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Factors Involved in Fruit Calcium Deficiency Disorders

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ABSTRACT

Understanding the mechanisms of calcium (Ca^{2+}) deficiency disorder development in plants has been a challenge for more than a 100 years. Previous studies support the hypothesis that Ca^{2+} deficiency disorders 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. Plant Ca^{2+} uptake can be determined by Ca^{2+} content and availability in the soil, root growth, activity of apoplastic and symplastic pathways of root Ca^{2+} uptake, as well as uptake competition between Ca^{2+} and other nutrients. Fruit Ca^{2+} uptake is determined by Ca^{2+} content in the xylem sap, and xylem/phloem ratio of fruit sap uptake, which is affected by the rates of leaf and fruit transpiration and growth. Calcium translocation to distal fruit tissue, containing the lowest fruit Ca^{2+} content and the highest susceptibility to Ca^{2+} deficiency disorders, is potentially dependent on the cell wall Ca^{2+}-binding capacity and symplastic Ca^{2+} uptake by the tissue at the peduncle end of the fruit, abundance of functional xylem vessels connecting peduncle and distal fruit tissues, as well as the hydrostatic gradient required for Ca^{2+} translocation towards the distal tissue. Cellular Ca^{2+} partitioning is defined by the activity of Ca^{2+} channels, Ca^{2+} ATPases, and Ca^{2+} exchangers present in cellular membranes, as well as the capacity of the cell wall to bind Ca^{2+}, and the formation of Ca^{2+} precipitates in different cellular compartments. Therefore,

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Ca^{2+} deficiency disorders in fruit may not be caused by a single factor, but most likely by a combination of mechanisms that lower Ca^{2+} concentration at a specific tissue and cell localization, leading to Ca^{2+} deficiency symptoms.

KEYWORDS: bitter pit (BP); blossom-end rot; calcium partitioning; calcium translocation; calcium uptake.

I. INTRODUCTION

Calcium deficiency disorders in plants have puzzled researchers for more than a 100 years, and still, little is known about the mechanisms involved (Ferguson and Watkins 1989; Saure 2001; Taylor and Locascio 2004). Although many years of research have shown that Ca^{2+} deficiency disorders in fruit, such as bitter pit (BP) in apple and blossom-end rot (BER) in tomato, pepper, and watermelon, can be induced by low fruit Ca^{2+} concentration, more recent evidence suggests that total fruit tissue Ca^{2+} content may not be the only cause of Ca^{2+} deficiency disorder development. Often, fruit with Ca^{2+} deficiency symptoms have equal or higher Ca^{2+} concentrations than sound fruit (Castro 1980; Nonami et al. 1995; Saure 1996, 2001; Ho and White 2005). In addition, no threshold fruit Ca^{2+} concentration has been determined to precisely predict Ca^{2+} deficiency disorder development, and no commercial treatment in use today is one hundred percent effective in controlling the development of these physiological disorders (Saure 1996, 2001, 2005; Ho and White 2005). New evidence suggests that Ca^{2+} deficiency disorders may also be triggered by abnormal cellular Ca^{2+} partitioning and distribution that leads to a cellular localized Ca^{2+} deficiency (Park et al. 2005; De Freitas et al. 2010, 2011a). It has also been proposed that Ca^{2+} deficiency disorders in different fruit species are triggered by conserved mechanisms (Saure 1996, 2001, 2005; Ho and White 2005). Here, potential mechanisms involved in Ca^{2+} deficiency disorders in fruit are discussed at the whole-plant, fruit, and cellular level.

II. PHYSIOLOGY OF CALCIUM DEFICIENCY IN FRUIT TISSUE

A. Visual Symptoms

At the fruit tissue level, Ca^{2+} deficiency symptoms begin with water-soaked tissue, followed by tissue disintegration and dehydration, which eventually appears as dark-brown and depressed lesions on the fruit surface (Simon 1978; Fuller 1980). BER affects the blossom-end tissue of the fruit and, in severe cases, may also spread to the entire fruit surface (Ho and White 2005). The BP symptom is a discrete pitting of the outer cortical flesh of the fruit, frequently just under the skin, such that collapse of the outermost cells causes small depressions in the skin. Pitting of the flesh is not always visible from the outside, and may occur deep into the flesh. The frequency of pitting is often greater towards the calyx end of the fruit (Ferguson and Watkins 1989).

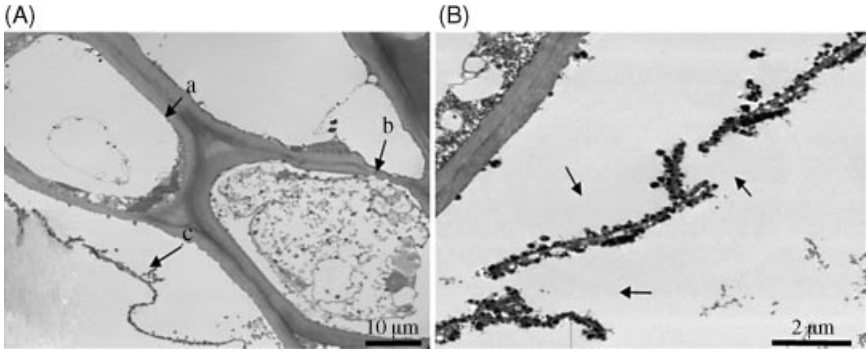

Fig. 3.1. Bitter pit (BP) symptom development in pitted apple fruit tissue (A and B). Arrows indicate healthy (a), plasmolyzed (b), and senescing (c) cells (A). Arrows indicate membrane disintegration during BP development (B). (De Freitas et al. 2010).

B. Ultrastructure

At the cellular level, Ca^{2+} deficiency symptoms start by leaky membranes that lead to cell plasmolysis, and membrane breakdown (Smock and Van Doren 1937; MacArthur 1940; Saure 1996; Ho and White 2005; De Freitas et al. 2010) (Fig. 3.1). BP cavities result from the collapse of several cells, and are bound by the remains of the original cell walls (Smock and Van Doren 1937; MacArthur 1940). Studies in tomato have shown that cells in the water-soaked area have disruption of the plasma membrane and tonoplast, wavy-shaped cell walls, broken down endoplasmic reticulum, and swollen plastids. In these studies, cells around necrotic BER tissue had normal internal structure, but the plasma membrane was detached from the cell wall, suggesting the occurrence of cell plasmolysis, which was not observed in other parts of fruit or in the cells of normal fruit (Suzuki et al. 2000).

C. Mineral and Biochemical Changes

Chemical and x-ray analysis have shown that apple fruit tissue with visual Ca^{2+} deficiency symptoms has higher Ca^{2+} concentration than healthy fruit tissue (Askew et al. 1960; Meyer et al. 1979; Chamel and Bossy 1981; Val et al. 2008). In accord with these results, Ford (1979) showed that ^{45}Ca fed to apple tree roots moved into pitted tissue in the fruit after the tissue began to disintegrate. In tomato fruit, it has been shown that during the stage of rapid fruit expansion, there is an increasing amount of Ca^{2+} bound to the plasma membrane in healthy fruit and a decreasing amount of Ca^{2+} bound to the plasma membrane of

plasmolyzed cells in fruit tissue with visual BER symptoms (Suzuki et al. 2003). In addition, the amount of Ca^{2+} bound to the plasma membrane increases as the distance from collapsed cells in the BER damaged tissue increased (Suzuki et al. 2003). The BP spots in apple fruit have been shown to contain high starch content, which has been suggested to be the result of cell death and loss of capacity for starch hydrolysis (Smock 1936). In addition, pitted tissue has been shown to have high concentrations of citric acid, reduced levels of oleic acid, and increased levels of linoleic acid (Ferguson and Watkins 1989). Leaves of tomato plants grown under low Ca^{2+} conditions showed lower levels of tocopherol and reduced superoxide dismutase activity, as well as higher content of malondialdehyde, which is a degradation product of lipid peroxidation (Schmitz-Eiberger et al. 2002). Other studies have shown an increase in proteins participating in antioxidant processes (ascorbate–glutathione cycle) and the pentose phosphate pathway in fruit with BER symptoms, suggesting that these two biochemical pathways may be acting as reactive oxygen species scavengers in BER-affected fruit to restrain the spread of the blackening to the whole fruit (Casado-Vela et al. 2005).

III. CALCIUM TRANSLOCATION AT THE WHOLE PLANT LEVEL

Calcium uptake from the soil and translocation to different organs in the plant, including the fruit, is controlled by different factors along the soil–root–fruit pathway. The factors controlling Ca^{2+} uptake and translocation to the fruit include Ca^{2+} content and availability in the soil, root growth, root Ca^{2+} uptake, Ca^{2+} competition with other nutrients in the root, as well as leaf and fruit competition for Ca^{2+} available in the xylem sap (Fig. 3.2).

