REVIEW

Insights into Blossom End-Rot Disorder in Tomato (*Solanum lycopersicum*)

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Abstract

Blossom end rot (BER) is mainly a calcium (Ca^{2+}) deficiency-related physiological disorder of fruits that affects various crop production worldwide including tomato (*Solanum lycopersicum*). The visible symptoms of BER include cell wall disintegration, cell plasmolysis, and water-soaked signs at the distal end of the fruits. During fruit development increase in cell expansion and a decrease in the transport of Ca^{2+} to the distal part of the fruits, can lead to the development of BER. It is hypothesized that insufficient Ca^{2+} is available for essential apoplastic and cytoplasmic functions during the cell expansion phase of fruits when the cellular Ca^{2+} demand exceeds the Ca^{2+} supply. Therefore, abnormal intracellular Ca^{2+} content or signals, cause weakening of cell walls, and a loss of cellular integrity, potentially leading to cell death and the outward manifestations of BER. The occurrence of BER in tomatoes is also influenced by environmental factors that affect the cellular growth of the fruits. These factors such as drought, high salinity, high temperature, insufficient xylem tissue development, phytohormones, and oxidative stress can influence the development of BER. The availability of a high-quality reference genome and whole genome sequencing allowed us to identify selected loci that can cause BER, facilitating genetic dissection and a deeper comprehension of the molecular mechanisms underlying this disorder. This review summarized the various factors and genes involved in BER development and management strategies.

Keywords Blossom End Rot · Calcium Deficiency · Abiotic Stress · Reactive Oxygen Species · Cell Wall · Pectin Methyl Esterase

Abbreviations

BER	Blossom End Rot
PME	Pectin methylesterase
ACO	1-Aminocyclopropne-1-Carboxylic acid oxidase
GAs	Gibberellins
ABA	Abscisic acid
GPX	Glutathione peroxidase
APX	Ascorbate peroxidase
GR	Glutathione reductase
MDHAR	Monodehydro ascorbate reductase
DHAR	Dehydro ascorbate reductase
AsA-GSH	Ascorbate- Glutathione

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Introduction

Today, as the world's population continuously grows, the demand for food is proportionally increasing (Lobell et al. 2009). Several agriculture-based companies and farmer's incomes depend on the production of edible crops, mainly from leguminous and solanaceous plants. One of the most common solanaceous edible fruits, the tomato (*Solanum lyopersicum*), is prevalent due to its high carotenoid content and antioxidant properties. Several physiological factors affect the growth and productivity of tomato crops, including biotic and abiotic stresses that can negatively impact yield and result in financial losses (Atkinson et al. 2011; Jolánkai et al. 2016).

BER is a physiological disorder that is influenced by multiple factors, affecting various vegetable crops such as eggplant (*Solanum melongena L.*) watermelon (*Citrullus lanatus (Thunb.*), pepper (*Capsicum annuum L.*), and tomato (*Solanum lycopersicum L.*) (Taylor and Locascio 2004; Zhang et al. 2019). BER can grow in any tomato-producing region of the world and has been shown to cause 50% yield reductions. BER symptoms first appear as a water-soaked area at the fruit's blossom end, typically, the area develops quickly, resulting in a blackened, dry, sunken leathery spot (Fig. 1a)

(Ho and White 2005; Topcu et al. 2022). BER can also affect the placenta's blossom end, the surrounding locular contents, and the outside wall. There is a limited variation of the disorder that manifests as an internal browning or blackening of the internal tissue within the adjacent parenchyma surrounding the immature seeds and the distal portion of the placenta (Ho and White 2005). These disorders primarily affect the fruits, but they can also damage other organs like leaves and flower buds (Barta and Tibbitts 2000; Francois et al. 2019). A variety of leafy vegetables experience tipburn due to defective Ca^{2+} distribution (Kuo et al. 1981; Su et al. 2019). Other vegetables, including celery and cauliflower, are also affected by illnesses that resemble BER (Aloni and Pressman 1987; Bianco et al. 2015). Bitter pit is a similar type of disorder that affects apple fruits (de Freitas et al. 2010).

BER disorder was originally described as "Black rot" and was poorly understood and thought to be caused by a parasitic organism from the late 1800s until the early 1940s (Taylor and Locascio 2004). However, the initial evidence indicated that BER is a Ca^{2+} deficiency disorder based on the following data: (i) BER usually occurs when the fruit's Ca^{2+} content is less throughout its tissues and (ii) the incidence of BER was significantly decreased when Ca^{2+} ferti-

lizer and sprays were applied to field and greenhouse tomatoes (Ho et al. 1993).

The total amount of Ca^{2+} concentration present on the earth's crust is approximately 3.6% while soils contain 1.37%, both play an important role in soil maintenance and stability (Hawkesford et al. 2011). Ca^{2+} is a key nutrient required for the cell wall structure, signalling and stress response, cell growth, plant tissue development, and membrane stability (Dodd et al. 2010). Plants primarily absorb Ca^{2+} from the soil and transfer it to the xylem of the root tissue and further transport in fruit or leaf tissue occurs via both apoplastic (extracellular space between cells) and symplastic (neighboring cells connected by plasmodesmata) pathways (Yang and Jie 2005) (Fig. 1b). In cells and subcellular organelles, Ca^{2+} uptake and transport are mainly facilitated by Ca^{2+} channels, Ca^{2+}-ATPase, and Ca^{2+}/H^+ antiporter, so Ca^{2+} concentration at the cellular and extracellular levels will be highly regulated (Yang and Jie 2005; Tuteja and Mahajan 2007; Stael et al. 2012; Pathak et al. 2021). It has been demonstrated that greater free Ca^{2+} in the apoplastic pool (1-10 mM) is necessary to maintain proper membrane structure and function, but cytosolic Ca^{2+} levels are maintained at low concentrations (less than 1 mM) in comparison to the apoplastic and organelle pools (Hanson
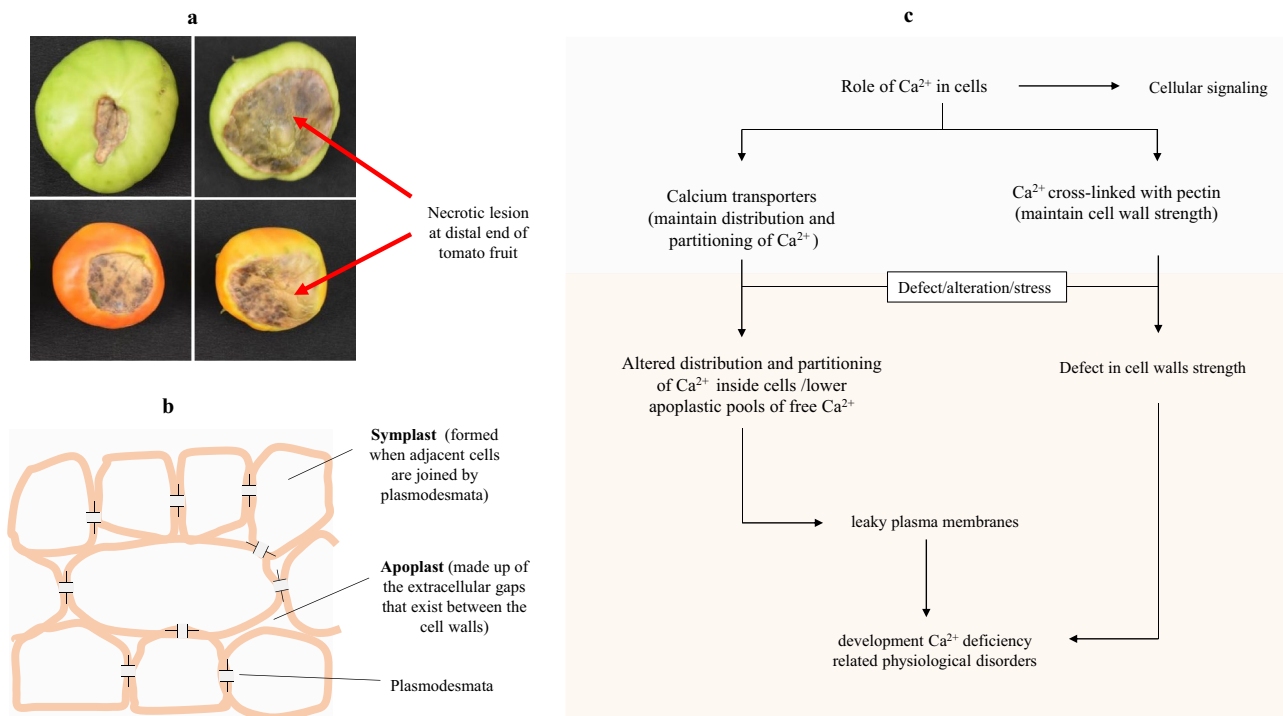

Fig. 1 **a** BER symptoms at the distal end of tomato fruits. The tomato fruit displays a sunken, leathery patch with a dark brown color as a sign of external blossom end rot (BER), marked with arrows **b** Symplasts are formed by connecting neighboring cells through plasmodesmata, whereas apoplasts are formed by extracellular gaps that exist between the cell walls of neighboring cells **c** A schematic model illustrating the basic role of Ca^{2+} in cells and how a defect/ stress/change in normal cellular functioning leads to the development of Ca^{2+} deficiency-related physiological disorders

1960; Plieth 2001). An increase in the basal Ca^{2+} concentration may result in anomalies in development and growth, increased rigidity of the fruit cell wall, and cellular toxicity while reduced basal Ca^{2+} concentrations have been linked to the breakdown of fruit cell walls and the emergence of many physiological diseases (Hocking et al. 2016). Ca^{2+} treatment could improve the fruit quality and yield in the postharvest Ca^{2+} treatment which reduces the physiological disorders (Irfan et al. 2013; Madani et al. 2016; Zhang et al. 2019).

Tomato fruit growth and development are predominantly associated with the cytosolic Ca^{2+} concentration. Several studies signify that the main cause of BER is a localized deficiency of Ca^{2+} in the distal fruit tissue, due to defective local calcium transport or imbalanced incorporation of Ca^{2+} with the pectin, a cell wall component (Adams and Ho 1993; Taylor and Locascio 2004; de Freitas et al. 2012a). During cellular growth, mainly two types of cellular processes regulate the effective concentration of cytoplasmic Ca^{2+} in cells. The first involves Ca^{2+} distribution and partition in the cell and apoplast using transporters, secondly, Ca^{2+} can create a link with cell wall components pectin, improving cell wall mechanical strength. However, in conditions of stress or Ca^{2+} deprivation, the functions of these two pathways are compromised, resulting in a failure in Ca^{2+} partitioning, decreased membrane selective permeability, and cellular content leakage, which influences the incidence of BER (Fig. 1c). The difference in Ca^{2+} concentrations between the proximal and distal regions of the fruit is linked to the development of BER during fruit growth; larger the difference, higher is the incidence of BER.

Research indicates that in healthy tomato fruit, Ca^{2+} binding to the cell membrane increases during rapid cell development, whereas in fruit damaged by BER, it decreases (Suzuki et al. 2003). Several studies suggest that even though the Ca^{2+} concentration in the fruit is adequate when rapidly growing fruit tissue requires Ca^{2+}, an inadequate Ca^{2+} supply to rapidly growing tissue leads to the development of BER (de Freitas et al. 2018; Ho and White 2005; Ikeda and Kanayama 2015). Fruit exhibiting BER symptoms have much less Ca^{2+} than comparable unaffected fruits, indicating that fruit produced by plants cultivated in lower Ca^{2+} environments have a higher incidence of BER than fruit grown in higher Ca^{2+} environments.

