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RESEARCH PAPER

Abscisic acid triggers whole-plant and fruit-specific mechanisms to increase fruit calcium uptake and prevent blossom end rot development in tomato fruit

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Abstract

Calcium (Ca) uptake into fruit and leaves is dependent on xylemic water movement, and hence presumably driven by transpiration and growth. High leaf transpiration is thought to restrict Ca movement to low-transpiring tomato fruit, which may increase fruit susceptibility to the Ca-deficiency disorder, blossom end rot (BER). The objective of this study was to analyse the effect of reduced leaf transpiration in abscisic acid (ABA)-treated plants on fruit and leaf Ca uptake and BER development. Tomato cultivars Ace 55 (Vf) and AB2 were grown in a greenhouse environment under Ca-deficit conditions and plants were treated weekly after pollination with water (control) or 500 mg l^{-1} ABA. BER incidence was completely prevented in the ABA-treated plants and reached values of 30–45% in the water-treated controls. ABA-treated plants had higher stem water potential, lower leaf stomatal conductance, and lower whole-plant water loss than water-treated plants. ABA treatment increased total tissue and apoplastic water-soluble Ca concentrations in the fruit, and decreased Ca concentrations in leaves. In ABA-treated plants, fruit had a higher number of Safranin-O-stained xylem vessels at early stages of growth and development. ABA treatment reduced the phloem/xylem ratio of fruit sap uptake. The results indicate that ABA prevents BER development by increasing fruit Ca uptake, possibly by a combination of whole-plant and fruit-specific mechanisms.

Key words: ABA, BER, calcium deficiency, leaf transpiration, stomatal conductance, xylem sap.

Introduction

Blossom end rot (BER) is a physiological disorder associated with plant water stress and low levels of Ca in fruit tissue that can negatively affect production of many vegetables and fruit, including tomato (White and Broadley, 2003; Saure, 2001; Taylor and Locascio, 2004). The symptoms of this disorder start with water-soaked tissue near the blossom end region that eventually darkens as cells die and can cover half of the fruit surface (Abdal and Suleiman, 2005). BER is believed to result from plasma membrane breakdown in response to low levels of free Ca in the apoplast, which is required for proper membrane function (Hanson, 1960; Zhao *et al.*, 1987; McLaughlin and Wimmer, 1999; Plieth, 2001; Saure, 2001; Suzuki *et al.*, 2003). Increasing overall fruit Ca uptake has been shown to reduce the risk of BER development (Ho and White, 2005). It has been suggested that fruit Ca uptake may be promoted by decreasing leaf transpiration (Adams and Ho, 1992; Guichard *et al.*, 2005), and/or by increasing the abundance of functional xylem vessels in the fruit (Ho *et al.*, 1993; Taylor and Locascio, 2004; Ho and White, 2005).

The pattern of water and nutrient flow to fruit and leaves in the plant has been extensively studied, and it is generally believed that Ca is transported within the plant exclusively in the xylem (Ferguson and Watkins, 1989; White and Broadley, 2003; Ho and White, 2005). Consequently, although phloem sap accounts for most of the water used for fruit growth and transpiration, xylem sap is the source of Ca required to prevent BER development (Ho *et al.*,

Abbreviations: ABA, abscisic acid; BER, blossom end rot; Ca, calcium; CaC, fruit Ca content; $[Ca]_{xylem}$, calcium concentration in xylem sap; DAP, day(s) after pollination; PSU, phloem sap uptake in the fruit; TFSU, total fruit sap uptake; Ts, fruit transpiration; VPD, vapour pressure deficit; XSU, xylem sap uptake in the fruit.

1987; Ho and White, 2005). It has been estimated that the majority (80%) of pedicel sap water flux to tomato fruit remains as part of expansive growth, whereas only 20% of the sap flux is lost by transpiration from the fruit (Araki *et al.*, 2000).

Because of the relatively high phloem water uptake (70–85% of fruit water uptake) and low transpiration of the fruit compared with leaves, it has been suggested that under high leaf transpiration conditions (high atmospheric vapour pressure deficit, VPD), most of the xylemic water and Ca will flow towards the leaves and away from the fruit (Ho, 1989; Adams and Ho, 1992; Araki *et al.*, 2000; Tadesse *et al.*, 2001; Taylor and Locascio, 2004; Guichard *et al.*, 2005). Consistent with this understanding, increasing fruit transpiration has been shown to be more effective in increasing fruit Ca uptake than increasing Ca concentration in the substrate (Paiva *et al.*,1998). Under such conditions, the term 'competition' has been extensively used to explain the partitioning of xylemic Ca between fruit and leaves (Clarkson, 1984; Ho, 1989; Ho *et al.*, 1993; McLaughlin *et al.*, 1999; Taylor and Locascio, 2004; Sharma *et al.*, 2006). If fruit Ca uptake occurs through the xylem, then the potential total fruit Ca uptake will simply be the product of xylem inflow to the fruit and xylem Ca concentration. It is clear that Ca in the soil does not, on its own, determine Ca availability to the fruit, because Abdal and Suleiman (2005) reported that tomato plants grown in calcareous soil can develop 50% BER in the fruit. Cell expansion and fruit growth is also dependent on water movement into the fruit and previous work has shown a reduction in fruit size and yield under high leaf transpiration conditions (Eguchi *et al.*, 2003; Guichard *et al.*, 2005; Magan *et al.*, 2008), but whether these effects are related specifically to high leaf transpiration, or generally to the occurrence of plant water deficit as a result of high transpiration, is not clear.

Calcium is thought to be delivered along with water exclusively via the xylem vessels to the expanding fruit cells (Ferguson and Watkins, 1989; Ho and White, 2005). The density of functional xylem elements decreases during fruit expansion and the number of functional xylem elements reduces towards the blossom end region of tomato fruit (Ho and White, 2005). The combination of accelerated cell expansion and poor abundance of functional xylem elements in the fruit is generally linked to BER development because of restricted Ca uptake and movement in the fruit (Ho and White, 2005; Peet, 2009). There is also evidence that low plant water status may be linked to a low number of functional xylem vessels in tomato fruit (Belda and Ho, 1993).

