Chapter 15

Calcium Deficiency Disorders in Plants

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Abstract

Physiological disorders, such as bitter pit in apple; blossom-end rot in tomato, watermelon, and pepper; tipburn in lettuce, cauliflower, artichoke; and blackheart in celery are believed to be triggered by Ca^{2+} deficiency and can strongly reduce crop quality and yield. These disorders are characterized by dark brown lesions on distal young and fast-growing tissue. In leafy vegetables, stunted growth and curling are also common symptoms. The conserved symptoms and factors leading to Ca^{2+} deficiency disorders suggest the existence of conserved mechanisms regulating these disorders in fruits and vegetables. Suggested mechanisms triggering these disorders are involved in the inhibition of Ca^{2+} accumulation or abnormal regulation of cellular Ca^{2+} partitioning in affected tissues. Interactions between Ca^{2+} and other nutrients in affected tissue have also been suggested to be involved. Although recent ideas have suggested that oxidative stress may play an important role in Ca^{2+} deficiency disorder development, they remain to be experimentally analyzed. Considering the complexity of Ca^{2+} deficiency

disorders, control strategies should first identify the genetic and environmental factors triggering mechanisms leading to symptom development. Based on the factors involved, specific approaches can be identified to effectively inhibit Ca^{2+} deficiency disorder development.

15.1 History of Ca^{2+} Deficiency Disorders

The symptoms of Ca^{2+} deficiency disorders in plants have been reported in the literature for more than a hundred years for different crop species (Jager, 1869; Galloway, 1888; Cobb, 1895; Brooks, 1914). Earlier named only as physiological disorders due to unknown causes, these disorders were first suggested to be the result of pathogen infection, toxicity, and plant stress conditions (Smith, 1926; Wedgworth et al., 1926; Chamberlain, 1933; Atanasoff, 1934; Carne and Martin, 1934). Later studies focusing on plant nutrient requirements revealed that growing plants under low or high levels of Ca^{2+} could increase or decrease the incidence of these disorders, respectively, which were then named Ca^{2+} deficiency disorders (Raleigh and Chucka, 1944; Bussler, 1962; Hewitt, 1963; Shear, 1975; Chiu and Bould, 1977). Further studies have been focused on practical methods to reduce or predict the incidence of Ca^{2+} deficiency disorders in crop plants (Ferguson and Watkins, 1989; Taylor and Locascio, 2004). More recent studies have improved our understanding of the mechanisms regulating Ca^{2+} deficiency disorders in plants, setting the stage for the development of more efficient control strategies (Ho and White, 2005; Saure, 2005; Freitas and Mitcham, 2012).

15.2 Role of Ca^{2+} as an Essential Plant Macronutrient

Significant improvements in our understanding of the mechanisms regulating Ca^{2+} deficiency disorders in plants must follow a better understanding of the roles of Ca^{2+} at the cellular level. Calcium has a large ion radius that facilitates ion dehydration and, consequently, binding to several anionic substances (Hauser et al., 1976; Jaiswal, 2001; Batistic and Kudla, 2010). This property allows Ca^{2+} to form noncovalent bonds within the pectin matrix of the cell wall, contributing to cell wall structure and strength (Marschner, 1995). One type of noncovalent bond, known as a coordination bond, is formed between Ca^{2+} and oxygen or nitrogen present in pectic polysaccharides (Marschner, 1995). Another type of noncovalent bond, known as an electrostatic bond, is formed when Ca^{2+} is attracted to a negatively charged group, such as carboxylate ($-COO^-$) on pectates and polygalacturonic acid (Marschner, 1995). The high abundance of Ca^{2+} in the cell wall has a strong

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effect not only on cell wall strength, but also on cell wall pH due to its cation effect on the cell wall medium (Demarty et al., 1984). In addition, Ca^{2+} has also been reported to affect the synthesis of cell wall polysaccharides, such as 1,3-β-glucan (Kauss, 1987; Brett and Waldron, 1996).

The high binding capacity of Ca^{2+} makes this ion an important signaling molecule in the cytosol (Hepler and Wayne, 1985; White and Broadley, 2003; Batistic and Kudla, 2010). Indeed, Ca^{2+} plays an important role in cytosolic signal transduction pathways involved in cell responses to a wide range of biotic and abiotic factors (Scrase-Field and Knight, 2003; White and Broadley, 2003). In the resting or quiescent state, cytosolic Ca^{2+} ranges between 100 and 200 nM, reaching about 2 μM under stimulus (Rudd and Franklin-Tong, 1999). Changes in cytosolic Ca^{2+} concentrations may take the form of single calcium transients (Knight et al., 1996), oscillations (McAinsh et al., 1995), or repeated spikes (Ehrhardt et al., 1996). The spatial and temporal characteristics of these stimuli-specific Ca^{2+} transients have become known as Ca^{2+} signatures (Scrase-Field and Knight, 2003). Specific Ca^{2+} signatures have been suggested to encode information about the type and severity of the input stimulus (Dolmetsch et al., 1997), which is then decoded by downstream components of the signaling pathway, eventually leading to the specific and appropriate cellular response (Scrase-Field and Knight, 2003). After raising cytosolic Ca^{2+} levels, the resting state must be reestablished to avoid Ca^{2+} toxicity and, potentially, cell death. At high concentrations in the cytosol, Ca^{2+} can become toxic due to its precipitation with inorganic phosphate and other ionic substances, as well as its competition for binding sites with other cations, such as Mg^{2+}, that are required for enzyme activation and proper cellular metabolism (Hepler and Wayne, 1985; Batistic and Kudla, 2010). For these reasons, cytosolic Ca^{2+} must be under strict biochemical and physiological control. After cytosolic oscillations, the resting state of cytosolic Ca^{2+} is reestablished by the activity of Ca-ATPases and H^+/Ca^{2+} exchangers, which are proteins that transport Ca^{2+} from the cytosol into the apoplast or into storage organelles in the cell (White and Broadley, 2003).

Calcium is needed at high concentrations inside cellular organelles so it is available to be loaded into the cytosol during signaling responses, and as a counterion to inorganic and organic anions (Jones and Bush, 1991; White and Broadley, 2003). The vacuole is the largest pool of Ca^{2+} in the cell, with 90%–95% of the cell's volume and 1–10 mM Ca^{2+} (Bush, 1995; White and Broadley, 2003). In the endoplasmic reticulum, Ca^{2+} concentration can range from 1 to 5 mM (Kendall et al., 1992). Chloroplasts and mitochondria also contribute, with the Ca^{2+} being stored inside these cellular organelles at concentrations ranging between 0.1–10 μM and 0.2–1.2 μM, respectively (Johnson et al., 1995; Logan and Knight, 2003). Inside these organelles, Ca^{2+} can be present as a free ion that can be used for cytosolic signaling responses or as a countercation to inorganic and organic anions forming complexes with substances such as organic acids, proteins, peptides, and

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other anions (Jones and Bush, 1991; White and Broadley, 2003). Studies have suggested that free Ca^{2+} present in each organelle is responsible for a specific cellular response, and that all organelles act in concert to shape a Ca^{2+} signaling response (Batistic and Kudla, 2010).

The high affinity of Ca^{2+} for anionic phosphate and carboxylate groups of lipids and proteins at the membrane surface makes Ca^{2+} an important regulator of membrane structure and function (Hanson, 1960; Hauser et al., 1976; Clarkson and Hanson, 1980; Legge et al., 1982; Kirby and Pilbeam, 1984; Picchioni et al., 1998; Jaiswal, 2001; Hirschi, 2004; Batistic and Kudla, 2010). Calcium binding reduces membrane fluidity by tightly packing the membrane lipids and proteins, which reduces the passive flow of monovalent ions such as H^+, Na^+, and K^+ (Williams, 1970; Jaiswal, 2001; White and Broadley, 2003; Plieth, 2005). Since cytosolic Ca^{2+} must be maintained at extremely low levels to avoid toxicity, apoplastic free Ca^{2+} has been suggested to be the most important pool to regulate proper membrane structure and function (Hanson, 1960; Steveninck, 1965; Wallace et al., 1966; Lund, 1970; Kirby and Pilbeam, 1984; Picchioni et al., 1998).

