Abscisic Acid Impacts Tomato Carotenoids, Soluble Sugars, and Organic Acids

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Abstract. Plant growth regulators (PGRs) are chemicals used on a wide range of horticultural crops. These exogenous chemicals, similar to endogenous plant hormones, regulate plant development and stimulate a desired growth response, such as control of plant height. One such PGR is abscisic acid (ABA), which has been used effectively to improve fruit quality, specifically sugars and phytonutrients. The purpose of this study was to examine the effects of exogenous applications of ABA on tomato (Solanum lycopersicum) fruit quality, such as carotenoids, soluble sugars and organic acids, and ABA on tomato leaf chlorophylls and carotenoids. Furthermore, this study compared how ABA and calcium (Ca) treatments together affect fruit quality and whether there are added benefits to treating plants with both simultaneously. ABA treatments proved effective in increasing tomato fruit soluble sugars and decreasing organic acid concentrations. This study demonstrated that ABA is a viable PGR to significantly improve tomato fruit quality, specifically pertaining to carotenoids, soluble sugar, and organic acid concentrations.

PGRs are synthetic chemicals that are used on a wide range of horticultural crops and regulate plant development to stimulate a desired metabolic response, such as controlling plant height. In the floriculture industry PGRs are typically used to control plant height and promote flower initiation or to delay bloom (Blanchard and Runkle, 2007; Curry and Erwin, 2012; Lewis et al., 2004). For example, uniconazole is used to inhibit plant growth and has been demonstrated to be effective in tomato (S. lycopersicum) transplant production (Shin et al., 2009). In addition, previous research has demonstrated that ABA can be used as a PGR in watermelon (Cucumis melo) and peppers (Capsicum annuum) to control transplant height (Shinsuke and Leskovar, 2014a, 2014b). In the nursery industry PGRs can improve crop quality by stimulating lateral branching, as a substitution for a cold storage requirement, and control plant height (Clough et al., 2001; Gibson and Whipker, 2003; Lattimer et al., 2003). Traditionally, in the fruit industry PGRs have been used for thinning flower blossoms to achieve larger fruit and to improve fruit firmness and nutritional quality (Greene et al., 2011; Jones et al., 1991; Meland et al., 2011). The PGR 6-benzyladenine, used in apple (Malus domestica) and pear (Pyrus communis) production, is effective in inducing flower thinning and increasing return bloom (Stopar et al., 2009).

Applications of PGRs can manipulate plant growth and development and has been evaluated for many years in horticultural crops. However, research in recent years focused on using PGRs to improve fruit quality parameters, such as soluble sugars, fruit color, and phytonutrients (Buran et al., 2012; Gonzalez et al., 2012; Gu et al., 2011; Zhang and Whiting, 2013). One such PGR is ABA, which has been used effectively to improve fruit quality, especially in grape (Vitis vinifera) production (Peppi et al., 2006). ABA has significantly increased soluble sugars in grapes, thereby improving fruit flavor. In addition, ABA also improved fruit color adding to the visual aesthetics and nutritional value (Quiroga et al., 2009). Previous research has demonstrated that ABA particularly increases anthocyanins (Cantin et al., 2007) and carotenoids (Barickman et al., 2014a) in fruit tissue. Based upon many studies, it has been suggested that anthocyanin compounds possess anti-inflammatory and anticarcinogenic activity (He and Giusti, 2010). In addition, carotenoids are powerful antioxidants linked to inhibiting cancers such as prostate (Giovannucci et al., 1995), skin (Gonzalez et al., 2003), and colon (Slattery et al., 1999). Organic acids, such as malic and citric, and soluble sugars, such as fructose and glucose, contribute to the characteristic tomato flavor. Sugar to acid ratios play an important part in tomato fruit flavor quality. In general, tomato fruit quality is determined by color, texture, and flavor (Azodanlou et al., 2003).

The improvement in fruit and vegetable quality parameters and the demand for healthier produce have sparked additional research in other horticultural crops. These studies indicated that in addition to PGRs, manipulating environmental factors might contribute to improve fruit quality parameters, specifically flavor and phytonutrients. Barickman et al. (2013) observed that manipulating mineral nutrients found in soils affected nutritional quality in Brassica species. They found that supplementing adequate selenium in fertilizer solutions maintained glucosinolate concentrations at beneficial levels for human nutrition. Therefore, manipulating environmental factors in addition to PGRs may significantly improve crop quality.