Abscisic acid (ABA) is a growth regulator involved in plant responses to biotic and abiotic stresses and perhaps the most studied role of ABA in plants is its regulation of stomatal closure, leaf transpiration, and plant water potential (Verslues and Zhu, 2007). Higher ABA levels in the leaves, more specifically in the guard cells, triggers a complex pathway leading to stomatal closure and reduction in water loss by transpiration (Garcia-Mata and Lamattina, 2003). Although tomato fruit have a small number of stomata at early stages of growth and development, the concentration of stomata in the fruit is much lower than in the leaves (Blanke, 1986). Therefore, treating tomato plants with ABA is a useful approach to specifically reduce leaf transpiration without significantly changing fruit transpiration, and to analyse the effect of lower leaf transpiration and higher plant water status on fruit Ca uptake and BER development. The hypothesis we have tested in this study is that tomato plants treated with ABA have lower leaf transpiration and higher plant water status, which increases fruit Ca content by increasing xylemic water and Ca flow into the fruit, eventually reducing BER development. The objective of this work was to analyse the effect of reduced leaf transpiration in ABA-treated plants on fruit and leaf Ca uptake and BER development.

Materials and methods

The processing tomato *Solanum lycopersicum* cultivar AB2 and fresh market tomato cultivar Ace 55 (Vf) were grown in 9.5 l pots containing organic substrate (0.33 peat, 0.33 sand, 0.33 redwood compost with 2.6 g kg^{-1}dolomite lime in a greenhouse environment in spring 2008 and 2009, respectively. The same treatments were applied to both cultivars. At full bloom, fully opened flowers were selected, tagged, and manually pollinated on each plant to monitor the chronological age of the fruit. One day after pollination (DAP), each plant was sprayed with 0.2 l of deionized water (control) or 500 mg l^{-1} ABA (Valent Biosciences, Libertyville, Illinois, USA), each solution containing 0.5 ml l^{-1} polysorbate 20 (Tween®20; Fisher Scientific, Fair Lawn, NJ, USA) as a surfactant. The combination of concentration and timing of ABA treatment was defined in preliminary experiments as the concentration and timing of ABA treatment that would maintain leaf stomatal conductance lower and stem water potential higher than control plants for the entire period of fruit growth and development. There were four single plant replications per treatment. The treatments were applied every 7 d to the same plants for 40 d in the cultivar AB2 and 45 d in the cultivar Ace 55 (Vf).

Before initiating the treatments, the plants were irrigated every day with a fertilizing solution containing N (102 mg l^{-1}), P (26 mg l^{-1}), K (124 mg l^{-1}), Ca (90 mg l^{-1}), Mg (24 mg l^{-1}), S (16 mg l^{-1}), Fe (1.6 mg l^{-1}), Mn (0.27 mg l^{-1}), Cu (0.16 mg l^{-1}), Zn (0.12 mg l^{-1}), B (0.26 mg l^{-1}), and Mo (0.016 mg l^{-1}). On the day of manual pollination, 20 g of slow release fertilizer containing N (150 g kg^{-1}), P (90 g kg^{-1}), K (120 g kg^{-1}), Mg (10 g kg^{-1}), S (23 g kg^{-1}), Fe (4.5 g kg^{-1}), Mn (0.6 g kg^{-1}), Cu (0.5 g kg^{-1}), Zn (0.5 g kg^{-1}), B (0.2 g kg^{-1}), and Mo (0.2 g kg^{-1}), but without Ca (Osmocote Plus®; Scotts-Sierra Horticultural Products Co., Marysville, OH, USA) was added to the pot for each plant. From this time forward, the plants were irrigated every other day with deionized water until saturation of the substrate. Tomato plants and fruit were analysed 3 d after each treatment was applied.

All plant and fruit evaluations were accomplished for both tomato cultivars. The only exception was the water-soluble apoplastic Ca concentration in fruit tissue, which

was evaluated only on the tomato cultivar Ace 55 (Vf). The plant and fruit evaluations are described below.

Plant parameters

Stem water potential was measured at midday during a 2 h period (1:00–3:00 p.m.) following methods outlined by McCutchan and Shackel (1992). Briefly, on each plant, one leaf directly connected to the main stem was enclosed in a reflective envelope to suppress leaf transpiration for ~20 min to allow the water potential of the leaf to equilibrate with the water potential of the stem at the point of attachment. Each bagged leaf was then excised and placed into a pressure chamber (PMS Instrument Company, Albany, OR, USA) for water potential measurement (Fulton *et al.*, 2001). Stomatal conductance was measured in two fully expanded leaves located on opposite sides of each tomato plant (fifth to seventh basipetally located leaves) at midday during a 2 h period (1:00–3:00 p.m.) using a steady-state porometer (LI-1600; LI-COR Biotechnology, Lincoln, NE, USA). Both stem water potential and stomatal conductance were analysed at 12, 24, 31, 38, and 45 DAP in Ace 55 (Vf) and at 12, 26, and 40 DAP in AB2 tomato plants.

To determine water loss over a 24 h period, plants in 9.5 l pots were irrigated early in the morning with 2 l of water. After draining, the pots were bagged with a plastic bag and weighed. Twenty-four hours later, the plants were weighed again and the volume of water lost to the atmosphere was calculated as the difference between the first and the last weight. Plant fresh weight was previously determined to be equal in water- and ABA-treated plants. Therefore, for simplification, the results are presented as weight of water lost per plant. Weekly water loss measurements were used to estimate the daily water loss, which was then used to calculate the total amount of water lost per plant. Root weight was determined by cutting the plant at the substrate level, washing the root, and weighing each root after drying at 20 °C for 1 h. Root weight was determined at 12, 24, 31, 38, and 45 DAP in Ace 55 (Vf) and at 12, 26, and 40 DAP in AB2 tomato plants.

Fruit parameters

Percentage BER incidence was determined by dividing the number of tagged fruits with BER symptoms by the total number of tagged fruit. Membrane leakage was determined in three fruit discs of 1 cm diameter and 0.7 cm thickness (~3 g each) cut from the blossom end of healthy fruit (without visible symptoms of BER) with a stainless steel cork borer, and sectioned with a double-bladed knife 1 mm under the skin. Each sample of three discs from three fruit represented one replication and was placed into a 50 ml conical tube on a rotary shaker in a mannitol solution (0.2 M) with similar water potential to the disc tissue (0.68 MPa), and the conductivity was recorded periodically over 6 h. After 6 h, the samples were frozen and thawed twice to determine total conductivity. The values were expressed as a percentage of the total conductivity (Saltveit, 2002). The tissue water potential was determined by incubating pericarp discs in solutions containing different concentrations of mannitol. The tissue water potential was determined based on the mannitol concentration that did not change tissue weight after incubation. Similar tomato fruit water potential was also found in previous studies (Guichard *et al.*, 2005).