15.3 Symptoms of Ca^{2+} Deficiency Disorders in Fruit

The visual symptoms of Ca^{2+} deficiency disorders are highly conserved across different fruit species (Figure 15.1, Table 15.1). The symptoms usually start as water-soaked tissue caused by plasma membrane breakdown that leads to cell death and tissue dehydration, resulting in dark brown and depressed lesions on the fruit surface (Simon, 1978; Fuller, 1980; Suzuki et al., 2000, 2003; Ho and White, 2005). Since the visual symptoms and causes are similar in different fruit species, it is believed that the mechanisms involved are also highly conserved across different species (White and Broadley, 2003; Saure, 2005).

The symptoms of Ca^{2+} deficiency can develop in different regions depending on the species (Figure 15.1, Table 15.1). In fruit, tissue susceptibility to Ca^{2+} deficiency disorders is believed to be determined by Ca^{2+} accumulation and cellular Ca^{2+} localization (Adams and Ho, 1993; Nonami et al., 1995; Marcelis and Ho, 1999). In that case, tissue that accumulates less Ca^{2+} is more susceptible to Ca^{2+} deficiency disorders than tissue with higher levels of Ca^{2+} content (Adams and Ho, 1993; Nonami et al., 1995; Marcelis and Ho, 1999). Some examples of Ca^{2+} deficiency disorders in fruit are described below.

15.3.1 Apple

The symptoms of Ca^{2+} deficiency disorder in apple, known as bitter pit (BP), start as water-soaked spots in the outer cortical flesh of the fruit, frequently

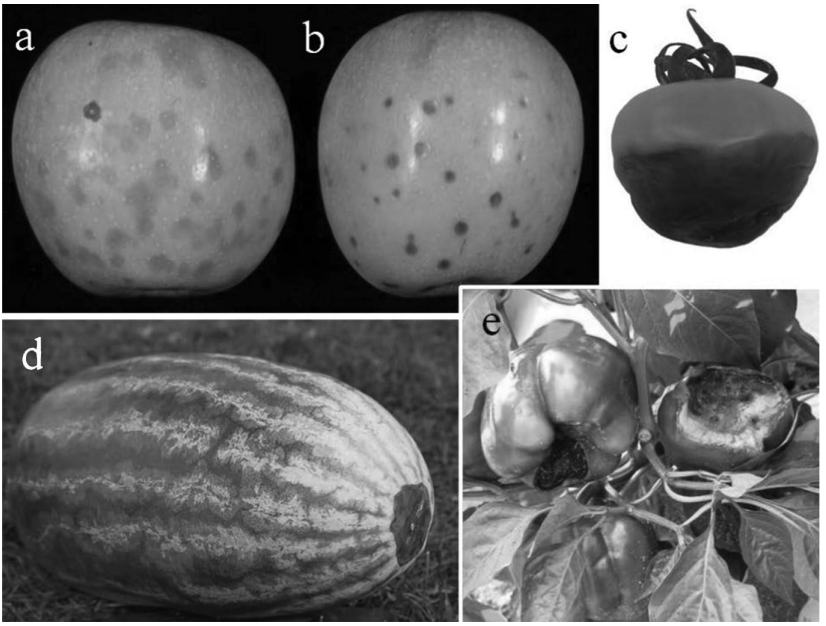

Figure 15.1 Calcium deficiency disorders in fruit: (a, b) apple with BP initial water-soaked symptoms that become dark brown, depressed on the fruit surface; (c) blossom-end rot symptoms in tomato fruit; (d) watermelon; and (e) bell pepper. (Panel c from Freitas and Mitcham, 2011a. Copyright © American Society of Plant Biologists, www.plantphysiol.org. Panel d from Ontario Crop Integrated Pest Management (IPM), Blossom-end rot, 2009, Queen's Printer for Ontario, http://www.omafra.gov.on.ca/IPM/english/cucurbits/diseases-and-disorders/blossom-end-rot.html. Copyright © 2015 Queen's Printer for Ontario, image courtesy of John G. Strang, Department of Horticulture, University of Kentucky. Panel e from Hochmuth, G.J., and Hochmuth, R.C., Blossom-end rot in bell pepper: causes and prevention, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, 2012, http://edis.ifas.ufl.edu/ss497#FIGURE%203. Copyright © 2015 University of Florida, Institute of Food and Agricultural Sciences.)

just under the skin (Figure 15.1a, Table 15.1). Later, cell dehydration and death result in the collapse of the outermost cells, causing small dark brown depressed lesions (pits) on the surface (Figure 15.1b). BP lesions have also been associated with vascular elements and in severe cases may coalesce to form larger necrotic areas (MacArthur, 1940). Although pitting of the flesh also occurs, symptoms are not always visible from the outside. The frequency of pitting is often greater toward the calyx end of the

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Table 15.1 Calcium Deficiency Disorders in Fruits: Symptoms and Mechanisms Involved

Fruit	*Disorder*	*Symptoms*	*Mechanisms*	*References*
Apple (A)	BP	Water-soaked spots (A) or tissue (T, W, P) that eventually becomes dark brown mainly (A) or always (T, W, P) at the distal fruit tissue	1. Reduction of total fruit Ca^{2+} concentration (A, T, W, P) 2. Reduction of Ca^{2+} concentration at specific cellular compartments (A, T) 3. Interaction between Ca^{2+} and other nutrients (N, K^{+}, Mg^{2+}) in the fruit (A) 4. Growth regulator homeostasis at the whole-plant and fruit-specific levels (A, T) 5. Environmental stress conditions (A, T, W, P) 6. Reduction of Ca^{2+} concentration that results in ROS accumulation and lipid peroxidation in the fruit (T, P)	Bangerth, 1979; Ferguson and Watkins, 1989; Saure, 1996; White and Broadley, 2003; Freitas et al., 2010, 2013
Tomato (T)	BER			Saure, 2001; White and Broadley, 2003; Taylor and Locascio, 2004; Ho and White, 2005; Saure, 2005; Freitas et al., 2011a, 2011b, 2012b, 2012c, 2014
Watermelon (W)				Maynard and Hopkins, 1999
Pepper (P)				Marcelis and Ho, 1999; Aktas et al., 2005; Hochmuth and Hochmuth, 2012

fruitw(Ferguson and Watkins, 1989). Fruit pitting usually takes place after harvest, but in severe cases, it can also develop before harvest (Ferguson and Watkins, 1989).

15.3.2 Tomato

In tomato fruit, Ca^{2+} deficiency disorder was named blossom-end rot (BER) due to its appearance: decay at the blossom end of the fruit (Figure 15.1c, Table 15.1). During BER development, blossom end tissue shows water-soaked symptoms that become dark brown on the fruit surface at later stages. In severe cases, BER can expand from the blossom end tissue toward the calyx end tissue, affecting the whole fruit (Ho and White, 2005; Freitas et al., 2011a). During BER development, death of fruit tissue can favor pathogen infection, which also contributes to the rot symptoms. The initial water-soaked symptoms are believed to be caused by high membrane leakage, leading to cell dehydration and death that trigger phenol oxidation and the dark brown color development (Suzuki et al., 2000, 2003; Ho and White, 2005). In tomato, Ca^{2+} deficiency symptoms usually occur early during fruit growth and development, when limited Ca^{2+} moves into rapidly expanding fruit (Ho and White, 2005).

