by the *t*-test (p<0.05).

However, after 120 min of incubation, fruits treated with 28-homocatasterone showed greater electrolyte leakage (Table 4). The application of 28-homocatasterone possibly increased membrane permeability, promoting greater Ca supply to the catalytic centers of the cytoplasm, making tissues less susceptible to injuries caused by Ca deficit in fruits.

Tomatoes treated with 28-homocatasterone had a lower incidence of BER than control tomatoes (Figure 1). The application of 28-homocatasterone reduced the occurrence of BER by 37%. BER occurrence is directly associated with Ca deficiency in fruits (VILLEGAS-TORRES et al. 2017).

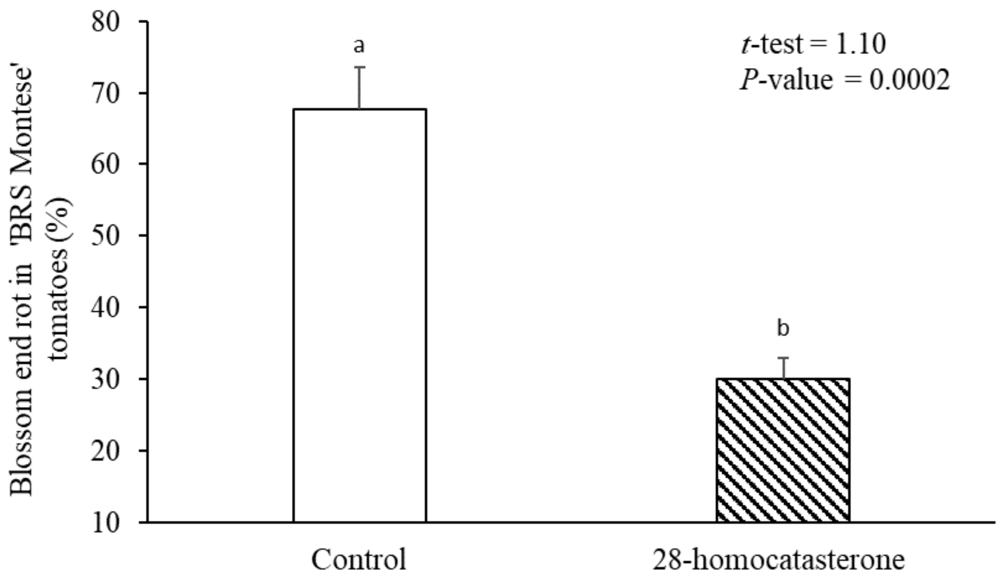

Figure 1. Incidence of blossom end rot in ‘BRS Montese’ tomatoes harvested 24 days after application of 28-homocatasterone at a concentration of 10^{-6} M. Vertical bars indicate the standard error of the mean.

Ca is present in the cell wall in the form of calcium pectate and acts as a binding component between phosphate and the carboxylic groups of the plasma membrane and proteins, providing stability and integrity of the cell membrane (TAIZ & ZEIGER 2017). Because of low Ca availability, the rapid fruit growth can compromise the integrity of membranes and cell walls, leading to disorganization, necrosis, and tissue death, as it occurs with BER incidence in tomatoes (VINH et al. 2018).

The application of 24-Epibrassinolide also inhibited the development of BER in 'BRS Montese' tomatoes (RIBOLDI et al. 2018). Although the studies and evidence of the effectiveness of brassinosteroids are recent, these hormones have a great potential use in agriculture, especially in the reduction of BER in tomatoes, contributing to the reduction of qualitative and quantitative production losses (GARCÍA-MARTÍNEZ & HERNÁNDEZ SILVA 2016).

CONCLUSIONS

The application of brassinosteroid 28-homocatasterone at a concentration of 10^{-6} M does not change the fresh mass and texture; however, it promotes the red color in 'BRS Montese' tomatoes.

The application of 28-homocatasterone at a concentration of 10^{-6} M increased the membrane permeability, the number of functional xylem vessels, and Ca concentration in the apoplast and reduced the incidence of BER in 'BRS Montese' tomatoes.

ACKNOWLEDGEMENTS

The authors thank the Federal University of Paraná for providing the phytoregulator. We also thank the teams of the Postharvest Physiology Laboratory (CAV/UDESC) and the Phytotechnics Laboratory of the Federal Institute of Santa Catarina (IFSC São Miguel do Oeste).

REFERENCES

ANDRADE IGV et al. 2020. Quality of Italian tomatoes grown with different forms of calcium application. Research, Society and Development 9: e-10191210837.

AYUB RA & REZENDE BLA. 2010. Contribuição do ácido giberélico no tamanho de frutos do tomateiro. Biotemas 23: 25-28.

BARTLETT MS. 1937. Properties of sufficiency and statistical tests. Proceedings of the Royal Society of London 160: 268-282.