Xylem function was measured in developing fruit. Fruit were harvested and held in sealed plastic bags for ~20 min with 100 ml of free water to reduce transpiration until the peduncle of each fruit was immersed in a solution of 1% Safranin-O at 20 °C under ⩽20% relative humidity. After 24h, fruit were cut into three equal sections at a 90° angle to the peduncle axis. The number of stained vascular bundles (xylem vessels) was counted in the placenta and pericarp tissues at the cut surfaces at the blossom and peduncle end regions of each fruit.

Water-soluble apoplastic Ca concentration in pericarp tissue was measured in 12 fruit discs of 1 cm diameter and 0.3 cm thickness (total ~11 g fresh weight) cut from the blossom end of the fruit with a stainless steel cork borer and sectioned with a double-bladed knife beginning 1 mm under the skin. Each sample of 12 discs, two discs from each of six fruits, represented one replication. After cutting, each disc was rinsed in deionized water for 10 s and blotted dry. Each disc was then placed in a funnel containing a flat acrylic membrane (1.2 cm diameter) with pore size 10–16 μm (Kimax®; Kimble, Vineland, NJ, USA). The funnel was then placed and sealed in a kitasato flask (Pyrex®, Lowell, MA, USA), and 10–15 Hg of vacuum was applied with a vacuum pump. A mannitol solution with similar water potential to the disc tissue (0.68 MPa) was slowly dripped over the entire disc surface (300 μl) and collected in the kitasato flask. After repeating the same procedure for all 12 fruit discs, the mannitol solution accumulated in the kitasato flask was used for Ca quantification, representing the water-soluble apoplastic Ca content. The entire procedure was accomplished at 4 °C. Cell damage was not detected under a light microscope Olympus SZH10 (America Inc., Lake Success, NY, USA) when samples were analysed before and after the extraction of water-soluble apoplastic Ca.

Mass balance approach to analysis of fruit and leaf Ca uptake

Calcium concentrations can differ within the fruit and between leaves, and therefore the calculations of tissue and plant Ca content are estimates of the actual amounts. To reduce variability in our study, Ca analysis was made using blossom end tissue of fruit without visible symptoms of BER harvested from the first and second clusters, and from the fifth to the seventh basipetally located fully expanded leaves on each plant. The fruit blossom end tissue was chosen because it is the place where BER develops. The first and second fruit clusters were used because most of the fruit growth and development in these clusters takes place before the beginning of plant senescence. The fifth to seventh basipetally located leaves were used because these were the

youngest fully expanded leaves on each plant at the beginning of ABA treatment.

The tissue was collected, frozen in liquid N_2, and freeze-dried. Samples were subjected to microwave acid digestion/dissolution and analysed for Ca by inductively coupled plasma atomic emission spectrometry (Meyer and Keliher, 1992). Total leaf and fruit Ca content per plant was calculated by multiplying the Ca concentration in the leaf or fruit dry weight (DW) by the total leaf or fruit DW per plant. Total leaf and fruit Ca accumulation per plant observed from 12 to 45 DAP [Ace 55 (Vf)] and 12–40 DAP (AB2) were calculated by subtracting the total leaf or fruit Ca content per plant observed at 45 DAP [Ace 55 (Vf)] or at 40 DAP (AB2) by the total leaf or fruit Ca content per plant observed at 12 DAP, respectively.

Leaf and fruit DWs were determined by harvesting, freeze drying, and then weighing leaf and fruit samples. Fruit dry matter was expressed as a percentage and was calculated by multiplying the fruit DW by 100 and dividing by its respective fresh weight. Average fruit fresh weight was calculated by dividing the total fresh weight of tagged fruit per replication by the number of tagged fruit in each replication. The total number of fruit and fresh fruit weight per plant was determined by counting and weighing both tagged and untagged fruit on each plant. Water loss per increase in plant and fruit DW was calculated based on the daily average total plant water loss divided by the daily average increase in the DW of shoots, leaves, and fruit (not root) from 12 to 45 DAP [Ace 55 (Vf)] or from 12 to 40 DAP (AB2). Plant water loss was determined as described before and the values obtained from 12 to 45 DAP [Ace 55 (Vf)] or from 12 to 40 DAP (AB2) were used to estimate the daily plant water loss. The average increase in DW was obtained by subtracting the DW observed at 45 DAP [Ace 55 (Vf)] or 40 DAP (AB2) by the value observed at 12 DAP and divided by the number of days between 12 and 45 DAP [Ace 55 (Vf)] or between 12 and 40 DAP (AB2), respectively. Calcium concentration in the xylem sap was calculated by dividing the total fruit plus leaf Ca content accumulated per plant from 12 to 45 DAP [Ace 55 (Vf)] or from 12 to 40 DAP (AB2) by the total water loss per plant observed during the same period in each cultivar.

Analysis of xylem and phloem sap uptake in tomato fruit

The analysis of xylem sap uptake (XSU) was based on the assumption that Ca uptake into the fruit takes place exclusively through the xylem (Ho and White, 2005). In this case,

$$XSU = CaC/[Ca]_{xylem}$$

Where, CaC=fruit Ca content and $[Ca]_{xylem}$= Ca concentration in the xylem sap.

The analysis of phloem sap uptake (PSU) was based on the assumption that total fruit water content accumulated from 12 to 45 DAP [Ace 55 (Vf)] or from 12 to 40 DAP (AB2) was supplied only by xylem and phloem vessels. Therefore, PSU into the fruit is equal to the total fruit sap uptake (TFSU) minus the XSU plus fruit transpiration (Ts). In this case,

$$PSU = TFSU - XSU + Ts$$

Based on previous studies, Ts=0.375 g of water fruit^{-1} d^{-1}, for the same tomato fruit developmental stage (Liu *et al.*, 2007). The values obtained for the calculations represent the daily averages obtained from 12 to 45 DAP [Ace 55 (Vf)] or from 12 to 40 DAP (AB2). The results were expressed as ml of sap uptake g^{-1} fruit DW d^{-1} by dividing the data by the average fruit DW.

Results

BER ranged from 30% to 45% in the water controls and was completely prevented in the ABA-treated plants (Fig. 1A, 2A). BER was present as early as 24 DAP in Ace 55 (Vf), and at 40 DAP in AB2. Membrane leakage was higher in tomato fruit treated with water than in fruit treated with ABA in both Ace 55 (Vf) and AB2 (Fig. 1B, 2B).

