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# Fruit shape regulates susceptibility of tomato to blossom-end rot

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**ABSTRACT.** : Calcium ( $\text{Ca}^{2+}$ ) is a nutrient in tomato plants, of which deficiency usually causes several problems including a physiological disorder known as blossom-end rot (BER) in the fruit. The objective of this study was to evaluate and identify morphological and physiological characteristics related to the susceptibility of tomato varieties to BER. The varieties studied were 'Amalia', 'IPA-6', 'M-82', 'Mara', and 'Nagcarlan', presenting different fruit formats. Physiological parameters that negatively correlated with BER were plant water potential, leaf area, plant dry mass, relationship between proximal/distal  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$  content in the proximal and distal portions of the fruit, and proximal  $\text{Ca}^{2+}$  content. Physiological parameters that positively correlated with BER were number of trichomes in the abaxial and adaxial leaf portions, leaf stomatal conductance, distal  $\text{Ca}^{2+}$  content bound to the cell wall, leaf transpiration, and fruit length. Our results showed that 'Mara' and 'Nagcarlan', 'Amalia' and 'IPA-6', and 'M-82' presented low, medium, and high susceptibility to BER, respectively. We also found that total fruit  $\text{Ca}^{2+}$  concentration, particularly in the distal fruit tissue, was not the only factor responsible for the development of BER; rather, the balance between factors that increase and decrease the susceptibility of each variety affected development of this disorder.

**Keywords:** Blossom-end rot, tomato varieties,  $\text{Ca}^{2+}$  disorder, Fruit length, Fruit  $\text{Ca}^{2+}$ , Cell wall-bound  $\text{Ca}^{2+}$ .

## Introduction

Calcium ( $\text{Ca}^{2+}$ ) is a nutrient for plant growth, participating in both metabolic and structural processes. More specifically,  $\text{Ca}^{2+}$  plays an important role in cell wall composition and membrane structure and integrity (White & Broadley, 2003), in addition to acting as a secondary messenger in the cytosol (Gilroy et al., 2016; Tuteja & Mahajan, 2007). When bound to pectins, calcium provides the structural maintenance of tissues, which in turn provides rigidity, responsible for protection against external agents. When bound to membrane phospholipids,

$\text{Ca}^{2+}$  maintains membrane stability, thereby regulating the primary intercellular exchange processes (Hepler & Winship, 2010).

In tomato fruit, typical symptoms of  $\text{Ca}^{2+}$  deficiency are known as blossom-end rot (BER). The characteristic symptoms of this disorder are initially an aqueous aspect, followed by death and darkening of the tissues in the distal region of the fruit (Riboldi, Gaziola, Azevedo, Freitas, & Castro, 2019). Studies suggest that BER occurs when  $\text{Ca}^{2+}$  flowing into the distal region of the fruit does not occur in synchrony with cell growth, causing abnormal cellular distribution of  $\text{Ca}^{2+}$ , which leads to cell death and subsequent tissue necrosis of the affected region (Freitas, Shackel, & Mitcham, 2011b; Ho, 1998; Saure, 2005).

The onset of BER may be related to environmental conditions, such as high temperatures and low relative humidity, which promote high transpiration rates (Riboldi, Araújo, Múrcia, Freitas, & Castro, 2018a). The movement of  $\text{Ca}^{2+}$  in the plant is connected to the movement of xylem sap. Therefore, because of transpiration rates, the distribution of calcium to the leaves is favored to the detriment of the fruit (Freitas et al., 2011b; Ho, Belda, Brown, Andrews, & Adams, 1993).  $\text{Ca}^{2+}$  reaching the leaves remains immobilized and is not translocated to the fruit. In situations of high growth rates, a deficient  $\text{Ca}^{2+}$  supply may not be able to meet physiological demands (Dražeta, Lang, Hall, & Volz, 2004; Ho & White, 2005). As a result, the occurrence of water deficit may affect translocation of  $\text{Ca}^{2+}$  within the plant (Guichard, Bertin, Leonard, & Gary, 2001).

Great genetic variability occurs among tomato genotypes for susceptibility to BER. Elongated fruits present greater susceptibility than that of rounded, flattened fruits and small cherry fruits (Riboldi, Araújo, Freitas, & Castro, 2018b). Studies suggest that this occurs because of the shape and size of the fruits, which influence the transport of  $\text{Ca}^{2+}$  to the distal region. Other factors, such as the venation pattern and degree of xylem maturation and functionality, also influence transport (Dražeta et al., 2004), and these factors may be related to growth velocity and blockage of xylem vessels (Freitas et al., 2011b).

Fruit susceptibility to BER is highly dependent on genotype, with varieties presenting high and low susceptibility to the disorder. However, only a few studies investigate which mechanisms act in each variety to inhibit or stimulate the development of this physiological disorder. Studies on BER should not be restricted only to approaches related to  $\text{Ca}^{2+}$  deficiencies but should also include other factors that contribute to the greater or lesser susceptibility to this disorder. Knowledge of the primary mechanisms regulating BER in each variety will allow the development of more specific and efficient control approaches.