15.3.3 Watermelon

Calcium deficiency disorder in watermelon is also known as BER due to its visual symptoms, as previously described for tomato. The symptoms are characterized by softening and shriveling of the fruit blossom end tissue, which eventually becomes dark brown, sunken, and leathery (Figure 15.1d, Table 15.1) (Maynard and Hopkins, 1999). Genotypes with elongated fruit have been reported to be more susceptible to BER than round fruit genotypes (Hammouda, 1987).

15.3.4 Pepper

The symptoms of Ca^{2+} deficiency disorder in pepper are quite similar to those of tomato fruit, which is also known as BER (Figure 15.1e, Table 15.1). The symptoms usually occur early during fruit growth and development, starting as water-soaked tissue in the blossom end region, which becomes brown, with necrotic areas at later stages (Marcelis and Ho, 1999). In severe cases, BER can expand to the entire pepper fruit (Aktas et al., 2005). Fruit tissue close to the BER symptoms tends to lose green color faster than the rest of the pepper. In addition, BER favors tissue infection with saprophytic

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fungi and soft-rot bacteria species that enhance the rot-like appearance (Hochmuth and Hochmuth, 2012).

15.4 Symptoms of Ca^{2+} Deficiency Disorders in Leafy Vegetables

Water movement into mature leaves takes place exclusively through the xylem, whereas water uptake into young, low-transpiring leaves takes place though both the phloem and xylem (Taylor and Locascio, 2004). Since Ca^{2+} is only mobile in the plant through the xylem, and the rate of xylem sap flow is controlled mainly by transpiration and growth rates, Ca^{2+} accumulation in old and mature leaves is much higher than in low-transpiring, young, and enclosed leaves (Saure, 1998). In the leaf, Ca^{2+} accumulation is higher at the base and lower in the tip tissue (Barta and Tibbitts, 2000). Therefore, the symptoms of Ca^{2+} deficiency in leafy vegetables usually take place at the tip of low-transpiring young and enclosed leaves, due to low Ca^{2+} accumulation triggered by low transpiration rates. In addition, high tipburn incidence is always associated with environmental conditions favoring plant growth (Collier and Tibbitts, 1982), which is believed to reduce young leaf Ca^{2+} concentration and increase leafy vegetables' susceptibility to Ca^{2+} deficiency disorders. The symptoms of Ca^{2+} deficiency disorders are highly conserved among leafy vegetables, which are characterized by stunted growth, curling, and the appearance of brown spots and necrosis associated with phenol oxidation of young leaves, leaf tips, and primarily meristematic cells (Figure 15.2, Table 15.2) (Bussler, 1962; Simon, 1978; Saure, 1998; Koike and Smith, 2010). Some examples of Ca^{2+} deficiency disorders in leafy vegetables are described below.

15.4.1 Lettuce

Calcium deficiency disorder in lettuce, known as tipburn, is characterized by dark brown lesions and necrosis on the margins of young developing leaf tips (Figure 15.2a, Table 15.2) (Saure, 1998; Koike and Smith, 2010). In some genotypes, tipburn is first seen on the small veins along the margin of young leaves. Inner and younger leaves are more susceptible to tipburn incidence than outer and older leaves (Koike and Smith, 2010).

15.4.2 Cauliflower

Calcium deficiency disorder in cauliflower, also called tipburn, is characterized by a light brown coloration and tip necrosis of young inner wrapper

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leaves that enclose the cauliflower head (Figure 15.2b, Table 15.2) (Rosen, 1990; Koike and Smith, 2010). In severe cases, Ca^{2+} deficiency symptoms can also result in curd discoloration due to secondary pathogen infection (Maynard et al., 1981).

15.4.3 Artichoke

Calcium deficiency disorder in artichoke is also known as tipburn and is characterized by brown discoloration and the death of leaf margins. In addition, immature flower buds can also develop black lesions along the

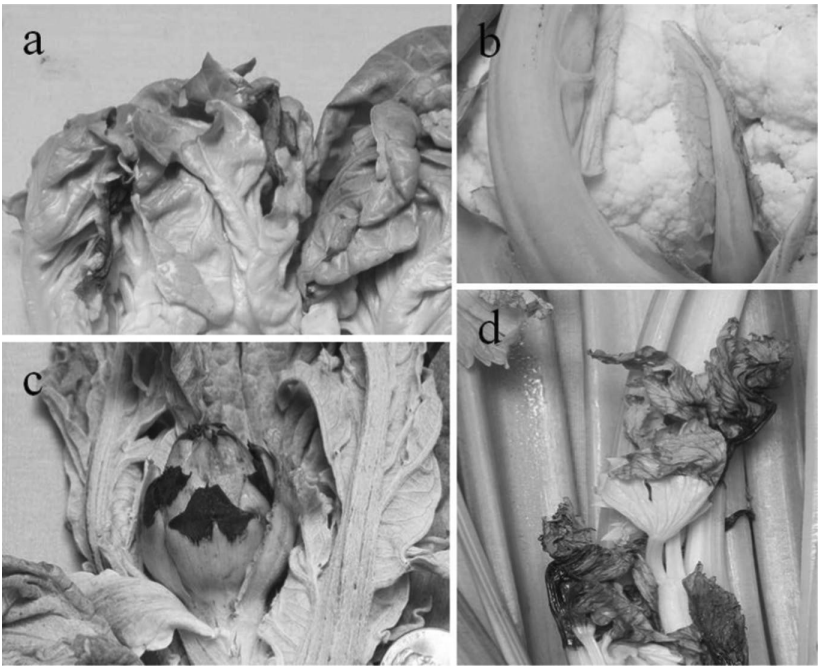

Figure 15.2 Calcium deficiency development in leafy vegetables. Tipburn development in (a) lettuce, (b) cauliflower, and (c) artichoke, and (d) blackheart in celery. (From Koike, S., and Smith, R., Calcium deficiency disorders hit vegetable crops in central coast, Regents of the University of California, Agriculture and Natural Resources, Oakland, CA, 2010, http://ucanr.org/blogs/blogcore/postdetail.cfm?postnum=3407. Copyright © 2015 Regents of the University of California, Agriculture and Natural Resources.)

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Table 15.2 Calcium Deficiency Disorders in Vegetables: Symptoms and Mechanisms Involved

Vegetable	*Disorder*	*Symptoms*	*Mechanisms*	*References*
Lettuce	Tipburn	Tip necrosis of young leaves and meristems	Low Ca^{2+} accumulation in young leaf tips and meristems	Saure, 1998; White and Broadley, 2003; Koike and Smith, 2010
Cauliflower				Rosen, 1990; Koike and Smith, 2010
Artichoke				Francois et al., 1991; Koike and Smith, 2010
Celery	Blackheart	Light to dark brown speckling, lesions and necrosis on the margins of developing leaf tips deep within the central growing region		White and Broadley, 2003; Koike and Smith, 2010

upper tips and edges of the flower bracts. These symptoms appear mainly on bracts in the inner whorls of the bud (Figure 15.2c, Table 15.2) (Francois et al., 1991; Koike and Smith, 2010). In severe cases, Ca^{2+}-deficient bracts can also be infected by pathogens (Francois et al., 1991).

15.4.4 Celery

The Ca^{2+} deficiency disorder in celery is known as blackheart and is characterized by light to dark brown speckling, lesions, and necrosis on the margins of developing leaf tips deep within the central growing region (Figure 15.2d, Table 15.2) (Koike and Smith, 2010). During plant growth and development, the blackheart symptoms may turn black and expand outward from the inner plant tissues (Koike and Smith, 2010).

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15.5 Potential Mechanisms Regulating Ca^{2+} Deficiency Disorders

After years of study, it is believed that plant susceptibility to Ca^{2+} deficiency disorders is affected by genetic and environmental factors regulating tissue Ca^{2+} content and cellular Ca^{2+} distribution (Figure 15.3) (Bangerth, 1979; Ferguson and Watkins, 1989; Francois et al., 1991; Saure, 1998, 2005; Taylor and Locascio, 2004; Ho and White, 2005; Freitas and Mitcham, 2012).