A. Calcium in the Soil

Calcium in the soil is present structurally bound to soil particles, loosely bound to negative charges in soil particles (cation exchange capacity), and soluble in the soil solution (McLaughlin and Wimmer 1999). Calcium soluble in the soil solution is the only form of Ca^{2+} available to plants, and is dependent on changes in the pool of bound Ca^{2+}, addition of Ca^{2+} fertilizers, and root Ca^{2+} uptake (McLaughlin and Wimmer 1999; Taylor and Locascio 2004). The movement of Ca^{2+} from the bound fractions into the soluble fraction is mostly affected by soil acidity. Increasing soil acidity is associated with both increased mineral weathering and increased availability of Ca^{2+} within the soil solution (McLaughlin

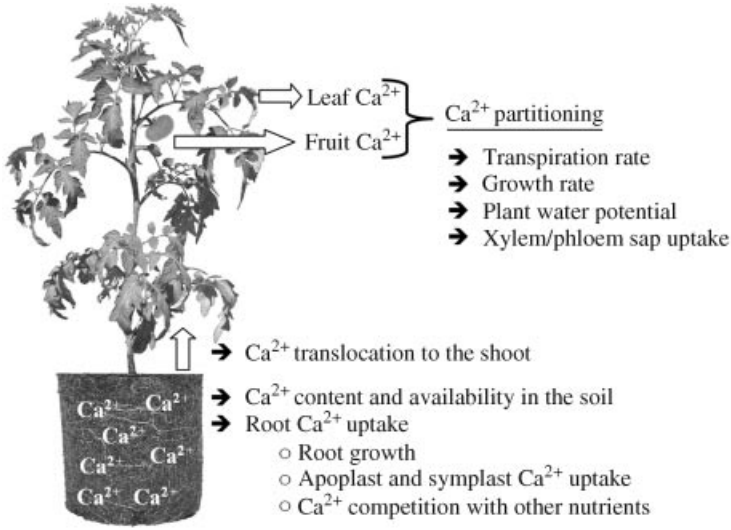

Fig. 3.2. Mechanisms involved in Ca^{2+} uptake, translocation, and partitioning in plants.

and Wimmer 1999). In a short-term period, low soil pH (<5.0) results in Ca^{2+} replacement on the cation-exchange sites by H^+ or other acidic cations and Ca^{2+} released into the soil solution, from which it may be absorbed by roots or lost by leaching (McLaughlin and Wimmer 1999; Taylor and Locascio 2004). As soil pH drops below 4.5, stores of Ca^{2+}-oxalate can become rapidly dissolved (McLaughlin and Wimmer 1999). In the long-term period, high acidity enhances mineral weathering, increasing Ca^{2+} concentration in the soil solution (Taylor and Locascio 2004). Although decreasing pH increases Ca^{2+} release into the soil solution, high levels of H^+ and other acidic cations have been shown to inhibit root Ca^{2+} uptake (McLaughlin and Wimmer 1999). The dynamic of Ca^{2+} changes from bound to soluble forms in the soil, Ca^{2+} leaching, and the rate of root Ca^{2+} uptake will determine the balance of Ca^{2+} available in the soil solution that can be used to nourish the plant and its organs during growth and development, and which will determine the probability of Ca^{2+} deficiency disorders developing in the plant.

B. Root Calcium Uptake

Calcium uptake in the root is to a large extent genetically controlled and is relatively minorly affected by Ca^{2+} supply to the root medium, provided that Ca^{2+} availability is adequate for normal growth (Kirkby and Pilbeam 1984). Genetically controlled factors such as the rate of root

growth and the activity of apoplastic and symplastic pathways of root Ca^{2+} uptake can potentially affect Ca^{2+} loading into the root xylem vessels and subsequent translocation to other parts of the plant.

Plants take up Ca^{2+} from the soil solution that is in contact with the root surface, which is determined by nutrient interception during root growth, mass flow, and diffusion of nutrients towards the root surface. Root interception has a much lower contribution in nutrient uptake than diffusion and mass flow processes (McLaughlin and Wimmer 1999). Calcium is transported in the soil towards the roots mainly through mass flow with water, which make plant water uptake from the soil, driven by transpiration and growth, an important process involved in root Ca^{2+} uptake (Ho et al. 1993). Higher rates of root growth can also increase plant Ca^{2+} uptake and reduce fruit susceptibility to Ca^{2+} deficiency disorders (Choi et al. 1997). However, at high root densities, the uptake rate of nutrients levels off, possibly caused by overlapping of the depletion zones of individual roots, resulting in interroot competition for nutrients (Fusseder et al. 1988).

From the root surface, Ca^{2+} can move towards the root endodermal cells through apoplastic or symplastic pathways. The apoplastic pathway is comprised of cell wall and intercellular spaces through which Ca^{2+} moves passively with water in response to the water potential gradient present across root cortical tissue (White 2001; Karley and White 2009). In the symplastic pathway, Ca^{2+} is taken up by epidermal cells and moves symplastically from cell to cell through plasmodesmata across root cortical tissue until it is actively loaded into the root xylem vessels (White 2001; Karley and White 2009). Since cytosolic Ca^{2+} is highly regulated and maintained at low concentrations (0.1–0.2 μM), it is believed that the root apoplastic Ca^{2+} represents the most important pathway for root Ca^{2+} uptake (White and Broadley 2003; Taylor and Locascio 2004; Karley and White 2009). In the apoplast, Ca^{2+} movement in the plant is not linear with the mass flow of water, but depends on adsorption and desorption of Ca^{2+} from active exchange sites within the cell walls (McLaughlin and Wimmer 1999). In that case, high cation exchange capacity of the cell wall matrix can potentially reduce apoplastic conductivity for Ca^{2+} ions in root cortical tissue. Root morphology varies along the main root axes and the presence of suberized endodermal cells, known as the casparian band, limits apoplastic movement of water and Ca^{2+} ions into the root vascular tissue (White 2001; Taylor and Locascio 2004; Karley and White 2009). For that reason, most of root Ca^{2+} uptake takes place through the root tip, and regions of secondary root growth, where differentiated endodermal cells have not been formed or where differentiated endodermal cell

have been broken by the growing secondary roots, respectively (White 2001; Taylor and Locascio 2004; Karley and White 2009).

Root Ca^{2+} uptake is dependent not only on Ca^{2+} availability in the soil, but also Ca^{2+} interaction with other nutrients during its movement from the soil into the plant. Although the mechanisms are still poorly understood, increasing the amount of cationic ions such as aluminum (Al^{3+}), manganese (Mn^{2+}), potassium (K+), sodium (Na^{+}), ammonium (NH_4^+), and magnesium (Mg^{2+}) in the soil solution have been reported to decrease (Bangerth 1979; Horst 1987; Taylor and Locascio 2004), whereas anionic ions such as nitrate and phosphate have been shown to increase plant Ca^{2+} uptake (Bangerth 1979). It has also been proposed that as the concentration of H^{+} increases in the soil, the rate of root uptake of Ca^{2+} is inhibited by impairment of net extrusion of H^{+} by plasma membrane ATPases, and by decreasing the loading of Ca^{2+} in the apoplast of root cortical cells (Schubert et al. 1990). Studies are required to better understand the complex nutrient interactions at the root uptake level, which will help in developing new approaches to improve plant Ca^{2+} uptake, and selecting crop plants more efficient in Ca^{2+} uptake and less susceptible to Ca^{2+} deficiency disorders.

C. Calcium Translocation to the Shoot

After having been apoplastically or simplistically loaded into the xylem vessels in the root, Ca^{2+} is translocated to the shoot with the mass flow of water in response to the more negative water potentials developed in leaves and fruit triggered by transpiration and growth (Ho 1989; Ho et al. 1993; De Freitas et al. 2011b). Xylem vessels form a very sophisticated plumbing system responsible for translocation of water and ions to sink organs in the plant. Although not well understood, evidence suggests that special transport systems exist at the interface between living cells and xylem vessels which allow intensive fluxes of ions and water into and out of the xylem (De Boer and Volkov 2003). Similar to the dynamics of Ca^{2+} movement in the root apoplast, Ca^{2+} movement in the xylem vessels from the root toward leaves or fruit is also dependent on the cation exchange capacity present in xylem cell walls along the translocation pathway (Taylor and Locascio 2004).

D. Calcium Partitioning Between Leaves and Fruit

Considering that Ca^{2+} is a signaling molecule in the cytosol, any living cells must maintain extremely low levels of free cytosolic Ca^{2+} to

avoid toxicity and cell death (White and Broadley 2003). For this reason, Ca^{2+} concentration inside living phloem cells is extremely low, which makes the phloem vessels incapable of contributing to growing leaves and fruit Ca^{2+} requirements (Taylor and Locascio 2004; Ho and White 2005). Indeed, it has been shown that Ca^{2+} movement in the plant toward leaves and fruit takes place exclusively through the xylem vessels, which are composed of dead cells at maturity (Ho et al. 1993; Taylor and Locascio 2004). In the xylem elements, leaf and fruit Ca^{2+} uptake is a passive phenomenon driven by transpiration and growth (Ferguson and Watkins 1989; Taylor and Locascio 2004; Ho and White 2005). The partitioning of Ca^{2+} flowing from the root toward leaves and fruit will then depend on Ca^{2+} content in the xylem sap (determined by the root Ca^{2+} uptake and cation-exchange capacity of xylem vessels), and rates of leaf and fruit transpiration and growth.