The objective of this study was to evaluate and identify morphological and physiological characteristics related to the susceptibility of tomato varieties to BER.

## Material and methods

### *Plant material, growth conditions, and application of treatments*

This study was conducted in greenhouses with the tomato varieties 'Amalia', 'Mara', 'Nagcarlan', 'IPA-6', and 'M-82', provided by the germplasm bank of the Department of Genetics of Luiz de Queiroz School of Agriculture. The plants were sown in trays with a 1:1 ratio of commercial substrate (Plantmax HT; Eucatex, Brazil) and expanded vermiculite, supplemented with 1 g L<sup>-1</sup> NPK at 10:10:10 and 4 g L<sup>-1</sup> limestone. Thirty days after planting, the seedlings were transplanted into individual 30-L pots containing the same substrate used for planting the seeds. Previous fertilization and cover fertilization were performed according to the recommendations for the crop (Jones Jr., 1998). The plants were fertilized every 20 days during growing and fructification periods with 10 g of slow release fertilizer containing N (16%), P<sub>2</sub>O<sub>5</sub> (8%), K<sub>2</sub>O (12%), MgO (2%), S (5%), Fe (0.4%), Cu (0.05%), Mn (0.06%), Zn (0.02%), B (0.02%), and Mo (0.015%), but without Ca (Basacote Plus; Compo Expert; Florianopolis, Santa Catarina State, Brazil). To stimulate the incidence of BER, no calcium was provided. The average maximum temperature was 32.5°C and the minimum 24.4°C. Relative humidity averages were 80% maximum and 55% minimum.

### *Incidence of BER and growth assessments*

The incidence of blossom-end rot (BER) was calculated according to the number of fruits with symptoms of rot in relation to the total number of fruits. The number of flowers was counted, and after pollination, the number of fruits was also counted, which were used to determine the percentage of fruit fixation. The fresh and dry mass of the plants was determined at the time of maximum plant growth, approximately 40 days after transplanting the seedlings. Biweekly measurements of height were conducted starting after transplantation and continuing until the maximum growth in height, totaling 4 measurements throughout the experiment.

### *Determination of stomata and trichome density*

Samples of the first pair of fully expanded leaves were collected for the analysis of epidermal impressions with the use of instant adhesive (Segatto et al., 2004). Sampling was performed in the median portion of the leaves. The numbers of stomata and trichomes were determined under an optical microscope, and the counting was performed from the scanned images of each field considered. The means of replicates (1 leaf per plant for each treatment) were calculated, and the stomata and trichome densities were expressed as numbers of stomata and trichomes per mm<sup>2</sup>, respectively.

### *Determination of leaf stomatal conductance, leaf transpiration, and SPAD index*

To determine leaf stomatal conductance and transpiration, a LI-COR-1600 model (LI-COR Biosciences, Lincoln, NE, USA) was used. The analyses were performed between 9 and 11 a.m. 10, 25, 40, and 55 days after pollination of the flowers from the first inflorescence, which corresponded to the periods of beginning of flowering, maximum flowering, beginning of fruiting, and maximum fruiting, respectively. Subsequently, the mean values of the entire period were used for data analysis.

Leaf stomatal conductance and transpiration were determined on mature leaves close to the first inflorescence. Leaf SPAD index was measured using a chlorophyll index meter (SPAD 502; Minolta Co. Ltd., Osaka, Japan). Leaves were selected from the middle third of the plants and measured 3 times to calculate the average SPAD index for each plant.

### *Determination of $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , and $\text{K}^+$ in leaves and fruit*

Fruit collection for determination of  $\text{Ca}^{2+}$  content was performed 30 days after pollination. The proximal and distal portions of whole fruits and leaves were used after oven drying at  $65^{\circ}\text{C}$  until a constant weight. Samples of mature leaves were removed from the region close to the fruits from the first inflorescence. To a sample of 500 mg of dry material, 6 mL of nitroperchloric acid (2:1) was added, followed by gypsum block digestion at  $240^{\circ}\text{C}$  and then completion to 15 g with distilled water. The determination was performed by atomic absorption, according to Malavolta, Vitti, and Oliveira (1997). The results are expressed in g of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$  per kg of tissue dry matter.

To determine  $\text{Ca}^{2+}$  bound to the wall, the pericarp tissue of the distal portion of the fruit was used, and the extraction of the cell wall was performed according to a method developed by Campbell, Huysamer, Stotz, Greve, and Labavitch (1990). The determination of  $\text{Ca}^{2+}$  was performed using the same methodology previously described for  $\text{Ca}^{2+}$  determinations in leaves and fruits.

### *Experimental design*

The experimental design was randomized blocks with four blocks and two plants per block. The results were submitted to analysis of variance and when significant, were submitted to the Tukey test at the 5% probability level. For the analysis of the percentage of blossom-end rot, the non-parametric Kruskal-Wallis test was performed at 5% probability. The Pearson correlation test was conducted for the variables at a 5% probability level.

