Influence of ABA on calcium binding in tomato fruit and its impact on fruit texture

Honor's Thesis Project

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Abstract:

This experiment tested the influence of ABA on calcium binding in tomato fruit and its impact on tomato fruit quality. Tomato plants were treated with 0, 50, and 500 $mg \cdot L^{-1}$. Calcium binding was evaluated in the proximal and distal portions of mature tomato fruit. Four solvents (water, sodium nitrate, acetic acid, and hydrochloric acid) were used to sequentially extract calcium from dried tomato tissue to detect differences in the partitioning of calcium in cellular components of the fruit. ABA treatment of plants resulted in more structural calcium in both the proximal and distal portions of the tomato fruit leading to a decrease in blossom end-rot (BER), increased shelf-life, and better quality tomatoes for processing and raw consumption.

Introduction:

Tomato, in terms of dollar value, is the second largest crop consumed in the world (Rao and Barringer 2005). Two thirds of the tomatoes produced are further processed in the food industry (Rao and Barringer 2005). For example, salsa is now the top-selling condiment in the US over ketchup (Rao and Barringer 2005) and fresh-cut tomatoes are of increasing importance in reducing preparation time in the foodservice industry (Magee et al 2003). For this use fruit quality is a high priority. One reason for the increase in tomato consumption is the nutritional value found in tomatoes, such as antioxidants and carotenoids such as lycopene which is important in cancer prevention (Fanasca et al 2006, Aghofack-Nguemezi and Tatchago 2010). Quality of the tomato starts at planting. Tomatoes are susceptible to physiological disorders such as blossom end rot (BER) and they ripen and senesce rapidly after harvest. BER and overall fruit quality are related to calcium nutrition and fruit calcium content (Park et al 2005). These issues can become problematic to growers trying to provide increased yields of high quality tomatoes to satisfy consumer demand.

Calcium is important in tomato fruit because it forms the structure of the cell wall and membrane (White and Broadley 2003, Aghofack-Nguemezi and Tatchago 2010). Calcium is found in the soil and is transported to the plant from the roots through the xylem (Marschner 1995, White and Broadley 2003). Calcium moves primarily in the transpiration stream by way of the xylem with very little movement in the phloem (Marschner 1995). This mechanism determines the amount of calcium entering the cell. In the plant cell, calcium ions will move across the cell, endoplasmic reticulum, and vacuole membranes causing specific cell responses (White and Broadley 2003). Most importantly, calcium ions bind with the negatively charged carboxylic group in pectin to form cross bridges in the cell wall and membrane complex (Magee et al 2003). These cross bridges inhibit the activity of pectinmethylesterase (PME) and polygalacturonase (PG) which are enzymes that break down pectin in the cell wall (Magee et al 2003). Too much calcium in the cytoplasm is lethal to the cell (Conway et al 1994). Thus, it is important that calcium be bound in the cell wall, the plasma membrane or in other cellular membranes, or be compartmentalized external to the cell. Calcium is usually more concentrated in the proximal section of the tomato fruit than in the distal tissue, a main reason for BER (Suzuki et al 2003).

Blossom-end rot (BER) is a primary physiological disorder in tomatoes because of the localization of calcium in the proximal tissue of the fruit, leaving the distal end with softer tissue and more susceptible to disease (Suzuki et al 2003, Ho and White 2005, Park et al 2005, Adams and Ho 1993). When calcium enters the plant it travels through the xylem, which is more prevalent at the proximal or leaf end than the distal or blossom end of the fruit (Ho et al 2003). In addition, BER is not found in wild tomatoes, so it is believed that rapid growth of tomatoes and the desire for larger tomatoes leads to BER as well (Ho and White 2005). Another study showed

that at the site of BER in tomato fruit, no calcium was found in the plasma membrane or wall of cells but as the distance increased from the BER site, so did the amount of calcium present (Suzuki et al 2003). Finally 50% of calcium treated crops still suffer from BER (Freitas et al 2011); therefore the problem of BER is not that calcium is not available but that it is moved to the wrong part of the tomato (Adams and Ho 1993).