15.5.1 Total Tissue Ca^{2+} Content

Calcium accumulation in leaf and fruit tissues is regulated at the whole plant and at the leaf- and fruit-specific levels (Figure 15.3). At the whole-plant level, Ca^{2+} movement into the leaf or fruit is determined first by Ca^{2+} availability to the plant and root Ca^{2+} uptake activity (Taylor and Locascio, 2004).

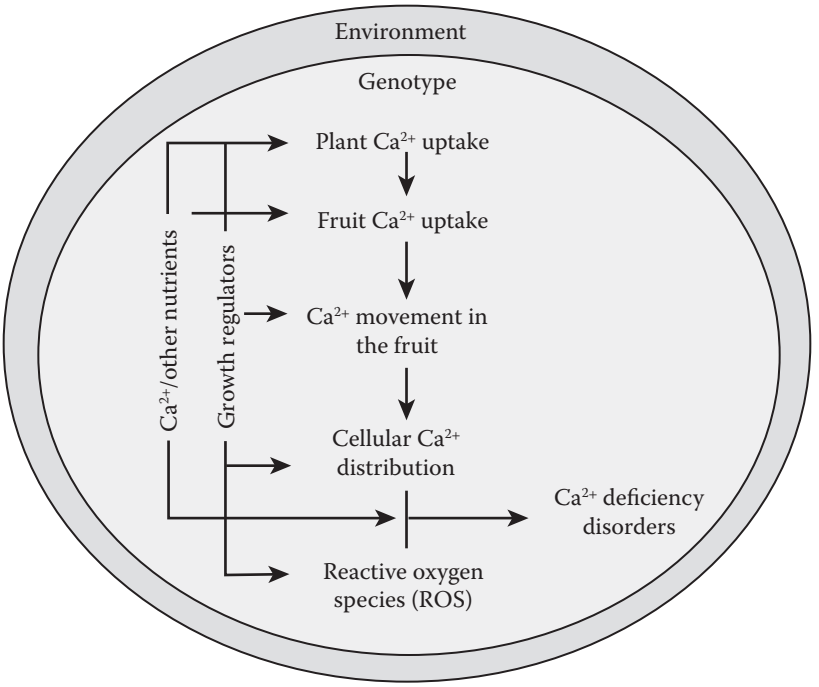

Figure 15.3 Potential mechanisms regulating Ca^{2+} deficiency disorders in fruit and vegetables.

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Most of the root Ca^{2+} uptake is believed to take place passively through the apoplast at the root tip and lateral root growth regions where the Casparian band is not present (White, 2001; Taylor and Locascio, 2004). In that case, root growth enhances Ca^{2+} uptake by increasing the number of root tips and lateral roots that lead to higher apoplastic Ca^{2+} movement into the root system (White, 2001). Although root Ca^{2+} uptake may also take place through the symplastic pathway, the complex mechanisms that have evolved to strictly regulate and maintain low levels of cytosolic Ca^{2+} suggest otherwise (White, 2001). In addition, root Ca^{2+} uptake is also affected by uptake competition between Ca^{2+} and other ions available in the soil, as will be discussed later in this chapter on section 15.5.3. (Bangerth, 1979; Ferguson and Watkins, 1989; Saure, 2005). Therefore, soil nutrient composition also influences root Ca^{2+} uptake and, consequently, leaf and fruit susceptibility to Ca^{2+} deficiency disorders.

After Ca^{2+} is taken up by the roots, it moves in the xylem vessels toward the leaves and fruit by mass flow in response to the water potential gradient generated by leaf and fruit transpiration and growth (Saure, 1998; Taylor and Locascio, 2004). Mature leaves have higher transpiration rates than young leaves and fruit, which explain their higher Ca^{2+} accumulation (Saure, 1998; Ho and White, 2005; Freitas et al., 2011b). Accordingly, low relative humidity increases Ca^{2+} uptake into mature high-transpiring leaves, but reduces in low-transpiring fruit and the inner leaves of leafy vegetables with a closed growing point (Olle and Bender, 2009). In addition, mature leaves receive water exclusively from the xylem, whereas young leaves and fruit receive water from both xylem and phloem vessels (Ho and White, 2005; Freitas et al., 2011b). Considering that Ca^{2+} moves in the plant only through the xylem, water uptake from the phloem further decreases the capacity to uptake Ca^{2+} through the xylem sap into young leaves and fruit, compared to mature leaves. Similar behavior is also observed in other sink organs, such as meristems that have low transpiration rates and receive water from both the phloem and xylem, making sink organs highly susceptible to Ca^{2+} deficiency disorders (Saure, 1998; Koike and Smith, 2010). Studies have shown that increasing transpiration of sink organs, without changing whole-plant transpiration, is more efficient to increase Ca^{2+} content in these organs than increasing Ca^{2+} availability in the soil (Tadesse et al., 2001). In addition, reducing whole-plant transpiration either by decreasing water vapor pressure deficit (WVPD) or by spraying the plant with abscisic acid (ABA) can reduce xylem sap movement toward mature leaves and increase xylemic flow toward low-transpiring fruit, which has been shown to increase fruit Ca^{2+} accumulation and decrease fruit susceptibility to Ca^{2+} deficiency disorders (Guichard et al., 2005; Freitas et al., 2011b, 2014; Barickman et al., 2014).

Plant water stress has been widely reported to increase Ca^{2+} deficiency disorder development in both low-transpiring leaves and fruit

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(Saure, 1998; Ho and White, 2005; Koike and Smith, 2010). Water stress, triggered by low water availability or high salinity conditions, reduces the water potential gradient between roots and low-transpiring leaves and fruit, consequently decreasing Ca^{2+} uptake and increasing tissue susceptibility to Ca^{2+} deficiency disorders (Taylor and Locascio, 2004; Freitas and Mitcham, 2012). This effect is further enhanced by Ca^{2+} immobility through the phloem, ensuring that the high levels of Ca^{2+} accumulated in older leaves are not redistributed to low-transpiring Ca^{2+}-deficient tissues (White and Broadley, 2003).

Although transpiration and growth are important factors determining xylem sap flow and Ca^{2+} uptake, the number of functional xylem vessels and the hydrostatic gradient required for xylem sap movement can also affect Ca^{2+} content in sink organs (Ho et al., 1993; Tadesse et al., 2001; Dražeta et al., 2004a; Bondada et al., 2005; Ho and White, 2005; Freitas et al., 2011b). Accordingly, studies have shown that the number of functional xylem vessels and Ca^{2+} accumulation in the fruit decrease simultaneously during fruit growth and development (Nonami et al., 1995). High numbers of functional xylem vessels have also been associated with high levels of fruit Ca^{2+} uptake and low fruit susceptibility to Ca^{2+} deficiency disorders (Freitas et al., 2011b). Besides a high number of functional xylem vessels, the hydrostatic gradient in the xylem vessels may also be required for xylem sap movement into distal fruit tissues (Bondada et al., 2005), contributing to Ca^{2+} accumulation and reducing susceptibility to Ca^{2+} deficiency disorders.

15.5.2 Cellular Regulation of Ca^{2+} Partitioning and Distribution

Mechanisms regulating cellular Ca^{2+} partitioning have recently been reported in the literature (Park et al., 2005; Conn et al., 2011; Freitas et al., 2011a; Wu et al., 2012). As previously described, Ca^{2+} is required at specific concentrations in each cellular compartment (White and Broadley, 2003). Therefore, abnormal changes in cellular Ca^{2+} partitioning can potentially result in a cell-localized Ca^{2+} deficiency, cell death, and Ca^{2+} deficiency symptom development (Figures 15.3 and 15.4). Indeed, studies have shown that abnormal regulation of cellular Ca^{2+} partitioning represents an important mechanism determining tissue susceptibility to Ca^{2+} deficiency disorders in plants (Park et al., 2005; Freitas et al., 2011a) (Figure 15.4).