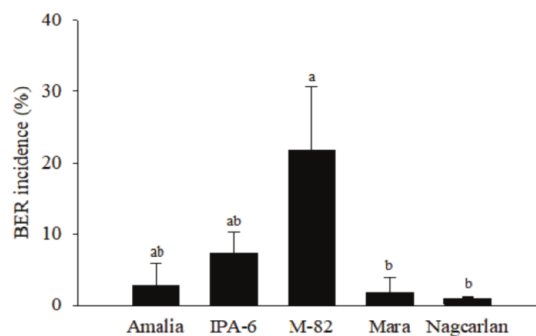
To identify patterns of variation among treatments, principal component analysis (PCA) was performed. For the selection of factors for

the analysis, the Kaiser criterion (Kaiser, 1960) was used for factors with their own value greater than 1. The analysis was performed through the FactoMineR bookstore (Husson, 2014) in the R Project Team (2014).

## Results

### *Percentage of blossom-end rot*

According to our results, the incidence of BER was low in ‘Mara’ and ‘Nagcarlan’, medium in ‘Amalia’ and ‘IPA-6’, and high in ‘M-82’ (Figure 1). The percentage of fruit fixation was highest in ‘Nagcarlan’, whereas ‘Amalia’ had the lowest amount of fruit at the end of the experiment (data not shown). The other varieties presented intermediate values of percentage of fruit fixation. Fruit of ‘Amalia’ was larger and flatter and that of ‘Nagcarlan’ smaller and rounder than fruits of the other varieties.



**Figure 1**

Blossom-end rot (BER) incidence in tomato varieties with low (‘Mara’ and ‘Nagcarlan’), medium (‘Amalia’ and ‘IPA-6’), or high (‘M-82’) susceptibility to BER. Fruit was harvested at 15 days after pollination. Averages with the same letter are not significantly different (Kruskal-Wallis, 5%). Data shown are the mean  $\pm$  standard deviation.

### *Correlation between physiological parameters and blossom-end rot*

In this section, only the physiological parameters that presented a correlation coefficient higher than ( $\pm$ ) 0.5 with BER incidence are considered for discussion, which are considered high according to the classification described by Cohen (1988).

Correlation analyses showed negative correlations between BER and water potential, leaf area, plant dry mass, proximal/distal  $\text{Ca}^{2+}$ , total  $\text{Ca}^{2+}$  in the proximal fruit tissue, and  $\text{K}^+$  in the distal and proximal fruit tissues (Table 1).

The physiological parameters of abaxial and adaxial trichomes, leaf stomatal conductance, cell wall-bound  $\text{Ca}^{2+}$  in distal fruit tissue, leaf transpiration, and fruit length showed a positive correlation with BER incidence (Table 1).

Table 1

Correlation analysis between physiological parameters and BER incidence to determine parameters potentially inhibiting ( $-R^2$ ) or triggering ( $+R^2$ ) BER in tomato varieties with low ('Mara' and 'Nagcarlan'), medium ('Amalia' and 'IPA-6'), or high ('M-82') susceptibility to BER.

Inhibiting BER	$R^2$	p-value	Triggering BER	$R^2$	p-value
Water Potential	-0.93	0.02	Adaxial Trichomes	+0.92	0.02
Leaf Area	-0.79	0.12	Abaxial Trichomes	+0.90	0.03
Plant Dry Mass	-0.76	0.13	Stomatal Conductance	+0.87	0.05
Fruit Proximal/Distal $Ca^{2+}$	-0.70	0.19	Fruit Distal Cell Wall $Ca^{2+}$	+0.80	0.10
Fruit Proximal $K^+$	-0.63	0.25	Transpiration	+0.77	0.13
Fruit Distal $K^+$	-0.58	0.31	Fruit Length	+0.61	0.28
Fruit Proximal $Ca^{2+}$	-0.50	0.39	Leaf $Mg^{2+}$	+0.49	0.40
Abaxial Stomata	-0.49	0.39	Fruit Cell Wall $Ca^{2+}$ /Distal $Ca^{2+}$	+0.54	0.35
Leaf $K^+$	-0.24	0.96	Leaf $Ca^{2+}$	+0.04	0.95
SPAD Index	-0.21	0.73	Fruit Distal $Ca^{2+}$	+0.02	0.97
Fruit Distal $Mg^{2+}$	-0.13	0.83	Fruit Proximal $Mg^{2+}$	+0.01	0.99
Adaxial Stomata	-0.09	0.88			

SPAD index = chlorophyll content index

### Parameters of plant growth

At the beginning of fruiting, sampling was performed to determine the leaf area (Figure 2a). The largest leaf area, greater than 2,500 cm<sup>2</sup>, was observed in 'Nagcarlan'. The other varieties presented values less than 2,000 cm<sup>2</sup>, and 'M-82' presented the smallest leaf area with a value less than 1,500 cm<sup>2</sup> (Figure 2a). Similarly, 'Nagcarlan' showed the highest dry mass per plant, with a mean value of 33 g, whereas 'M-82' had the lowest plant dry weight, which was 16 g (Figure 2b).