The total leaf DW per plant observed at 45 DAP in Ace 55 (Vf) or at 40 DAP in AB2 plants treated with ABA was similar to the total leaf DW per plant observed in water-treated plants (Tables 1A, 2A). The tomato cultivar AB2 had lower total leaf DW per plant than the cultivar Ace 55 (Vf) (Tables 1A, 2A). The total fruit DW per plant at 45 DAP in Ace 55 (Vf) or at 40 DAP in AB2 was 1.40× or 1.12× higher, respectively, in ABA-treated plants than in water-treated plants (Tables 1B, 2B). The total fruit DW per plant was lower in AB2 than in Ace 55 (Vf) tomato plants (Tables 1B, 2B). Total fruit Ca accumulation per plant from 12 to 45 DAP in Ace 55 (Vf) or from 12 to 40 DAP in AB2 was 10.30× or 2.46× higher, respectively, in ABA-treated fruit compared with water-treated fruit (Tables 1C, 2C), whereas the total leaf Ca accumulation per plant observed in ABA-treated plants was 0.54× [Ace 55 (Vf)] or 0.47× (AB2) the value observed in water-treated plants (Tables 1D, 2D). The sum of total fruit plus total leaf Ca content per plant accumulated from 12 to 45 DAP in Ace 55 (Vf) or from 12 to 40 DAP in AB2 plants treated with ABA was, respectively, 0.55× or 0.48× the Ca accumulation observed in water-treated plants during the same period of time (Tables 1E, 2E). The total fruit plus leaf Ca content per plant was lower in AB2 than in Ace 55 (Vf) tomato plants (Tables 1E, 2E). The total water loss per plant observed from 12 to 45 DAP in Ace 55 (Vf) or from 12 to 40 DAP in AB2 plants treated with ABA was, respectively, 0.57× or 0.48× the water loss observed in water-treated plants (Tables 1F, 2F). Total water loss per plant was lower in AB2 than in Ace 55 (Vf) tomato plants (Tables 1F, 2F). As a result of the similar effect of ABA on both water loss and plant Ca content, the calculated Ca concentration in the plant xylem sap was similar in water- and ABA-treated plants in both tomato cultivars (Tables 1G, 2G). The cultivar AB2 had higher Ca concentration in the plant xylem sap than the cultivar Ace 55 (Vf) (Tables 1G, 2G).

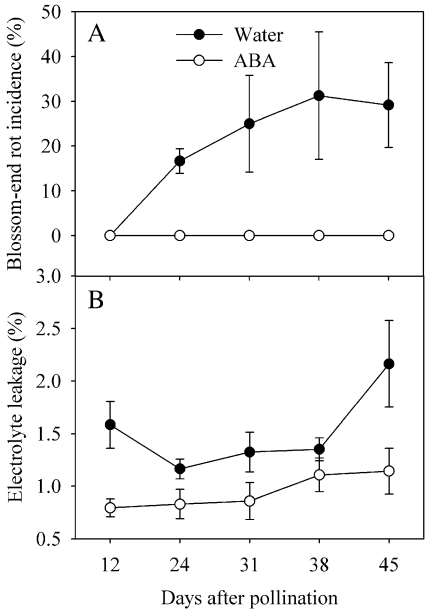

Fig. 1. BER incidence (A) and membrane leakage (B) of Ace 55 (Vf) tomato fruit. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

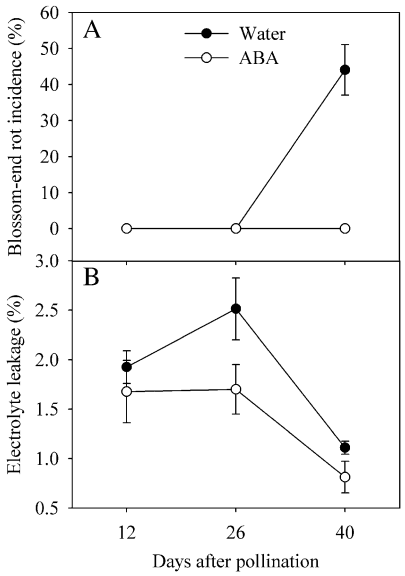

Fig. 2. BER incidence (A) and increase in membrane leakage (B) of AB2 tomato fruit. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

The mass balance approach to analyze leaf Ca uptake based on leaf stomatal conductance revealed that the total leaf Ca accumulation from 12 to 45 DAP [Ace 55 (Vf)] or from 12 to 40 DAP (AB2) per ABA-treated plant was, respectively, 0.55× or 0.42× the value estimated for water-treated plants (Tables 1I, 2I). The Ca concentration observed per leaf DW at 45 DAP in Ace 55 (Vf) or at 40 DAP in AB2 plants treated with ABA was, respectively, 0.59× or 0.46× the Ca concentration observed per leaf DW in water-treated plants (Tables 1J, 2J). The total leaf Ca content per plant observed at 45 DAP in Ace 55 (Vf) or at 40 DAP in AB2 plants treated with ABA was, respectively, 0.58× or 0.37× the Ca content observed in water-treated plants (Tables 1K, 2K). Total leaf Ca content per plant was lower in AB2 than in Ace 55 (Vf) tomato plants (Tables 1K, 2K). The estimated fruit water uptake to reach the observed total fruit Ca accumulation per plant from 12 to 45 DAP in Ace 55 (Vf) or from 12 to 40 DAP in AB2 plants was 10.6× or 2.5× higher in ABA-treated fruit, respectively (Tables 1L, 2L).

In both tomato cultivars, the analyses of XSU and PSU revealed that ABA-treated fruit had lower PSU and higher XSU than water-treated fruit (Tables 3, 4). ABA-treated fruit also had a lower phloem/xylem ratio of sap uptake than water-treated fruit (Tables 3, 4).

Stem water potential was consistently higher in ABA-treated plants than in water-treated plants during fruit growth and development of Ace 55 (Vf) and AB2 tomatoes (Fig. 3A, B). In both cultivars, the average leaf stomatal conductance was lower in ABA-treated plants than in water-treated plants during fruit growth and development (Tables 1H, 2H).

At the time of BER development in both tomato cultivars, Ca concentration was higher in blossom end fruit tissue on plants treated with ABA than in blossom end fruit tissue on plants treated with water (Fig. 4). ABA treatment maintained higher apoplastic water-soluble Ca concentrations in the pericarp tissue of Ace 55 (Vf) fruit (Fig. 5).

The number of Safranin-O-stained vascular bundles was higher during early stages of fruit growth and development, decreasing thereafter in both tomato cultivars (Figs 6, 7). Fruit treated with ABA showed higher numbers of stained vascular bundles in the placenta and pericarp tissue early in fruit development, both at the blossom end and at the peduncle end of the fruit (Figs 6, 7). ABA-treated fruit also showed a more regular and homogeneous distribution of stained vascular bundles in the placenta and pericarp tissue, compared with the water-treated fruit (data not shown).