Abiotic stress-induced BER has an influence on tomato output, and ineffective management measures increase the problem in terms of the frequency and severity of BER (Hagassou et al. 2019). The tomato susceptibility to BER also varies among cultivars, generally large-sized tomatoes are more susceptible to BER than small-sized tomatoes (Ho and White 2005). Variability appears to be caused by differences in fruit growth rate, Ca^{2+} content, and fruit xylem tissue development. Because BER only affects the top truss, which contains the fruits, it affects both green and ripe tomatoes, it is becoming a serious issue in tomato cultivars (Adams and Ho 1992). Abiotic stresses, phytohormones, high salinity, low humidity, high temperature, inadequate xylem tissue development, unfavorable moisture, and oxidative stress are additional factors that contribute to the BER disorder in the distal end of tomato fruits in addition to the Ca^{2+} deficiency (Taylor and Locascio 2004; Hagassou et al. 2019). This review summarizes the various factors that induce BER in tomato fruit, and different BER management techniques.

Symptoms and Occurrence of BER

The external symptoms of BER in fruit tissue begin with discolored, water-soaked, necrotic tissue at the distal end, which is associated with the collapse of epidermal and subepidermal parenchyma, following turgor loss which ultimately manifests as dark-brown and depressed abscesses on the fruit surface (Spurr 1959; Marcelis and Ho 1999; Ho and White 2005) (Fig. 1a). The first sign or external symptoms of BER in tomato fruits usually occur within two weeks of fruit set, when young fruit's relative growth rate is at its maximum (Spurr 1959; Adams and El-Gizawy 1988), however, the symptoms of BER in tomato fruit can potentially emerge later in development, at five weeks after pollination (Marcelis and Ho 1999; Rached et al. 2018; de Freitas et al. 2018). Cells in the water-soaked area have interruption of the plasma membrane and tonoplast (vacuolar membrane), wavy-shaped cell walls, broken endoplasmic reticulum, and swollen plastids. BER signs often appear externally on the pericarp near the distal end of the placenta, while they can also persist internally in the tissue and remain undetected (Adams and Ho 1992; Ho and White 2005). It is believed that internal BER is a mild form or an earlier stage of the development of external BER. Both are assumed to be the outcome of cellular death and the spilling of its contents into the extracellular space. After induction, the fruit's BER-affected patches often get bigger, turn into brown necrotic regions that cover a significant area of the fruit, and occasionally, they even infect the whole fruit. Soon after pollination, the symptoms could get worse and the fruit would never grow to its full size. Cells in the zone of necrotic BER tissue revealed normal internal structure, but the cell membrane was detached from the cell wall, indicating cell plasmolysis, which was not detected in other sections of the fruit or normal fruit cells (Taylor and Locascio 2004; Ho and White 2005).

Calcium Partitioning and Induction of BER

The plant cell wall contains 60–70% of the Ca^{2+} in plant tissue, making it the largest Ca^{2+} pool (Aghdam et al. 2012). Ca^{2+} is a well-known essential nutrient involved in many metabolic processes and signaling in plants. It also functions as a secondary messenger in diverse organisms and is critical for the stability and integrity of the cell wall and cell membrane (Gao et al. 2019). The uptake and transportation of Ca^{2+} from the soil to the shoot, fruit, and leaves of the plant depends upon the availability of Ca^{2+} content in the soil. The root takes up Ca^{2+} and is transported to the shoot and fruit utilizing apoplastic as well as symplastic pathways (Pathak et al. 2021). To cross the Casparian strip, it must enter the cytosol of endodermal cells via Ca^{2+}-channel proteins and then be exported back into the apoplast via Ca^{2+}-ATPases (Pittman et al. 2011; Pandey and Sanyal 2021). In tomatoes, the Ca^{2+} influx into fruit cells after vascular unloading has been postulated to occur via cyclic nucleotide-gated channel (CNGC) proteins (Thor 2019).

In plants, Ca^{2+} is utilized to perform mainly three primary functions throughout fruit growth and development (a) the partitioning and distribution of Ca^{2+} concentration in subcellular compartments, cytosol, and apoplast compartments, controls the activation of various proteins and signaling (b) Ca^{2+} interacts with the cell wall component pectin, forming a crosslinking that makes cell walls rigid. (c) Ca^{2+} also contributes significantly to cell membrane integrity by binding proteins and phospholipids in the plasma membrane (Hepler and Winship 2010). Furthermore, various types of biotic or abiotic stresses trigger signaling cascades in which calcium acts as a secondary messenger. Ca^{2+} enters into the cytosol from higher concentration compartments (apoplast, organelles) via channel proteins to induce an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]cyt$) involved in various downstream signaling pathways. However, if Ca^{2+} is transported out of the cytosol by Ca^{2+}-ATPases or Ca^{2+}/H^+-antiporters in the plasma or organelle membranes then it terminates the downstream signaling events (Fig. 2).
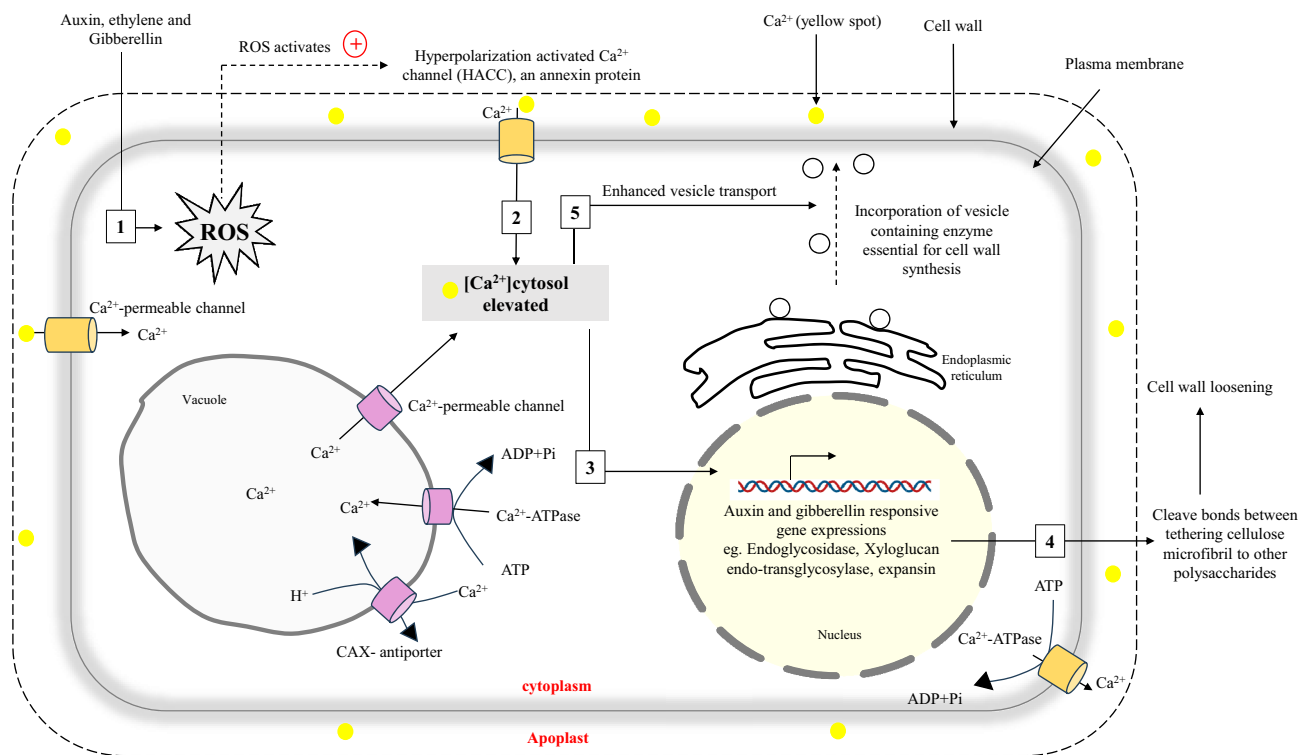

Fig. 2 Coordination between cytosolic Ca^{2+} concentration and cellular expansion during tomato fruit development. The various subcellular Ca^{2+} transporter involved in transport leads to the accumulation or depletion of Ca^{2+} depending on stimuli in plant cells. Similarly, in the vacuole localized Ca^{2+}-permeable channel, Ca^{2+}-ATPase is involved in increasing cytosolic Ca^{2+}, while CAX (Ca^{2+}/H^+ antiporter) is involved in the storage of Ca^{2+} from the apoplastic region to the vacuole. (1) Treatment with phytohormone causes the production of ROS, which activates hyperpolarization-activated Ca^{2+} channel (HACC) proteins (denoted by a circled positive symbol) (2) HACC proteins involved in Ca^{2+} transport from apoplast to cytoplasm region lead to an increase in cytosolic Ca^{2+} (3) Ca^{2+} increases the expression of phytohormone responsive, cell wall modifying, and expansin genes (4) Expression of various genes responsible for hydrolyzing the bond between cellulose microfibril and other polysaccharides, causing cell wall loosening (5) Increased cytosolic Ca^{2+} causes enhanced incorporation of vesicle-containing enzymes required for cell wall synthesis

Because of the poor phloem movement of Ca^{2+}, the transportation of Ca^{2+} mainly depends upon the xylemic and transpirational water flow (Bukovac and Wittwer 1957; Montanaro et al. 2012). During the early stage of fruit development, Ca^{2+} plays an important role in cell division, responsible for the formation of cell plates and cellular metabolism. In the subsequent stage of fruit development, Ca^{2+} plays an important role in cell–cell interaction (Hocking et al. 2016). The accumulation of Ca^{2+} in tomato fruit is regulated by growth rates and transpiration. The tomato fruit develops at a high rate in its early stages, as compared to later stages, this suggests that Ca^{2+} is essential for the early stages of fruit development (Gao et al. 2019). Since, the xylem is the in-charge of supplying Ca^{2+} to aerial sink organs such as fruit, where it accumulates, the fruit may have localized Ca^{2+} shortages as a result of reduced xylem Ca^{2+} mobility or an abnormality in xylem development (White 2001; Kumar et al. 2015).

As the fruit develops, xylem vessels transport Ca^{2+} to the fruit tissues, these vessels are smaller and less in numbers near the fruit's blossom end, than they are near its proximal end. The xylem:phloem ratio also decreases toward the fruit's distal end. Additionally, during the crucial stage of rapid fruit cell expansion, the xylem network in the pericarp tissue grows while just two single working strands remain in the placental tissue. These characteristics are thought to reflect the anatomical origin of the distant placental tissues linked to the initial appearance of BER (Riboldi et al. 2022). Ca^{2+} therefore contributes to cell growth, and aberrant $[Ca^{2+}]$cyt signals may arise from a local deficiency of accessible Ca^{2+}. During the later phases of fruit ripening, the phloem plays an important role in the transport of water, sugar, and basic nutritional inputs to most species (Dražeta et al. 2004).

One of the first indications of BER observed in tomato fruit is cell membrane rupture. This could happen if the $[Ca^{2+}]$apoplast around a cell was depleted too much, or it could be caused by increasing Ca^{2+} flow into the vacuole, a Ca^{2+} storage organelle that acts as a Ca^{2+} sink, demonstrating a strong inverse relationship between the incidence of BER and the apoplastic Ca^{2+} concentration in the distal half of tomato fruits tissues. Low calcium levels in the plant could lead to necrosis and curling in the leaf, bitter pit, BER, fruit cracking, and other physiological disorders during the development of fruit (Combrink 2013). A previous study suggests that the application of Ca^{2+} in the grapes enhances the flesh density and decreases the occurrence of *Botrytis cinerea* in grapes (Ciccarese et al. 2013; Gao et al. 2019). Another study suggested that the altered activity of Ca^{2+} channels, Ca^{2+}/H^+-exchanger, Ca^{2+}-ATPase, and Ca^{2+} sensors or inappropriate cellular Ca^{2+} partitioning and distribution in the apoplast of cells in early fruit developmental stages can induce BER (Ho and White 2005; de Freitas and Mitcham 2012).

