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Review

Why calcium deficiency is not the cause of blossom-end rot in tomato and pepper fruit – a reappraisal

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ABSTRACT

From a review of the relevant literature it is concluded that Ca^{2+} deficiency is not the cause but a result of blossom-end rot (BER) in tomato and pepper fruit. Actually, a depletion of the apoplastic pool of water-soluble Ca^{2+} in fruit has been observed only after the symptoms of BER were already visible, whereas in fruit at the early stages of BER development, the distribution and concentration of Ca^{2+} was still similar to that in healthy fruit. The actual causes of BER are obviously the effects of abiotic stress, e.g. by salinity, drought, high light intensity, heat, and ammonia nutrition, resulting in an increase of reactive oxygen species (ROS), high oxidative stress and finally cell death. Cell death results in a disintegration of the plasma membrane and tonoplast and a breakdown of the endoplasmic reticulum, thus not following but preceding ion leakage, including Ca^{2+} leakage, and loss of turgor. Bioactive gibberellins (GAs) reduce the accumulation of Ca^{2+} but increase the susceptibility to stress and the risk of BER, while abscisic acid (ABA) has the opposite effect. Ca^{2+} stabilizes cell structures and may thus limit cell expansion. It is usually sufficiently available for plant development and therefore Ca^{2+} deficiency is rare in nature. Application of GA biosynthesis inhibitors, such as prohexadione-Ca, and of GA antagonists, such as ABA, may completely inhibit the development of BER even at very low availability of Ca^{2+}. With this approach, a better understanding and a more efficient control of BER in tomato and pepper fruit is envisaged.

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1. Introduction

Blossom-end rot (BER) in fruit of tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) is characterized by enhanced permeability and deterioration of cell membranes, with subsequent loss of turgor and leakage of cell liquids. Since Lyon et al. (1942) reported that BER was most prevalent and severe when the contents of Ca^{2+} in the nutrient solution and in the fruit was very low, BER is generally considered a 'calcium-related disorder', resulting from a localized inadequacy of Ca^{2+} due to various environmental factors (Shear, 1975). Recently, De Freitas and Mitcham (2012) in an extensive review have supported the hypothesis that BER, like bitter pit (BP) in apple, is not only related to but *caused by* fruit calcium deficiency, i.e. that this disorder "can be triggered by mechanisms that reduce plant Ca^{2+} uptake from the soil, fruit Ca^{2+} uptake from the plant, and Ca^{2+} translocation within the fruit, and also result in abnormal regulation of cellular Ca^{2+} partitioning". However, at the same time De Freitas et al. (2012) also stated that the mechanisms triggering BER are still not well understood after being studied for more than 100 years, and that they are among the most complex and challenging processes in plants.

On the other hand, Nonami et al. (1995) suggested that Ca^{2+} deficiency in the fruit may *not* be the direct cause of the occurrence of BER in tomato, because fruit that had just started having

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BER had a similar distribution and concentration of Ca^{2+} ions as normal fruit. Ho and White (2005) stated that no absolute, critical fruit Ca^{2+} concentration for the occurrence of BER has been identified, and that BER may occur in fruit with apparently adequate tissue Ca^{2+} concentrations. They agreed that predicting and preventing the occurrence of BER in glasshouse tomatoes based on measurements of their Ca^{2+} status was not always effective.

Because a relationship between Ca^{2+} deficiency and the occurrence of BER is not always obvious (Petersen and Willumsen, 1992; Nonami et al., 1995; Saure, 2001; Aktas et al., 2005), the function of Ca^{2+} deficiency in the development of BER remains disputed. Therefore, it has been suggested that it may not be the total Ca^{2+} content of the fruit, but an abnormal cellular Ca^{2+} partitioning and distribution that leads to a cellular localized Ca^{2+} deficiency and thus to BER (Ho and White, 2005; De Freitas and Mitcham, 2012). However, recent research rather indicates a central function of stress in BER development.

2. Stress and BER: the causes of cell death

In an earlier review on the causes of BER (Saure, 2001), it has been outlined that neither insufficient Ca^{2+} nutrition alone nor the influence of stress alone reliably cause symptoms of BER. Rather, an interaction between increased susceptibility to stress and subsequent stress was supposed to be the prerequisite for an occurrence of BER. However, the mechanism by which various stresses under certain conditions may cause BER, remained obscure. Meanwhile, considerable progress has been achieved. In the last years, several investigations have contributed to a better understanding on how various stress factors, such as salinity, drought, high light intensity, heat, and ammonia nutrition, cause BER and contribute to Ca^{2+} deficiency.