In both tomato cultivars, ABA treatment tended to reduce the relative dry matter content of the tomato fruit (Fig. 8A). Both cultivars showed higher fruit fresh weight in response to ABA treatment (Fig. 8B). The total number of fruits per plant increased in the cultivar Ace 55 (Vf), but decreased in AB2 in response to ABA treatment (Fig. 9A). The total fruit fresh weight per plant nearly doubled in Ace 55 (Vf), but did not change in AB2 in response to ABA treatment (Fig. 9B). In both cultivars, total plant fresh weight was about the same in both ABA and water treatments (data not shown). ABA treatment reduced the water loss per plant plus fruit DW accumulated over 24 h in both Ace 55 (Vf) and AB2 tomato cultivars (Fig. 10).

Discussion

Mechanisms involved in BER prevention by ABA

The tomato cultivars Ace 55 (Vf) and AB2 are both susceptible to BER development. ABA treatment completely prevented BER development in both cultivars grown under

Table 1. Analysis of Ace 55 (Vf) tomato leaf and fruit Ca uptake

Line	Parameter	Water	SD[a]	ABA	SD	ABA:water
Whole plant (leaf+fruit)						
A	Total leaf DW plant^{-1} at 45 DAP (g)	284.5 a	±42.7	279.6 a	±38.1	0.98
B	Total fruit DW plant^{-1} at 45 DAP (g)	64.6 b	±12.1	92.3 a	±13.2	1.40
C	Total fruit Ca accumulation plant^{-1} observed from 12 to 45 DAP (45–12 DAP) (mg)	4.3 b	±1.3	44.3 a	±9.1	10.30
D	Total leaf Ca accumulation plant^{-1} observed from 12 to 45 DAP (45–12 DAP) (mg)	3341.7 a	±586.3	1815.9 b	±342.1	0.54
E	Total fruit+leaf Ca accumulation plant^{-1} observed from 12 to 45 DAP (Line C+Line D) (mg)	3346.0 a	±587.1	1860.2 b	±344.7	0.55
F	Total water loss plant^{-1} observed from 12 to 45 DAP (l)	38.6 a	±6.3	22.0 b	±3.4	0.57
G	Ca concentration estimated in the plant xylem sap from 12 to 45 DAP (Line E/Line F) (mg l^{-1})	86.6 a	±22.1	84.5 a	±15.2	0.97
Leaf						
H	Average stomatal conductance observed from 12 to 45 DAP (mmolm^{-2}s^{-1})	176.6 a	±32.7	98.1 b	±28.2	0.56
I	Relative leaf Ca accumulation plant^{-1} estimated from 12 to 45 DAP (Line G×Line H)	–	–	–	–	0.55
J	Ca concentration observed leaf DW^{-1} at 45 DAP (mg g^{-1})	18.2 a	±2.3	10.8 b	±1.9	0.59
K	Total leaf Ca content plant^{-1} observed at 45 DAP (mg)(Line A×Line J)	5177.9 a	±958.8	3019.6 b	±786.1	0.58
Fruit						
L	Water movement into the fruit estimated from 12 to 45 DAP (Line C/Line G) (ml)	49.6 b	±9.6	524.2 a	±105.1	10.60

Means with similar letters in each line are not significantly different (P=0.05) according to Duncan's test.
[a] ± standard deviation (SD), n=4 for water and ABA treatment.

Table 2. Analysis of AB2 tomato leaf and fruit Ca uptake

Line	Parameter	Water	SD[a]	ABA	SD	ABA:water
Whole plant (leaf+fruit)						
A	Total leaf DW plant^{-1} at 40 DAP (g)	85.8 a	±23.5	71.1 a	±21.6	0.82
B	Total fruit DW plant^{-1} at 40 DAP (g)	28.1 a	±4.2	31.6 a	±5.4	1.12
C	Total fruit Ca accumulation plant^{-1} observed from 12 to 40 DAP (40–12 DAP) (mg)	5.8 b	±1.1	14.3 a	±4.1	2.46
D	Total leaf Ca accumulation plant^{-1} observed from 12 to 40 DAP (40–12 DAP) (mg)	1357.5 a	±383.7	641.7 b	±98.8	0.47
E	Total fruit+leaf Ca accumulation plant^{-1} observed from 12 to 40 DAP (Line C+Line D) (mg)	1363.3 a	±384.2	656.0 b	±121.4	0.48
F	Total water loss plant^{-1} observed from 12 to 40 DAP (l)	10.3 a	±3.1	5.04 b	±1.1	0.48
G	Ca concentration estimated in the plant xylem sap from 12 to 40 DAP (Line E/Line F) (mg l^{-1})	132.3 a	±28.4	130.1 a	±26.3	0.98
Leaf						
H	Average stomatal conductance observed from 12 to 40 DAP (mmolm^{-2}s^{-1})	178.5 a	±27.9	77.0 b	±24.2	0.43
I	Relative leaf Ca accumulation plant^{-1}estimated from 12 to 40 DAP (Line G×Line H)	–	–	–	–	0.42
J	Ca concentration observed leaf DW^{-1} at 40 DAP (mg g^{-1})	27.8 a	±4.2	12.7 b	±3.6	0.46
K	Total leaf Ca content plant^{-1} observed at 40 DAP (mg) (Line A×Line J)	2385.2 a	±448.5	902.9 b	±183.2	0.37
Fruit						
L	Water movement into the fruit estimated from 12 to 40 DAP (Line C/Line G) (ml)	43.8 b	±8.2	109.9 a	±17.1	2.50

Means with similar letters in each line are not significantly different (P=0.05) according to Duncan's test.
[a] ± standard deviation (SD), n=4 for water and ABA treatment.

conditions of restricted Ca. The results obtained point to possible mechanisms by which ABA treatment can increase Ca concentration in the blossom end tissue of tomato fruit. One mechanism may be related to the decrease in the ratio of phloem/xylem fruit water uptake. Another mechanism is the higher abundance of functional xylem vessels that reached the blossom end tissue of the tomato fruit, allowing more Ca to be translocated to this region. In all cases, the higher total Ca accumulation in the blossom end tissue of tomato fruit contributed to higher Ca concentration in the apoplast, which has been shown to be required for proper membrane function (Hanson, 1960; Minorsky, 1985; Plieth, 2001; Suzuki *et al.*, 2003), eventually resulting in the lower membrane leakage and prevention of BER observed in ABA-treated fruit. The different patterns of ion leakage observed in the two tomato cultivars may possibly result from differences in membrane composition and/or membrane lipid biosynthesis in response to stress conditions (Welti *et al.*, 2002). ABA treatment appears to indirectly prevent BER by increasing the total and apoplastic Ca content in the blossom end tissue of tomato fruit.