A hormone found in tomato fruit, abscisic acid (ABA), is used to move calcium to the tomato fruit. ABA is used mainly during environmental stress (Setha 2012, Waterland et al 2010). ABA reduces the xylem activity of leaves and increases phloem activity, moving more calcium to the tomato fruit rather than the leaf tissue (de Freitas et al 2011). ABA also increases xylem activity in the distal tissue, moving more calcium where the fruit is most susceptible to BER (de Freitas et al 2011). Additionally, ABA causes a higher concentration of calcium in the apoplast which assists in membrane functionality (de Freitas et al 2011). It can thus be hypothesized that ABA would increase calcium levels in tomato fruit to prevent BER and increase quality.

Tomato fruit after harvest faces many obstacles to maintain cell wall integrity (and thus fruit quality) including shipping, handling, delivery, heat treatment, passage through pipes and pumps, and transportation inside packaging (Rao and Barringer 2005, Park et al 2005). Because of a tomatoes unique texture and palatability, consumer demand makes a firm cell wall necessary in fresh fruit, diced fruit, and diced and whole canned tomato fruit (Magee et al 2003). The food industry has relied heavily on $CaCl_2$ to compensate for calcium deficiency in the cell wall, but cost of this application, the risk for postharvest fungal infection, the limited fruit area effected, and consumer detection of added calcium off flavors is not ideal (Park et al 2005, Magee et al

2003). This treatment also doesn't prevent BER which decreases crop yield. The use of ABA to partition calcium to the tomato fruit would increase quality and quantity of the tomato crop.

The objective of this experiment is to test the effect of ABA on the partitioning of calcium between the leaf and fruit and within the tomato fruit in order to produce a better product. The impact of total calcium levels on the proximal and distal tissue of the fruit and the cellular location of calcium within these two regions of the fruit tissue will be measured in order to determine the cellular location of the additional calcium that enters the fruit.

Materials and Methods:

Tomatoes were grown under greenhouse conditions as described by Barickman et al (2013). "Mountain Fresh Plus" tomato seeds were germinated in Pro-Mix BX soilless medium, then transferred after 30 days to 11-L Dutch Pots filled with Sunshine® Pro Soil Conditioner and fertilized with a complete nutrient solution with 180 $mg \cdot L^{-1}$ N, 93 $mg \cdot L^{-1}$ P, 203.3 $mg \cdot L^{-1}$ K, and three levels of calcium as described by Barickman et al (2013).

During fruit development, tomatoes were treated with 3 treatment levels of calcium (60, 90, and 180 $mg \cdot L^{-1}$). In addition, tomato plants received one of three ABA treatments or a 0% ABA control treatment. The treatments were: 1) a 0 ABA foliar spray with water only, 2) ABA foliar spray at 500 $mg \cdot L^{-1}$, 3) ABA 50 $mg \cdot L^{-1}$ soil drench or 4) both the ABA foliar spray at 500 $mg \cdot L^{-1}$ and the ABA soil drench at 50 $mg \cdot L^{-1}$ (Barickman et al 2013). There were three replications of each treatment.

After harvesting, tomatoes were divided into proximal and distal tissues sections. Fresh weight was taken, then the tissue was dried at 40°C for approximately one week. Once dry, tissue was weighed for dry weight and then ground. Ground tissue was weighed for (0.2 g) and transferred to a 15 mL polyethylene tube.

The experimental design was a 3 by 4 by 2 factorial with three complete replications. The factors were three calcium nutrition levels, four ABA treatments, and two areas of the tomato fruit. The following four fractions were extracted in order to determine the amount of calcium present in different cellular location within the fruit tissue. Extraction were: 1) water; extracting water soluble calcium, 2) sodium nitrate; extracting exchangeable calcium on proteins and pectin, 3) acetic acid; for calcium phosphates and carbonates, and 4) hydrochloric acid; for calcium oxalate.