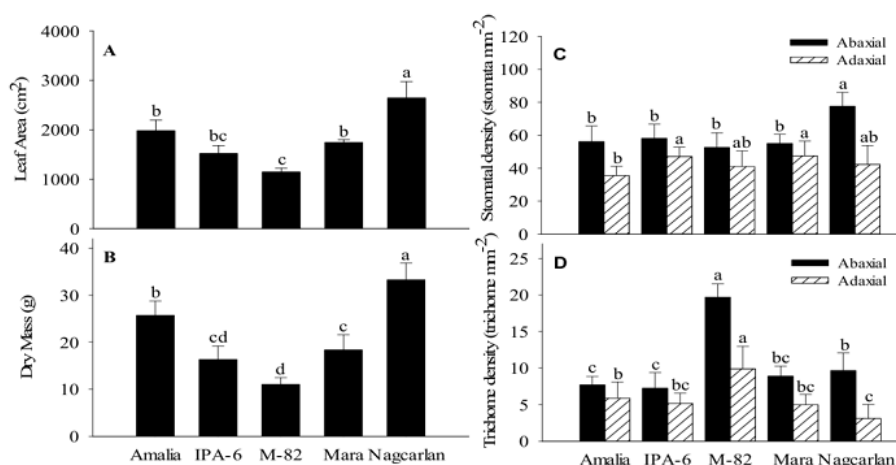


Figure 2

Leaf area per plant (A) and plant dry mass (B) at maximum growth (60 days after planting), leaf stomatal density (C) and trichome density (D) of tomato varieties with low ('Mara' and 'Nagcarlan'), medium ('Amalia' and 'IPA-6'), or high ('M-82') susceptibility to BER. The averages with the same letter are not significantly different (Tukey 5%). Data shown are the mean  $\pm$  standard deviation.

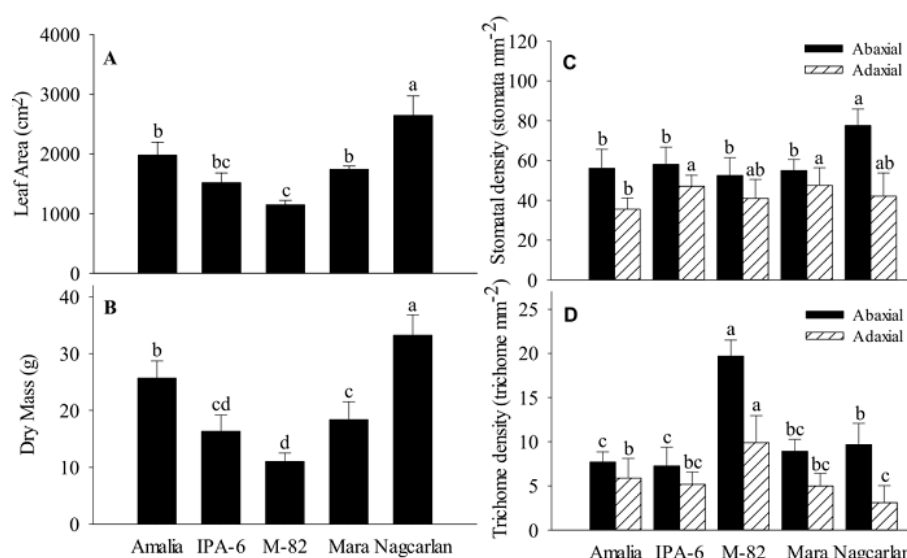


*Stomata and trichome density*

'Nagcarlan' showed the highest stomatal density in the abaxial portion of the leaves (Figure 2c). 'Amalia', 'M-82', and 'Nagcarlan' presented the lowest stomatal density in the adaxial portion (Figure 2c). 'M-82' showed the highest number of trichomes in the abaxial part, and 'Amalia', 'IPA-6', and 'Mara' had the lowest values (Figure 2d). In the adaxial portion, 'M-82' also showed the highest number of trichomes and 'IPA-6', 'Mara', and 'Nagcarlan' the smallest (Figure 2d).

*Concentration of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$  in plant and fruit*

Leaf nutrient analysis revealed that the highest  $\text{Ca}^{2+}$  concentration was observed in 'Nagcarlan', compared with that of 'Mara' (Figure 3a). For leaf  $\text{Mg}^{2+}$ , 'IPA-6' showed a higher concentration than that of 'Mara'. In relation to leaf  $\text{K}^+$ , 'Amalia' presented a higher concentration than that of 'M-82' and 'Nagcarlan' (Table 2).



**Figure 3**

Leaf  $\text{Ca}^{2+}$  (A), fruit  $\text{Ca}^{2+}$  (black bars are proximal tissue, and striped bars are distal tissue) (B), cell wall-bound  $\text{Ca}^{2+}$  in fruit distal tissue (C), and percentage of total fruit  $\text{Ca}^{2+}$  bound to the cell wall (D) in tomato varieties with low ('Mara' and 'Nagcarlan'), medium ('Amalia' and 'IPA-6'), or high ('M-82') susceptibility to BER. The averages with the same letter are not significantly different (Tukey 5%). Data shown are the mean  $\pm$  standard deviation.