Studies have shown that mutant plants lacking the expression Ca^{2+}/proton antiporters *CAX1* and *CAX3* in the tonoplast have higher apoplastic free Ca^{2+} content than wild-type plants, suggesting that *CAX* genes

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are indeed important mechanisms regulating cellular Ca^{2+} partitioning (Conn et al., 2011). Accordingly, attempts to increase fruit Ca^{2+} content by enhancing the expression of a tonoplast *CAX* resulted not only in higher fruit Ca^{2+} accumulation, but also in higher fruit susceptibility to Ca^{2+} deficiency disorder (Park et al., 2005) (Figure 15.4). In these studies, the *Arabidopsis CAX1* gene without the N terminus regulatory region (*sCAX1*) was inserted into the tomato genome under the control of the cell cycle promoter *cdc2a* (Park et al., 2005). The *cdc2a*::*sCAX1* construct resulted in high *sCAX1* expression during cell division, which produces a constitutively active protein (Park et al., 2005). The *sCAX1*-transformed fruit had about twofold higher total tissue Ca^{2+} content and higher vacuolar Ca^{2+} accumulation than wild-type fruit (Figure 15.4). However, *sCAX1*-expressing fruit also showed lower cytosolic and apoplastic Ca^{2+} concentrations that possibly resulted in the observed higher plasma membrane leakage, cell plasmolysis, and 100% incidence of Ca^{2+} deficiency symptoms in fruit, compared to wild-type fruit that did not develop Ca^{2+} deficiency symptoms (Freitas et al., 2011a) (Figure 15.4). Coexpression of an endoplasmic reticulum Ca^{2+} binding protein known as calreticulin has been shown to mitigate Ca^{2+} deficiency symptoms observed in *sCAX1*-expressing fruit (Wu et al., 2012). Although the authors suggested that

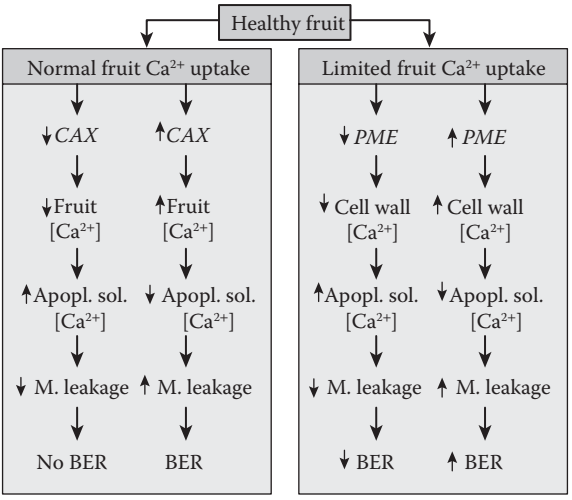

Figure 15.4 Potential mechanisms regulating cellular Ca^{2+} distribution and fruit and vegetable susceptibility to Ca^{2+} deficiency disorders. *CAX, Ca^{2+}/proton antiporters; PME, pectin methylesterase;* Apopl. sol., apoplastic soluble; M. leakage, membrane leakage.

coexpression of the endoplasmic reticulum calreticulin reduced fruit susceptibility to Ca^{2+} deficiency disorders by altering the cellular Ca^{2+} content and distribution within the plant matrix (Wu et al., 2012), more studies are required to further understand the mechanisms involved. It is possible that calreticulin could mitigate Ca^{2+} deficiency symptoms in *sCAX1*-expressing fruit by mechanisms unrelated to cellular Ca^{2+} distribution (Qiu et al., 2012).

Plants have evolved with cell walls as defensive barriers, conduits for information, and sources of signaling molecules and developmental cues (Bacic et al., 1988; O'Neill et al., 1990; Carpita and Gibeaut, 1993; Ridley et al., 2001). Cell wall composition and, consequently, its Ca^{2+} binding capacity can vary widely from plant to plant (Sorensen et al., 2010). Accordingly, dicotyledeonous plants have higher cell wall Ca^{2+} binding capacity and Ca^{2+} requirements than monocotyledonous plants (Islam et al., 1987; Kirkby and Pilbeam, 1984; White and Broadley, 2003). Considering that the cell wall contains, on average, 70% of the total plant tissue Ca^{2+} content (Demarty et al., 1984; Freitas et al., 2010), small changes in cell wall Ca^{2+} binding capacity can potentially have a great impact on Ca^{2+} availability in other pools of the cell. Indeed, recent studies suggest that increasing cell wall Ca^{2+} binding capacity can reduce the levels of Ca^{2+} available in other pools in the cells, resulting in a cell-localized Ca^{2+} deficiency, cell death, and Ca^{2+} deficiency symptom development (Freitas et al., 2012b) (Figure 15.4). Silencing tomato fruit *pectin methylesterase* (*PME*) genes reduced the number of carboxyl groups in the cell wall matrix, and therefore reduced cell wall Ca^{2+} content (Freitas et al., 2012c) (Figure 15.4). These *PME*-silenced fruit had increased apoplastic water-soluble Ca^{2+}, lower membrane leakage, and reduced susceptibility to Ca^{2+} deficiency disorders (Figure 15.4). These studies suggest that regulation of cellular Ca^{2+} distribution, either by the activity of enzymes involved in cellular Ca^{2+} transport or by Ca^{2+} binding to the cell wall, is a potential mechanism regulating tissue susceptibility to Ca^{2+} deficiency disorders (Figure 15.4).

15.5.3 Other Nutrients

15.5.3.1 Nitrogen

Nitrogen (N) is commonly applied at high rates in the soil to stimulate plant growth and yield (Bramlage and Weis, 2004). However, excessive N levels might increase fruit susceptibility to Ca^{2+} deficiency disorders (Figure 15.3) (Raese and Drake, 1997; Dris et al., 1998). Besides the total amount of N, the form of N that is used can also influence fruit quality. Soil fertilization with ammonium rather than nitrate nitrogen substantially

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reduced Ca^{2+} accumulation in apples and greatly increased the incidence of BP (Ludders, 1979), since ammonium is antagonistic to Ca^{2+} uptake by roots (Shear and Faust, 1971). However, since ammonium can be rapidly converted to nitrate in the surface layers of the soil, it is believed that the total amount of N being applied to the soil is a much more important concern to Ca^{2+} deficiency disorders than is the form in which N is being applied (Bramlage et al., 1980).

High N fertilizer doses lead to substantial vegetative growth (Bramlage and Weis, 2004). Since Ca^{2+} moves with water in the transpiration stream, greater leaf area will shunt more water and Ca^{2+} to the leaves rather than fruit, reducing the Ca^{2+} content in the fruit (Saure, 2005). Nitrogen fertilization also has an indirect effect on Ca^{2+} deficiency disorder development by increasing fruit size (van Schreven, 1961; Ho and White, 2005).

Fruits with high levels of N also show higher respiration and ethylene production rates after harvest, reflecting advanced ripening and senescence stages (Fallahi et al., 2010), and this possibly accelerates the mechanisms leading to Ca^{2+} deficiency disorders (Lötze and Theron, 2006; Lötze et al., 2010).

15.5.3.2 Potassium and Magnesium

Excessive potassium (K^+) and magnesium (Mg^{2+}) fertilization has been found to increase the incidence of BP in apples (Ferguson and Watkins, 1989) and BER in tomatoes (Ho et al., 1993) (Figure 15.3). Concentrations of K^+, Mg^{2+}, and Ca^{2+} in roots and mature leaves of cultivated plants are generally high, but although K^+ is readily redistributed within the plant, concentrations of Mg^{2+}, and especially Ca^{2+}, are often low in phloem-fed tissues such as fruits and young leaves (Karley and White, 2009). In addition, at the onset of cell expansion in fruits, xylem dysfunction often begins and might lead to reduced fruit uptake of Ca^{+2}, while the function of the phloem remains unchanged relative to the xylem flow, favoring the supply of K^+ (and to a lesser extent Mg^{2+}) (Lang, 1990; Dražeta et al., 2004a).