ABA reduces leaf Ca uptake

The growth regulator ABA is widely known to reduce leaf transpiration under water stress conditions (Verslues and Zhu, 2007). Leaf transpiration has been suggested to affect leaf and fruit Ca uptake (Ho and White, 2005), which may also be influenced by ABA. Our results show that, for both cultivars, ABA reduction in total leaf Ca accumulation per

Table 3. Analysis of phloem and xylem fruit sap uptake in water (control) and ABA-treated fruit cultivar Ace 55 (Vf) from 12 to 45 DAP

Treatment	Sap uptake (ml g fruit DW^{-1} day^{-1})				Phloem/xylem	
	Phloem	SD[a]	Xylem	SD	Ratio	SD
Water	8.57 a	0.19	2.65 b	0.18	3.25 a	0.30
ABA	4.99 b	0.15	6.78 a	0.16	0.74 b	0.04

Means followed by the same letter in a column are not significantly different (P=0.05) according to Duncan's test.
[a] ± standard deviation (SD), n=4 for water and ABA treatment.

Table 4. Analysis of phloem and xylem fruit sap uptake in water (control) and ABA-treated fruit cultivar AB2 from 12 to 40 DAP

Treatment	Sap uptake (ml g fruit DW^{-1} day^{-1})				Phloem/xylem	
	Phloem	SD[a]	Xylem	SD	Ratio	SD
Water	9.09 a	0.08	1.87 b	0.09	4.86 a	0.25
ABA	7.28 b	0.04	4.35 a	0.05	1.68 b	0.02

Means followed by the same letter in a column are not significantly different (P=0.05) according to Duncan's test.
[a] ± standard deviation (SD), n=4 for water and ABA treatment.

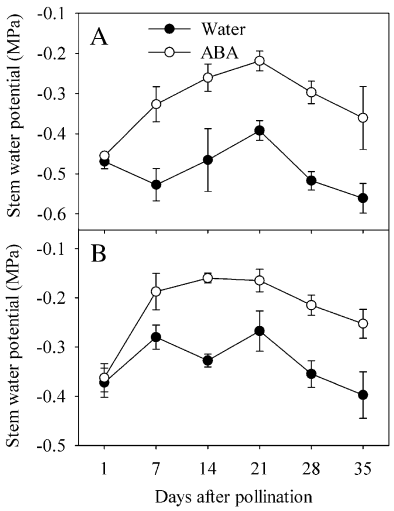

Fig. 3. Stem water potential in Ace 55 (Vf) (A) and AB2 (B) tomato plants. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

plant (0.47–0.54× of water-treated plant observed within 12–45 DAP), in total leaf Ca content per plant (0.37–0.58× of water-treated plant observed at 40–45 DAP), and in Ca concentration per leaf DW (0.46–0.59× of water-treated plant observed at 40–45 DAP) were similar to the relative ABA reduction in leaf Ca accumulation (0.42–0.55× of water-treated fruit) per plant estimated based on Ca concentration in the xylem sap and leaf conductance in each cultivar during fruit growth and development (within 12 to 45 DAP).

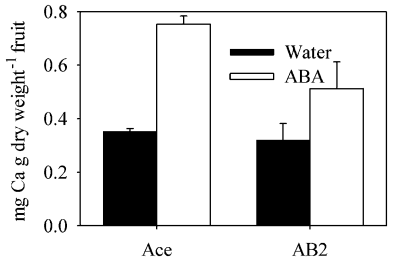

Fig. 4. Calcium concentration in the blossom end tissue of healthy Ace 55 (Vf) and AB2 tomato fruit at 45 and 40 DAP, respectively. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

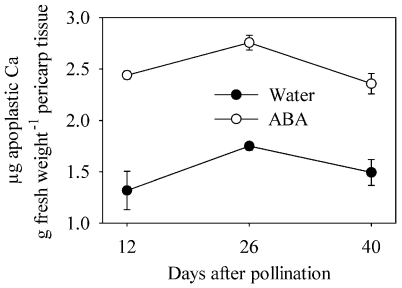

Fig. 5. Apoplastic Ca concentration in fresh pericarp tissue of Ace 55 (Vf) tomato fruit. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

It is relevant to note that the Ca concentration in the xylem sap of water and ABA-treated plants, as well as total leaf DW in water and ABA-treated plants were about the same between treatments in each cultivar. Therefore, our results show that ABA treatment decreased leaf Ca uptake proportionally to the reduction in leaf conductance in each cultivar, which supports the idea that overall Ca uptake into the plant is determined primarily by leaf transpiration and that leaves passively accumulate Ca (Taylor and Locascio, 2004; Ho and White, 2005).

ABA increases fruit Ca uptake

Fruit Ca uptake in response to ABA treatment appears to be more complex than in the leaves. ABA-treated fruit had 10.3× or 2.4× higher total fruit Ca accumulation per plant than water-treated fruit during fruit growth and development (within 12–45 DAP) in Ace 55 (Vf) or AB2, respectively. These results suggest that ABA treatment maintains higher fruit Ca uptake during later stages of fruit growth and development (after 12 DAP), when fruit Ca uptake would normally be greatly reduced (Ho and White, 2005). Since water and ABA-treated plants had similar Ca concentration in xylem sap, the 10.3× [Ace 55 (Vf)] or 2.4× (AB2) higher fruit Ca content per plant observed in ABA-treated plants represents ~10.3× [Ace 55 (Vf)] or 2.4× (AB2) higher xylemic water and Ca movement into fruit tissue. Although ABA-treated plants had 1.4× [Ace 55 (Vf)] and 1.2× (AB2) higher total fruit DW per plant than water-treated plants, the results show that ABA treatment