The influence of stress on the occurrence of BER in pepper and tomato fruit is based in part on an increased activity of NAD(P)H oxidase – an oxygen radicals generating enzyme – and on increased production of reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals and singlet oxygen (O_2) in the fruit apoplast, i.e. in the free diffusional space outside the plasma membrane (Aktas et al., 2003, 2005; Turhan et al., 2006; Mestre et al., 2012). This happens especially in the most sensitive developmental stage at the beginning of rapid fruit growth and development, and mainly in the fruit placental tissue close to the fruit tip in non-affected fruit (Aloni and Karni, 2001). ROS are known to trigger cell death, which is characterized by a progressive loss of membrane integrity, resulting in swelling of the cytoplasm and release of cellular constituents (Van Breusegem and Dat, 2006). This agrees with morphological changes in tomato fruit being affected by BER, as described by Suzuki et al. (2000, 2003): Inside the cells, these changes consist of a disruption of the plasma membrane and tonoplast, a wavy-shaped cell wall, breakdown of endoplasmic reticulum and swollen plastids, leading to a rapid vacuolation of parenchyma cells, epidermal cells and sub-epidermal parenchyma cells in the pericarp of the fruit, and consequently to high electrolyte leakage. This includes the loss of Ca^{2+} ions, which may explain the lower Ca^{2+} concentrations mainly in the apoplast (De Freitas et al., 2012).

3. Phytohormones and BER: the susceptibility to stress

A certain amount of stress, caused by either a single or an interaction of several environmental factors, does not always result in a corresponding degree of BER (Saure, 2001). Rather, a sequence of luxuriant growth, promoting high susceptibility to BER, and subsequent severe stress appears to be required in order to trigger cell death. Mainly two kinds of phytohormones appear to interfere specifically with stress, in opposite directions: Bioactive gibberellins (GAs) and abscisic acid (ABA).

3.1. Gibberellins

For the promotion of vigorous growth, an important factor is high availability of bioactive GAs; either endogenously produced as a result, e.g. of excessive nitrogen supply, disturbed root/shoot balance, or poor yield, or being externally applied. In the tomato pericarp, the physiologically most important bioactive GAs are presumably GA_1 and GA_{20} (Bohner et al., 1988). The concentration of GA_1 in tomato pericarp is highest early during fruit development. At this time, fruit growth by cell expansion is rapid, especially at the blossom-end. This is the phase of greatest risk of BER. Whereas GA concentrations are highest, the concentration of Ca^{2+} is strictly reduced in this period (Goodwin, 1978); plants have developed mechanisms for restricting the transport of Ca^{2+} to fruits to allow for rapid cell expansion and high membrane permeability (Marschner, 1995). However, Marschner (1995) warned that high growth rates may increase the risk that the tissue content of Ca^{2+} falls below the critical level required for cell wall stabilization and membrane integrity. In this case, spraying with Ca^{2+}-salts onto the fruits may help to reduce the incidence of BER, as observed by several authors (*cf* Saure, 2001; Ho and White, 2005). Yet, obviously the main cause of BER remains the increased level of physiological active GAs together with severe stress, but not Ca^{2}-deficiency: Chiu and Bould (1976) reported that at low Ca^{2+} supply BER did not occur when plants were growing slowly.

An antagonism between Ca^{2+} and vegetative growth had already been observed by Lyon et al. (1942): Low Ca^{2+} in the nutrient medium resulted in the most extensive root systems and the most succulent vines, indicating high GA activity. In this case, low supply of Ca^{2+} may have caused the high incidence of BER more indirectly via increased GA activity.

A promotion of BER incidence in tomato by application of GAs has been stated first by Bangerth (1973) and confirmed by Castro and Malavolta (1977). An intensive investigation over 2 years has been carried out by De Freitas et al. (2012): Weekly treatments with GA_{4+7} increased the membrane permeability of fruit tissue and reduced apoplastic water-soluble Ca^{2+} content in pericarp tissue. However, total water-soluble Ca^{2+} content did not differ among treatments in these experiments. The incidence of BER in fruit treated with GA_{4+7} reached nearly 100%, as compared to 20–40% after control treatments with water. In contrary, treatments with prohexadione-Ca (Apogee), a GA biosynthesis inhibitor, resulted in 0% BER, even at very low Ca^{2+} content in the substrate.