In fruit, the highest concentration of  $\text{Ca}^{2+}$  in the proximal portion was observed in 'Nagcarlan' and 'IPA-6', compared with that in 'Mara' and 'M-82' (Figure 3b). For the distal portion, the highest concentration of  $\text{Ca}^{2+}$  was observed in 'Amalia' and 'IPA-6' in relation to 'Mara' (Figure 3b). Regarding  $\text{Mg}^{2+}$  in the proximal portion, the highest concentration was observed in 'Mara' in relation to 'Amalia' (Table 2).



However, in the distal portion, 'IPA-6', 'Mara', and 'Nagcarlan' had higher  $Mg^{2+}$  concentrations than that of 'Amalia'. 'Mara' had higher  $K^+$  concentrations than those of 'IPA-6' and 'M-82' in the proximal portion and in relation to 'M-82', in the distal portion (Table 2). The concentration of cell wall-bound  $Ca^{2+}$  was highest in 'M-82' and lowest in 'Amalia' (Figure 3c), with no significant difference among the other varieties (Figure 4c). The percentage of total  $Ca^{2+}$  bound to the cell wall was highest in 'M-82' and lowest in 'Amalia' (Figure 3d).

**Table 2**

Leaf and fruit  $Mg^{2+}$  and  $K^+$  concentrations in tomato varieties with low ('Mara' and 'Nagcarlan'), medium ('Amalia' and 'IPA-6'), or high (M-82) susceptibility to BER. Leaf and fruit were harvested at 15 days after pollination. Fruit was analyzed at the proximal and distal tissues.

Varieties	Leaf	Fruit tissue		Leaf	Fruit tissue	
		Proximal	Distal		Proximal	Distal
Amalia	4.7 ab*	1.5 b	1.5 b	38.0 a	39.3 abc	41.2 ab
IPA-6	5.9 a	1.8 ab	1.9 a	34.2 ab	34.3 c	38.4 ab
M-82	5.3 ab	1.7 ab	1.6 ab	28.1 bc	36.1 bc	38.0 b
Mara	4.1 b	2.1 a	1.7 a	33.3 abc	44.4 a	40.0 a
Nagcarlan	4.9 ab	1.5 b	1.7 a	24.6 c	41.4 ab	36.7 ab
CV%	13.62	14.55	8.06	12.38	6.94	6.03

\*The averages followed by same letter are not significantly different (Tukey 5%).

### *Physiological parameters*

Leaf stomatal conductance was highest in 'M-82' and 'IPA-6' (Table 3). 'Amalia' and 'Mara' presented the lowest values, and 'Nagcarlan' presented an intermediate value of leaf stomatal conductance. Leaf transpiration rates followed a similar pattern to that of leaf stomatal conductance behavior (Table 3), and 'M-82' and 'IPA-6' had the highest leaf transpiration rates of the period, and only 'Mara' presented a significantly reduced mean value in the period (Table 3).

Leaf water potential was highest (more positive) in 'Amalia', 'Nagcarlan', and 'Mara'. 'IPA-6' had an intermediate value, and 'M-82' had the lowest value (Table 3). The SPAD index did not present significant differences among the varieties studied, with an average index of 45 for the varieties (data not shown).

Table 3

Leaf stomatal conductance ( $g_s$ ), leaf transpiration rate (E), and leaf water potential ( $\Psi_w$ ) in tomato varieties with low ('Mara' and 'Nagcarlan'), medium ('Amalia' and 'IPA-6'), or high ('M-82') susceptibility to BER.

Varieties	$g_s$ (cm s <sup>-1</sup> )	E (mg cm <sup>-2</sup> s <sup>-1</sup> )	$\Psi_w$ (MPa)
'Amalia'	0.97 ± 0.10 c	12.27 ± 1.50 ab	- 1.13 ± 0.04 cd
'IPA-6'	1.27 ± 0.21 ab	14.12 ± 1.90 a	-1.35 ± 0.04 b
'M-82'	1.39 ± 0.25 a	14.15 ± 1.90 a	-1.54 ± 0.05 a
'Mara'	0.90 ± 0.23 c	11.36 ± 2.10 b	-1.17 ± 0.03 c
'Nagcarlan'	1.05 ± 0.15 bc	12.32 ± 1.70 ab	-1.01 ± 0.03 d
CV%	9.8	7.89	2.4

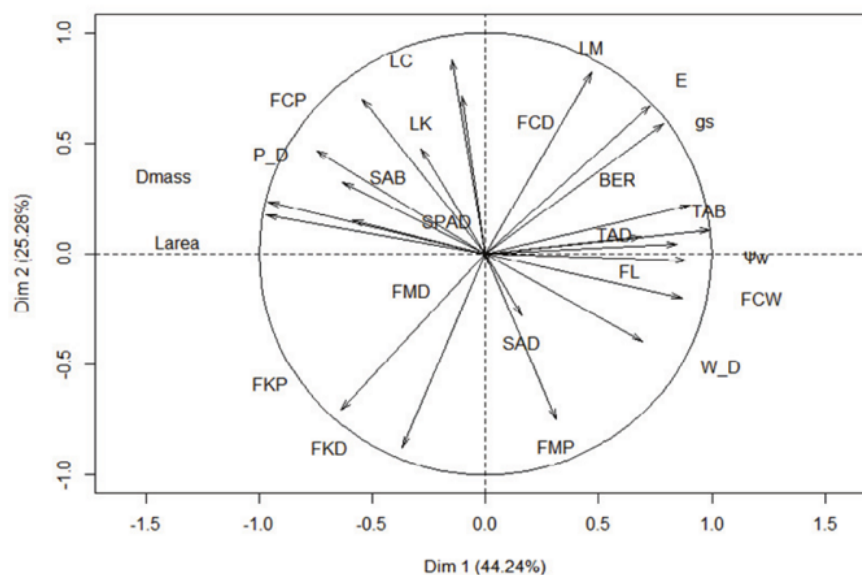
\*The averages followed by the same letter are not significantly different (Tukey 5%).