High K^+/Ca^{2+}, Mg^{2+}/Ca^{2+}, or $(K^+ + Mg^{2+})/Ca^{2+}$ ratios in the fruit have been used to predict BP in apples (Vang-Petersen, 1980; Ferguson and Watkins, 1989; Argenta and Suzuki, 1994; Nachtigall and Freire, 1998; Lanauskas and Kvikliene, 2006; Amarante et al., 2006a, 2006b, 2013). Potassium and Mg^{2+} compete with Ca^{2+} for binding sites at the plasma membrane surface, but these elements cannot replace the role of Ca^{2+} in membrane structure and stability (Schonherr and Bukovac, 1973; Yermiyahu et al., 1994). Consequently, less Ca^{2+} will be bound to the plasma membrane, which will become leakier, eventually leading to plasmolysis,

membrane breakdown, and cell death, during the manifestation of Ca^{2+} deficiency disorders (Freitas et al., 2010, 2013).

15.5.3.3 Boron

Boron (B) is a micronutrient element that plays a key role in reproductive processes such as pollen germination and pollen tube growth (Dickinson, 1978), and application of B increases fruit set and yield in B-deficient apple and pear trees (Wojcik and Wojcik, 2003; Wojcik and Treder, 2006; Wojcik et al., 2008). Boron has a synergistic effect with Ca^{2+} and helps to increase the Ca^{2+} concentration in fruit (Dickson et al., 1973; Bramlage et al., 1980; Wojcik et al., 1999; Wojcik and Wojcik, 2003; Bramlage and Weis, 2004) and reduce BP incidence in apples (Faust and Shear, 1968; Granelli and Ughini, 1990; Wojcik et al., 1999; Sen et al., 2010) and internal browning in pears (Wojcik and Wojcik, 2003; Mielke and Chaplin, 2008) (Figure 15.3). Boron might suppress the activity of indole-3-acetic acid (IAA) oxidase (Marschner, 1995), leading to an increase in IAA that promotes acropetal movement of Ca^{2+} (Dela Fuente et al., 1986) and stimulates xylem tissue differentiation in the pedicel of the fruit (Dražeta et al., 2004b), thereby improving Ca^{2+} uptake by fruits. Soil fertilization with B also increased the dry weight of fine roots in apple trees (Wojcik et al., 2008), where most of the Ca^{2+} uptake is believed to take place (White, 2000, 2001; Taylor and Locascio, 2004). This might also contribute to increased Ca^{2+} content of leaves and fruits in plants fertilized with B. Boron also plays an important role in maintaining both the structural and functional properties of membranes (Cakmak and Römheld, 1977), thereby reducing internal browning disorders in pear fruit (Xuan et al., 2001, 2005; Mielke and Chaplin, 2008).

15.5.3.4 Phosphorus

Mulder (1952) reported that BP in apple fruit was associated with low P content. However, several authors reported that BP was associated with high P content (Brown, 1926; Oberly and Kenworthy, 1961; Sharples, 1964). The high P content of the affected fruit is not surprising if one takes into account the observation that mineral elements move into the pitted tissue (Chamel and Bossy, 1981).

Apple trees supplied annually at bloom with P (with 20 g P per tree as ammonium polyphosphate, and receiving adequate fertigation applications of N, K+, and B) had fruit with reduced incidence of water core (also a Ca^{2+} deficiency disorder) at harvest, higher resistance to browning of cut slices, reduced membrane leakage, and elevated antioxidant content after cold-air storage (Neilsen et al., 2008). This indicates a role for P in the maintenance of apple fruit membrane stability and cellular energetics that might aid in

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reducing the incidence of Ca^{2+} deficiency disorders in apples (Bramlage et al., 1980) (Figure 15.3).

15.5.4 Reactive Oxygen Species

It has been suggested that Ca^{2+} deficiency is not the cause of BER, but a result of it, with BER caused by stress conditions leading to high levels of reactive oxygen species (ROS) that disintegrate cellular membranes, resulting in BER symptoms in fruit tissue (Saure, 2014). Indeed, previous studies have shown higher levels of ROS, such as superoxide radicals, hydroxyl radicals, and singlet oxygen (O_2), in fruit tissue with BER (Aktas et al., 2003, 2005; Turhan et al., 2006; Mestre et al., 2012). However, an extensive study focusing on the relationship between oxidative metabolism and BER development revealed that reducing fruit Ca^{2+} concentration also reduced the activity of the main enzymes responsible for ROS detoxification, leading to H_2O_2 accumulation, lipid peroxidation, and BER symptom development (Mestre et al., 2012). Therefore, Ca^{2+} can inhibit BER development directly by binding to phospholipids and proteins on cellular membranes, and by stimulating the activity of enzymes required for ROS detoxification. Considering that environmental stress conditions can increase ROS levels independent of fruit Ca^{2+} content (Saure, 2001), it is possible that neither Ca^{2+} nor ROS alone can fully explain Ca^{2+} deficiency disorder development, but the interaction between Ca^{2+} and ROS concentrations in the tissue. In addition, studies show that regulation of cellular Ca^{2+} distribution plays an important role in fruit susceptibility to BER (Freitas et al., 2011a, 2012b), suggesting that the combined Ca^{2+}/ROS effect on Ca^{2+} deficiency disorders may also be cellularly compartmentalized in the apoplast. In that case, Ca^{2+} deficiency disorders may develop only if Ca^{2+} levels in the apoplast are not able to counteract the ROS effects on membrane lipid peroxidation (Figure 15.3). Although these studies aided our understanding of the mechanisms involved in Ca^{2+} deficiency disorders, future studies should better characterize the combined effects of different Ca^{2+}/ROS ratios in different cellular compartments on Ca^{2+} deficiency disorders.

15.5.5 Growth Regulators

Although many growth regulators have been associated with Ca^{2+} deficiency disorders through specific mechanisms (Saure, 1996, 1998, 2001, 2005; Ho and White, 2005), the final tissue susceptibility to these disorders is likely determined by the combined effects of various growth regulators on total tissue Ca^{2+} accumulation and cellular Ca^{2+} distribution (Freitas and Mitcham, 2012) (Figure 15.3).

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15.5.5.1 Growth Regulators Affecting Total Tissue Ca^{2+} Content

15.5.5.1.1 Growth Regulators Influencing Root Growth and Ca^{2+} Uptake Activity Root Ca^{2+} uptake can be determined by a growth regulator's effect on root growth and activity. Studies have suggested that basipetal auxin transport and root auxin content are responsible for increases in root growth and activity (Dewitte and Murray, 2003; Yang et al., 2004; Chapman and Estelle, 2009). Accordingly, maintaining auxin transport and increasing root auxin content have been shown to increase root activity and Ca^{2+} uptake, resulting in higher leaf and fruit Ca^{2+} accumulation (Steenkamp and de Villiers, 1979; Yang et al., 2004). Although cytokinins have been reported to promote cell differentiation in the roots (Chapman and Estelle, 2009), treating plants with cytokinins can also increase root Ca^{2+} uptake by enhancing root affinity for Ca^{2+} (Yang et al., 2008). Gibberellins (GAs) and ABA have been shown to inhibit cell division and growth by enhancing the transcription of cell cycle inhibitors (Wang et al., 1998; Achard et al., 2009). Accordingly, GAs have been suggested to reduce root Ca^{2+} uptake, decreasing Ca^{2+} accumulation in fruit, meristems, and leaves (Cohen and Greene, 1989; Monge et al., 1994; Saure, 2005). These studies show that root growth and activity are tightly regulated by several growth regulators (Ubeda-Tomás et al., 2012), affecting root Ca^{2+} uptake, plant Ca^{2+} content, and consequently, leaf and fruit susceptibility to Ca^{2+} deficiency disorders.