Ca^{2+} transport proteins are found in plant cells, and research on these proteins has developed in *Arabidopsis thaliana* (Hirschi et al. 1996). Ca^{2+}-ATPases are integral membrane proteins found largely in the plasma membrane that transfer Ca^{2+} to the apoplast in plant cells utilizing ATP despite considerable concentration gradients. *CAXs* (Cation exchangers) are membrane proteins that transfer Ca^{2+} in exchange for proton ($H^{+)}$) or sodium (Na^+) gradients generated by major transporters, which can disturb Ca^{2+} homeostasis (Hirschi 1999; Pittman and Hirschi 2001) (Fig. 2). In yeast (*Saccharomyces cerevisiae*) mutants, two *Arabidopsis* Ca^{2+}/H^+ antiporter genes, *CAX1* and *CAX2*, can act as vacuolar Ca^{2+} transporters (Hirschi et al. 1996). *Arabidopsis thaliana* expresses the Ca^{2+}/H^+ antiporter *CAX1* (Cation Exchanger 1), which is found in the vacuolar membrane. In the *sCAX1* encoded version, the full-length gene is truncated at the N-terminus and lacks its regulatory region, resulting in a constitutively active Ca^{2+}/H^+-antiporter (de Freitas et al. 2011; Pittman and Hirschi 2001). When *sCAX1* was expressed in tomatoes, most of the fruit displayed BER symptoms (Cheng et al. 2005). This is because the overexpression of the *sCAX1* gene in the tomato plant causes Ca^{2+} to move from the apoplastic region to the vacuolar region of the cell, which lowers the Ca^{2+} level in the apoplastic region and causes membrane disintegration, a BER symptom, even in the presence of a "sufficient" Ca^{2+} concentration (de Freitas et al. 2011).

Even though there is enough Ca^{2+} in every part of the plant, transgenic tomato plants that have overexpressed *Arabidopsis CAX1* and *CAX2* exhibit severe Ca^{2+} deficiency-like symptoms like tip-burn and/or BER. Previous research has shown that Ca^{2+}/H^+-antiporter *sCAX2A*, a mutant variation of the *sCAX2* gene, causes increased Ca^{2+} accumulation in tomatoes; additionally, the incidence of BER was the same in both the wild-type control line and the *sCAX2A*-expressing lines (Chung et al. 2010). This conclusion was supported by the fact that numerous transmembrane Ca^{2+}-exchanger genes are involved in Ca^{2+} transport and the upregulation of one of these genes may cause the downregulation of other genes to preserve the Ca^{2+} flow into cellular storage organelles.

A similar study created transgenic plants expressing *sCAX1* and *CAX4* and discovered that the Ca^{2+} content of the transgenic plants was significantly higher than that of the vector controls, conversely, only the tomatoes expressing *sCAX1* had a significantly higher incidence of BER (Park et al. 2005). The *sCAX1*-expressing tomato had lower apoplastic and cytosolic Ca^{2+} concentrations than the wild-type tomato due to excessive Ca^{2+} accumulation in the vacuole. These results clearly showed that elevated *sCAX1* expression decreases Ca^{2+} mobility, which leads to the development of BER in fruits. Through simultaneous expression of *sCAX1* and calreticulin (CRT), a Ca^{2+}

binding protein located in endoplasmic reticulum, a study was able to correlate the frequency of Ca^{2+} accumulation with deficiency disorders in plants (Wu et al. 2012). The $sCAX$ + CRT-expressing and $sCAX$-expressing lines' Ca^{2+} concentrations, however, did not differ noticeably. These results showed that $sCAX1$ + CRT-expressing tobacco and tomato lines exhibited lower levels of Ca^{2+} deficiency symptoms (leaf tip necrosis and BER) than the line expressing $sCAX1$ alone. Table 1 provides an overview of research that looked into the genes responsible for transporting Ca^{2+} and their role in the emergence of disorders related to Ca^{2+}.

Another important component to which Ca^{2+} contributes is the fruit's cell membrane. The phosphate and carboxylate groups found in the phospholipids and proteins, in the cell membrane provide a bridge that Ca^{2+} crosses, reducing the permeability of the membrane and boosting its integrity. In strawberries and apricots, Ca^{2+} therapy delays fruit ripening and reduces cell wall disintegration (Figueroa et al. 2012; H. Liu et al. 2017). When applied to pear fruit, calcium chloride ($CaCl_2$) decreases permeability in the cell membrane, controls metabolism in the cell membrane, and lessens lipid peroxidation in the cell membrane, which is brought on by reactive oxygen species (ROS). Moreover, the efficacy of the plant's various Ca^{2+} sinks might influence Ca^{2+} accumulation in the tomato and produce Ca^{2+}-related physiological disorders such as blossom end rot (BER) (Ho and White 2005; Kumar et al. 2015). In conclusion, Ca^{2+} is a critical nutrient for cell wall structure, cell growth, plant tissue development, and membrane function, and it also acts as a counter-cation inside storage organelles, so Ca^{2+} concentrations at the cellular and extracellular levels will be highly regulated. Increased Ca^{2+} concentrations can cause cellular toxicity, increased rigidity of the fruit cell wall, and developmental and growth defects. Ca^{2+} deficiency can cause fruit cell walls to collapse and the development of a variety of physiological disorders including induction of BER in tomato fruits. In postharvest Ca^{2+} treatment, which lowers physiological abnormalities, fruit quality, and yield may be improved.

Calcium Interaction with de-Esterified Pectin and Induction of BER

The plant cell wall contains three layers the middle layer or middle lamella, the inner or secondary wall, and the outer layer, also known as the primary wall. The primary cell wall and middle lamella are primarily composed of cellulose microfibril which are crosslinked by branched polysaccharides such as hemicelluloses, and pectins (Cosgrove 2005; Lampugnani et al. 2018). The biosynthesis of pectins takes place in the Golgi apparatus, after that it is transferred to the fruit's cell wall (Lampugnani et al. 2018). The role of Ca^{2+} is very important for the cell wall during the development of the tomato fruit by binding with the de-esterified pectin which is present in the middle lamella of the plant cell (de Freitas et al. 2012a). During the growth period of the tomato fruit, the secreted pectin undergoes some modification, which is caused by the pectin methyl esterase, after which Ca^{2+} will interact with the carboxy-terminal of the demethylated pectin, where the interaction of Ca^{2+} and pectin leads to the thickening of the tomato fruit cell wall, when the interaction of pectin and Ca^{2+} changes, the stability of the cell wall collapses, resulting in BER.

Pectins are a highly diverse and complex family of heteropolysaccharides that are enriched in α-1,4 galacturonic acid (a sugar acid derived from galactose) subunits as principal components (Harholt et al. 2010). For example, homogalacturonans (HGs) are α-(1–4)-linked D-galacturonic acid linear chains polymer. The other structural type of pectin includes rhamnose/galacturonan disaccharide repeat rhamnogalacturonan-I (Mohnen 2008). The backbone pectin galacturonan residues can be decorated with other oligosaccharides such as α-(1,5)- arabinans and β-(1,4)-galactans with their methyl-esterified or acetyl derivatives. Highly esterified HGs are delivered to the wall where they undergo selective de-esterification, which facilitates polymer crosslinking via Ca^{2+} (Fig. 3).

A wide variety of branching and linear side chain derivatives of pectins are also observed, and they together constitute the pectin structural classes xylogalacturonan, rhamnogalacturonan-I & II, and apiogalacturonan (Zdunek et al. 2021). Rhamnogalacturonan-II is the most complex pectin, with up to 12 different sugar residues and over 20 different connection types. The expression of several enzymes that contribute to pectin modifications such as polygalacturonases and pectin methylesterases during growth and development contributes to its structural complexity, which implies that pectin may have several potential interactions and functional roles in plants (Bonnin et al. 2014). Pectin methylesterases can demethylesterify HGs, which affects cell wall flexibility because this change is required for HG cross-linking to other pectic polysaccharides and cell wall proteins. Pectin methylesterase inhibitors enhance pectin methylesterification and therefore wall loosening by inhibiting pectin methylesterases (Caffall and Mohnen 2009).

Pectin methylesterase (PME) is an enzyme found in the cell wall that modifies the binding of Ca^{2+} to the pectic acid in the cell wall (Micheli 2001). Pectin methyl esterase causes fluctuations in the amount of pectin secreted by tomato fruits during their growth phase (Micheli 2001). The amount of PME activity and the availability of Ca^{2+} in the apoplast has a direct effect on the strength and expansion of the cell wall. PMEs de-esterify the pectin polymers found in fruit cell walls. This results in the formation of a negative carboxyl group on which positive Ca^{2+} will bind. This interaction between Ca^{2+} and the pectin polymer resembles an

Table 1 An overview of studies on tomato that used genetic analysis to identify the genes responsible for Ca^{2+} deficiency disorders