### Principal component analysis (PCA)

Principal component analysis was used to reduce the dimensionality of the database and determine the relation of the components to BER, with a total of four dimensions explaining 100% of the database variability (Figure 4).

The first component explained 44.24% of the variability of the database, and the main variables that contributed to this component were leaf water potential, SPAD chlorophyll index, abaxial stomata, adaxial trichomes, leaf area, dry mass, leaf stomatal conductance, leaf transpiration, Ca<sup>2+</sup> concentration in the cell wall in the distal portion of the fruit, K<sup>+</sup> in the proximal portion of the fruit, BER, fruit length, percentage of total Ca<sup>2+</sup> bound to the cell wall in the distal portion of the fruit, and the relation between proximal and distal Ca<sup>2+</sup> in the fruit. These were the components that had the greatest relation to BER.

The second component explained 25.28% of the variability of the database, and the main variables were Ca<sup>2+</sup> in the proximal and distal portions of the fruit, Mg<sup>2+</sup> and K<sup>+</sup> in the proximal portion of the fruit, K<sup>+</sup> in the distal portion of fruit, and Ca<sup>2+</sup> and Mg<sup>2+</sup> in the leaves. The third component explained 17.74% of the variability of the database, and the variables were stomata in the adaxial portion and Mg<sup>2+</sup> in the distal portion of the fruit. The fourth component explained 12.73% of the variability of the database, and the main variables were abaxial trichomes and leaf K<sup>+</sup>.



**Figure 4**

Relations among variables (PCA) shown by scatter plot of the first two principal components based on traits. The variables are BER - blossom-end rot, Dmass - dry mass, E - transpiration, FCD - fruit distal  $\text{Ca}^{2+}$ , FCP - fruit proximal  $\text{Ca}^{2+}$ , FCW - fruit distal cell wall  $\text{Ca}^{2+}$ , FL - fruit length, FMD - fruit distal  $\text{Mg}^{2+}$ , FMP - fruit proximal  $\text{Mg}^{2+}$ , FKD - fruit distal  $\text{K}^+$ , FKP - fruit proximal  $\text{K}^+$ ,  $g_s$  - stomatal conductance, Larea - leaf area, LC - leaf  $\text{Ca}^{2+}$ , LK - leaf  $\text{K}^+$ , LM - leaf  $\text{Mg}^{2+}$ ,  $\Psi_w$  - leaf water potential, P\_D - fruit proximal  $\text{Ca}^{2+}$  and distal  $\text{Ca}^{2+}$  relation, SAB - density of abaxial stomata, SAD - density of adaxial stomata, SPAD - SPAD index, TAB - density of abaxial trichomes, TAD - density of adaxial trichomes, and W\_D - fruit distal cell wall-bound  $\text{Ca}^{2+}$  and distal  $\text{Ca}^{2+}$  relation.

## Discussion

### *Susceptibility of varieties to blossom-end rot*

BER manifested differently among the varieties evaluated. Considering the reduced percentage of BER, in addition to the high growth rates of 'Nagcarlan' under favorable conditions for the appearance of the disorder, this variety was classified as tolerant to BER. By contrast, 'M-82' presented high levels of BER incidence, in addition to reduced growth rates, and was classified as highly susceptible to the disorder. The other varieties had an intermediate degree of tolerance to BER. Based on these results, 'Mara' and 'Nagcarlan', 'Amalia' and 'IPA-6', and 'M-82' were classified as low, medium, and high susceptible to BER, respectively.

### *Physiological parameters that inhibit blossom-end rot*

The physiological parameters negatively correlated with the incidence of BER were considered inhibitors of the disorder in the varieties studied. High water potential inhibited BER. A drastic reduction in leaf water potential leads to a reduction in the transport of xylem sap to the upper

parts of the plant, reducing  $\text{Ca}^{2+}$  transport to fruit and consequently increasing the incidence of BER. In less severe situations in which the transport of xylem sap to the upper parts of the plant is not significantly reduced, a reduction in the leaf stomatal conductance may favor the transport of  $\text{Ca}^{2+}$  to the fruit.

