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**Physiological and morphological mechanisms regulating blossom-
end rot in tomato fruits**

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Thesis presented to obtain the degree of Doctor in
Science. Area: Plant Physiology and Biochemistry

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RESUMO

Mecanismos fisiológicos e morfológicos que regulam a podridão apical em frutos de tomate

O Ca^{2+} é um nutriente para o desenvolvimento de plantas, e sua deficiência causa mal desenvolvimento celular em frutos de tomate, resultando em desordem fisiológica conhecida como podridão apical ou *blossom-end rot* (BER). Estudos sobre esta desordem fisiológica não são recentes e são focados principalmente na deficiência de Ca^{2+} nos tecidos e na interação entre planta e meio ambiente. No entanto, novas pesquisas têm se baseado na interação hormonal e nos mecanismos oxidativos como reguladores desta desordem fisiológica nos frutos. Hormônios como giberelinas, ácido abscísico e brassinosteróides têm sido implicados tanto na ativação quanto na inibição dos sintomas de BER. A aplicação de ácido abscísico e epibrassinolideo diminuiu a incidência de BER, reduzindo a concentração de Ca^{2+} nas folhas e aumentando-as nos frutos. A aplicação de ácido abscísico provocou diminuição na transpiração, o que explica a mudança no fluxo de seiva e cálcio no xilema que conduz da folha para fruto. O epibrassinolideo, por outro lado, aumentou a resposta antioxidante, diminuindo as concentrações de peróxido de hidrogênio e aumentando as de ácido ascórbico, ascorbato peroxidase, catalase e superóxido dismutase nos frutos. A seleção de genótipos mais tolerantes ao aparecimento de BER e a identificação de fatores que os tornam resistentes, são ferramentas importantes no processo de seleção de novas variedades. Neste trabalho, foram estudados muitos genótipos para compreender a relação entre a forma do fruto e o ambiente de crescimento sobre a incidência de BER. De acordo com os resultados obtidos, genótipos alongados são mais susceptíveis a podridão apical.

Palavras-chave: Podridão apical; Ácido Abscísico; Brassinoesteroides; Formato de frutos; Frutos alongados

ABSTRACT

Physiological and morphological mechanisms regulating blossom-end rot in tomato fruits

Ca^{2+} is a nutrient for plant development, and its deficiency causes poor cellular development in tomato fruits, resulting in a physiological disorder known as blossom-end rot (BER). Studies on this physiological disorder are not recent and mainly focused on Ca^{2+} deficiency in tissues and on the interaction between plant and the environment. However, new research has been based on hormonal interaction and oxidative mechanisms in fruits. Hormones such as gibberellins, abscisic acid, and brassinosteroids have been implicated in both activation and inhibition of BER symptoms. The application of abscisic acid and epibrassinolide decreased fruit susceptibility to BER. Abscisic acid reduces leaf transpiration, inhibiting xylem sap and calcium flow into the leaves and increasing into the fruit. Epibrassinolide increased fruit antioxidant responses, decreasing hydrogen peroxide and increasing ascorbic acid content, as well as increasing ascorbate peroxidase, catalase, and superoxide dismutase activities. In addition, the selection of varieties more tolerant to BER and the identification of factors that make new varieties resistant are important tools in the selection of new varieties. In this study, many varieties were evaluated in order to understand the relationship between fruit shape and growing environment on BER incidence in the fruit. According to the results, elongated fruit varieties showed higher susceptibility to BER.

Keywords: Blossom-end rot; Abscisic acid; Brassinoesteroids; Fruit shape; Elongated fruits

1. INTRODUCTION

Ca^{2+} is a plant nutrient, which has structural and metabolic roles required for proper growth and development processes. In the soil, this nutrient has low mobility, competing with other cations, such as Al^{3+} , H^+ , K^+ , Mg^{2+} , Mn^{2+} , Na^+ , NH_4^+ , for root uptake (Taylor; Locascio, 2004).

Transport of Ca^{2+} by plants occur via transpiratory current; dissolved in water, this nutrient is mobilized from the roots to the aerial part of the plant, mainly high-growth and intense transpirations zones as meristems, leaves, and fruits (Ho et al., 1993). Nonetheless, there are several barriers for Ca^{2+} and other ions to reach all of these tissues. Its translocation occurs exclusively via xylem and is determined by transpiration and growth rates of different plant organs (De Freitas et al., 2011). Thus, transpiration occurring on the leaf surface indirectly help water and nutrients such as Ca^{2+} rise to growing tissues (Guichard et al., 2005). Once Ca^{2+} reaches these tissues, however, it becomes less mobile.