A preliminary experiment was conducted to determine the number of extractions of each solvent required to maximize the extraction for that solvent, and it was determined that after 2 extractions there was no significant difference in the amount of calcium extracted from that fraction. Thus each tissue was double extracted with each solvent and the two extracts were combined for analysis. Using the procedure stated by Himelrick (1981), dried tissue was vortex with 10 mL of boiling reverse osmosis water, centrifuged at 4400 rpm for 10 minutes, then the decant liquid was poured into a 50 mL polyethylene tube. The second extraction was added to the same 50 mL tube. Tomato tissue was then mixed twice with 1N sodium nitrate ($NaNO_3$), then 1N acetic acid (HAc), and then 2N hydrochloric acid (HCl) (Himelrick 1981). Into separate 15 mL polyethylene tubes, 5 mL of each sample was filtered with a 0.45 μm PTFE filter. Calcium content was determined on an inductively coupled plasma mass spectrometer (Barickman et al 2013).

Results:

There was no significant difference in the amount of calcium extracted based on the levels of calcium added (Table 1).

There were differences among the ABA treatments. The spray and root treatment resulted in more calcium in the tomato fruit than in the non-treated fruit ($P \leq 0.05$). The spray treatment resulted in more calcium than the root treatment ($P \leq 0.05$) (Table 2). The control treatment extracted a mean value of 23353 mg/100g dry weight (DW) of dry tomato tissue. The ABA spray treatment contained 28101 mg/100g DW. The ABA root treatment resulted in 22940 mg/100g DW and the ABA spray and root application treatment resulted in 28803 mg/100g DW.

The proximal tissue contained more calcium than the distal tissue ($P \leq 0.001$) (Table 3). The mean value of calcium extracted from the proximal end was 31373 mg/100g DW while calcium content of the distal end of the fruit was 20226 mg/100g DW. The distal tissue from the fruit in the ABA spray treatment had more calcium than the distal tissue of the fruit from the 0% ABA (control) and the ABA root application treatments ($P \leq 0.05$).

There was a significant ($P \leq 0.001$) difference in calcium concentrations among the different extraction solvents. Water, sodium nitrate, and acetic acid extracted higher amounts of calcium than hydrochloric acid (Table 4). The water, sodium nitrate, and acetic acid extracted water soluble calcium, exchangeable calcium on proteins and pectin, and calcium phosphates and carbonates respectively (Himelrick 1981). The hydrochloric acid extracted calcium oxalates (Himelrick 1981). Water extracted 30% of the calcium, sodium nitrate 33%, acetic acid 32% and hydrochloric acid 4%.

Discussion:

Treatment of tomato plant with ABA foliar spray and root treatment resulted in the highest calcium concentration in the tomato fruit. Most of the calcium extracted was water soluble, exchangeable on proteins and pectin, and part of phosphates and carbonates. The latter two forms of calcium are basic structural compounds in the tomato fruit (Hosoda and Kato 2000,

White and Broadley 2003). Calcium oxalate was found as well, which forms a tight structure in the cell wall and gives the tomato fruit and firmer texture (Bouropoulos et al 2001). Compared to the untreated control, this suggests that an ABA spray and root treatment will increase the amount of calcium in the tomato fruit and therefore affect the texture.

Himelrick (1981), reported that air-dried tomatoes contained about 50% water soluble calcium with the other 50% bound in structural components. Our data indicated that the ABA treatments increased the amount of calcium in the structural fraction of tomato tissue by 20% compared to untreated tomato tissue. Thus, ABA treatments not only increased the amount of calcium in tomato fruit it also increased the amount of calcium in structural components of tomato tissue in both the proximal and distal fruit tissue.

As found in most previous reports on tomatoes, the proximal tissue contained more calcium than the distal tissue. However the calcium content was also increased in the distal tissue where BER normally forms. This increase in calcium in the distal portion of the tomato fruit resulted in a decrease in the incidence of BER (Barickman et al 2013). This decrease in BER is likely due to the increase in calcium where the calcium is needed in the fruit (distal end), and strengthening the cell wall components of the tomato fruit because of the addition of ABA. This suggests that the addition of ABA to tomato plants increases tomato firmness while also decreasing the chance of BER.