ALCANTARA-CORTES JS et al. 2019. Principales reguladores hormonales y sus interacciones en el crecimiento vegetal. Nova 17: 109-129.

DRAŽETA L et al. 2004. Causes and Effects of Changes in Xylem Functionality in Apple Fruit. Annals of Botany 93: 275-282.

FAQUIN V. 2005. Nutrição mineral de plantas. 1.ed. Lavras: UFL.

FREITAS ST et al. 2010. Cellular approach to understand bitter pit development in apple fruit. Postharvest Biology and Technology 57: 6-13.

FREITAS ST et al. 2014. Calcium partitioning and allocation and blossom-end rot development in tomato plants in response to whole-plant and fruit-specific abscisic acid treatments. Journal of Experimental Botany 65: 235-247.

FREITAS ST et al. 2011. Dynamic alternations in cellular and molecular components during blossom-end rot development in tomatoes expressing CAX1, a constitutively active Ca_2^+/H^+ antiporter from *Arabidopsis*. Plant Physiology 156: 844-855.

FREITAS ST et al. 2017. Transcriptome approach to understand the potential mechanisms inhibiting or triggering blossom-end rot development in tomato fruit in response to plant growth regulators. Journal of Plant Growth Regulation 37: 183-198

FUKUDA H. 2004. Signals that control plant vascular cell differentiation. Nature Reviews Molecular Cell Biology 5: 379-391.

GARCÍA-MARTÍNEZ I & HERNÁNDEZ SILVA E. 2016. Brasinoesteroides en la agricultura. Revista Mexicana de Ciencias Agrícolas 7: 451-462.

MIQUELOTO A et al. 2018. Mechanisms regulating fruit calcium content and susceptibility to bitter pit in cultivars of apple. Acta Horticulturae 1194: 469-474.

NAGATA N et al. 2001. Brassinazole, an inhibitor of brassinosteroid biosynthesis, inhibits development of secondary xylem in Cress plants (*Lepidium sativum*). Plant and Cell Physiology 42: 1006-1011.

OLLE M & WILLIAMS IH. 2017. Physiological disorders in tomato and some methods to avoid them. The Journal of Horticultural Science and Biotechnology 92: 223-230.

PEET MM. 2009. Physiological disorders in tomato fruit development. Acta Horticulturae 821: 151-160.

PLANAS-RIVEROLA A et al. 2019. Brassinosteroid signaling in plant development and adaptation to stress. Development 146: e-151894.

RAIOLA A et al. 2014. Enhancing the Health-Promoting Effects of Tomato Fruit for Biofortified Food. Mediators of Inflammation 2014: 1-16.

RAMOS AP et al. 2019. Effects of an Auxin and a brassinosteroid on physical, chemical and biochemical attributes of “Galaxy” apples. Ciência Rural 49: e-20180311.

REITZ NF et al. 2021. Differential effects of excess calcium applied to whole plants vs. excised fruit tissue on blossom-end rot in tomato. Scientia Horticulturae 290: e-110514.

RIBOLDI et al. 2018. 24-Epibrassinolide Mechanisms Regulating Blossom-End Rot Development in Tomato Fruit. Journal of Plant Growth Regulation 38: 812-823.

SAITO M et al. 2018. BES1 and BZR1 Redundantly Promote Phloem and Xylem Differentiation. Plant and Cell Physiology 59: 590-600.

SAS INSTITUTE. 2002. Getting started with the SAS learning edition. Cary: SAS Institute Inc.

SAURE MC. 2005. Calcium translocation to fleshy fruit: its mechanism and endogenous control. Scientia Horticulturae 105: 65-89.

SHAPIRO SS & WILK MB. 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591-611.

SOLDATELI FJ et al. 2020. Crescimento e produtividade de cultivares de tomate cereja utilizando substratos de base ecológica. Colloquium Agrariae 16: 1-10.

SONG WP et al. 2018. Ca Distribution Pattern in Litchi Fruit and Pedicel and Impact of Ca Channel Inhibitor, La^{3+}. Frontiers in Plant Science 8: 1-11.

SOUSA VF et al. 2011. Irrigação e fertirrigação em frutíferas e hortaliças. 1.ed. Brasília: Embrapa Informação Tecnológica.

TAIZ L & ZEIGER E. 2017. Fisiologia e Desenvolvimento Vegetal. 6.ed. Porto Alegre: Artmed. 888p.

VILLEGAS-TORRES OG et al. 2017. Relación del calcio con las enfermedades de los cultivos. Investigación Agropecuaria 4: 77-86.

VINH TD et al. 2018. Comparative Analysis on Blossom-end Rot Incidence in Two Tomato Cultivars in Relation to Calcium Nutrition and Fruit Growth. The Horticulture Journal 87: 97-105.