Transpiration rates and mass accumulation per plant are much higher in leaves than in fruit (Ho and White 2005; De Freitas et al. 2011b). Water uptake in mature leaves come exclusively from xylem vessels, while phloem flow is directed from leaves toward sink organs such as fruit (Ho and White 2005). In the fruit, phloem represents 76–83% and xylem represents 17–24% of peduncle water uptake at early stages of growth and development (De Freitas et al. 2011b). At later stages, the xylem contribution to fruit water uptake decreases due to loss of xylem functionality and reduction of the water potential gradient that leads to xylemic water uptake (Ho et al. 1993; Drazeta et al. 2004; Bondada et al. 2005; De Freitas et al. 2011b). Accordingly, previous studies showed that reducing leaf transpiration by decreasing atmospheric vapor pressure deficit or triggering stomatal closure can increase plant water potential which reduces the tension in the xylemic water column connecting leaves to fruit. This condition favors a higher xylem/phloem ratio of fruit sap uptake (De Freitas et al. 2011b), which increases fruit Ca^{2+} content and reduces fruit susceptibility to Ca^{2+} deficiency disorders (Ho 1989; Paiva et al. 1998; Araki et al. 2000; Bertin et al. 2000; Tadesse et al. 2001; Taylor and Locascio 2004; Guichard et al. 2005; Sharma et al. 2006; De Freitas et al. 2011b). Although the xylem/phloem ratio of fruit water uptake can be manipulated to increase fruit Ca^{2+} uptake, the sum of xylemic water required for transpiration and growth is much higher in leaves than in fruit, which explain the much higher Ca^{2+} concentration frequently observed in the leaves compared to the fruit (Ho 1989; De Freitas et al. 2011b).

IV. CALCIUM AT THE FRUIT LEVEL

The mechanisms involved in plant Ca^{2+} uptake and translocation to the fruit represent the first levels of factors that could affect total fruit Ca^{2+} concentration and fruit susceptibility to Ca^{2+} deficiency disorders. At the fruit level, Ca^{2+} movement from the peduncle towards the distal fruit tissue is also affected by different mechanisms, which define Ca^{2+} concentration in the distal-end tissue and fruit susceptibility to Ca^{2+} deficiency disorders (Fig. 3.3). In the fruit, total tissue Ca^{2+} concentration decreases from the peduncle towards to the distal end tissue (Lewis and Martin 1973; Nonami et al. 1995). Accordingly, distal fruit tissue is more susceptible to Ca^{2+} deficiency disorders than fruit tissue at the peduncle region and Ca^{2+} deficiency symptoms usually begin in the distal tissue, eventually spreading to the whole fruit in severe cases (White and Broadley 2003; Ho and White 2005). The reason for such fruit Ca^{2+} distribution is not well understood, but different mechanisms can potentially be involved such as cell wall Ca^{2+}-binding capacity and symplastic Ca^{2+} uptake in the peduncle end tissue, abundance of functional xylem vessels from peduncle to distal fruit tissue, as well as the driving force required for Ca^{2+} translocation from peduncle to distal fruit tissue (hydrostatic gradient) (Fig. 3.3).

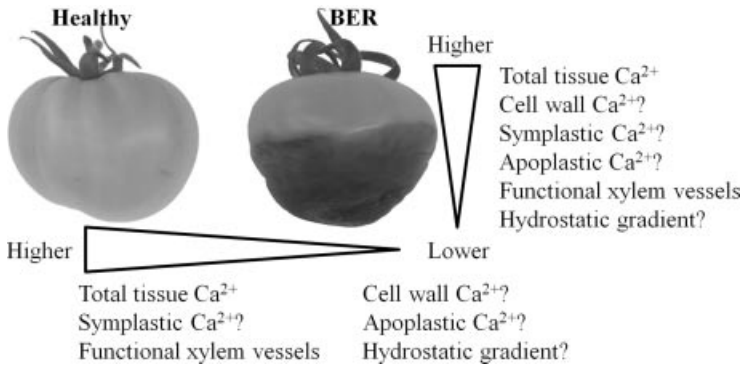

Fig. 3.3. Mechanisms involved in the regulation of fruit Ca^{2+} partitioning. Tomato fruit without (healthy) and with visible Ca^{2+} deficiency symptoms known as blossom-end rot (BER). Question tag "?" means that there is no research information available for that factor.

A. Cell Wall and Symplastic Calcium

Calcium ion is highly attracted by negatively charged particles (White and Broadley 2003). Part of the Ca^{2+} reaching the fruit through the xylem vessels will bind to negatively charged particles present in the cell wall of the xylem vessels and apoplastic environment in the peduncle end tissue of the fruit (Ralet et al. 2001; Herbette and Cochard 2010). Previous studies have shown that the cell wall represents about 60–75% of the total fruit tissue Ca^{2+} content (Demarty et al. 1984; De Freitas et al. 2010). Another fraction of the Ca^{2+} reaching the fruit through the xylem vessels will be taken up into the symplast and will be stored in cellular organelles such as the vacuole, which occupies about 90% of the cell's volume and contains 1–10 mM Ca^{2+} (Bush 1995; White and Broadley 2003). Calcium binding to the cell wall and/or taken up into cellular storage organelles at the peduncle end tissue of the fruit can potentially lower the water-soluble apoplastic Ca^{2+} (Conn et al. 2011; De Freitas et al. 2011a,b) and xylemic Ca^{2+} concentration at the peduncle end tissue of the fruit. Lowering xylemic Ca^{2+} concentration in the peduncle end tissue can potentially limit the amount of Ca^{2+} translocated to the distal end tissue of the fruit, which can increase fruit susceptibility to Ca^{2+} deficiency disorders.

B. Functional Xylem Vessels

The abundance of functional xylem vessels in the fruit has been shown to be higher in the peduncle end tissue, and lower in the distal end tissue. In addition, the number of functional xylems vessels has been shown to decrease in both peduncle and distal end tissues during fruit growth and development (Ho et al. 1993; Drazeta et al. 2004; Ho and White 2005; De Freitas et al. 2011b). Similarly, Ca^{2+} concentration is higher at the peduncle end and decreases towards the distal end of the fruit. Calcium concentration also decreases in both peduncle and distal end tissues during fruit growth and development (Ho et al. 1993; Drazeta et al. 2004; Ho and White 2005; De Freitas et al. 2011b). The abundance and distribution of functional xylem vessels are then well correlated with the Ca^{2+} concentration in the peduncle end and distal end tissues during fruit growth and development, suggesting the involvement of xylem vessels in defining fruit susceptibility to Ca^{2+} deficiency disorders (Ho et al. 1993; Drazeta et al. 2004; Ho and White 2005; Peet 2009; De Freitas et al. 2011b).

C. Hydrostatic Gradient Required for Calcium Translocation in the Fruit

Previous studies have shown that a high abundance of functional xylem vessels in the fruit may not necessarily result in high xylemic water and potential Ca^{2+} movement into the distal end of the fruit if the hydrostatic (tension) gradient between the peduncle and distal fruit tissue is not present (Bondada et al. 2005). The authors suggest that significant changes in the pattern of solute partitioning between the fruit symplast and the apoplast may affect the hydrostatic gradient between peduncle and distal fruit tissue, which reduces the translocation of xylemic water into the distal tissue. They also suggested that diurnal patterns in symplast/apoplast solute partitioning in fleshy fruit may explain the low xylemic contribution to the water budgets of fruit.

The mechanisms involved in Ca^{2+} translocation in the fruit acting together or individually can limit Ca^{2+} translocation towards the distal end of the fruit, which in combination with the higher rates of cell expansion (Cheniclet et al. 2005) can potentially result in lower Ca^{2+} concentration in the tissue and increased fruit susceptibility to Ca^{2+} deficiency disorders. Previous studies have already shown that the abundance of functional xylem vessels is well correlated with Ca^{2+} concentration at the distal end tissue and fruit susceptibility to Ca^{2+} deficiency disorders (Ho et al. 1993; Ho and White 2005; De Freitas et al. 2011b). However, future studies are required to better understand the role of cell-wall binding of Ca^{2+}, symplastic Ca^{2+} uptake, and the role of the hydrostatic gradient between the peduncle and distal end tissue on Ca^{2+} translocation to the distal end tissue and fruit susceptibility to Ca^{2+} deficiency disorders.

V. CALCIUM AT THE CELLULAR LEVEL

The total fruit tissue Ca^{2+} content can influence the Ca^{2+} concentration in different pools in the cell and, consequently, fruit susceptibility to Ca^{2+} deficiency disorders. However, the exact Ca^{2+} concentration in each cellular pool of Ca^{2+} is highly regulated by mechanisms that control cellular Ca^{2+} partitioning (Fig. 3.4). These mechanisms controlling cellular Ca^{2+} partitioning have recently been shown to be the final level of regulation of fruit susceptibility to Ca^{2+} deficiency disorders (Park et al. 2005; De Freitas et al. 2011a). In these studies, Ca^{2+} deficiency disorders were triggered in fruit tissue with high Ca^{2+} content by specifically modifying cellular Ca^{2+} partitioning (Park et al. 2005; De Freitas et al.

2011a). The regulation of cellular Ca^{2+} partitioning is determined by cell wall Ca^{2+}-binding capacity, activity of Ca^{2+} channels, Ca^{2+} ATPases, and Ca^{2+} exchangers present in cellular membranes, as well as the formation of Ca^{2+} precipitates in the cell that immobilizes Ca^{2+} in specific cellular compartments (Fig. 3.4).