| S. No	Candidate genes	Plant materials	Ca^{2+} deficiency-related disorder	Major comparison	References
1	Expression of Arabidopsis sCAX1 vacuolar H^+/Ca^{2+} exchanger	Tomato (Lycopersicon esculentum Mill. cultivars Red Cherry)	Increased incidence of BER	Wild-type vs. sCAX1 expressing line	(Park et al. 2005)
2	sCAX2A (Ca^{2+} antiporter)	S.lycopersicum cv. Ailsa Craig (AC)	No BER,	Wild type vs. sCAX2A-expressing plants	(Chung et al. 2010)
3	sCAX1 (Ca^{2+} antiporter)	S.lycopersicum. cultivar Ace 55 (Vf)	BER	Wild-type vs. sCAX1-expressing plants	(de Freitas et al. 2011)
4	Co-expression of Maize ER specific Ca^{2+} binding protein Calreticulin (CRT) and sCAX1 (Ca^{2+} antiporter and Calreticulin	sCAX1-expressing tobacco and tomato	Decrease in BER as compared to sCAX1-expressing plants Solanum lycopersicum 'Rubion'	sCAX1-expressing plants vs. sCAX1 and CRT-co-expressing plants	(Wu et al. 2012)
5	PME	Tomato (S.lycopersicum)	BER	Wild-type vs. PME-silenced plant	(de Freitas et al. 2012a)
6	CAX3 and Ca^{2+}-ATPase1 (Ca^{2+} antiporter)	Tomato (S.lycopersicum) cultivar 'Ace 55 (Vf)' and AB2	Gibberellin 4+7 treatment-induced BER, GA biosynthesis inhibitor Apogee had 0% BER development	Gibberellin (GA) treatments (Control (water), GA4+7, and prohexadione-calcium (GA biosynthesis inhibitor))	(de Freitas et al. 2012b)
7	The IL8-3 region of the S. pennellii genome introgressed into S. lycopersicum chromosome 8	Tomato (S.pennellii)	BER incidence was less in IL8-3 vs. the S. lycopersicum parent (M82d)	IL8-3 vs. the S. lycopersicum parent (M82d	(Uozumi et al. 2012)
8	Ca^{2+} antiporter (CAX3, 4, and 6 Solyc12g055750) Ca^{2+}-ATPase (Ca^{2+}-ATPase2 Ca^{2+}-ATPase3, Solyc04g016260 Solyc07g0026740 Solyc09g082870 Solyc10g079300)	Tomato (S.pennellii)	Higher expression in BER line IL8-3	BER-resistant introgression line (IL8-3) vs. the parent ('M82'), which is a BER-susceptible cultivar	(Ikeda et al. 2017)
9	SlGLR1.1 and SlGLR3.5 (Ca^{2+} channel) expressed in Arabidopsis	A.thaliana	Curl and deformed leaves	Wild type vs. SlGLR1.1 over expressing plant vs. SlGLR3.5 overexpressing plant	(Aouini et al. 2012)
10	A QTL located in chromosome 7	Tomato (Solanum lycopersicum cv. 'Moneymaker' ×S. pimpinellifolium accession TO-937	Tip burn	Temperature treatment Genetic mapping was conducted using 168 RILs and 52 Introgression Line (IL))	(Gonzalo et al. 2020)
11	35 candidate genes inhibiting BER and 7 candidate genes triggering BER	Tomato (Solanum lycopersicum L.) pericarp	BER	Growth regulator treatments (Control (water), GA4+7, prohexadione-calcium (GA biosynthesis inhibitor), and ABA	(de Freitas et al. 2018)
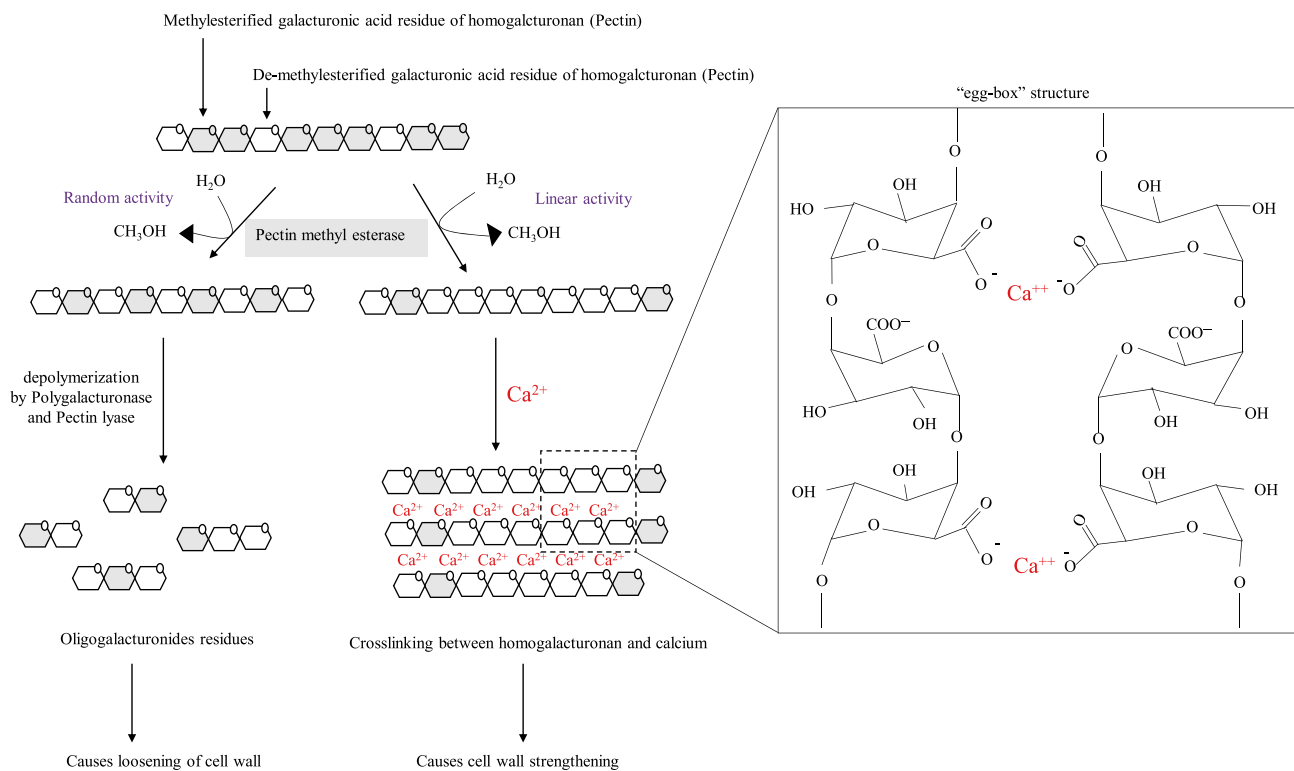
Fig. 3 Diagrammatic representation illustrating the function of the enzyme pectin methyl esterase in controlling the mechanical strength of cell walls. PMEs can act in two ways: either randomly, activating cell wall hydrolases like polygalacturonases (PG) and pectin lyase to cause the cell wall to loosen, or linearly, creating blocks of free carboxyl groups in homogalacturonan (pectin) that interact with bivalent ions (Ca^{2+}) to cause the cell wall strengthening. The cross-linking and interaction between Ca^{2+} and the pectin polymer is depicted in an "egg box" model. An expanded version of the "egg box" can be seen in the inset. (Gray hexagon-methylated and, White hexagon-demethylated residue of galacturonic acid)

"egg-box" which provides stability and integrity to the cell wall (Fig. 3). This cross-linking between the pectin and Ca^{2+} forms the structural part of the fruit and it also inhibits the BER and pathogen susceptibility in tomato fruit (de Freitas et al. 2012a, b; Hocking et al. 2016; Zhang et al. 2019; Gao et al. 2019). On the other hand, BER results from the collapse of the cell wall due to a change in the interaction between pectin and Ca^{2+} (de Freitas et al. 2012a). Tomato fruit differs from leaves in that its xylem moves more slowly and experiences less transpiration. This causes the fruit to become deficient in Ca^{2+}, which can result in many physiological illnesses.

A previous study also evaluated the effects of PME expression, the amount of Ca^{2+} bound to the cell wall, and the esterified pectin concentration on tomato fruit during BER development (de Freitas et al. 2012a). The expression of PME genes (*PMEU1, LOC544090, LOC544289, Les.9028, Les.10790,* and *Les.10560*), was analyzed in the tomato fruit (*Lycopersicon esculentum*) cultivar "Rutgers" and demonstrated that the key period for BER development in tomato fruit is exactly coincident with an increase in PME expression and activity. The research reveals a considerable decrease in the incidence of BER in PME-silenced tomato fruits that have less Ca^{2+} bound to the cell wall and exhibited higher total and apoplastic water-soluble Ca^{2+} concentrations in the pericarp. The total tissue, Ca^{2+} concentrations of wild-type and PME-silenced fruit were equivalent; however, the wild-type fruit had more membrane leakage, one of the early symptoms of BER, and reduced water-soluble apoplastic Ca^{2+} content. The study's findings suggest that the concentration of apoplastic water-soluble Ca^{2+} influences fruit sensitivity to Ca^{2+} deficient illnesses (de Freitas et al. 2012a).

In summary, the $[Ca^{2+}]$cyt can regulate the rapid cell expansion that occurs during the early stages of tomato fruit growth and development. Because expansion growth is a Ca^{2+}-dependent process that incorporates cell wall biosynthetic material into the cell wall and plasma membrane, a higher $[Ca^{2+}]$cyt has been linked to growth in a variety of cell types. A simplified and schematic representation of the regulation of cell growth and expansion by apoplastic and cytoplasmic Ca^{2+} is presented in Fig. 4a. The early stages of tomato fruit growth, when cells expand quickly leads to an increase in the relative volume of their vacuoles.
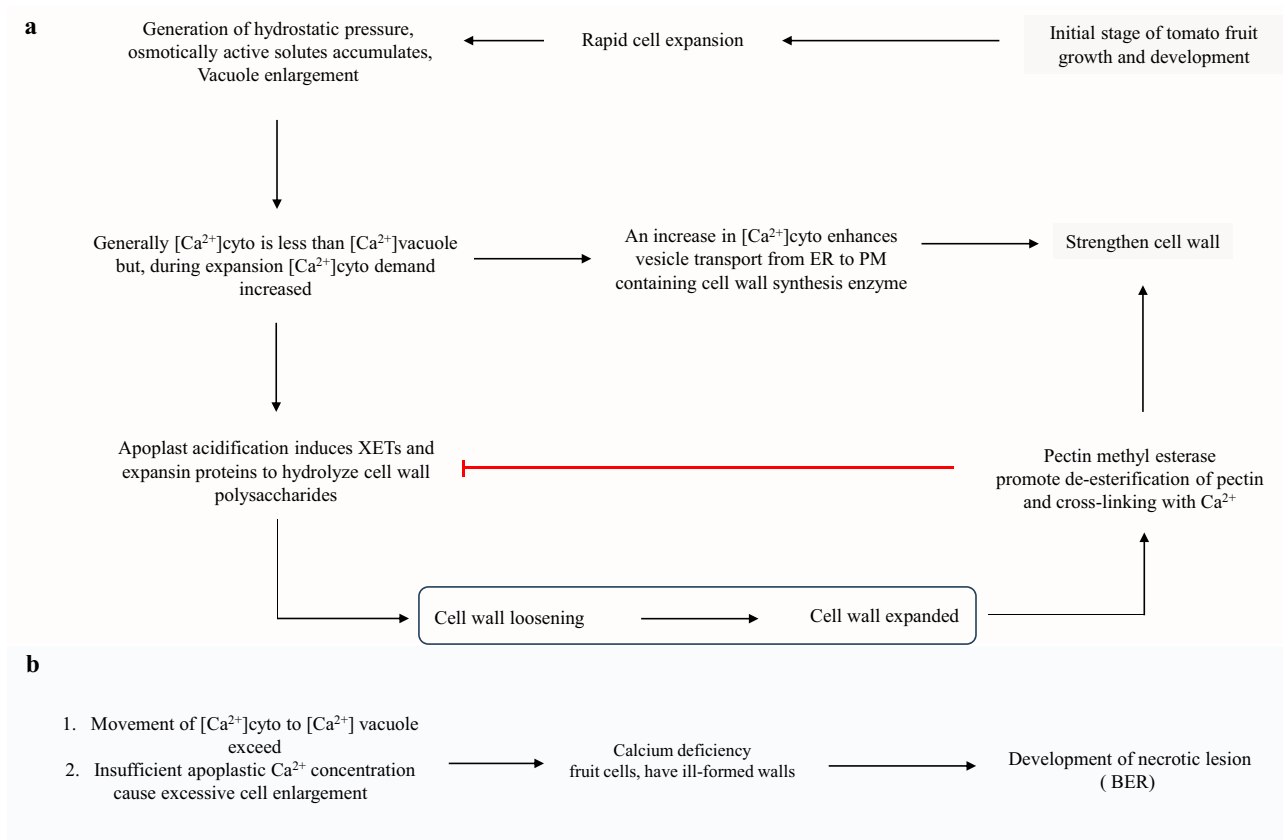
a

| Generation of hydrostatic pressure, osmotically active solutes accumulates, Vacuole enlargement | ← | Rapid cell expansion | ← | Initial stage of tomato fruit growth and development |

Generally $[Ca^{2+}]$cyto is less than $[Ca^{2+}]$vacuole but, during expansion $[Ca^{2+}]$cyto demand increased → An increase in $[Ca^{2+}]$cyto enhances vesicle transport from ER to PM containing cell wall synthesis enzyme → Strengthen cell wall

Apoplast acidification induces XETs and expansin proteins to hydrolyze cell wall polysaccharides —⊣ Pectin methyl esterase promote de-esterification of pectin and cross-linking with Ca^{2+}

Cell wall loosening → Cell wall expanded

b

1. Movement of $[Ca^{2+}]$cyto to $[Ca^{2+}]$ vacuole exceed
2. Insufficient apoplastic Ca^{2+} concentration cause excessive cell enlargement
→ Calcium deficiency fruit cells, have ill-formed walls → Development of necrotic lesion (BER)

Fig. 4 A simplified and schematic illustration of how apoplastic and cytoplasmic Ca^{2+} regulates tomato fruit cell growth and expansion (a). Cells expand rapidly during the early stages of tomato fruit growth and development, resulting in an increased cellular demand for calcium. Under normal conditions, which govern cell wall loosening and expansion, however, further expansion is controlled by pectin-methyl esterase by de-esterifying and interacting with Ca^{2+}. The schematic model is based on a previous study (Ho and White 2005).. (**b**) Excessive cell enlargement is caused by defects in the Ca^{2+} movement between the $[Ca^{2+}]$cyto and $[Ca^{2+}]$vacuole, as well as an insufficient apoplastic Ca^{2+} concentration. As a result of a Ca^{2+} deficiency in the fruit cells and an ill-formed cell wall, BER develops. The schematic model is based on a previous study (Ho and White 2005)

Appropriate maintenance of $[Ca^{2+}]$apoplast and $[Ca^{2+}]$cyt, along with regulated solute accumulation that is osmotically active, are necessary for cell expansion. As the osmotically active solutes accumulate, hydrostatic pressure is produced as a result of vacuolar enlargement. In typical circumstances, the $[Ca^{2+}]$cyt is smaller than the $[Ca^{2+}]$apoplast. However, the internal Ca^{2+} level rises during cell expansion due to the cytoplasm's elevated Ca^{2+} level brought on by the apoplasts' import of Ca^{2+} into the cytosol and the vacuoles' efflux of Ca^{2+}. The apoplast acidification caused by this elevated Ca^{2+} causes xyloglucan endo-transglycosylases/hydrolase (XETs) and expansin protein to hydrolyze the link between cellulose microfibril with other polysaccharide components of the cell wall. This leads to a loosening of the cell wall and cell expansion. Pectin methyl esterase promotes pectin de-esterification and cross-linking with Ca^{2+}, which strengthens the cell wall as the wall expands. Pectins also seem to prevent proteins like expansin proteins and xyloglucan endo-transglycosylases/hydrolase (XETs)

from accessing their substrates, which can alter cell wall rigidity. Furthermore, as cytosol Ca^{2+} concentration rises, vesicles containing enzymes and materials for cell wall synthesis are transported from the endoplasmic reticulum to the plasma membrane at an enhanced rate, further strengthening the cell wall (Fig. 4a).