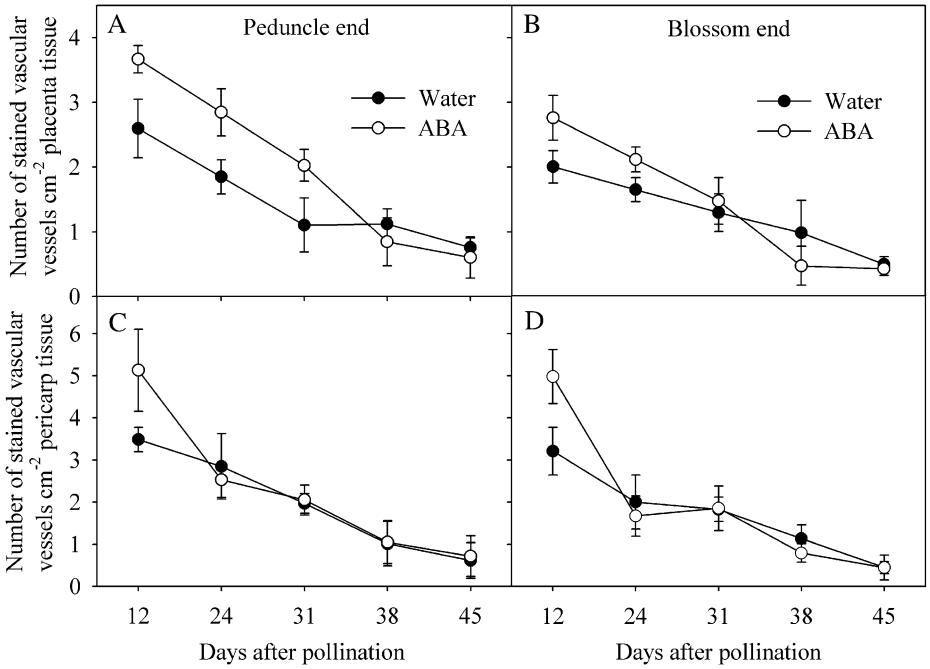

Fig. 6. Number of stained vascular bundles in the placenta (A and B) and pericarp (C and D) tissues at the peduncle (A and C) and blossom end regions (B and D) of Ace 55 (Vf) tomato fruit. Vascular bundles were stained with 1% Safranin-O. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

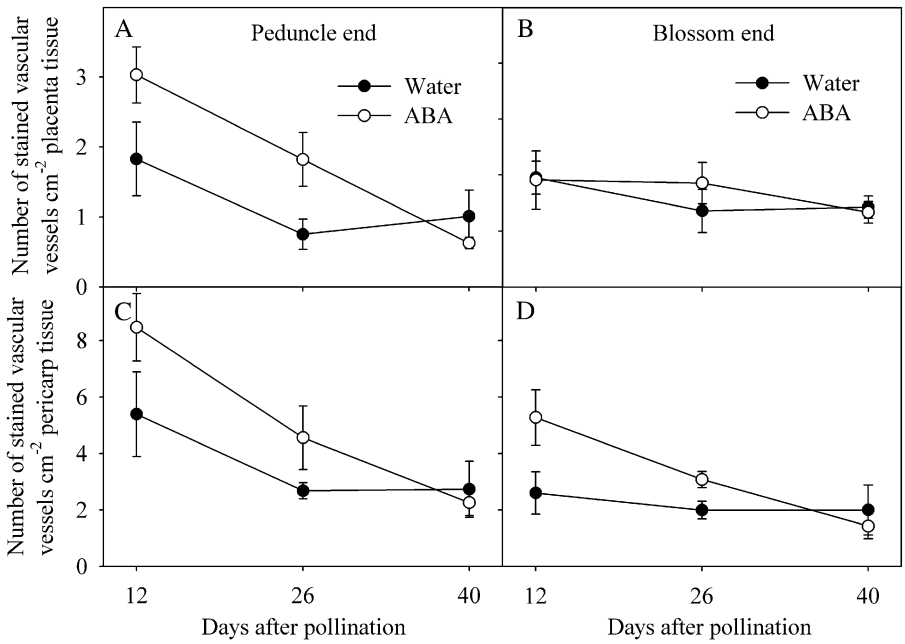

Fig. 7. Number of stained vascular bundles in the placenta (A and B) and pericarp (C and D) tissues at the peduncle (A and C) and blossom end regions (B and D) of AB2 tomato fruit. Vascular bundles were stained with 1% Safranin-O. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

decreased the whole-plant xylemic water and Ca flow into the leaves and increased flow into the fruit.

ABA decreases phloem/xylem ratio of fruit water uptake

Fruit water balance is the result of water influxes through the phloem and xylem vascular tissues, fruit growth, and water efflux by transpiration. The phloem represents ~70–85% of total fruit water uptake under non-water-stress conditions, but can be the only source of water to the fruit under high water deficit and VPD conditions (Ho *et al.*, 1987; Araki *et al.*, 2000, 2004; Guichard *et al.*, 2001, 2005; Eguchi *et al.*, 2003). Xylem water uptake into the fruit is higher at early stages of growth, but decreases thereafter, possibly due to

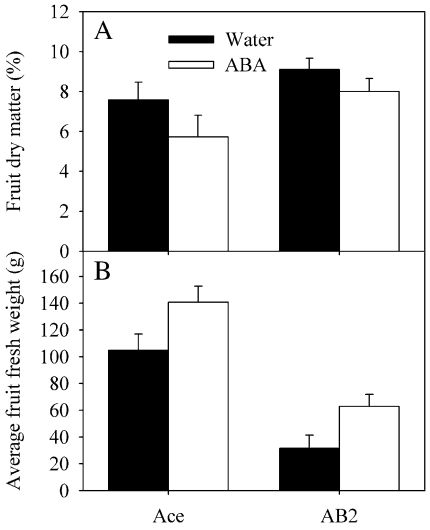

Fig. 8. Fruit dry matter (A) and average fresh weight of tagged fruit (B) of Ace 55 (Vf) tomato at 45 DAP and AB2 at 40 DAP. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

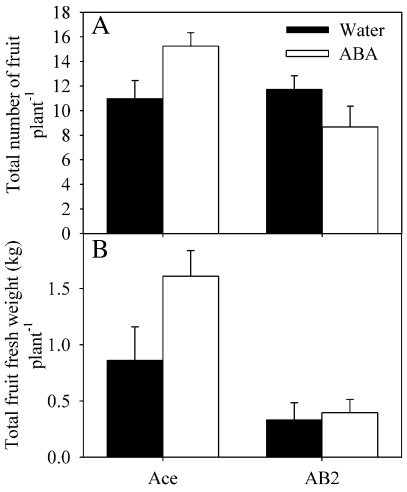

Fig. 9. Total number of fruit per plant (A), and total fruit fresh weight per plant (B) of tomato cultivars Ace 55 (Vf) at 45 DAP and AB2 at 40 DAP. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

a reduction in the number of conducting xylem vessels in the fruit and low fruit transpiration (Ho *et al.*, 1987; Araki *et al.*, 2000; Ho and White, 2005). Although fruit water influx becomes more phloemic than xylemic during growth and development, xylemic water is the only source of Ca to the fruit (Ho *et al.*, 1987; Ho and White, 2005). Therefore approaches to increase fruit Ca concentration should focus on increasing xylemic water uptake to the fruit by maintaining a higher number of functional xylem vessels in the fruit, increasing fruit transpiration, and decreasing the phloem/xylem ratio of fruit water uptake.