A. Apoplastic Calcium

The apoplastic Ca^{2+} is represented by the water-soluble Ca^{2+}, as well as plasma membrane and cell wall bound Ca^{2+} (Ho and White 2005). The level of apoplastic, water-soluble Ca^{2+} is dependent on Ca^{2+} movement across the plasma membrane, as well as plasma membrane and cell wall Ca^{2+}-binding capacity (Fig. 3.4). Calcium movement across the plasma membrane is controlled by the activity of Ca^{2+} channels that allow Ca^{2+} flow from the apoplast into the cytosol, as well as Ca^{2+} exchangers and Ca^{2+} ATPases that move Ca^{2+} ions from the cytosol into the apoplast (White and Broadley 2003). In the apoplast, soluble Ca^{2+} is known to bind to phosphate and carboxylate groups of phospholipids and proteins at the membrane surface, which is required for proper plasma membrane structure and function (Hanson 1960; Clarkson and Hanson 1980; Legge et al. 1982; Kirkby and Pilbeam 1984; Picchioni et al. 1998; Hirschi 2004). Previous studies have shown that apoplastic

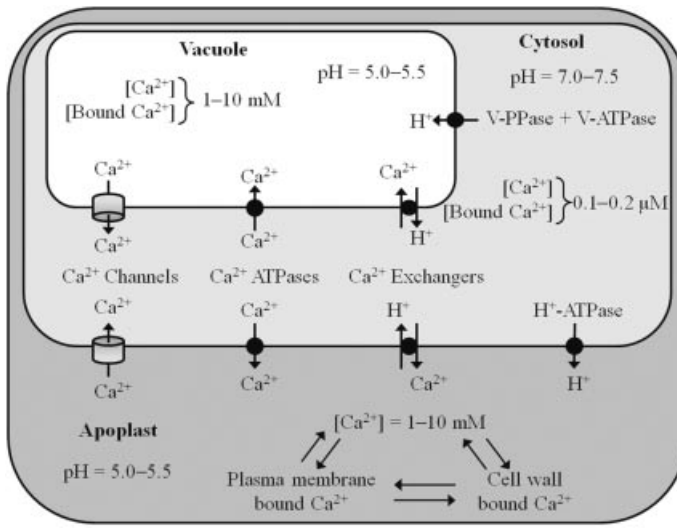

Fig. 3.4. Mechanisms involved in the regulation of cellular Ca^{2+} partitioning.

levels of water-soluble Ca^{2+} must be maintained at certain thresholds to avoid excessive membrane leakiness and damage (Hanson 1960; Kirkby and Pilbeam 1984; Picchioni et al. 1998). Accordingly, immersing protoplasts in solutions containing EDTA resulted in leaky plasma membranes, and solutes were lost from the cytoplasm to the apoplast (Steveninck 1965). These changes could be completely and immediately reversed through the addition of Ca^{2+} to these solutions. In addition, Ca^{2+} replacement from its binding sites on the plasma membrane by other ions have also been suggested to result in plasma membrane damage (Wallace et al. 1966; Lund 1970). Although the plasma membrane binds Ca^{2+} to maintain proper structure and function, it's actual capacity to bind Ca^{2+} is not well understood and can potentially have a small effect on the total water-soluble Ca^{2+} present in the apoplast.

The cell wall contains most of the total fruit tissue Ca^{2+} (Demarty et al. 1984; De Freitas et al. 2010) which makes it an important regulator of cellular Ca^{2+} partitioning. In the cell wall, Ca^{2+} can form two kinds of noncovalent bonds within the pectin matrix. One noncovalent bond, known as a coordination bond, is formed when several oxygen or nitrogen atoms of a carbon compound donate unshared electrons to form a bond with Ca^{2+} ions. As a result, the positive charge on the Ca^{2+} is neutralized (McLaughlin and Wimmer 1999). Another noncovalent bond, known as an electrostatic bond, is formed because of the attraction between Ca^{2+} and negatively charged groups such as carboxylate ($–COO^-$) groups present on polygalacturonic acid chains (Caffall and Mohnen 2009). Unlike the situation in coordination bonds, Ca^{2+} ions in an electrostatic bond retain their positive charge (Fig. 3.5) (Caffall and Mohnen 2009). Pectic polysaccharides, such as polygalacturonic acid, are synthesized and secreted to the apoplast in a highly methylesterified form, which is then deesterified by pectin methylesterases (PMEs), creating the carboxylate groups that strongly bind Ca^{2+} (Fig. 3.5) (Ralet et al. 2001; Micheli 2001; Caffall and Mohnen 2009). Deesterified pectin chains in parallel orientation can be bridged by Ca^{2+}, forming a structure with high affinity for this ion known as an "egg-box" structure (Fig. 3.5) (Ralet et al. 2001; Caffall and Mohnen 2009). Future studies should further elucidate the effect of mechanisms controlling Ca^{2+} movement across the plasma membrane, as well as Ca^{2+} binding to the cell wall on fruit tissue susceptibility to Ca^{2+} deficiency disorders.

B. Organellar Calcium

Many cellular organelles are storage compartments for Ca^{2+} ions. The vacuole represents the biggest pool of Ca^{2+} in the cell, as it often

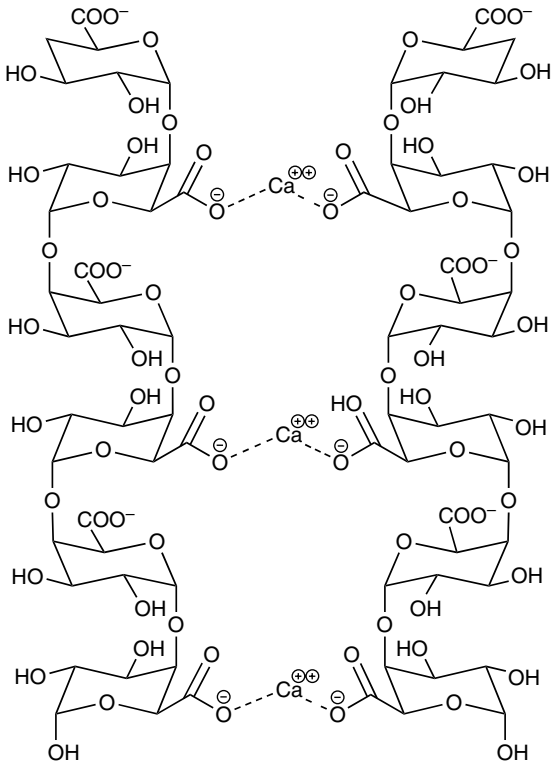

Fig. 3.5. The egg-box model of Ca^{2+} cross-linking in the pectic homogalacturonan (HG) polysaccharide. (Caffall and Mohnen 2009).

accounts for 90–95% of the cell's volume and contains 1–10 mM Ca^{2+} (Bush 1995; White and Broadley 2003). The endoplasmic reticulum has been reported to have 1–5 mM Ca^{2+} (Kendall et al. 1992). The chloroplast and mitochondria may also act as Ca^{2+} stores, with Ca^{2+} concentrations between 0.1 to 10 μM and 0.2 to 1.2 μM, respectively (Johnson et al. 1995; Logan and Knight 2003). In the nucleus, steady-state Ca^{2+} concentrations have been shown to range from 0.1 to 0.2 μM, and nuclear Ca^{2+} concentrations as high as 1.6 μM have been reported during signaling responses (Pauly et al. 2001; Reddy et al. 2011).

The Ca^{2+} content inside cellular organelles is determined by the activity of Ca^{2+} channels that reduce, and Ca^{2+} exchangers and Ca^{2+} ATPases that increase the concentration of this ion in these organelles (White and Broadley 2003; Clapham 2007). The dynamics of Ca^{2+} movement into cellular organelles by the combined activity of Ca^{2+}

channels, Ca^{2+} exchangers, and Ca^{2+} ATPases have been suggested to be highly regulated (Malho et al. 1998; Cessna and Low 2001; White and Broadley 2003; Clapham 2007). It has been shown that protoplasts of tobacco cells cultivated in a liquid media can trigger organellar Ca^{2+} release into the cytosol under low apoplastic Ca^{2+} concentrations, which has been suggested to be the response of apoplastic Ca^{2+} sensors (Cessna and Low 2001), and may work to reestablish proper apoplastic Ca^{2+} concentrations. In addition, capacitative Ca^{2+} entry through the plasma membrane into the cytoplasm has been shown to be regulated by the extent to which the intracellular Ca^{2+} stores are filled. Calcium entry ceases when the stores are full, but recommences as soon as store Ca^{2+} is discharged (Malho et al. 1998). A variety of ways have been found to induce capacitative Ca^{2+} entry and these include Ca^{2+}-mobilizing agonists, inositol 1,4,5-trisphosphate, cyclic ADP ribose, ionophores or cyclopiazonic acid, or simply placing the cells under Ca^{2+}-free conditions. Basically, any signal which empties the Ca^{2+} stores sets the process of refilling by capacitative Ca^{2+} entry into operation (Malho et al. 1998). Although the Ca^{2+} content inside storage organelles has been reported to be highly regulated, few studies have shown that an altered regulation of the mechanisms involved in Ca^{2+} movement into the these organelles can have a great effect on fruit tissue susceptibility to Ca^{2+} deficiency disorders (Park et al. 2005; De Freitas et al. 2011a). In these studies, transgenic tomato fruit expressing a constitutively functional tonoplast Ca^{2+}/H^{+} exchanger (sCAX1) had twofold higher tissue Ca^{2+} concentration, and a higher Ca^{2+} accumulation inside the vacuole than wild-type fruit. The *sCAX1*-expressing fruit also had lower cytosolic and apoplastic Ca^{2+} concentrations, which resulted in higher plasma membrane leakage, cell plasmolysis, and 100% of fruit with Ca^{2+} deficiency symptoms, compared to wild-type fruit that did not develop Ca^{2+} deficiency symptoms. Other studies have shown that loss-of-function mutants lacking expression of both *CAX1* and *CAX3* (tonoplast *Ca^{2+}/H^{+}exchanger 3*) have threefold greater apoplastic free Ca^{2+} in leaf tissue than wild-type plants (Conn et al. 2011). These studies show that mechanisms regulating Ca^{2+} content inside storage organelles play an important role in the regulation of cellular Ca^{2+} partitioning and fruit susceptibility to Ca^{2+} deficiency disorder. In addition, a high fraction of organellar Ca^{2+} is precipitated with organic and inorganic substances, being unavailable to other cellular functions (Jones and Bush 1991; Arnott and Webb 2000; White and Broadley 2003; Lersten and Horner 2006; Prychid et al. 2008). Studies show that many plant species precipitate Ca^{2+} into crystals, such as Ca^{2+} oxalate crystals, in a cell controlled manner (Arnott and Webb 2000; Volk et al.