GAs may promote BER, and cell death in general, by various mechanisms, e.g.

- by down-regulating ROS-scavenging enzymes (enzymatic antioxidants), such as superoxide dismutase, catalase, and ascorbate peroxidase, thus rendering these cells sensitive to oxidative damage and cell death (Kwak et al., 2006);
- by stimulating the destruction of the growth-inhibiting DELLA proteins, which normally cause ROS levels levels to remain low under environmental stress and in this way delay cell death and promote stress tolerance (Achard et al., 2008).

However, detailed information on the mechanisms by which less GA-signaling contributes to enhanced stress tolerance, is still scarce (Colebrook et al., 2014). From a practical point of view, the fact is important that GA-signaling can be reduced, e.g. by root restriction (Bar-Tal and Pressman, 1996; Karni et al., 2000), by application of the growth-retarding triazoles paclobutrazol

(Lurie et al., 1994) and uniconazole (Wang and Gregg, 1990; Wui and Takano, 1995; Saito et al., 2006), and by ABA (see next chapter)

3.2. Abscisic acid

As an antagonist to GAs, ABA is known to reduce the susceptibility of plants to stress, e.g. by promoting the transport of Ca^{2+} to fruits, thus interfering with rapid cell expansion and reducing membrane permeability. In an attempt to better understand the mechanisms regulating Ca^{2+} partitioning and allocation in tomato plants and fruit under water stress, De Freitas et al. (2011, 2014) subjected tomatoes to ABA treatments. Weekly spraying of whole plants with ABA increased Ca^{2+} accumulation in the blossom-end tissue, resulted in higher tissue- and water-soluble Ca^{2+} concentrations in the fruit and reduced the incidence of BER from 30–45% down to 0%, despite very low Ca^{2+} in the rooting zone, but the Ca^{2+} concentration in the xylem sap of water- and ABA-treated plants remained about the same. The authors emphasized that under conditions of low apoplastic Ca^{2+} the plasma membrane may become leaky, leading to cell plasmolysis, cell death and thus to BER. However, they did not consider the possibility that increased stress may cause leaky cell membranes, cell plasmolysis and cell death, i.e. the symptoms of BER, *followed* by a loss of apoplastic Ca^{2+}.

4. Conclusions

Hepler (2005) pointed out that Ca^{2+} participates in myriad life processes and is involved in nearly all aspects of plant development. However, this does not mean that Ca^{2+} is always actively involved, and in an earlier review on BER it has been concluded that Ca^{2+} cannot longer be considered a primary or an independent factor in the development of BER (Saure, 2001).

Most reports on Ca^{2+} deficiency being the cause of BER are based on observations of fruit in which the symptoms of BER are already visible. In fruit which were just going to develop BER, Ca^{2+} deficiency did not yet occur (Nonami et al., 1995), and in fruit with symptoms of BER the content of Ca^{2+} was only reduced in the affected, but not in comparable healthy tissue (Suzuki et al., 2003; Mestre et al., 2012). Actually, the development of BER requires several steps: Stress increases the production of ROS; ROS causes lipid peroxidation with an increase in the leakiness of membranes, leading to rapid vacuolation of parenchyma cells and the loss of ions, including water-soluble apoplastic Ca^{2+}. These are typical symptoms of BER. Accordingly, the final deficiency of Ca^{2+} can only be considered a result, but not the cause of BER.

Similarly, the hypothesis that ABA reduces the incidence of BER by promoting Ca^{2+} accumulation, must be reconsidered. The increase of Ca^{2+} after application of ABA must be rather attributed to the fact that ABA counteracts the leakiness of cell membranes and the subsequent loss of Ca^{2+} ions in the course of BER development, by preventing an increase of GAs. The promotion of BER development by GAs is also completely suppressed by GA biosynthesis inhibitors such as prohexadione-Ca (Apogee), but with minor effects on the content of Ca^{2+} (De Freitas et al., 2012). This contradicts again the hypothesis that Ca^{2+} deficiency is the cause of BER.

Considering Ca^{2+} deficiency as the main cause of BER and other 'Ca^{2+}-related disorders', such as bitter pit in apple and tipburn in leafy vegetables, has long prevented a better understanding and a more effective control of these disorders. With the new approach of Ca^{2+} deficiency being not the cause but a result of BER, new ways are opened up to control BER more effectively, by reducing the susceptibility to stress and the severity of stress through

- proper selection of suited production sites,
- improved management practices, and by
- breeding and selecting stress-resistant cultivars.

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