Our results show that ABA treatment increased xylemic water uptake and decreased phloemic water uptake into the fruit on a DW basis, resulting in ~4.4× [Ace 55 (Vf)] and

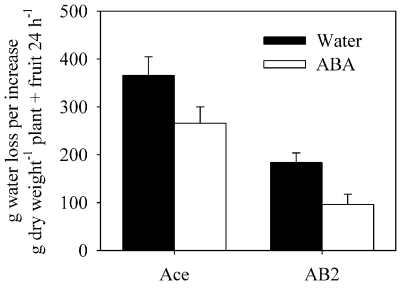

Fig. 10. Plant water loss per leaf and fruit DW accumulation over 24 h in Ace 55 (Vf) and AB2 tomato. Vertical bars indicate means ± standard deviation, n=4 for water and ABA treatment.

2.9× (AB2) lower phloem/xylem ratio of fruit water uptake than water-treated plants. Perhaps, by specifically reducing leaf transpiration, ABA treatment maintained higher stem water potential and decreased tension in the xylem, allowing more xylemic water and Ca movement into the low-transpiring fruit, as suggested in other studies (Ho, 1989; Adams and Ho, 1992; Araki *et al.*, 2000; Eguchi *et al.*, 2003; Guichard *et al.*, 2005). Similarly, the relationship between the incidence of BER and conditions of osmotic or water stress have been suggested to be due to a reduction in Ca transport to the fruit (Guichard *et al.*, 2001; Ho and White, 2005; Karlberg *et al.*, 2006; Magan *et al.*, 2008). Our results show that ABA treatment increased plant Ca usage efficiency not only by reducing total plant Ca uptake, but also by stimulating higher Ca accumulation in the fruit and preventing BER development. In addition, higher abundance of functional vascular bundles may also be involved in the prevention of BER development.

ABA increases the abundance of functional vascular bundles in tomato fruit

The models by which we can currently explain vascular pattern formation are limited and in-depth studies are required to understand the mechanisms involved in xylem and phloem development in plants (Koizumi *et al.*, 2000; Sachs, 2000; Aloni, 2001). It is believed that growth regulators are involved in vascular tissue development, but their roles are still unclear (Nelson and Dengler 1997; Saure, 2001). Our results show that tomato fruit treated with ABA have a higher number of stained vascular bundles through which water and Ca can move from the plant to the fruit and to the blossom end region in the fruit. It is possible that ABA, in conjunction with other growth regulators, triggers a cascade of events leading to xylem vessel development in the fruit (Aloni, 2001). Alternatively, the higher number of stained vascular bundles observed in tomato fruit treated with ABA may be a response to the maintenance of a hydrostatic gradient in the apoplast, which is responsible for the dye movement from the peduncle to the blossom end region in the fruit (Bondada *et al.*, 2005; Chatelet *et al.*, 2008).

Previous studies have shown that water flow becomes progressively more phloemic and less xylemic during fruit growth and development (Drazeta *et al.*, 2004; Bondada

et al., 2005; Chatelet *et al.*, 2008). Considering that fruit Ca uptake is believed to be exclusively dependent on xylemic water flow to the fruit, the increase in abundance of functional xylem vessels or the maintenance of the hydrostatic gradient in tomato fruit by ABA is fundamental to increased Ca accumulation and prevention of BER development in tomato fruit (Ferguson and Watkins, 1989; Dichio *et al.*, 2003, White and Broadley, 2003; Ho and White, 2005).

Cultivar susceptibility to BER development

The water-treated plants of tomato cultivar AB2 showed BER incidence only at 40 DAP, whereas the water-treated plants of tomato cultivar Ace 55 (Vf) started developing BER at 24 DAP. The lower susceptibility of AB2 tomato plants to BER development may be explained by the lower total fruit and leaf weight observed in this cultivar compared with Ace 55 (Vf), reducing the demand for available Ca in the substrate. In addition, high crop load has been suggested to increase fruit competition for Ca and the incidence of BER (Taylor and Locascio, 2004). Similarly, higher leaf area and leaf transpiration can lead to lower plant water potential, higher plant water stress, lower Ca transport to the fruit, and higher BER incidence (Ho and White, 2005). In addition, the average root fresh weight of AB2 control plants (78.0 $\pm$ 9.3 g from 12 to 40 DAP) was higher than the average root fresh weight of Ace 55 (Vf) control plants (40.6 $\pm$ 6.5 g from 12 to 45 DAP). This result suggests that AB2 plants were more efficient in extracting Ca from the organic substrate used in our study. Higher root Ca uptake can also explain the higher values of Ca concentration estimated in the plant xylem sap of AB2 plants compared with Ace 55 (Vf) plants. Therefore, smaller tomato plants with lower crop load and bigger root systems are potentially less susceptible to BER development because of lower fruit and leaf competition for Ca, higher root Ca uptake, higher Ca concentration in the xylem sap, and consequently higher total fruit Ca accumulation per plant as observed in AB2 control plants (5.8 mg from 12 to 40 DAP) compared with Ace 55 (Vf) control plants (4.3 mg from 12 to 45 DAP).

ABA is a potential tool for commercial prevention of BER and plant water loss

Although there are many years of research on BER development, it is still a major problem for the tomato industry, decreasing fruit yield and quality, and the return on the effort and investments spent during production. BER incidence as high as 50% of fruit production has been reported in calcareous soils (Abdal and Suleiman, 2005), showing that tomato plant susceptibility to BER may be a result of inefficient plant Ca uptake and translocation to the fruit. Our results show that in a greenhouse environment ABA treatment can increase fruit Ca concentration and completely prevent BER development in tomato plants grown under low Ca conditions, despite reducing total plant Ca uptake. Therefore, ABA is a potential tool to increase plant Ca use efficiency, reducing plant requirements for Ca nutrition, and preventing an important problem in tomato fruit production. In addition, ABA treatment also increased water use efficiency of tomato plants by decreasing leaf transpiration and also decreasing water loss per gram of plant and fruit DW. Accordingly, other studies have shown that tomato plants overexpressing a gene encoding 9-*cis*-epoxycarotenoid dioxygenase, the enzyme that catalyses a key rate-limiting step in ABA biosynthesis, not only increased ABA production, but also had higher transpiration efficiency, resulting in plants that required less water for growth and development (Thompson *et al.*, 2007; Tung *et al.*, 2008). Although our results show that ABA completely prevented BER development and increased plant water use efficiency, the concentration and timing used in this study are not compatible with current commercial practice. Therefore, future studies should identify the optimum ABA concentration and timing of application that can effectively prevent BER development and increase plant water use efficiency under commercial conditions.

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