2002). Although still not well understood, the mechanisms responsible for Ca^{2+} crystals formation have also been suggested to affect cellular Ca^{2+} partitioning (Volk et al. 2002), which adds another layer of complexity to the regulation of cellular Ca^{2+} distribution. Future studies are required to analyze if the mechanisms that regulate the dynamics of Ca^{2+} movement and precipitation into cellular organelles can be modified by specific stimuli that leads to the development of Ca^{2+} deficiency disorders in fruit tissue.

C. Cytosolic Calcium

The level of cytosolic Ca^{2+} is believed to be under strict biochemical and physiological control. In the "resting" or quiescent state, cytosolic Ca^{2+} is maintained in the range between 100 to 200 nM, but can increase up to 2 μM under stimulus (Rudd and Franklin-Tong 1999). After Ca^{2+} enters the cytosol through activated Ca^{2+} channels, down its electrochemical gradient, high capacity and low affinity Ca^{2+} exchangers are believed to be the most important mechanism to lower cytosolic Ca^{2+} levels. Then, the activity of high affinity and low capacity Ca^{2+} ATPases are suggested to be the most important mechanism for further lowering the cytosolic Ca^{2+} levels to the resting state (White and Broadley 2003; Clapham 2007). Failure of Ca^{2+} exchangers and Ca^{2+} ATPases to lower cytosolic Ca^{2+} concentration to the resting state has been shown to result in toxicity and cell death (Cunningham and Fink 1996). However, enhanced activity of a tonoplast Ca^{2+} exchanger (sCAX1) that transports Ca^{2+} from the cytosol into the vacuole has been shown to lower both apoplastic and cytosolic Ca^{2+} concentrations, resulting in plasma membrane damage, cell death, and Ca^{2+} deficiency symptoms in the fruit (Park et al. 2005; De Freitas et al. 2011a).

D. Calcium Signaling

Changes in cytosolic Ca^{2+} levels play a key role in signal transduction pathways involved in cell responses to a wide range of environmental, developmental, and growth stimuli in an equally wide range of tissues and cell types (Scrase-Field and Knight 2003). The pattern in cytosolic Ca^{2+} changes are shaped by the combined activity of Ca^{2+} channels, Ca^{2+} exchangers, and Ca^{2+} ATPases (White and Broadley 2003). These cytosolic Ca^{2+} changes can take the form of single 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 cytosolic Ca^{2+} changes 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; Dodd et al. 2010; Kudla et al. 2010; Reddy et al. 2011). Similar to the cytosol, Ca^{2+} signatures in the nucleus have also been reported to lead to specific cellular responses to different stimuli (Pauly et al. 2001). The translation of Ca^{2+} signatures takes place through Ca^{2+}-binding proteins such as Calmodulins (CaMs) and their related Calmodulin like proteins (CMLs), calcium-dependent protein kinases (CDPKs), and the Calcineurin-B like (CBL) proteins (White and Broadley 2003). After binding Ca^{2+}, these proteins undergo structural and/or enzymatic changes that activate downstream pathways leading to specific cellular responses to different stimuli (White and Broadley 2003).

The inability of cells to properly respond to specific Ca^{2+} signatures can lead to cell death and symptoms of Ca^{2+} deficiency disorders in fruit tissue. Studies have shown that apple fruit treated with CaM antagonists, such as fluphenazine, chlorpromazine, and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, had higher BP incidence than untreated fruit (Fukumoto and Nagai 1983; Fukumoto et al. 1987). Higher susceptibility to Ca^{2+} deficiency disorders has been shown to be associated with higher amounts of Ca^{2+}-binding proteins such as CaMs in fruit tissue (Cocucci et al. 1983), which could be a mechanism to maintain proper Ca^{2+} signaling responses under lower cytosolic Ca^{2+} conditions (De Freitas et al. 2011a). According to these studies, low levels of free Ca^{2+} in the apoplast, and possibly inside storage organelles, could limit the flow of Ca^{2+} into the cytosol in response to different stimuli, resulting in cells being unable to respond properly to biotic and abiotic challenges (Ho and White 2005; De Freitas et al. 2011a). In addition, cytosolic Ca^{2+}-binding proteins are known to be the key regulators of exocytosis and plasma membrane restructuring processes (Picchioni et al. 1996, 1998; Battey et al. 1999). In this case, reduction in the activity of Ca^{2+}-binding proteins could result in membrane damage, cell death, and Ca^{2+} deficiency disorders. Therefore, considering that Ca^{2+} concentrations in the apoplast and inside storage organelles are much higher than in the cytosol, the apoplastic and organellar pools of free Ca^{2+} have important roles as sources of Ca^{2+} for cytosolic Ca^{2+} signaling responses, exocytosis, and membrane-restructuring processes, which have been suggested to affect fruit tissue susceptibility to Ca^{2+} deficiency disorders.

VI. ROLE OF ABIOTIC STRESS IN CALCIUM DEFICIENCY DISORDERS

Many environmental and mineral factors have been shown to affect whole plant Ca^{2+} uptake, Ca^{2+} translocation to the fruit, the role of Ca^{2+} in the cell, and fruit susceptibility to Ca^{2+} deficiency disorders. These environmental factors are drought and salinity, relative humidity, shoot and root temperatures, light, and mineral imbalance conditions (Ho and White 2005).

A. Drought and Salinity

Drought and salinity conditions have been shown to stimulate Ca^{2+} deficiency disorders in fruit (Hwan et al. 1997; Dorji et al. 2005; Yu et al. 2006), and this can be enhanced by low relative humidity conditions (Ho 1989; Adams and Holder 1992; Bertin et al. 2000; Li et al. 2001; Ho and White 2005). The conserved effect of drought, salinity, and low relative humidity on increasing fruit susceptibility to Ca^{2+} deficiency disorders could be the result of plant water stress, which has been suggested to limit xylemic Ca^{2+} movement into the fruit (Adams and Ho 1993; White 2001; Ho and White 2005). In addition, drought and salinity have been shown to reduce the number of functional xylem vessels in the fruit, potentially decreasing fruit Ca^{2+} uptake (Belda and Ho 1993; Ho et al. 1993; Belda et al. 1996; Davies et al. 2000). Fruit grown under saline conditions have also been shown to have high levels of reactive oxygen species (ROS) in the apoplast at the time of BER development (Aktas et al. 2005). Accordingly, other studies have shown increasing hydrogen peroxide levels in fruit tissue with increasing salinity levels in the roots, which was highly correlated with increasing incidence of Ca^{2+} deficiency disorder (Turhan et al. 2006). Based on these results, drought, salinity, and low relative humidity can potentially increase plant water stress, reducing fruit Ca^{2+} uptake and increasing ROS levels in fruit tissue, which causes plasma membrane damage that leads to Ca^{2+} deficiency symptoms development in the fruit.

B. Light and Temperature

The effects of high light and air temperatures on the reduction of fruit Ca^{2+} concentrations usually take place simultaneously. Both high light and air temperature conditions have been shown to accelerate fruit expansion, perhaps due to increased photosynthetic rates and increased photo-assimilated supply to the fruit (Ho and White 2005).

Rapid fruit expansion can potentially dilute fruit Ca^{2+} content and increase fruit susceptibility to Ca^{2+} deficiency disorders (DeKock et al. 1982; Wui and Takano 1995; Bertin et al. 2000). Previous studies have suggested that Ca^{2+} deficiency disorders can be induced in the rapidly expanding distal fruit tissue when its demand for Ca^{2+} exceeds the immediate xylem supply (Ho et al. 1993; Ho and White 2005). Accordingly, Ca^{2+} deficiency disorders have been observed to increase with higher light intensity and air temperature conditions during fruit growth and development (Gerard and Hipp 1968; Ho and Grimbly 1988; Ho 1989; Ho et al. 1993; Hwan et al. 1998). In addition, increasing light and air temperatures can potentially reduce RH, which has been shown to enhance fruit susceptibility to Ca^{2+} deficiency disorders (Adams and Ho 1993).

Root temperature has also been shown to regulate plant and fruit Ca^{2+} uptake (Adams and Ho 1993). In these studies, root Ca^{2+} uptake was enhanced at temperatures from 14°C to 26°C, and was reduced at lower and higher temperatures (Adams and Ho 1993; Ho 1999).

C. Mineral Imbalance

The levels of other mineral nutrients can influence fruit susceptibility to calcium deficiency disorders. At the level of root uptake, interactions between Ca^{2+} and other nutrients can affect fruit Ca^{2+} uptake (Taylor and Locascio 2004; Ho and White 2005). High levels of N increase shoot growth, which has been suggested to enhance Ca^{2+} movement towards the leaves and away from the fruit possibly as a result of higher leaf transpiration rates at the whole plant level (Pill and Lambeth 1980; Ikeda and Osawa 1988; Ho et al. 1999; Ho and White 2005). High levels of N are also known to trigger rapid fruit and cell expansion, which can potentially result in further dilution of the limited Ca^{2+} content that moves into the fruit (Bar-Tal et al. 2001; Saure 2001).