The occurrence of high diurnal temperatures increases leaf transpiration rates that may induce water deficiency in plants, which affects several physiological processes under low soil water content (Tsukaguchi, Kawamitsu, Takeda, Suzuki, & Egawa, 2003); in tomato, water deficiency affects leaf water relations and root hydraulic conductivity (Morales et al., 2003). Such susceptibility is associated with increased competition between leaves and fruit for the  $\text{Ca}^{2+}$  in the xylem sap translocated to the upper parts of the plant (Tables 2 and 3). With the occurrence of high transpiration rates due to high temperatures,  $\text{Ca}^{2+}$  transport to the leaves increases, increasing the incidence of BER. This fact is also favored by low transpiration rates of the fruit (Freitas et al., 2011b; Ho & White, 2005; Riboldi et al., 2018a).

'Nagcarlan' and 'Mara', with low susceptibility to BER and resistance to high temperatures, presented the most positive values of leaf water potential. By contrast, 'M-82' presented the most negative value of leaf water potential (Table 3), which coincided with high susceptibility to the disorder and sensitivity to high temperatures (Paupière et al., 2017). The degree of tolerance to high temperatures apparently improves the water relations of the plant, which directly influences the transport of xylem sap to the upper parts and consequently the allocation of  $\text{Ca}^{2+}$  to the fruit.

Dry mass also inhibited BER. The dry mass analysis showed that 'Nagcarlan' presented the highest and 'M-82' the lowest dry mass values. In general, the other varieties showed intermediate behavior regarding the growth and accumulation of biomass. Similar behavior was observed in relation to the leaf area; this is an important factor regulating BER, as large leaf area provides a larger transpiration surface, which favors  $\text{Ca}^{2+}$  accumulation into the leaves (Ho et al., 1995). However, this behavior is not always the case, because plants such as 'Nagcarlan' are more adaptable at elevated temperatures (Paupière et al., 2017) and showed reduced transpiration rates and high water potential under the conditions of the experiments, resulting in lower BER incidence.

The ratio between proximal and distal  $\text{Ca}^{2+}$  was also related to inhibition of BER. Observing the concentrations of  $\text{Ca}^{2+}$  in both the distal and proximal portions alone, we noted that the values were very similar between the varieties and were inferior only in 'Mara' tomato fruit. Thus,  $\text{Ca}^{2+}$  concentrations in these varieties did not fully explain the appearance of BER. However, for the ratio between proximal and distal  $\text{Ca}^{2+}$ , the values closest to 1 were those observed for 'Nagcarlan' and 'IPA-6' tomatoes, indicating an efficient transport of the nutrient along the fruit. 'M-82' tomato had the lowest  $\text{Ca}^{2+}$  partition ratio between the two fruit portions, showing a less efficient transport along the fruit, which coincided with the highest BER incidence.

Mg<sup>2+</sup> and K<sup>+</sup> content compete with Ca<sup>2+</sup> for sites of uptake and translocation (Taylor & Locascio, 2004). In 'Mara' tomato, the lowest Ca<sup>2+</sup> levels were observed in the two fruit sections, although high values of Mg<sup>2+</sup> and K<sup>+</sup> were also observed in this variety. However, the incidence of BER in this variety was low, suggesting that Ca<sup>2+</sup> content in the proximal and distal tissues did not fully explain BER incidence.

The distribution of stomata on the abaxial and adaxial leaf surfaces was also negatively correlated with BER. 'Nagcarlan' tomatoes showed the most stomata on the abaxial leaf surface, which possibly contributed to reducing transpiration rates, improving the water relations of the plant under high temperatures and favoring increases in fruit Ca<sup>2+</sup> uptake.

The effect of the improvement in water relations on reducing BER incidence may be related to the reduction in generation of reactive oxygen species (ROS), which may increase fruit susceptibility to BER (Schmitz-Eiberger & Noga, 2003; Saure, 2014). Thus, the varieties that are more tolerant to stress conditions should present lower susceptibility to BER.

#### *Physiological parameters that stimulate blossom-end rot*

The physiological parameters positively correlated with BER were considered to stimulate the incidence of the disorder in the fruit. Leaf stomatal conductance was positively correlated with BER. 'M-82' and 'IPA-6' presented high values of leaf stomatal conductance and high BER incidence. The same pattern was observed in relation to transpiration, which correlated positively with BER. Stomata regulate leaf transpiration and consequently whole-plant water loss (Farber, Attia, & Weiss, 2016). Accordingly, high leaf transpiration rates can increase leaf Ca<sup>2+</sup> content and decrease Ca<sup>2+</sup> flow into the fruit, which has been linked to an increase in BER incidence (Freitas, McElrone, Shackel, & Mitcham, 2014).

Although 'Nagcarlan' presented the lowest BER incidence, the variety was one of those that showed low leaf stomatal conductance, in contrast to expectation, and high levels of leaf Ca<sup>2+</sup>. Possibly, each variety has a different pattern of response between factors triggering and inhibiting BER incidence. These results suggest that Ca<sup>2+</sup> content in leaves and fruit is not the only factor regulating fruit susceptibility to BER.

The concentration of cell wall-bound Ca<sup>2+</sup> in the distal portion of the fruit also correlated positively with BER. Although significant differences were not observed for the 'Nagcarlan' and 'IPA-6' tomatoes, the variety 'M-82', which had a high percentage of BER, presented high values in relation to the varieties 'Mara' and 'Amalia'. The binding of Ca<sup>2+</sup> to the cell wall limits the availability for other cellular activities (Conn et al., 2011; Freitas, Padda, Wu, Park, & Mitcham, 2011a; Freitas et al., 2011b). Therefore, varieties with high levels of Ca<sup>2+</sup> bound to the cell wall might be expected to have increased susceptibility to BER.