Ca^{2+} is associated with abnormal regulation of its partitioning in different cellular compartments during BER development. The binding of Ca^{2+} to negatively charged lipids maintains the structural integrity of the plasma membrane. In the case of cytosolic depletion of Ca^{2+}, membranes become semi-permeable, resulting in the "leaky" state of charged solutes and cellular death. In addition, rapid fruit growth associated with increased pectin biosynthesis and de-esterification may lead to altered Ca^{2+} distribution and partitioning within the cell, resulting in lower apoplastic Ca^{2+} concentrations that damage membrane integrity and structure and increase fruit vulnerability to BER development. Furthermore, the plasma membrane and walls

surrounding cells in BER fruit that collapse during rapid cell expansion have lower Ca^{2+} concentrations (Fig. 4b).

Phytohormones Mediated BER Development

Phytohormones are signal molecules produced by plants in extremely low concentrations. Plant hormones regulate all aspects of plant growth and development, including pathogenic defense and tolerance to stress. Several studies have shown a strong correlation between the occurrence of Ca^{2+} deficiency disorders in plants, especially in the early stages of fruit development, and phytohormones (Gao et al. 2019). Many physiological processes have been reported to involve Gibberellins (GA) in cellular Ca^{2+} partitioning and distribution, moreover it has been shown to increase the expression of CAX and Ca^{2+}-ATPase in the membranes of rice aleurone cells (Bush et al. 1989; Gilroy and Jones 1992; Chen et al. 1997). Consequently, in plants treated with GA, the concentration of water-soluble apoplastic Ca^{2+} dropped while the frequency of BER increased (de Freitas et al. 2012b).

A transcriptomics study was carried out to determine the possible mechanisms of inducing or preventing BER development in tomato fruit in response to phytohormones such as gibberellins (GA) and abscisic acid (ABA) or the GA biosynthesis inhibitor "Apogee". From 26 to 40 days after pollination, the incidence rate of BER was negligible in Apogee and ABA-treated plants, but high in GA-treated plants (de Freitas et al. 2018). They have the opposite effect when it comes to Ca^{2+} uptake: whereas GA can lower Ca^{2+} concentration, increasing stress sensitivity and the likelihood of BER, ABA increases Ca^{2+} translocation into the fruit, inhibiting the occurrence of BER (de Freitas et al. 2018). During the early growth and development of the tomato fruits, the GAs is high, whereas the level of Ca^{2+} is low. This high level of GAs is responsible for the rapid cell expansion which causes the blocking of xylem tissue in the distal end of the fruit, which is later developed as BER. The application of GAs in fruit decreases the Ca^{2+} content in the apoplastic region of the fruit and increases the function of the Ca^{2+}/H^+ exchanger and Ca^{2+}-ATPase transporter which are present in the tonoplast plays an important role in the transport of Ca^{2+} into the storage organelle such as vacuole, which induces BER in tomato fruit. The use of GAs also raises ROS levels while decreasing the expression of many antioxidant genes like catalase, peroxidase, and superoxide dismutase (Fath et al. 2001).

Abscisic acid (ABA) has the opposite effect, reduced cell expansion, and strengthened cell structures. ABA promotes Ca^{2+} mobility into the fruit, leading to higher and water-soluble extracellular Ca^{2+} concentrations that stop the spread of BER in the fruit tissue. Furthermore, combined treatment of ABA and Apogee (an inhibitor of GA biosyn-

thesis) can inhibit the development of BER by increasing the Ca^{2+} level in the fruit tissues (Heuvelink and Körner 2001). The foliar application of ABA increases the Ca^{2+} accumulation in the shoot and the xylem tissue at the distal end of the tomato fruit which reduces the incidence of BER. ABA treatment directly on fruit does not affect xylem sap flow rates or Ca^{2+} movement into the fruit, but it did raise water-soluble apoplastic Ca^{2+} concentrations and somewhat reduce fruit susceptibility to BER. During the fruit's early growth and development, ABA treatment increased the number of functional xylem vessels, which facilitated the translocation of Ca^{2+} toward the tissue close to the blossom end. The blossom-end tissue experienced a greater accumulation of Ca^{2+} following the whole-plant ABA treatment in contrast to the fruit-specific ABA treatment. This could be because the ABA treatment also increased the fruit's flow rate of xylem sap and its absorption of Ca^{2+}-containing xylem sap. Fruit's susceptibility to BER development decreased, membrane leakage decreased, and concentrations of water-soluble apoplastic Ca^{2+} increased as a result of this accumulation. In this scenario, selecting new tomato cultivars with enhanced ABA biosynthesis for reduced fruit susceptibility to BER may be an option in addition to conserving water.

A disruption in the fruit's polar auxin transport could also influence the development of BER. It's interesting to think that a decrease in xylogenesis and a subsequent poorer transport of calcium to the distal tissue could be the cause of this phenomenon. A high Ca^{2+} concentration rate in apples and pears decreases ethylene production by inhibiting the 1-aminocyclopropane-1- carboxylic acid oxidases (ACO) which is a precursor for the biosynthesis of ethylene (Gao et al. 2019). Ca^{2+} regulates the cell wall degrading enzymes such as cellulase, pectin esterase, pectin lyase, β- and galactosidase. If pectin degradation occurs, the free Ca^{2+} increases, leading to the loss of cell–cell interaction and fruit softening (Sasanuma and Suzuki 2016). It has also been suggested that ethylene plays a role in BER induction. However, it's also possible that ethylene and other "stress" factors that raise ROS production could affect the occurrence of BER by activating hyperpolarization-activated Ca^{2+} channels (HACCs), an annexin protein, which would raise $[Ca^{2+}]cyt$ and cause rapid cell expansion (Fig. 2, follow steps 1, 2, 3). A summary of genes involved in the induction or inhibition of BER upon phytohormone treatment is given in Table 2.

Oxidative Stress Induces BER Development

Previous research has examined the potential link between oxidative stress and BER incidence in tomato fruits to identify a major cause of BER development other than Ca^{2+} deficiency (Schmitz-Eiberger et al. 2002). Oxidative stress induced by reactive oxygen species (ROS) can result from any circumstance that disrupts the redox balance within cells (De Gara

Table 2 List of genes potentially involved in modulation of BER. (Data adapted from the previous transcriptomic study (de Freitas et al. 2018)

A	Genes down-regulated in higher BER fruit and potentially inhibiting BER development		
	Genes involved in biological process	**Name of gene(s)**	**Protein encoded by gene(s)**
1	Transcriptional regulation	*SRO2*	Radical-induced cell death-one 2
		DREB	Dehydration-responsive element-binding protein
		ERF109 and *ERF010*	Ethylene-responsive factors
		CBF1	C repeat/dehydration-responsive element binding factor 1
2	Hormone signaling	*MeJA-est*	methyl jasmonate esterase
		WAT1	Walls are thin 1
		AREB	ABA-responsive element-binding protein
3	Redox reactions	*TIC32*	Translocon at the inner envelope membrane of chloroplasts 32
		Class III-ADH	Alcohol dehydrogenase class-3
4	Protein metabolism	*FBXL2*	F-box/LRR-repeat protein 2
		PIIF	Wound-induced proteinase inhibitor I prepropeptide
		SRP7s	Signal recognition particle 7 s
		UBLCP1	Ubiquitin-like domain-containing CTD Phosphatase
		CBL	Calcineurin B-like molecules), CIPK 1 and 2 (interacting protein kinase 1 and 2)
5	Lipid metabolism	*GDSL*	Esterase's/lipases,
		AAE7	Acetate/butyrate-CoA ligase
		LTP1	Lipid Transfer Protein 1
6	Cellular defense response	*JRL19*	Jacalin-related lectin 19
		STO	Salt tolerance protein
		PRP6	Pathogenesis-related protein 6
7	Cellular detoxification	*LGL*	Lactoylglutathione lyase-like
8	Nutrient metabolism	*TAT*	Tyrosine aminotransferase
		MTP1	Metal tolerance protein 1
		CCH	Transport protein Copper Chaperone
B	**Genes up-regulated in higher BER fruit and potentially inhibiting BER development**		
	Genes involved in biological process	**Name of gene(s)**	**Protein encoded by gene(s)**
1	Transcriptional regulation	*ZAT11* and *ZAT12*	Zinc finger transcription factor)
2	Hormone signaling	*TIFY10A*	Repressors of JA signalling
3	Redox reaction	*Rboh*	Respiratory Burst Oxidase Homolog
		AO	L-Ascorbate oxidase
4	Protein metabolism	*SOBIR1*	Leucine-rich repeat receptor-like
			Protein kinase
5	Cell wall modification	*PME3*	Pectinesterase inhibitor 3

et al. 2010; Schieber and Chandel 2014). ROS overproduction is one of the main reasons for decreased productivity, damage, and plant death in response to environmental stress. Superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are the most dangerous ROS produced by plants because of their capacity to cross membranes and accumulate at varying degrees within cells (Sharma et al. 2012; Turhan et al. 2006). Membrane fluidity is often reduced as a result of lipid peroxidation caused by the formation of free oxygen radicals and H_2O_2, which can exacerbate membrane leakage and tissue degradation (Dhindsa et al. 1981; Chen and Yu 1994; Alché 2019) Both

Ca^{2+} and ROS signaling are interconnected secondary messengers that respond to a variety of environmental stresses. Ca^{2+} controls ROS production, whereas ROS controls Ca^{2+} homeostasis (Kobayashi et al. 2007; Görlach et al. 2015; Marcec et al. 2019).