15.5.5.1.2 Growth Regulators and Xylem Vessel Development Since Ca^{2+} moves in the plant exclusively through the xylem vessels, higher numbers of functional xylem vessels can favor Ca^{2+} movement into leaves and fruit, potentially reducing the susceptibility of these organs to Ca^{2+} deficiency disorders (White and Broadley, 2003; Saure, 2005). Growth regulators are known to control vascular tissue differentiation (Aloni, 1987). Xylem vessel differentiation is triggered by basipetal auxin transport in the plant, leaf, and fruit (Bustan et al., 1995; Saure, 2005), which can be enhanced by the presence of cytokinins (Aloni, 2001). Accordingly, plants treated with the auxin transport inhibitor 2,3,5-triiodobenzoic acid have reduced Ca^{2+} uptake into sink tissues (Banuelos et al., 1987; Cutting and Bower, 1989). Other studies suggest that xylem differentiation is triggered by high auxin/GA ratios, whereas low ratios have been associated with the development of phloem (Aloni, 1987, 2001; Saure, 2005). Gibberellins are also known to trigger cell expansion, which has been suggested to result in the constriction of xylem vessels during stages of rapid fruit expansion, leading to reduced fruit Ca^{2+} uptake and increased fruit susceptibility to Ca^{2+} deficiency disorders (Dražeta et al., 2004a; Saure, 2005; Freitas et al., 2012a). Accordingly, treating tomato plants with GA has been shown to decrease, whereas treating plants with prohexadione-calcium, a GA biosynthesis

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inhibitor, has been shown to maintain the number of functional xylem vessels during fruit growth and development, which was highly correlated with fruit Ca^{2+} uptake and susceptibility to Ca^{2+} deficiency disorders (Freitas et al., 2012a). Treating tomato plants with ABA has also been shown to maintain higher numbers of functional xylem vessels and higher xylem/phloem ratios of water uptake into the fruit, which resulted in higher Ca^{2+} uptake and lower fruit susceptibility to Ca^{2+} deficiency disorders (Freitas et al., 2011b, 2014; Barickman et al., 2014).

15.5.5.1.3 Growth Regulators Controlling Transpiration and Growth Leaf transpiration is known to be regulated by stomatal opening in response to ABA content in the leaf (Li et al., 2000). Studies have shown that treating whole plants with ABA can specifically trigger leaf stomatal closure, decreasing whole-plant leaf transpiration without affecting fruit transpiration (Freitas et al., 2011b, 2014). Leaf stomatal closure decreased xylem sap and Ca^{2+} uptake into mature leaves and increased xylem sap and Ca^{2+} uptake into the fruit, reducing fruit susceptibility to Ca^{2+} deficiency disorders under Ca^{2+} stress conditions (Freitas et al., 2011b, 2014). Accordingly, conditions that favor high mature leaf transpiration, plant water loss, and plant water stress have been shown to reduce fruit xylem sap and Ca^{2+} uptake and increase fruit susceptibility to Ca^{2+} deficiency disorders (Abdal and Suleiman, 2005; Guichard et al., 2005; Freitas et al., 2011b, 2014; Barickman et al., 2014).

Fruit and leaf growth rates are determined by the combined processes of cell division and expansion, which are controlled by growth regulator homeostasis in the tissue (Gillaspy et al., 1993). Studies have suggested that plant organs are usually more susceptible to Ca^{2+} deficiency disorders when higher growth rates are combined with lower tissue Ca^{2+} uptake, which leads to dilution of tissue Ca^{2+} content (Taylor and Locascio, 2004; Ho and White, 2005; Saure, 2005). Accordingly, distal leaf and fruit tissues are believed to have higher susceptibility to Ca^{2+} deficiency disorders because of their higher growth rates and lowest total Ca^{2+} content (Saure, 1998, 2005; Barta and Tibbitts, 2000). Gibberellins trigger cell expansion (Gillaspy et al., 1993; Achard et al., 2009), which has been suggested to decrease Ca^{2+} uptake and dilute Ca^{2+} content in leaves and fruit, increasing tissue susceptibility to Ca^{2+} deficiency disorders (Saure, 1998, 2005). The inhibition of Ca^{2+} deficiency disorders by ABA has also been attributed to its antagonistic effect on GA responses that limit leaf and fruit Ca^{2+} uptake (Saure, 1998; Freitas et al., 2011b; Freitas and Mitcham, 2012).

Amarante et al. (2003) have shown that spraying apple trees at full bloom with increasing concentrations (up to 20 mg L^{-1}) of thidiazuron (TDZ; N-phenyl-N′-1,2,3-thiadiazol-5-ylureia), which has cytokinin-like activity, to promote fruit set, increases vegetative growth and consequently

reduces Ca^{2+} concentrations in the fruit. According to the authors, the intense promoting growth effect of TDZ on vegetative tissues (terminal buds and growing leaves) might increase the accumulation of nutrients into these organs, especially Ca^{2+}, to the detriment of growing fruit. On the other hand, Greene (1995) reported a higher BP incidence at harvest when apple trees were sprayed 18 days after full bloom with 15 mg L^{-1} TDZ, to promote fruit thinning. Therefore, the treatment with TDZ to reduce crop load might promote fruit growth and dilute fruit Ca^{2+} content, increasing the risk of BP (Greene et al., 1990; Elfving and Cline, 1993; Greene, 1993).

15.5.5.2 Growth Regulators Influencing Cellular Ca^{2+} Distribution

At the cellular level, growth regulators have been demonstrated to affect tissue susceptibility to Ca^{2+} deficiency disorders by regulating cellular Ca^{2+} partitioning and possibly by modulating cell responses to symptom development (Ho and White, 2005). Both auxins and GAs are known to trigger cell enlargement and tissue expansion (Saure, 1996, 2001, 2005; Perrot-Rechenmann, 2010). The combined effect of high cell enlargement rates and restricted tissue Ca^{2+} uptake triggered by auxin and GAs could result in excessive cell expansion, leading to increased membrane leakage and Ca^{2+} deficiency symptom development (Ho and White, 2005). Recently, studies have shown that spraying tomato plants with GAs or a GA biosynthesis inhibitor (prohexadione-calcium) resulted in 100% or 0% incidence of Ca^{2+} deficiency symptoms, respectively (Freitas et al., 2012a). More detailed analyses revealed that GA treatment increased the expression of genes coding for Ca^{2+} transport proteins that lead to Ca^{2+} movement into cellular storage organelles, reduced apoplastic water-soluble Ca^{2+}, and increased fruit tissue membrane leakage (Freitas et al., 2012a). These data suggest that GAs trigger abnormal cellular Ca^{2+} distribution that increases tissue susceptibility to Ca^{2+} deficiency disorders. ABA is believed to act as an antagonist of many GA responses (Saure, 2001). Indeed, studies have shown that ABA not only increases total fruit tissue Ca^{2+} uptake, but also may act as a GA antagonist, maintaining higher levels of apoplastic water-soluble Ca^{2+} and decreasing fruit susceptibility to Ca^{2+} deficiency disorders (Freitas et al., 2011b, 2014).

Ethylene is known to accelerate fruit ripening and senescence-related processes, and possibly accelerate the mechanisms leading to Ca^{2+} deficiency symptom development (Lötze and Theron, 2006; Lötze et al., 2010). Ethylene could accelerate Ca^{2+} deficiency disorders by increasing the expression and activity of PMEs, increasing Ca^{2+} binding to the cell wall and reducing Ca^{2+} availability in other pools in the cell (Freitas et al., 2010, 2013). Later, during fruit ripening and softening, increasing

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the activity of cell wall–degrading enzymes could release Ca^{2+} from the cell wall matrix back into other pools in the cell, reducing tissue susceptibility to Ca^{2+} deficiency disorders (Freitas et al., 2010). Accordingly, Ca^{2+} deficiency symptom development is usually restricted to a short period of time during apple development, which is highly correlated with an increase in PME expression and activity (Freitas et al., 2010). Previous studies have also shown that ethylene increases plasma membrane permeability (Candan et al., 2008), which could also enhance tissue susceptibility to Ca^{2+} deficiency disorders under conditions of low Ca^{2+}. Other growth regulators not mentioned here may also affect plant tissue susceptibility to Ca^{2+} deficiency disorders. However, the mechanisms involved remain to be explored and understood.