The density of trichomes, both in the adaxial and abaxial leaf portions, was also positively correlated with BER incidence. Notably, 'M-82'

tomato plants, which had the highest leaf trichome density, also had the highest percentage of BER incidence. Trichome density in the 'M-82' variety might be a response to elevated temperatures as a form of protection against excess water loss via stomata, as observed by Gianfagna, Carter, and Sacalis (1992).

Fruit length was also positively related to BER. The 'Nagcarlan' and 'Mara' varieties had smaller fruit (3.5 and 5.0 cm, respectively) and lower percentage of BER incidence than those of the other varieties. Varieties with more elongated fruit, 'M-82' (7.0 cm) and 'IPA-6' (7.5 cm), presented high BER incidence, with the highest incidence and severity of this disorder observed in 'M-82'. The relationship between BER and fruit shape is not fully understood, but BER may be more common in elongated fruit (Ho & White, 2005). A possible explanation for this behavior is the fact that the xylem connection in such fruit is inefficient in bringing  $\text{Ca}^{2+}$  from the proximal to the distal fruit regions, restricting  $\text{Ca}^{2+}$  accumulation in the distal tissues where BER symptoms develop (Freitas, Amarante, & Mitcham, 2016).

#### *Summary of the parameters regulating blossom-end rot development in different tomato varieties*

Several studies emphasize the importance of total  $\text{Ca}^{2+}$  concentration in the distal portion of fruit as the primary factor involved in the incidence of BER in tomato. However, speculation is that this physiological disorder is controlled by several factors, not only the nutrient content in the fruit but also the factors that affect its distribution between leaves and fruit, such as leaf stomatal conductance and leaf transpiration. In fact, our results identified the roles of several factors acting either to inhibit or trigger BER in different varieties, in addition to tolerance to high temperatures, as observed by other authors (Saure, 2014).

$\text{Ca}^{2+}$  transport in the xylem to the aerial parts is a central factor for the partition of the nutrient between leaves and fruit. The primary factors affecting  $\text{Ca}^{2+}$  partition are leaf transpiration and leaf stomatal conductance. High values for such factors are accompanied by an increase in the transport of  $\text{Ca}^{2+}$  to the leaves and consequently, by a reduction in the availability of the nutrient to the fruit. Therefore, this reduction may act as a trigger of BER (Abdal & Suleiman, 2005; Guichard, Gary, Leonardi, & Bertin, 2005; Paiva, Martinez, Casali, & Padilha, 1998).

Water relations of plants are modulated by leaf stomatal conductance and transpiration and therefore, by the transport of water to the aerial parts, which also affects the incidence of BER. The influence of water potential on the incidence of BER is also dependent on the intensity of the water deficit, and in situations with a great decrease in this parameter, a drastic reduction occurs in the transport of sap to the aerial parts and consequently a reduction in  $\text{Ca}^{2+}$  transport to the fruit. Note that the morphological characteristics of leaves that affect the transpiration rates, such as leaf area, stomatal density, and trichomes, also affect  $\text{Ca}^{2+}$



transport to the leaves, indirectly affecting the incidence of BER in fruit, as mentioned previously.

The partition of  $\text{Ca}^{2+}$  in fruit also affected the incidence of BER. The balance between proximal and distal  $\text{Ca}^{2+}$ , in addition to the content of cell wall-bound  $\text{Ca}^{2+}$ , may differently affect the incidence of the disorder. In general, high values of distal  $\text{Ca}^{2+}$ , accompanied by low concentrations of cell wall-bound  $\text{Ca}^{2+}$  in the distal portion, may inhibit the development of BER.

Finally, based on the morphological characteristics of the fruit of the varieties analyzed, the genetic components that affect the physiological and morphological characteristics of fruits may also influence the  $\text{Ca}^{2+}$  partitioning in them and consequently the incidence of BER. Larger and more elongated fruits, such as those of 'M-82' and 'IPA-6', or those with a deficient conducting vessel system may have inefficient distribution of  $\text{Ca}^{2+}$  between the different parts of the fruit, thereby showing increased incidence of BER.

## Conclusion

The varieties evaluated in this experiment showed differences in relation to the possible factors regulating BER development in the fruit. Varieties with smaller and/or flattened fruit, such as 'Nagcarlan', 'Amalia', and 'Mara', presented a lower incidence of BER than that of the other varieties. Conversely, the elongated variety 'M-82' presented the highest incidence of BER.

Physiological conditions of plants, such as low transpiration and low leaf water potential, could help susceptible varieties, e.g., 'M-82', to maintain higher levels of  $\text{Ca}^{2+}$  in the fruit. Future studies should focus on identifying new elongated varieties that are more resistant to BER in unfavorable conditions, such as high temperature and drought, to understand the physiological mechanisms by which they present increased tolerance to BER development.

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