During abiotic and biotic stress ROS generated by the plasma membrane NADPH oxidase, by-products of mitochondrial, endoplasmic reticulum, and chloroplast respiratory chain (Turhan et al. 2006; Jiang et al. 2011). The balance between ROS generation and detoxification determines whether ROS is a threat to cells or plays a role in response signaling (Ayer et al. 2014). Plant cells can
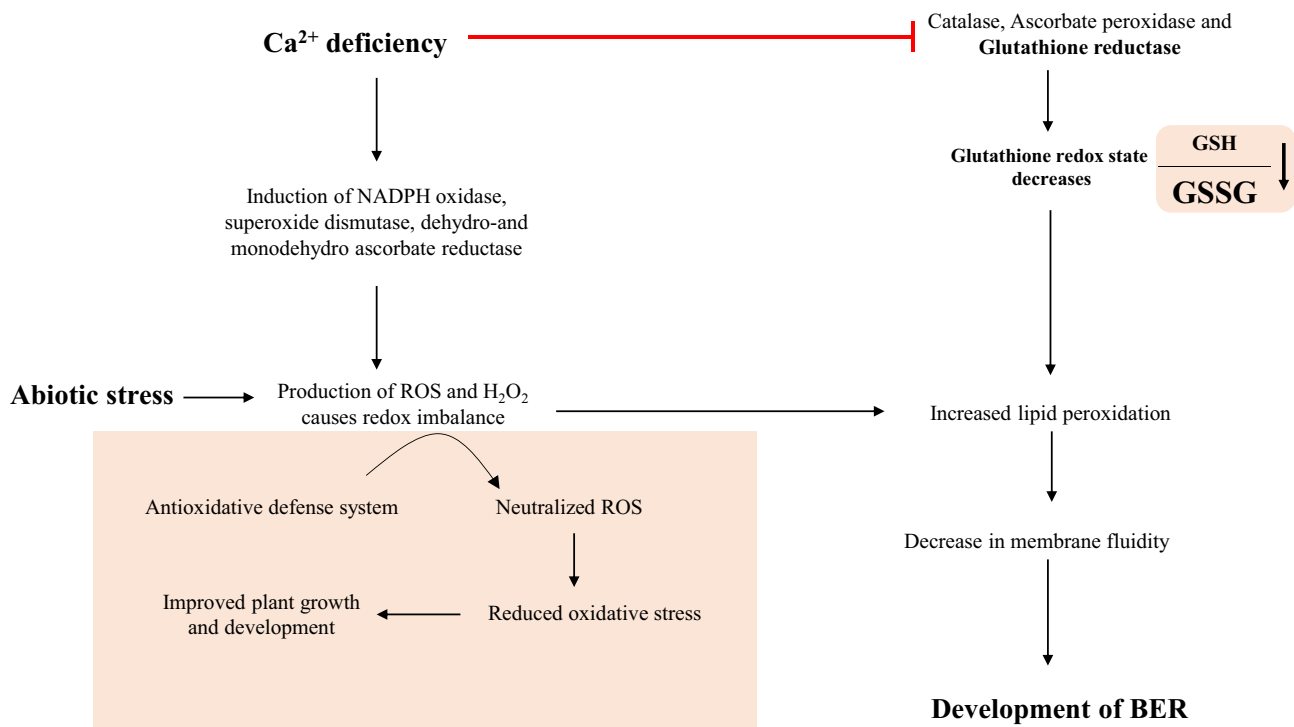
Fig. 5 Increased membrane lipid peroxidation and BER formation in fruit cells are caused by an increase in oxidative stress molecules (ROS), which alter the cellular redox level because of Ca^{2+} deficiency or abiotic stress. Ca^{2+} deficiency inhibits several enzymes, including catalase, ascorbate peroxidase, and glutathione reductase, and induces NADPH oxidase, superoxide dismutase, and dehydro- and mono-dehydro ascorbate reductase. These conditions cause an increase in the production of reactive oxygen species (ROS) and a decrease in cellular redox, both of which have an impact on the development of BER via lipid peroxidation

reduce the negative effects of ROS by utilizing enzymatic and non-enzymatic antioxidant pathways (Fig. 5) (Das and Roychoudhury 2014). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), monode-hydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) are the example of enzymatic anti-oxidants. Non-enzymatic antioxidants are ascorbic acid (vitamin C), α- Tocopherols, glutathione, carotenoids, and phenolic compounds such as flavonoids, tannins, lignin, and hydroxycinnamate esters which are abundant in the plant tissue (Ahmad et al. 2010; Ayer et al. 2014; Topcu et al. 2022). The Ascorbate–Glutathione pathway, which consists of four major enzymes (APX, MDHAR, DHAR, and GR) and two antioxidants Ascorbate–Glutathione (AsA- GSH), is important in the detoxification of ROS in plants (Foyer and Noctor 2011). BER is associated with excessive ROS and unbalanced glutathione homeosta-sis which causes lipid and protein peroxidation, enzyme inhibition, and cellular membrane leakage (Mestre et al. 2012). Tomatoes cultivated in Ca^{2+}-deficiency environ-ments have increased BER occurrence which is related to the increased expression of NADPH oxidase and SOD (Mestre et al. 2012). Similarly, peppers cultivated under

high saline environments have elevated ROS accumulation in the apoplast because of elevated NADPH oxidase activ-ity (Aktas et al. 2005). Conversely, tomatoes cultivated in Ca^{2+}-deficient environments exhibit down-regulation of numerous antioxidant genes, including CAT, APX, and GR (Gong and Li 1995; Schmitz-Eiberger et al. 2002; Yang and Poovaiah 2002). As a result, ROS is regarded as an important factor in the onset and progression of BER (Fig. 5) (Van Breusegem and Dat 2006; Sharma et al. 2012; Topcu et al. 2022). According to studies, GSH is essential for shielding plants from the majority of envi-ronmental stresses in multiple ways (Chakraborty et al. 2021). The likelihood of this physiological disorder is dependent on the degree of GSH homeostasis and its cellular concentration, both of which are influenced by GR activity (Mestre et al. 2012). Tomato cultivars with naturally high ascorbate and anti-oxidant levels during the most susceptible stage of BER are more resilient to the disorder than those with lower levels of antioxidants, regardless of fruit Ca^{2+} concentration. Additionally, BER does not always affect the whole fruit. This could be due to higher lignification, antioxidants, and oxidative stress-related proteins, which prevent BER from spreading to surrounding healthy tissues (Mestre et al. 2012; Rached

et al. 2018; Reitz and Mitcham 2021; Topcu et al. 2022). Antioxidants and ROS-generating enzymes work together to greatly accelerate the development of BER, which is made worse by low Ca^{2+} concentration and abiotic stress (Aloni et al. 2008; Rached et al. 2018).

A proteomic approach to BER in tomato (*Lycopersicon esculentum M.*) fruits revealed the induction of proteins from the pentose phosphate pathway as well as specific antioxidant (ascorbate–glutathione cycle) enzymes, implying that these two metabolic pathways limit the blackening of the entire fruit by acting as scavengers of ROS in BER-affected fruits (Casado-Vela et al. 2005). Furthermore, a comparative transcriptome study in tomato fruits from an introgression line found a correlation between higher fruit ascorbic acid concentration and high expression of pectinesterase family proteins and two polygalacturonases related to pectin degradation. This is primarily accomplished by increasing flux through the L-galactonic acid pathway, which is driven by pectin degradation and may be triggered by ethylene (Di Matteo et al. 2010). According to a prior study, pepper (*Capsicum annuum L.*) fruit that is susceptible to BER may exhibit alterations in apoplastic pH, a decrease in apoplastic AsA concentration, and an increase in apoplastic ascorbate oxidase activity as a contributing factor to the development of BER symptoms (Aloni et al. 2008). One of the most popular processing tomatoes in California, USA, HM 4885, had an 85% BER incidence, which contributed to catalase activity suppression, resulting in higher ROS accumulation (Reitz and Mitcham 2021). In conclusion, decreased activity of the ROS detoxification pathway can result in massive H_2O_2 deposition, lipid peroxidation, and membrane degradation, DNA breakdown, protein degradation resulting in increased BER incidence (Fig. 5).

Abiotic Stress-Mediated BER Induction

Even though Ca^{2+} deficiency is a main contributor, BER can be induced by a variety of abiotic stresses. Numerous studies demonstrate the connection between BER and abiotic stressors like excessive salinity, drought, relative humidity, shoot and root temperatures, high light levels, and mineral imbalance (Taylor and Locascio 2004; Ho and White 2005). The induction of BER by stress factors does not rule out the possibility of Ca^{2+} involvement, because changes in cytosolic Ca^{2+} ([Ca^{2+}]cyt) will most likely play a role in coordinating the cellular reactions to all of these stressors. Many studies have been conducted to examine the impact of abiotic stresses on the Ca^{2+} transport to the fruit, particularly in the distal end of the fruit, and has been found to regulates plant Ca^{2+} uptake, Ca^{2+} motility to the fruit, Ca^{2+} role in the cell, and fruit susceptibility to Ca^{2+} deficiency disorders.

High light and high air temperature conditions speed up fruit expansion, which may be related to higher photo-synthetic rates and photoassimilated fruit delivery. Plants exposed to high temperatures and light levels experience a reduction in cell differentiation, an alteration in membrane permeability, and an increase in ROS production, which ultimately results in lipid peroxidation within the plant cell (Ho and White 2005; Roeber et al. 2021; Sachdev et al. 2021). The ideal range for root temperature to transfer Ca^{2+} into a plant is between 15 °C and 27 °C. Root temperature also controls the amount of Ca^{2+} that enters the plant. The amount of Ca^{2+} transported to the plant is reduced if this temperature changes (de Freitas and Mitcham 2012).

Some nutrients are resistant to absorption while others are not. High soil monovalent cation concentrations, such as potassium (K^+), magnesium (Mg^+), sodium (Na^+), and ammonium (NH_4^+), inhibit divalent cation Ca^{2+} uptake, increasing the frequency of BER. Increased NH_4^+ concentration in the nitrate/ammonium ratio, for example, reduced Ca^{2+} absorption and increased BER formation. Fruits obtained from a BER-resistant cultivar exhibited a strong link between boron (B^+) and Ca^{2+} concentrations in the distal region of each fruit, whereas susceptible accessions exhibited no association between the two elements. In this scenario, the relationship between boron (B^+) and Ca^{2+} could disclose a role in cell wall pectin structural stabilization (Watanabe et al. 2021). High nitrogen (N) levels promote shoot growth, which has been linked to increased Ca^{2+} transport towards the leaves and away from the fruit, presumably due to higher leaf transpiration rates at the whole plant level (Ho and White 2005). High quantities of nitrogen are also known to cause rapid fruit and cell development, which may result in further diluting of the fruit's limited Ca^{2+} content which can lead to BER (de Freitas and Mitcham 2012). K^+ is found to be involved in cell-expansion-related processes, high levels of K^+ may stimulate rapid plant and fruit growth, which could reduce fruit Ca^{2+} uptake, dilute fruit Ca^{2+} content, and increase fruit sensitivity to BER. Ca^{2+} might theoretically be replaced by large quantities of K^+ and Mg^{2+} at the plasma membrane surface, but not for its function in membranes, this can cause a leaky plasma membrane and increases the incidence of BER in fruit tissue (de Freitas and Mitcham 2012).

To adapt to different abiotic stressors and survive in low light, they must be able to sense both the quality and quantity of light and respond appropriately (Roeber et al. 2021). Climate change also exposes plants to more unfavorable environmental conditions, which reduces fitness and crop production. Drought and salinity have been shown to promote Ca^{2+} deficiency disorders in fruits (Bashir et al. 2021; Manishankar et al. 2018). The effect of drought, salinity, and low relative humidity on enhancing fruit sensitivity to Ca^{2+} deficit-related disorders may be due to plant water stress, which has been proposed as a means of restricting xylemic Ca^{2+} mobility into the fruit. Furthermore, drought and salinity have been shown to decrease the number of

functional xylem vessels in fruit, lowering fruit Ca^{2+} uptake. Both drought and salinity produce ROS in the plant which is the major cause of developing BER in the tomato fruit (Ho and White 2005). Fruit Ca^{2+} concentrations are typically lowered simultaneously by the effects of excessive light and air temperatures. Plants exposed to high temperatures and light cause their cells to become less differentiated, alter the permeability of their membranes, and produce more ROS, which in turn causes lipid peroxidation in the plant's cells. Alteration in temperature also causes inhibition of Ca^{2+} uptake by the plant and influences the development of BER. A summary of various abiotic factor-mediated cellular alterations that lead to the development of BER in tomatoes is provided in Fig. 6a.