High levels of K^+ and Mg^{2+} have also been reported in fruit tissue with Ca^{2+} deficiency symptoms (De Freitas et al. 2010). Potassium is known to be involved in cell-expansion-related processes (Elumalai et al. 2002), suggesting that high levels of K^+ could favor rapid plant and fruit growth leading to a reduction in fruit Ca^{2+} uptake, dilution of fruit Ca^{2+} content, and high fruit susceptibility to Ca^{2+} deficiency disorders. Cationic ions such as K^+ and Mg^{2+} are known to compete with Ca^{2+} for binding sites at the plasma membrane (Schonherr and Bukovac 1973; Yermiyahu et al. 1994). Therefore, high levels of K^+ and Mg^{2+} could potentially replace Ca^{2+} at the plasma membrane surface, but not its role in membrane structure and function (Schonherr and Bukovac

1973; Yermiyahu et al. 1994), which could lead to a leaky plasma membrane and increased fruit tissue susceptibility to Ca^{2+} deficiency disorders.

Since N, K^+, and Mg^{2+} can affect fruit Ca^{2+} uptake and the role of Ca^{2+} at the cellular level, it has been suggested that nutrient concentration ratios such as N/Ca^{2+}, K^+/Ca^{2+}, Mg^{2+}/Ca^{2+}, and $[K^+ + Mg^{2+}]/Ca^{2+}$ are usually more precise parameters to predict fruit susceptibility to Ca^{2+} deficiency disorders than total fruit Ca^{2+} concentration alone (Dris et al. 1998; Lanauskas and Kvikliene 2006; De Freitas et al. 2010).

VII. ROLE OF GROWTH REGULATORS IN CALCIUM DEFICIENCY DISORDERS

Growth regulators control many cellular processes in plants that can affect Ca^{2+} uptake, translocation, and partitioning at the cellular level. A few studies have shown that growth regulators affect fruit susceptibility to Ca^{2+} deficiency disorders. However, more studies are required to further understand the mechanisms.

A. Auxins

Studies show that auxin produced in the seeds and basipetally transported in the fruit are required for fruit Ca^{2+} uptake and reduction of fruit susceptibility to Ca^{2+} deficiency disorders (Oberly 1973; Bangerth 1973, 1976; Banuelos et al. 1987; Brown and Ho 1993). In these studies, treating fruit with auxin transport inhibitors such as 2,3,5-triiodobenzoic acid (TIBA) and chlorofluorenolmethyl ester (CME) inhibited basipetal transport of auxin, particularly in 10–12-day-old tomato fruit, and simultaneously restricted the acropetal transport of Ca^{2+} into the fruit, potentially increasing fruit susceptibility to Ca^{2+} deficiency disorders (Banuelos et al. 1987). In addition, artificially induced parthenocarpic fruit of apple, pear, and tomato, as well as seeded fruit treated with TIBA, frequently showed low Ca^{2+} content and symptoms of Ca^{2+} deficiency disorders, further supporting the idea that auxins, produced by the seeds and basipetally transported in the fruit, play a significant role in fruit Ca^{2+} uptake and susceptibility to Ca^{2+} deficiency disorders (Stahly and Benson 1970, 1976; Benson and Stahly 1972; Oberly 1973; Bangerth 1976). Accordingly, other studies also showed that applying auxin to the stigma of nonpollinated sweet pepper flowers resulted in a 30% reduction in fruit susceptibility to BER incidence (Heuvelink and Korner 2001).

Reduction in shoot Ca^{2+} uptake in lettuce and avocado flower panicles and fruitlets has also been observed in response to auxin transport inhibitor treatments (Banuelos et al. 1988; Cutting and Bower 1989). In addition, acropetal Ca^{2+} movement has also been shown to affect basipetal auxin transport (Tang and Fuente 1986; Allan and Rubery 1991; Allan et al. 1993), suggesting a cross-communication between auxin and Ca^{2+} transport in the plant.

Although evidence shows that auxin transport affects fruit susceptibility to Ca^{2+} deficiency disorders, the mechanisms involved are not well understood. It is possible that basipetal auxin movement in the plant increases root activity, enhancing plant and fruit Ca^{2+} uptake, and reducing fruit susceptibility to Ca^{2+} deficiency disorders (Yang et al. 2004). Alternatively, continuous development of new vascular tissues is required for xylemic Ca^{2+} movement into fruit tissues undergoing growth and development. The differentiation of xylem vascular tissue along the plant is induced and controlled by longitudinal streams of auxin (Saure 2005; Yoshida et al. 2009). Previous studies reported that a polar auxin transport inhibitor, 1-*N*-naphthylphthalamic acid (NPA) decreased intracellular auxin levels and prevented tracheary element differentiation, but that additional auxin, 1-naphthaleneacetic acid (NAA), overcame this inhibition (Fukuda 2004; Yoshida et al. 2009). Therefore, reducing the levels of auxin and its transport can potentially reduce the rates of xylem vessels development and Ca^{2+} transport into growing and developing fruit tissues, and consequently increase fruit susceptibility to Ca^{2+} deficiency disorders.

Others hypothesize that the interaction between auxin and Ca^{2+} at the cellular level determines fruit susceptibility to Ca^{2+} deficiency disorders (Ho and White 2005). Based on this hypothesis, during the process of auxin-induced cell growth (Perrot-Rechenmann 2010), low levels of cytosolic Ca^{2+} could result in abnormal auxin-induced signaling responses, and insufficient apoplastic Ca^{2+} concentrations could lead to excessive cell enlargement, both cases leading to cell death and Ca^{2+} deficiency symptom development in growing fruit (Ho and White 2005). Future studies should focus on the mechanisms by which auxin transport increases fruit Ca^{2+} uptake, as well as on the mechanisms through which the cellular interaction between auxin and Ca^{2+} affect fruit susceptibility to Ca^{2+} deficiency disorders.

B. Gibberellins (GAs)

Early studies have showed that treating tomato plants with GAs increased fruit susceptibility to Ca^{2+} deficiency disorders by 35%

(Bangerth 1973; Castro 1980). Recent studies showed that tomato plants grown under low Ca^{2+} conditions and treated weekly with GAs reached 100% BER incidence at about 50 days after pollination (De Freitas et al. 2011c). In these studies, tomato plants treated with a gibberellin biosynthesis inhibitor (Prohexadione-Ca) did not develop BER, and plants treated with water exhibited up to 30% BER incidence (De Freitas et al. 2011c). In addition, BER incidence was highly correlated with increased plasma membrane leakage, low levels of water-soluble apoplastic Ca^{2+}, and high expression of putative vacuolar *H^+/Ca^{2+}transporters and Ca^{2+}ATPases* in fruit tissue (De Freitas et al. 2011c). These results suggest that GAs affect cellular Ca^{2+} partitioning and distribution, which leads to higher fruit susceptibility to BER. Since GAs trigger many cellular processes, other mechanisms are also expected to be involved.

High levels of physiologically active GAs have been proposed to inhibit fruit Ca^{2+} uptake (Saure 1996, 2001, 2005). Rapid cell and fruit expansion, triggered by GAs, has been suggested to constrict functional xylem vessels in the fruit, reducing Ca^{2+} uptake and translocation in the fruit (Drazeta et al. 2004; Ho and White 2005). It has also been proposed that high GA levels can reduce xylem vessel formation and a high auxin/GA ratio could promote the differentiation of xylem at the expense of phloem formation (Aloni 2001), which could thus contribute to improved Ca^{2+} translocation into fruit tissues (Saure 2005). Studies in our laboratory have shown that treating tomato plants and fruit with a GA biosynthesis inhibitor, prohexadione-Ca, successfully maintained a higher abundance of functional xylem vessels in the peduncle and distal end tissues of tomato fruit, which was highly correlated with greater Ca^{2+} concentration in these tissues and lower fruit susceptibility to Ca^{2+} deficiency disorder (De Freitas et al. 2011c). Another possible explanation for the effect of GAs is that rapid cell and fruit expansion dilutes fruit Ca^{2+} concentration, which may enhance fruit susceptibility to Ca^{2+} deficiency disorders (Saure 2001, 2005; Ho and White 2005).

If these ideas are valid, reducing GA levels by various means may be a possible strategy to increase fruit Ca^{2+} uptake and translocation to the distal fruit tissue, reducing fruit susceptibility to Ca^{2+} deficiency disorders. Similar to auxin, during GA-induced cell growth, appropriate cytosolic and apoplastic levels of Ca^{2+} may be required to avoid abnormal GA-induced signaling responses and excessive cell expansion that can lead to Ca^{2+} deficiency symptom development in expanding fruit (Ho and White 2005). Further studies are required to prove the validity of these hypotheses.