This mobility is directly linked to the role it plays in plant. Most Ca^{2+} in tissues is attached to the cell wall. Its structural role is related to the stabilization of pectin chains. Thus, Ca^{2+} is bound to de-esterified pectin molecules that act as reinforcement to the cellulosic chains in various plant tissues (De Freitas et al., 2012a).

In addition, Ca^{2+} plays an important role in the stabilization of membranes. It has been shown that, in tomato fruit, during the rapid expansion phase, there is an increasing amount of Ca^{2+} bound to the plasma membrane in healthy fruits and a decreasing amount in membranes of plasmolyzed cells from fruit tissues with visible blossom-end rot (BER) symptoms (Suzuki et al., 2003).

Cytosolic Ca^{2+} has the function of signaling regulator, in which several proteins, such as calmodulins, nutrient pumps and exchangers are involved. Thus, Ca^{2+} concentrations within the cytoplasm should be maintained at very low levels, since any oscillation can trigger metabolic alteration, leading to cellular responses and even to cell death. In this case, most Ca^{2+} is stored within the vacuoles and other cellular organelles, where it also plays an important role in charge balance (Ho; White, 2005).

Symptoms of Ca^{2+} deficiency may be easily observed in plants. In leaves, the most classic symptoms are the burning of the edges of new leaves, disorder also

known as tipburn in lettuce (Uno et al., 2016). However, these damages are better known in fruits, whose classic symptom is the softening of the distal region of the fruits and posterior necrosis, better known as BER in tomato. In apples, the disorder caused by Ca^{2+} deficiency is known as bitter pit (De Freitas et al., 2010).

Damages resulting from this physiological disorder are enormous, especially in fruit, since the final product ends up being commercially depreciated. Many studies have attempted to understand the main mechanisms regulating BER in plants. Initially, the idea was that Ca^{2+} concentration in soil and tissues, as well as the balance between Ca^{2+} and other nutrients would trigger the BER symptoms (Castro, 1980; Ho, White, 2005). However, many studies show that, even under high Ca^{2+} conditions in both soil and fruit, there may be a high incidence of BER (De Freitas et al., 2011, De Freitas et al., 2014).

Environmental factors, especially water stress, low relative humidity, and high temperatures, very common in summer and in greenhouse conditions, are other factors that lead to a high incidence of BER. Thus, excess transpiration may displace water flow with higher intensity mainly to the leaves, in detriment of the fruits, mainly by modifying the water potential of the whole plant, depriving fruits, which do not transpire as intensely as leaves, from Ca^{2+} accumulation.

However, in recent years, studies have focused on other factors that could potentially regulate BER (De Freitas et al., 2014, De Freitas et al., 2016, De Freitas et al., 2017). Endogenous factors, such as hormones, fruit types and formats, morphological differences between proximal and distal portions, and antioxidant activity in fruits have led researchers to suggest new crop management approaches to control or minimized BER incidence.

There are several hormones that cause changes in plant growth and in this case the hormones gibberellin and abscisic acid have been quite studied when it comes to BER. The use of gibberellin was shown to promote plant growth (Serrani et al., 2007), however it was noted that there was an increase in the incidence of BER (De Freitas et al., 2012b). The mechanism by which gibberellins increase the incidence of BER are linked to accelerated growth and absence of Ca^{2+} , during fruit growth (Castro, 1980).

In addition, there may be an imbalance of some enzymes activity responsible for maintaining cell wall integrity, such as pectin methylesterase (De Freitas et al., 2012a). Pectin methylesterase activity is directly related to an increase in the

incidence of BER, since these enzymes increase de-esterification of pectins, increasing the amount of cell wall-bound Ca^{2+} , and decreasing the pool of free and soluble Ca^{2+} responsible for membrane stabilization (De Freitas et al., 2012a).

The abscisic acid (ABA) has been gaining ground in research lately, since it promotes a better response of the plant to adverse cultivation conditions, reducing transpiration and promoting activation of the plant's antioxidant system. In addition, ABA is able to promote a better redistribution of Ca^{2+} in fruits, mainly by promoting better functionality of the xylem vessels, which carry water and nutrients to the distal portion of fruits. This region typically presents the lowest levels of Ca^{2+} and the first symptoms of BER (De Freitas et al., 2011).