In conclusion, the majority of environmental conditions that influence the incidence of BER disrupt the link between the rate of cell expansion and Ca^{2+} buildup in growing fruit. Similarly, Ca^{2+} sequestration in expanding vacuoles of a fruit cell during a period of low Ca^{2+} supply may deprive the cytoplasm or apoplast of Ca^{2+} required for intracellular signaling or cellular function. This may be exacerbated by specific mineral stressors that inhibit calcium absorption or promote the synthesis of organic acids that chelate Ca^{2+}. Decreases in $[Ca^{2+}]$apoplast can lead to altered responses to environmental or developmental stimuli that are triggered by Ca^{2+} influx; modifications in plasma membrane permeability that result in uncontrollable solute fluxes and solute leakage; and compromised cell wall properties that lead to premature cell expansion and structural weakness. This would ultimately lead to an irreversible cell death (Fig. 6b).

Genetic Factor's Contribution towards BER

The genomic techniques that, with the aid of more extensively accessible genomic resources for tomatoes, can be utilized to look into the genetic mechanisms supporting BER. A simple approach would be to use association analysis to correlate phenotypic data with allelic diversity by surveying allele variation in the entire population (Caballero and Rodríguez-Ramilo 2010). The chromosomal loci influencing the occurrence of BER can also be identified using genomic regions that contribute to variation in quantitative trait locus (QTL) or quantitative phenotype analyses (Corre and Kremer 2003; Powder 2020). This will provide insights into the physiologi-
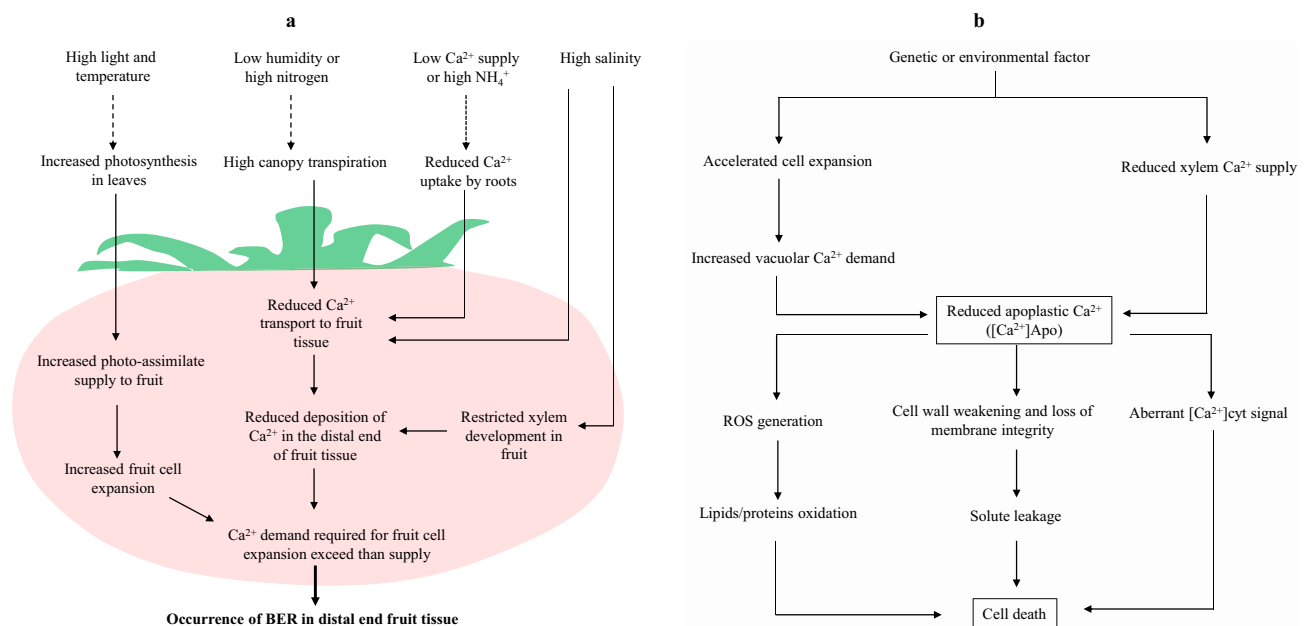

Fig. 6 **a** A simplified model for the impact of various abiotic stress factors involved in the occurrence of BER in distal fruit tissue. Blossom end-rot is promoted by environmental factors such as high light and temperature, low humidity, or high nitrogen, low Ca^{2+} supply or high ammonium, and high salinity. A common mechanism is a decrease in local Ca^{2+} content in the distal end of tissue, which affects the Ca^{2+} demand required for fruit cell expansion and induces the occurrence of BER. The schematic model is adapted and modified from a previous study (Ho and White 2005). **b** A schematic representation of how different environmental and genetic factors cause tomato fruit to develop blossom-end rot (BER), which results in cell death. In the distal section of young tomato fruits, environmental and genetic factors facilitate the rapid expansion of cells with enhanced delivery of Ca^{2+} to vacuoles from the cytosol. Reduced apoplastic Ca^{2+} concentration is the result of an imbalance between Ca^{2+} supply and Ca^{2+} demand brought on by increased cell expansion. This has an impact on cytosolic Ca^{2+} signals, ROS generation, compromised cell wall, and membrane integrity which can affect solute leakage which ultimately leads to cell death. The schematic model is adapted and modified from a previous study (Ho and White 2005)

cal mechanisms causing BER and will direct future breeding efforts and research. Previous studies using tomato introgression lines (ILs) provided the first information about the genetic foundations of BER. The genomic segments of *Solanum pennellii* LA716 that comprise ILs were introduced into *Solanum lycopersicum* cv. M82 (Eshed and Zamir 1995). Of these lines, IL8–3 has a lower BER incidence as compared to the parent M82, and fine mapping this region revealed a segment of 610 kb regions containing 78 genes (Uozumi et al. 2012; Watanabe et al. 2021). These results suggested that IL8-3 may harbor gene(s) affecting Ca^{2+} concentration and growth rate in the early stages of fruit development because of the higher Ca^{2+} concentration in the distal portion of the fruit and the initially slower growth rate in the BER-resistant line.

Additional examination of these IL8–3 lines ten days after flowering also showed that M82 and IL8–3 differed from each other in the expression of several genes related to Ca^{2+} transport, such as Ca^{2+}/H^{+}-exchanger (CAX), Na^{+}/Ca^{2+}-exchanger (NCX) Ca^{2+}-ATPase, and Ca^{2+}-channel however, none of these genes were mapped to the region where IL8–3 is located on chr08 (Ikeda et al. 2017). Possibly the genes located in the 610 kb region of IL8-3 controls, Ca^{2+} transport-related genes in other chromosomes, could explain these findings. Similarly IL5-4, which is situated on chr05, BER differences were also noted; however, in this case, the BER differences are more pronounced in the IL than in the control M82 (Matsumoto et al. 2021). Through the enrichment of SNPs linked to the trait, molecular markers to map BER loci in the population are created by employing the QTL seq approach (Topcu et al. 2021). Populations resulting from crosses between *Solanum lycopersicum var. cerasiforme* (SLC) *and S. lycopersicum var. lycopersicum* (SLL) contained four loci: BER3.1 and BER3.2 on chr03, BER4.1 on chr04, and BER11.1 on chr11 (Topcu et al. 2021). BER3.2 and BER11.1 were fine-mapped to 1.58 and 1.13 Mb, respectively, even though BER11.1 had previously been mapped in a different population derived from SLL cv Ailsa Craig and SLL cv Kentucky Beefsteak (Prinzenberg et al. 2021). According to the findings, BER3.2 is most likely related to FW3.2/KLUH, the fruit weight gene that was found to be segregating in one of the populations.

A comparison of large vs mid-sized tomato cultivars revealed that the difference in BER susceptibility is most likely driven by changes in genetic factors, which may be closely related to potential fruit size and mostly determines water-soluble Ca^{2+} delivery in the tissue. The size of the tomato fruit and the occurrence of BER are co-related to each other (Heuvelink and Körner 2001; Ho et al. 2015), because the incidence of BER does not occur in the small tomato fruits that don't have the phase of rapid cell expansion, but it affects the large fruits that have the phase of rapid cell expansion (Topcu et al. 2022). The susceptibility of BER is different in different sizes of fruit. Except for FW3.2/

KLUH, five loci in tomatoes have been identified as being involved in the genetic basis of BER studies: Chr 03, Chr 04, Chr 05, Chr 08, and Chr 11. The function of these various loci could be examined using a biotechnological method, which can offer fresh perspectives on BER developments.

The tomato gene Cell Size Regulator (FW11.3/CSR) causes cells to enlarge, which increases fruit weight (Mu et al. 2017). FW11.3 near-isogenic lines (NILs) carrying the derived allele of CSR had a significantly higher BER incidence than FW11.3 NILs carrying the wild-type allele, indicating that FW11.3/CSR may be involved in the development of BER. The relationship between BER and these fruit weight genes is most likely indirect rather than causative, as many tomato types with the derived fruit weight alleles are resistant to BER. In addition to fruit size, elongated fruit forms are more prone to BER than round-fruited cultivars (Grandillo et al. 1996; Ku et al. 2000; Riboldi et al. 2018). A small number of genes—SUN, OVATE, OFP20, and FS8.1—are responsible for controlling the elongated fruit form of tomatoes. Among these genes, the minimal BER incidence is associated with the fs8.1 round fruit allele (Ku et al. 2000; Liu et al. 2002; Xiao et al. 2008; Wu et al. 2018). Furthermore, the variations in OVATE mutation-carrying San Marzano or banana legs with the SUN mutation have a very high potential for BER (Riboldi et al. 2018).

Prevention and Control Strategies for BER

Increasing crop yield under target conditions should always begin with the adoption of so-called good agricultural practices (Kılıç et al. 2020). Even though the exact mechanism responsible for BER onset remains unknown, many management strategies have been employed to prevent and control the frequency of BER, beginning with an assessment of the characteristics of the soil, proper irrigation, Calicum spray, utilizing BER resistant genetic variety, and possible use of genetically engineered plants. These methods could be paired with efficient irrigation strategies that are dependent on the water's quality, growth regulators, and maintaining balanced nutrition which are crucial components in preventing abiotic stressors. Figure 7 provides an overview of the several approaches that can be used to avoid and manage BER.