C. Abscisic Acid (ABA)

Abscisic acid is well known for its effect on stomata leading to reduced leaf transpiration (Verslues and Zhu 2007), but not for its effect on Ca^{2+} deficiency disorders in fruit. Previous studies have shown that reducing leaf transpiration (by increasing relative humidity) and reducing plant water stress can be more effective to increase fruit Ca^{2+} uptake and reduce fruit susceptibility to Ca^{2+} deficiency disorders than increasing Ca^{2+} availability in the soil (Paiva et al. 1998; Abdal and Suleiman 2005; Guichard et al. 2005). It has been suggested that leaves and fruit compete for xylemic water and nutrients in the plant and the final rates of xylem uptake is determined by growth and transpiration rates of these organs (Clarkson 1984; Ho 1989; Ho et al. 1993; Passam et al. 2007). It is well known that leaves have much higher transpiration rates than fruit, which can also be confirmed by the much higher Ca^{2+} concentration observed in leaf tissue compared with fruit tissue (Clarkson 1984; Ho 1989; De Freitas et al. 2011b). However, this scenario may be changed by increasing ABA levels in the leaves, which can specifically trigger stomatal closure, reducing leaf transpiration and increasing plant water potential (Verslues and Zhu 2007; De Freitas et al. 2011b). Accordingly, BER has been shown to be completely prevented by spraying tomato plants weekly with ABA, whereas plants sprayed with water developed 30–45% BER (De Freitas et al. 2011b). In these studies, the ABA treatment reduced leaf stomatal conductance and leaf Ca^{2+} accumulation by half, while it increased fruit Ca^{2+} accumulation by up to 10 fold, compared to the water treatment (De Freitas et al. 2011b). The ABA treated fruit also had higher numbers of functional xylem vessels, higher xylem/phloem ratio of water uptake, higher levels of water-soluble apoplastic Ca^{2+}, and lower plasma membrane permeability which potentially resulted in the observed decrease in fruit susceptibility to BER (De Freitas et al. 2011b). It has also been proposed that ABA could act as an antagonist of GA responses at the cellular level (Saure 2001). This hypothesis has been supported by our studies where ABA had a similar effect to a GA-biosynthesis inhibitor, prohexadione-Ca, on the inhibition of BER development in tomato fruit grown under low Ca^{2+} conditions (De Freitas et al. 2011b,c). Therefore, treating tomato plants with ABA may be a useful approach to reduce fruit susceptibility to Ca^{2+} deficiency disorders though different mechanisms.

D. Cytokinins

Previous studies have shown that spraying apple trees with increasing concentrations of thidiazuron (TDZ; *N*-phenyl-*N*′-1,2,3-thiadiazol-5-

ylureia), which has cytokinin-like activity, results in decreasing concentrations of Ca^{2+} in the fruit (Amarante et al. 2003). Accordingly, other studies have reported higher BP incidence at harvest when apple trees were sprayed 18 days after full bloom with 15 mg L^{-1} of TDZ (Greene 1995). The reduction in fruit Ca^{2+} concentration and increase in fruit susceptibility to BP has been attributed to the thinning affect of cytokinins applied to the canopy of apple trees (McLaughlin and Greene 1984; Elfving 1989, 1991; Elfving and Cline 1993; Greene and Autio 1989; Greene et al. 1990), which reduces crop load and increases fruit size, diluting the Ca^{2+} content in the fruit (Greene 1993).

On the other hand, a few studies have shown that high levels of cytokinins in roots can increase the Ca^{2+} uptake rate by plants and the Ca^{2+} concentration in the shoot (Shear 1975; Yang et al. 2008). It is possible that cytokinins increase root Ca^{2+} uptake and translocation to the shoot by increasing the abundance of functional xylem vessels in the plant. Xylem vessel differentiation is controlled by the basipetal flow of auxins in the plant, but the responsiveness of the cambium to auxin is increased by the cytokinins produced in the roots (Aloni 2001). Higher Ca^{2+} translocation to the shoot and delays in plant senescence previously reported in response to cytokinins (Dong et al. 2008) could potentially lead to extended and greater fruit Ca^{2+} uptake, reducing fruit susceptibility to Ca^{2+} deficiency disorders. However, cytokinins are also known for their antagonistic effect on root growth, which is counterbalanced by auxins (Chapman and Estelle 2009). Based on this evidence, cytokinins may reduce or increase Ca^{2+} concentration in fruit tissue and fruit susceptibility to Ca^{2+} deficiency disorders depending on its concentration and location in the plant at specific stages of growth and development.

E. Ethylene

Fruit with lower Ca^{2+} concentration have been reported to have higher ethylene production, as well as faster ripening and senescence (Ferguson 1984), suggesting a negative interaction between Ca^{2+} ions and ethylene responses (Ferguson 1984; Wang et al. 2006). Although ethylene can potentially play a role in fruit susceptibility to Ca^{2+} deficiency disorders, the mechanisms involved remain poorly understood and future studies are required to further understand the antagonistic control points between Ca^{2+} ions and ethylene. When applied in the orchard two to 3 weeks before harvest, ethylene was shown to decrease apple fruit susceptibility to BP (Schumacher and

Fankhauser 1972; Pfammater and Dessimoz 1974), which has been suggested to be the result of advancing fruit maturation (Marcelle and Clijsters 1978). However, when applied after harvest, ethylene has been used to accelerate BP development (Lötze and Theron 2006; Lötze et al. 2010). The induction of Ca^{2+} deficiency symptoms during storage of apple fruit with ethylene could be explained by an acceleration of fruit ripening and senescence, which may also accelerate the events leading to Ca^{2+} deficiency symptoms development. After harvest, ethylene is known to regulate the expression and activity of cell wall enzymes (Bennett and Labavitch 2008), and an increase in ethylene could affect the dynamics of Ca^{2+} binding to the cell wall and fruit susceptibility to Ca^{2+} deficiency disorders. Our studies have shown that PMEs activity in apple fruit increases the fraction of total fruit tissue Ca^{2+} bound to the cell wall and potentially decreases free Ca^{2+} content in the apoplast leading to higher fruit susceptibility to Ca^{2+} deficiency disorders (De Freitas et al. 2010). Other studies have shown an increase in ethylene biosynthesis in fruit with initial Ca^{2+} deficiency symptoms (Taylor and Locascio 2004). During Ca^{2+} deficiency symptom development, increasing tissue damage is probably the reason for an increase in ethylene biosynthesis, which is known to be involved in stress response pathways (Cao et al. 2007). In addition, ethylene is known to increase plasma membrane permeability (Candan et al. 2008), which may enhance the effect of lower apoplastic Ca^{2+} on plasma membrane leakage and increase tissue susceptibility to Ca^{2+} deficiency disorders.

F. Brassinosteroids (BRs)

Although there are no studies relating BRs to Ca^{2+} deficiency disorders, their role in plants suggest that BRs could potentially be involved. Brassinosteroids have been shown to induce stress tolerance in plants (Schenabel et al. 2001), and to increase cell viability under stress conditions by increasing the cellular capacity to scavenge ROS (Liu et al. 2009). ROS, such as free oxygen radicals and hydrogen peroxide, cause lipid peroxidation and membrane damage, which can then lead to cell death (Dhindsa et al. 1981; Fridovich 1986; Moran et al. 1994). Plant cells treated with a BR known as 24-epibrassinolide were shown to increase the activity of antioxidative enzymes such as ascorbate peroxidase, catalase, peroxidase, and superoxide dismutase, as well as to enhance the synthesis of antioxidant substances such as ascorbic acid and reduced glutathione, resulting in lower contents of ROS and lipid peroxidation in the cells (Liu et al. 2009).

ROS have been suggested to contribute to Ca^{2+} deficiency symptom development in fruit tissue (Schmitz-Eiberger and Noga 2003; Casado-Vela et al. 2005; Aktas et al. 2005; Turhan et al. 2006). In this context, BR could increase fruit tissue capacity to scavenge ROS, which could prevent or reduce fruit susceptibility to Ca^{2+} deficiency symptom development. Brassinosteroids are also known for their function on xylem vessel development. Plants grown in a medium containing brassinazole, a specific BRs biosynthesis inhibitor, were shown to have slight predominance of phloem differentiation and remarkable inhibition in the development of secondary xylem, indicating that BRs play an important role in xylem development (Nagata et al. 2001). Based on this evidence and the fact that Ca^{2+} is a xylem mobile nutrient, higher levels of BRs, or proper BRs homeostasis with other growth regulators, could favor xylem development and Ca^{2+} translocation in the plant and fruit, possibly increasing fruit Ca^{2+} uptake, and reducing fruit susceptibility to Ca^{2+} deficiency disorders.

G. Jasmonates (JAs)

Applications of methyl jasmonate (MJ) at 2 weeks interval starting at 119 days after full bloom resulted in higher BP incidence in apple fruit at harvest, 172 days after full bloom (Rudell et al. 2005). In this study, similar Ca^{2+} concentrations were observed between MJ treated and nontreated fruit. The authors attributed the effect of MJ on BP incidence to a possible interaction between JA and GAs during fruit growth and development (Rudell et al. 2005). However, other studies show that MJ treatment increases the contents of chlorogenic acid, cyanidin, quercetin, and phloretin glycosides in apple fruit (Rudell et al. 2002). It is believed that the characteristic browning symptoms of Ca^{2+} deficiency disorders in fruit is the result of phenol oxidation (Dekock et al. 1980; Casado-Vela et al. 2005). Therefore, a high accumulation of phenolic and toxic compounds in fruit tissue could potentially favor BP development and explain the observed higher fruit susceptibility to this disorder in response to MJ treatment. Jasmonates have also been reported to trigger the accumulation of ROS in the cell, disrupting organellar membranes, and resulting in apoptosis which is a programmed cell death. Although JAs' role in these processes are possibly related to mechanisms of plant defense against the spread of biotic challenges (Reinbothe et al. 2009), it is possible that similar mechanisms could also be involved in the development of Ca^{2+} deficiency symptoms in fruit.