15.5.5.3 Growth Regulator Effect on Oxidative Metabolism

It has recently been suggested that GAs increase the susceptibility to Ca^{2+} deficiency disorders by downregulating ROS scavenging enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase (Kwak et al., 2006; Saure, 2014), and by stimulating the destruction of the growth-inhibiting DELLA proteins, which normally cause ROS levels to remain low under environmental stress (Achard et al., 2008; Saure, 2014). Since ABA is an antagonist to GAs, it has been suggested that ABA inhibition of GA-induced Ca^{2+} deficiency disorders can be due to its effect on increasing plant and fruit stress tolerance (Saure, 2014).

Growth regulators such as brassinosteroids have been reported to increase plant stress tolerance by increasing the activity of ROS scavenging enzymes (Schenabel et al., 2001; Liu et al., 2009). Although there are no studies showing the effect of brassinosteroids on Ca^{2+} deficiency disorders, the role of these growth regulators suggests a possible inhibition of fruit susceptibility to Ca^{2+} deficiency disorders by enhancing ROS scavenging (Freitas and Mitcham, 2012). Similarly, salicylic acid has also been shown to make plants more tolerant to high salinity conditions, reducing membrane permeability of leaf tissue (Stevens et al., 2006). Considering that salinity conditions are known to increase ROS levels in plants, salicylic acid, similar to brassinosteroids, can act on ROS scavenging and may have an inhibitory effect on Ca^{2+} deficiency disorders. Other studies have shown that high levels of methyl jasmonate increase fruit susceptibility to Ca^{2+} deficiency disorders (Rudell et al., 2005) and fruit phenol content (Rudell et al., 2002). Since Ca^{2+} deficiency symptoms involve phenol oxidation (Dekock et al., 1980; Casado-Vela et al., 2005), methyl jasmonate may increase fruit susceptibility to Ca^{2+} deficiency disorders by increasing phenol content that favors phenol oxidation in fruit tissue (Freitas and Mitcham, 2012).

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15.6 Possible Control Strategies

The information presented in this review demonstrates that fruit and leafy vegetable susceptibility to Ca^{2+} deficiency disorders is determined by genetic and environmental factors affecting tissue Ca^{2+} content and cellular Ca^{2+} distribution. Therefore, prior to developing control strategies, one should first identify the genetic and environmental factors leading to limited tissue Ca^{2+} content or abnormal cellular Ca^{2+} distribution in order to develop specific strategies that can effectively control Ca^{2+} deficiency disorders.

15.6.1 At the Tissue Level

Adequate soil Ca^{2+} availability and root Ca^{2+} uptake are usually the first strategy to ensure Ca^{2+} accumulation in leaf and fruit tissue. Selection of genotypes and rootstocks with higher root Ca^{2+} uptake activity can potentially reduce susceptibility of new crop plants to Ca^{2+} deficiency disorders. Adequate use of other nutrients can avoid root uptake competition with Ca^{2+} or excessive vegetative growth that leads to higher Ca^{2+} movement into mature leaves and away from low-transpiring young leaves and fruit. Calcium uptake and movement into low-transpiring young leaves and fruit is negatively correlated to plant water stress. Therefore, adequate plant watering can also facilitate root Ca^{2+} uptake and movement into low-transpiring young leaves and fruit.

Although Ca^{2+} is believed to move in the plant exclusively through the xylem vessels, the development of technologies that allow Ca^{2+} movement through the phloem could allow Ca^{2+} movement from older leaves into low-transpiring young leaves and fruit, which receive most of their water from the phloem. One example of such a technology was developed for boron (B), which is also considered to be phloem immobile or to have only limited phloem mobility in higher plants (Brown and Hu, 1996). Studies revealed that B is mobile in the phloem of some plant species when it forms stable complexes with sorbitol (Brown and Hu, 1996). Based on these studies, tobacco plants were engineered to synthesize high amounts of sorbitol, which resulted in a marked increase in B mobility in the plant and increased plant growth and yield when grown with limited or interrupted soil B supply, compared to wild-type plants (Brown et al., 1999). Analyses revealed that transgenic plants were able to remobilize B present in mature leaves through the phloem into sink organs, which was not observed in wild-type plants (Brown et al., 1999). A similar approach might also be developed for Ca^{2+} mobility in the phloem, decreasing plant susceptibility to Ca^{2+} deficiency disorders.

Plant treatments with growth regulators, such as ABA and GA biosynthesis inhibitors, have demonstrated their ability to maintain higher

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numbers of functional xylem vessels during fruit growth and development, and can be used commercially to favor fruit Ca^{2+} uptake and decrease fruit susceptibility to Ca^{2+} deficiency disorders. In addition, selection of new fruit and leafy vegetable genotypes that maintain high numbers of functional xylem vessels during growth and development can also potentially favor higher Ca^{2+} accumulation and reduce fruit and young leaf susceptibility to Ca^{2+} deficiency disorders. Besides the higher number of functional xylem vessels, the hydrostatic gradient is also required for xylem sap and Ca^{2+} movement in the fruit. In that case, techniques that favor fruit transpiration, such as reduced wax deposition and shoot and mature leaf pruning, as well as techniques that reduce mature leaf transpiration, such as reductions in WVPD, can favor fruit Ca^{2+} uptake and reduce fruit susceptibility to Ca^{2+} deficiency disorders.

15.6.2 At the Cellular Level

Selection of new crop genotypes with lower Ca^{2+} precipitation inside storage organelles or lower Ca^{2+} binding to the cell wall has been suggested to reduce fruit susceptibility to Ca^{2+} deficiency disorders. Breeding programs could use marker-assisted selection of new genotypes that require lower amounts of Ca^{2+} for proper tissue growth and development. In addition, treating plants with growth regulators, such as ABA and GA biosynthesis inhibitors, has been suggested to favor an adequate cellular Ca^{2+} distribution that could be used commercially to reduce fruit susceptibility to Ca^{2+} deficiency disorders.

15.7 Final Considerations and Future Research Needs

More than a hundred years of study on Ca^{2+} deficiency disorders has revealed the complexity of the mechanisms involved. It is reasonable to consider that many additional mechanisms remain undiscovered. For example, although the effects of individual growth regulators have been tested on plant susceptibility to Ca^{2+} deficiency disorders, future research should focus on growth regulator homeostasis and on the network of responses determining fruit susceptibility to Ca^{2+} deficiency disorders.

Although recent studies have shown the effect of xylem and the xylem/phloem ratio on Ca^{2+} uptake into low-transpiring sink organs, more detailed studies are required to better understand the mechanisms regulating Ca^{2+} movement in the plant. These studies may help the development of technologies that increase the efficiency of Ca^{2+} movement in plants and into low-transpiring young leaves and fruit, which can be used to reduce crop plant's susceptibility to Ca^{2+} deficiency disorders.

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The regulation of cellular Ca^{2+} distribution has been recently suggested to be the final step in the control of tissue susceptibility to Ca^{2+} deficiency disorders. Therefore, better understanding of the mechanisms regulating cellular Ca^{2+} distribution can potentially reveal powerful approaches to control plant tissue susceptibility to Ca^{2+} deficiency disorders. In addition, future studies should also better characterize the combined effects of Ca^{2+}/ROS ratios in different cellular compartments on Ca^{2+} deficiency disorders.

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