There are several pools of Ca^{2+} in fruit tissue, including: proximal, distal, wall-attached, organelles-attached, cytoplasmic, and apoplastic Ca^{2+} , and an adequate balance among different pools is necessary to avoid BER incidence. More precisely, apoplastic Ca^{2+} concentration is considered the main regulator of BER incidence, since it is always available and arrives rapidly in regions where growth is more active and there is a greater need for Ca^{2+} (De Freitas et al., 2014).

Brassinosteroids, still poorly studied, have similar effects as ABA, being able to suppress the negative effects of Ca^{2+} deficiency in tissues. Their mode of action is still not well understood, but it is speculated that they activate antioxidant defenses in plants, inhibiting membrane lipid peroxidation (Schnabel et al., 2001, Liu et al., 2009).

Finally, a factor that is practically non-existent in studies is the influence of fruit format on the incidence of BER. There is popular knowledge that elongated fruits, represented mainly by the 'San Marzano', have high rates of BER incidence (Elmer, Ferrandino, 1991) and that BER is smaller in rounded and flattened shape, or even cherry (Ho; White, 2005). Nevertheless, the reason why these varieties are highly susceptible is still not well understood. Fruit shape may cause difficulties for the transport of Ca^{2+} along the fruit to the distal portion. As the distance from the proximal to the distal portion is greater in elongate fruits, the susceptibility of these fruits to BER is also generally greater. In addition, functionalities of xylem vessels in these varieties as well as xylem branching along the fruit tissue may also influence the appearance of BER symptoms.

Studies suggest that Ca^{2+} transport along the fruit may be limited by the loss of xylem vessels functionality during growth and development (De Freitas et al., 2014), possibly due to the accumulation of substances, embolism, and loss of

hydrostatic gradient (Bondada et al., 2005). As Ca^{2+} transport is coordinated by transpiratory current and carried to the fruits via xylem, it is necessary that its xylem vessels are functional, mainly in the distal portion. Consequently, Ca^{2+} may reach growing tissues, inhibiting the appearance of BER.

Therefore, it is clear that BER is not exclusively related to the concentration of Ca^{2+} in tissues, but to a combination of whole plant and fruit specific factors that cause this disorder. Morphological factors, together with physiological ones, determine the movement and redistribution of Ca^{2+} in plant tissues, promoting accumulation in fruits and inhibiting the appearance of BER. The main objectives of this study are to better understand the main factors regulating the susceptibility of tomatoes to BER, as well as to propose possible crop management approaches to reduce BER incidence and severity.

2. 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.

The varieties evaluated in this experiment showed differences in relation to the factors possibly regulating BER development in the fruit. Varieties with smaller and/or flattened fruits such as 'Nagcarlan', 'Amalia' and 'Mara' presented lower incidence of BER. In other way, elongated variety 'M-82', presented higher incidence of BER.

In other way, elongated tomato varieties 'San Marzano' and 'Banana Legs' showed higher susceptibility to BER, whereas 'Roma' and 'Mini-Roma' had lower susceptibility to the disorder. In elongated varieties, like San Marzano, the environmental conditions should be maintained favorable to Ca^{2+} accumulation in the fruits, such as low leaf transpirations and less negative leaf water transpiration.

Future studies should try to understand the motives by which in distal part, xylem, loses its functionality and why more Ca^{2+} remains bounded to cell wall. BER incidence in elongated tomato varieties is not only triggered by exogenous factors or nutrient concentrations, but also by a combination of exogenous and endogenous factors.

Brassinosteroids are still not commercially used in agriculture, but there is a great potential for using it, mainly in stress conditions. Studies should be carried out to optimize doses, timing of applications and possible interactions with other hormones. Furthermore, it is necessary to better understand which mechanisms control the enzymatic activity, increasing soluble Ca^{2+} and decreasing Ca^{2+} .

In this study, EBL inhibited BER development in 'BRS Montese' tomato fruit. EBL maintained higher soluble Ca^{2+} and lower cell wall bound Ca^{2+} contents in fruit tissue.

EBL increase ascorbic acid content and decrease hydrogen peroxide contents, as well as increased the activity of the three main antioxidant enzymes known as ascorbate peroxidase, catalase, and superoxide dismutase in fruit tissue.

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.

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