H. Salicylic Acid (SA)

While no studies could be found related to SA effects on Ca^{2+} deficiency disorders in plants, SA has been shown to increase plant tolerance to high salinity conditions, which are known to increase fruit susceptibility to Ca^{2+} deficiency disorders (Aktas et al. 2005; Ho and White 2005; Turhan et al. 2006). In these studies, SA applied via root drenching increased tomato plant survival and relative shoot growth rates compared to untreated plants grown under high-salinity conditions (Stevens et al. 2006). The SA application reduced membrane permeability of leaf tissue by 44% in plants grown at 150 mM NaCl and by 32% in plants grown at 200 mM NaCl, compared to untreated plants, indicating possible protection of cellular membranes (Stevens et al. 2006). A possible relationship between SA and Ca^{2+} deficiency disorders should be investigated.

I. Growth Regulator Homeostasis

Although many growth regulators have been shown to potentially affect fruit susceptibility to Ca^{2+} deficiency disorders, the evidence together suggests that these disorders are likely affected by the combined effect of different growth regulators at the whole plant, fruit, and cellular level. In that case, specific growth regulator homeostasis in different plant tissues and at different developmental stages could potentially regulate plant and fruit Ca^{2+} uptake, Ca^{2+} translocation to distal fruit tissues, cellular Ca^{2+} partitioning, plasma membrane structure and integrity, and consequently fruit susceptibility to Ca^{2+} deficiency disorders (Fig. 3.6). Previous studies showed that complex growth regulator interactions are responsible for specific cellular responses (Santner et al. 2009). For example, the expression of many genes required for auxin biosynthesis is under the control of ethylene (Stepanova et al. 2005; Tao et al. 2008). Similarly, the expression of genes encoding 1-aminocyclopropane-1-carboxylate synthase, involved in ethylene biosynthesis, is regulated by auxin (Tsuchisaka and Theologis 2004). Overlap in growth regulator responses have also been reported for auxin and brassinosteroids (Santner et al. 2009). In addition, the DELLA proteins have been suggested to be a common cross-talk node for several interacting growth regulators such as GAs, auxins, ABA, and ethylene (Santner et al. 2009). Although interactions between growth regulators have already been identified, future studies are required to explore the effect of such interactions on Ca^{2+} deficiency disorder development in fruit tissue.

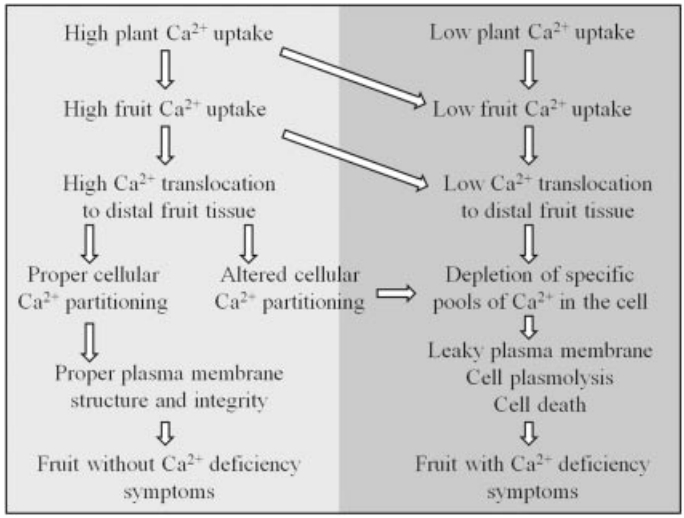

Fig. 3.6. Proposed mechanisms controlling fruit susceptibility to Ca^{2+} deficiency disorders. Fruit susceptibility to Ca^{2+} deficiency disorders is determined by a combination of factors controlling plant and fruit Ca^{2+} uptake, Ca^{2+} translocation to distal fruit tissue, and cellular Ca^{2+} partitioning.

VIII. PROPOSED MECHANISMS CONTROLLING FRUIT SUSCEPTIBILITY TO CALCIUM DEFICIENCY DISORDERS

Previous studies have shown that increasing plasma membrane leakage, cell plasmolysis, and death are characteristic symptoms of Ca^{2+} deficiency disorders in tissues of different plant species (Suzuki et al. 2000, 2003; De Freitas et al. 2010, 2011a). It has been previously suggested that soluble apoplastic Ca^{2+} is required to bind to phospholipids and proteins present in the plasma membrane, which contributes to proper membrane structure and integrity (Hanson 1960; Clarkson and Hanson 1980; Legge et al. 1982; Kirkby and Pilbeam 1984; Picchioni et al. 1998; Hirschi 2004). Here, we propose that low water-soluble Ca^{2+} concentration in the apoplast of fruit tissue leads to development of deficiency disorders. Low apoplastic Ca^{2+} is determined by the combined result of whole plant Ca^{2+} uptake from the soil, fruit Ca^{2+} uptake from the plant, Ca^{2+} translocation to distal end tissues in the fruit, and proper regulation of Ca^{2+} partitioning at the cellular level (Fig. 3.6).

Low plant Ca^{2+} uptake from the soil will limit downstream events of fruit Ca^{2+} uptake, Ca^{2+} translocation to distal fruit tissues, and maintenance of Ca^{2+} levels in the apoplast that is required for proper plasma

membrane structure and function, increasing the probability of Ca^{2+} deficiency disorder development in fruit tissue (Fig. 3.6). High plant Ca^{2+} uptake from the soil favors the potential for downstream events to result in higher apoplastic Ca^{2+} and lower fruit tissue susceptibility to Ca^{2+} deficiency disorders. However, in some cases, despite high plant Ca^{2+} uptake the final apoplastic Ca^{2+} concentration in the fruit tissue will not be sufficient to prevent Ca^{2+} deficiency disorder development, as this will be determined by the downstream events leading to fruit Ca^{2+} uptake, Ca^{2+} translocation to distal fruit tissues, and regulation of cellular Ca^{2+} partitioning (Fig. 3.6). Similarly, low fruit Ca^{2+} uptake will limit Ca^{2+} translocation within the fruit, and result in lower levels of apoplastic Ca^{2+} in distal fruit tissues, increasing the probability of Ca^{2+} deficiency disorder development (Fig. 3.6). High fruit Ca^{2+} uptake will favor downstream events leading to higher apoplastic levels of Ca^{2+} in distal tissues and lower fruit susceptibility to Ca^{2+} deficiency disorders. However, under high fruit Ca^{2+} uptake conditions, apoplastic Ca^{2+} concentration in distal fruit tissues and fruit susceptibility to Ca^{2+} deficiency disorders will be determined by the downstream events leading to Ca^{2+} translocation to distal fruit tissues, and regulation of cellular Ca^{2+} partitioning (Fig. 3.6). Low Ca^{2+} translocation in the fruit will limit the apoplastic levels of Ca^{2+} in distal fruit tissues and will increase the probability of Ca^{2+} deficiency incidence in the fruit (Fig. 3.6). High Ca^{2+} translocation in the fruit will favor higher levels of apoplastic Ca^{2+} in distal tissues. However, the concentration of apoplastic Ca^{2+} in distal fruit tissue required to suppress Ca^{2+} deficiency disorders ultimately will be determined by the mechanisms that regulate cellular Ca^{2+} partitioning (Fig. 3.6). As previously reported, fruit tissue with high total Ca^{2+} content can develop Ca^{2+} deficiency symptoms if cellular Ca^{2+} partitioning is not properly regulated (Park et al. 2005; De Freitas et al. 2011a). Therefore, mechanisms that regulate cellular Ca^{2+} partitioning are the final and the most important control level of Ca^{2+} deficiency disorders in fruit tissue with adequate levels of total Ca^{2+} content. In such condition, sudden changes on cellular Ca^{2+} partitioning in response to environmental factors and/or growth regulators could lead to a cell localized Ca^{2+} deficiency, cell death, and Ca^{2+} deficiency symptoms development in fruit tissue.

For each genotype and environmental conditions, the combined activity of all the mechanisms that determine the concentration and cellular localization of Ca^{2+} in fruit tissue will define fruit susceptibility to Ca^{2+} deficiency disorders. Therefore, for each genotype and environmental conditions, reliable strategies to prevent Ca^{2+} deficiency disorders should focus first on identifying the bottlenecks limiting total

Ca^{2+} uptake and/or proper regulation of cellular Ca^{2+} partitioning in fruit tissue. After identifying the bottlenecks leading to Ca^{2+} deficiency disorders, specific control strategies can be effectively accomplished.

Many studies have already identified a high number of environmental factors that affect total fruit tissue Ca^{2+} uptake, as previously described (Bangerth 1979; Ferguson and Watkins 1989; Saure 2001; Taylor and Locascio 2004; Ho and White 2005). In addition, previous studies have shown possible factors that could be manipulated to increase total fruit Ca^{2+} uptake, as well as Ca^{2+} translocation to distal fruit tissues (Ho 1989; Guichard et al. 2005; Ho and White 2005; De Freitas et al. 2011b). However, little is known about the mechanisms regulating cellular Ca^{2+} partitioning that affect fruit tissue susceptibility to Ca^{2+} deficiency disorders. Therefore, future studies are still required to better understand these mechanisms, which represent the final control level of fruit susceptibility to Ca^{2+} deficiency disorders, and as such, a powerful tool to prevent these physiological disorders in fruit tissue.

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