Improve the Soil Qualities

Both directly and indirectly, biochemical mineral and biological soil factors (symbiotic bacteria and fungi) influence the availability of nutrients, which impacts the prevalence of BER. Since tomato is a crop that is only moderately sensitive to soil salinity, the only practical way to avoid BER and significant output losses is to remove salt from the root zone using a process known as "reclamation" (Shaygan and
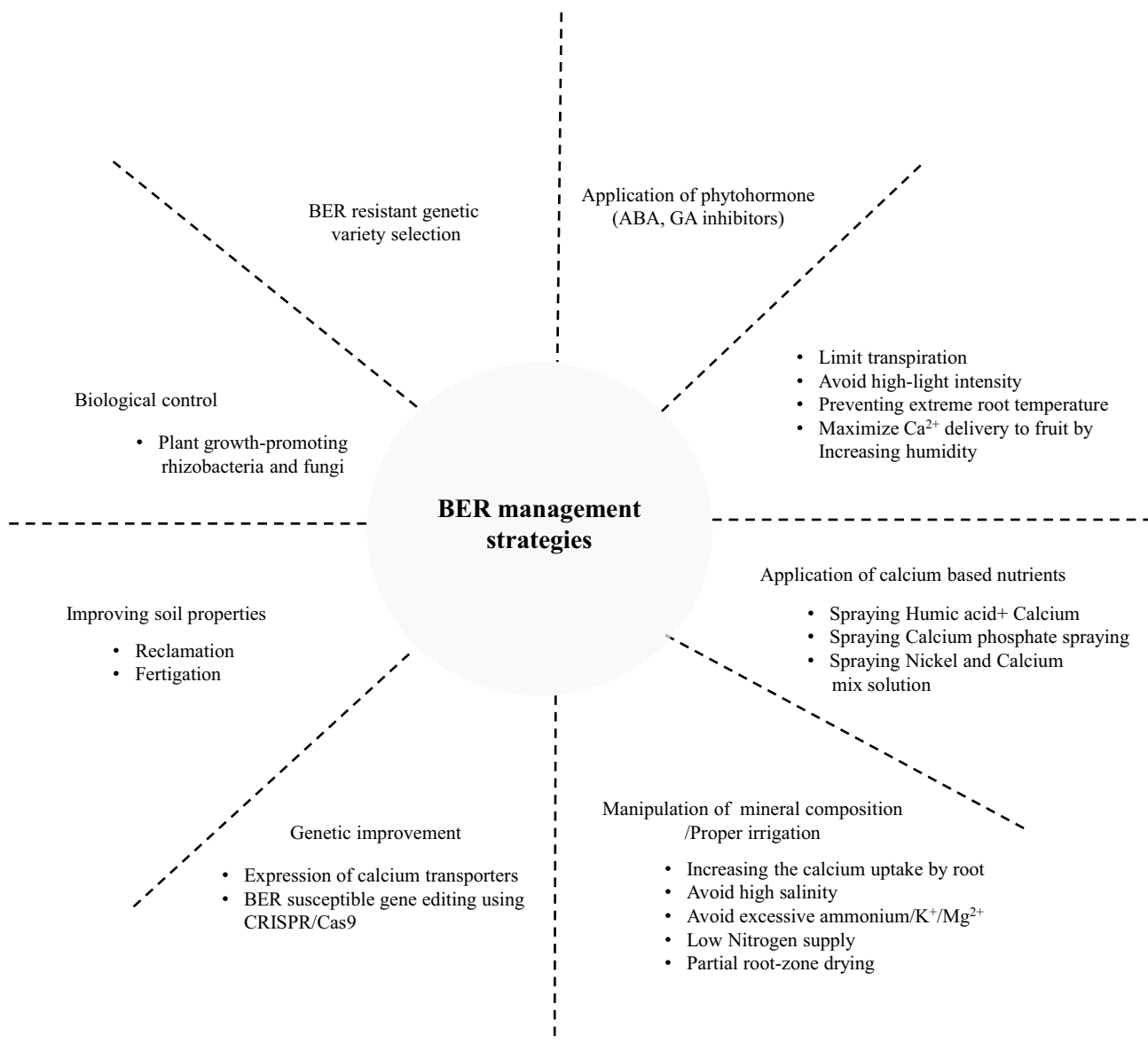
Fig. 7 Various management strategies that can be used to reduce BER in tomatoes. Calcium-based nutrients, BER-resistant genetic variety selection, and genetic improvements are examples of major technologies that can be used effectively to reduce BER

Baumgartl 2022). In addition, a widely used farming practice "fertigation" the process of applying fertilizers with irrigation water, improves the timing and availability of nutrients while making it simple to regulate the concentration of the fertilizer (Machado and Serralheiro 2019). Drip irrigation, which reduces inputs and delivers nutrients to the root zone, is used in the most efficient fertigation systems. This helps to reduce the effects of salt stress and prevent soil salinization (Costa et al. 2018). According to earlier research, there may be relationships between the rhizosphere microbiome, enzyme activity (mainly phosphatase, catalase, and invertase), and BER in tomatoes (Shao et al. 2018). The incidence rate of tomato BER was

significantly inversely related to the relative abundance of soil microorganisms but positively related to the abundance of bacteroides.

Proper Irrigation

An adequate water supply is required to maximize the quality and productivity of tomato crops (de Pascale et al. 2011; Halli et al. 2021). Conversely, BER occurrence may significantly increase in response to a high water supply (Zhai et al. 2016). A reduced irrigation technique known as partial root-zone drying (PRD) irrigates only half of the root system. PRD can lower the incidence of BER in tomato

plants and increase water use efficiency without appreciably affecting yields (Jovanovic and Stikic 2018; Iqbal et al. 2020). Many tomato-growing regions around the world struggle with high salinity levels in irrigation water, which have an adverse effect on fruit quality and yield. Irrigation with saline water is highly associated with lower yields, primarily due to a corresponding drop in fruit weight and a notable rise in the percentage of fruit with BER symptoms (Jha et al. 2017; El-Mogy et al. 2018). To avoid waterlogging and root hypoxia, which both lower fruit yields, tomatoes grown in saline irrigation require less frequent watering (Ide et al. 2022; Daniel and Hartman 2023).

Application of Calcium-based Nutrition

The most popular and initial agronomic technique to prevent BER development has been controlling the soil's Ca^{2+} accessibility. Soils containing high concentrations of monovalent cations, such as K^+, Na^+, and NH_4^+, can have a detrimental effect on the intake of Ca^{2+} (Hagassou et al. 2019). Consequently, these results imply that calcium is an essential component for the plant that can lower the frequency of BER in tomato fruit. The foliar application of 2% $CaCl_2$ decreased the incidence of BER and produced a large yield of tomatoes (Mazumder et al. 2021). Tomato fruit BER incidence is reduced by exogenous application of calcium nitrate or calcium liquid formulation (Syahren et al. 2012). Applying phosphorous solution to tomato plants increases their uptake of Ca^{2+}, reduces the incidence of BER in tomato fruit, and promotes vegetative growth and fruit yield (Alam et al. 2017). Similarly, applying a humic acid and calcium mixture helps tomato plants grow less stressed in saline water and lessens the likelihood of BER in tomato fruit (Kazemi 2014; Kataoka et al. 2017). Furthermore, applying nickel solution to tomato plants raises fruit yield, lowers BER incidence, and increases Ca^{2+} accumulation in the shoots (Macedo et al. 2022). Moreover, the application of ascorbic acid decreases the incidence of BER in tomato fruit, because it acts as an antioxidant that protects the plant tissue from ROS (Rached et al. 2018). In actuality, BER can be avoided by decreasing canopy management and enhancing Ca^{2+} transport toward fruit transpiration or through canopy Ca^{2+} sprays (Wani et al. 2021; Karlsons et al. 2023).

Application of Phytohormones

During the early fruit development, the amount of GAs is high which promotes rapid cell expansion, due to rapid cell expansion the obstruction of xylem tissue occurs which can lead to BER in tomato fruits (Topcu et al. 2022). The exogenous application of GA inhibitor decreases the incidence of BER in tomato fruit (de Freitas et al. 2012b). In addition, the root and foliar application of ABA increases the Ca^{2+} accu-

mulation in the tomato shoots and fruits, it also increases the xylem tissue development at the distal end of the tomato fruit which reduces the incidence of BER.

BER-Resistant Variety Selection

Certain tomato cultivars are more prone to BER than others such as cherry tomatoes have not been reported to have BER, but plum and pear-shaped tomatoes are more prone than round-fruited varieties. This has led to the hypothesis that the varied ways in which phloem-borne leaf assimilate and xylem-borne calcium are delivered to the distal end of the fruit in response to the growth environment are linked to the vulnerability of cultivars to BER. Fruit from BER-susceptible cultivars does, in fact, typically contain less calcium than fruit from non-susceptible cultivars, especially right after anthesis (Franco et al. 1994). In a previous study, to elucidate the relationship between BER incidence and oxidative stress, two BER-resistant cultivars, "Managua RZ" and "House Momotaro," and one BER-susceptible cultivar, "Reiyoh," were cultivated under salinity or standard nutrient solution (control) conditions. It was suggested that, in response to BER-inductive growth conditions, BER-resistant cultivars showed a larger increase in their ROS scavenging capacity, represented by ascorbate than BER-susceptible cultivars (Rached et al. 2018). Thus, choosing cultivars resistant to BER may be another strategy to reduce BER. Like this, Marmande-type tomato varieties had a higher prevalence of BER than cherry, cocktail, or round tomato varieties, which indicates that the genetic architecture of fruit is important (Hagassou et al. 2019). Grafting tomato plants could be a viable solution to mitigate the effects of salt and drought stress (He et al. 2009). To avoid BER, rootstock breeding with improved nutrient absorption, tolerance to salt stress, and fruit quality can be used (King et al. 2010).

Conclusions

Ca^{2+} is essential for fruit growth and development because it regulates various cellular activities and metabolism. BER is a Ca^{2+} deficiency disorder that primarily affects fruit tissues at the blossom end. Plants with altered $[Ca^{2+}]$ cyt homeostasis play a role in the onset of BER. BER is induced despite an adequate Ca^{2+} level in tomato fruits due to a defect in Ca^{2+} distribution and partitioning by the Ca^{2+}/H^+-exchangers, Ca^{2+}-ATPase, and Ca^{2+}-channels and a defective distribution of the xylemic vessel at the distal end of the fruit. Deregulated expression of an *Arabidopsis* Ca^{2+}/H^+ antiporter (*sCAX1*) raises total Ca^{2+} in crops but may cause yield losses because of symptoms resembling a Ca^{2+} deficiency. Given that *sCAX1* expression can significantly increase Ca^{2+} accumulation in edible

crop tissues (such as lettuce, tomato, potato, and carrot), the use of *sCAX1* for Ca^{2+} biofortification has been thoroughly studied in a variety of horticultural crop species. Ca^{2+} deficiency can also influence the degree of methylation of homogalacuronan pectin in the cell wall, which modulates apoplastic Ca^{2+} level and governs membrane stability and BER appearance. Any abiotic factors, that increase cell expansion and decrease the apoplastic Ca^{2+} level in the cell wall can cause the development of BER in tomato fruits. The primary antioxidant defense mechanism is suppressed by Ca^{2+} deficiency or oxidative stress, resulting in an excess of ROS. The accumulation of ROS leads to increased lipid peroxidation, resulting in increased membrane leakage and the development of the BER in tomato fruits.

Because physiological and environmental factors both have a significant impact on the incidence of BER, many agricultural settings find it difficult to manage this disorder under field and greenhouse growth conditions. Soil quality-improving techniques can help to reduce the induction of BER. However, to reduce BER, more emphasis must be placed on utilizing crop germplasm's genetic diversity. Uniform soil moisture using proper mulching techniques, a suitable drainage system, appropriate irrigation, soil quality improvement, proper irrigation, and balanced application of fertilizer are all effective ways to manage BER. In addition, there are other tactics like resistant cultivar selection, enhancing Ca^{2+} uptake, and application of ABA and GA inhibitors that also help to reduce the incidence of BER in tomato fruits. It is anticipated that genetic research and the identification of genes linked to BER in tomato fruit will advance our knowledge and mechanism of BER in tomatoes. Meanwhile, increasing the apoplastic Ca^{2+} concentration in susceptible fruit tissue should provide a simple reliable, practical solution for the prevention of BER in tomatoes. Biofertilizers are also can be used to diminish the effect of climatic change on crops. Novel approaches for crop improvement in many vegetables are becoming possible as genetic studies are shedding more and more light on the underlying genes that cause BER. For example, downregulating or eliminating BER susceptibility tomato genes through CRISPR/Cas9 mediated gene or promoter editing should lead to commercially produced varieties that are more resistant to BER. Consequently, it is expected that the toolkit for suppressing BER will expand, offering breeders fresh methods to produce varieties more resistant to this often-fatal physiological disorder.

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Declarations

Ethical Approval This article does not contain any studies with human participants or animals by any of the authors.

Consent for Publication Written informed consent for publication was obtained from all the authors.

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