Because there was no difference in the amount of calcium added to the plant, it may be possible to add a lower amount of calcium during growth decreasing costs and off flavors found in previous studies on tomatoes dipped in a calcium salt solution (Magee et al 2003). However, it is also possible that there was no statistical difference in the amount of calcium in the fruit among calcium treatments because of the large variation in calcium content from fruit to fruit.

This would indicate that more replications should be done in order to get a better statistical estimation of the true means of the calcium treatments. The levels of calcium in the fruit may indeed be significantly different among the calcium treatments once this variation is accounted for statistically. In a separate experiment it was found that the calcium content of the tomato fruit did differ among the calcium treatments and that the 180 ppm calcium treatment had 14% more total fruit calcium than the 90 or 60 ppm treatments (Barickman et al 2013). The ABA treatments did result in more calcium in both the proximal and distal tissues of the fruit rather than just adding excess to the proximal portion of the tomato fruit. This could indicate a possible increase in total calcium in the fruit that allowed for more movement into the distal tissues once the proximal tissues were saturated.

The ABA treatments resulted in more calcium in tissues in the distal end of tomato fruit which would strengthen the cell wall components in the fruit. This resulted in a decrease in the common disease ruining tomato crops-BER (Suzuki et al 2003, Ho and White 2005, Park et al 2005, Adams and Ho 1993). Strengthening cell wall components also increases quality in tomato fruit. Stronger cell structure results in better texture for consumers, longer shelf-life for ripe tomatoes, and stability throughout any processing of the tomato fruit and/or shipment of tomatoes across the country (Rao and Barringer 2005, Park et al 2005).

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Tables:

Table 1. Mean values of calcium in "Mountain Fresh Plus" dried tomato fruit tissue grown in a greenhouse and treated with 3 amounts of calcium added during growth

Calcium added ($mg \cdot L^{-1}$)	Calcium extracted ($mg \cdot L^{-1}$)
60	24835.00
90	24703.00
180	27859.00
P-Value	ns
Standard Error of Mean	7538.58

ns- not significant

Table 2. Mean values of calcium in "Mountain Fresh Plus" dried tomato fruit tissue grown in a greenhouse and treated with ABA as a foliar treatment, soil drench, or both

ABA treatment ($mg \cdot L^{-1}$)	Calcium extracted ($mg \cdot L^{-1}$)
Control (0)	23353.00
Foliar Spray (500)	28101.00
Soil Drench (50)	22940.00
Foliar + Soil (500+50)	28803.00
P-Value	*
Standard Error of Mean	7585.45

* $P \leq 0.05$

Table 3. Mean values of calcium in the proximal and distal ends of "Mountain Fresh Plus" dried tomato fruit tissue grown in a greenhouse and treated with calcium and ABA

	Calcium extracted ($mg \cdot L^{-1}$)
Proximal	31373.00
Distal	20226.00
P-Value	***
Standard Error of Mean	7483.97

*** $P \leq 0.001$

Table 4. Mean values of calcium in "Mountain Fresh Plus" dried tomato fruit tissue grown in a greenhouse and extracted with water, sodium nitrate, acetic acid and hydrochloric acid after drying

	Calcium Extracted ($mg \cdot L^{-1}$)
Water	31245.00
Sodium Nitrate	34181.00
Acetic Acid	33186.00
Hydrochloric Acid	4584.26
P-Value	***
Standard Error of Mean	7559.10

*** $P \leq 0.001$

Table 5. Mean value of calcium concentration in mg/100g DW of "Mountain Fresh Plus" dried tomato tissue in the proximal (P) and distal (D) portions of the tomato fruit when treated with 0, 50, and 500 $mg \cdot L^{-1}$ of ABA and 60, 90, and 180 $mg \cdot L^{-1}$ of calcium

	ABA treatment ($mg \cdot L^{-1}$)							
	Control		**Foliar Spray**		**Soil Drench**		**Foliar+Soil**	
Calcium treatment ($mg \cdot L^{-1}$)	**P**	**D**	**P**	**D**	**P**	**D**	**P**	**D**
60	27796	15508	34528	25782	23946	15430	34328	18987
90	26285	16032	27049	17405	31352	16994	37001	21398
180	21840	21336	29281	25873	30618	18323	39318	27150