The purpose of this study was to examine the effects of exogenous applications of ABA on tomato fruit quality parameters, such as carotenoids, soluble sugars, and organic acids. This study also examined the effects of ABA on tomato leaf chlorophylls and carotenoids. ABA treatments were applied foliarly and through the root because previous research indicated that both exogenous applications can be effective in improving fruit quality (Barickman et al., 2014a, 2014b, 2014c, 2014d). In addition, Ca fertilizer concentrations were manipulated because Ca treatments can affect tomato fruit quality parameters (de Freitas et al., 2013). Strengthening cell walls and plant tissue is largely due to Ca bound as pectate in the middle lamella. Application of Ca during plant growth may improve firmness in fruits by increasing the number of pectin cross linkages in the middle lamella and cell wall thus increasing fruit quality (Marschner, 1995). Previous research has also indicated that ABA treatments affect the uptake, partitioning, and distribution of Ca in vegetative and fruit tissue in tomato (Barickman et al., 2014b, 2014c, 2014d). Bastias et al. (2011) indicated that endogenous levels of ABA affects the modulation of organic acids and sugar content in tomato fruits. Furthermore, previous research conducted by Barickman et al. (2014a) indicated that exogenous application of ABA to tomato plants can affect the carotenoid concentrations in fruit tissue. Thus, since differing levels of Ca and endogenous and exogenous levels of ABA affect fruit quality, this study compared how the combination of ABA and Ca treatments affect vegetative tissue and fruit quality parameters and whether there are added benefits to treating plants with both simultaneously.

Materials and Methods

Plant culture and harvest. Seeds of ‘Mountain Fresh Plus’ tomato (Johnny’s Selected Seed, Waterville, ME) were sown...
into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in greenhouse conditions (Knoxville, TN; 35°N lat.) at 25/20 ± 3 °C (day/night). Natural photoperiod and intensity of sunlight for tomato production in the greenhouse were supplemented with 24 individual 1000 W high pressure sodium lights under a 16-h photoperiod. The lights delivered an average of 900 μmol·m⁻²·s⁻¹ over the entire photoperiod. Light intensity readings were taken at 1.22 m off the ground. At 30 d after seeding, the plantlets were transferred to 11-L Dutch pots (Tek Supply, Dyersville, IA) filled with Sunshine Pro Soil Conditioner (Sungro Horticulture, Agawam, MA). Tomato plants were grown hydroponically with a tomato fertilization program developed at the University of Tennessee. Elemental concentrations of the nutrient solutions were (mg·L⁻¹): nitrogen (N; 180), phosphorus (P; 93.0), potassium (K; 203.3), magnesium (Mg; 48.6), sulfur (S; 96.3), iron (Fe; 1.0), boron (B; 0.25), manganese (Mn; 0.25), zinc (Zn; 0.025), copper (Cu; 0.01), and molybdenum (Mo; 0.005). There were two identical experiments conducted. The first experiment was conducted in Fall 2012 and replicated in Spring 2013. Experimental design was a randomized complete block with a 3×4 factorial which consisted of six blocks and two replications, with each block, with individual pots representing an experimental unit. Ca was applied as calcium nitrate at three different treatment levels of 60, 90, and 180 mg·L⁻¹. Ca treatments were applied to the plants via the irrigation lines and were initiated at time of transplanting the tomato plants into the containers. The one control and three ABA treatments were applied either as no ABA, foliar, root, or as a combination of a foliar and root applications. For foliar ABA applications, treatments consisted of deionized (DI) water control (0 mg ABA-L⁻¹) or 500 mg ABA·L⁻¹ spray application. For ABA root applications, treatments consisted of a DI water control (0 mg ABA-L⁻¹) or 50 mg ABA·L⁻¹ applied via the irrigation lines. ABA spray treatments were applied once weekly until dripping from the foliage, while root applications were applied four times per day with the irrigation cycle. ABA treatments were initiated that anthesis and applied to tomato plants for 12 weeks. Fruit were harvested when red ripe. Subsequently, fruit were sorted by the use of United States Department of Agriculture tomato color for red ripe (USDA, 1975) and size classification into extra-large, large, medium and small (USDA, 2007). Tomato fruit with blossom-end rot were categorized separately. Fruit from each treatment were separated by replication and weighed for biomass. At least three fruit from each of two clusters from individual plants for each experimental unit were chosen randomly, frozen and prepared for organic acid, soluble sugars, and carotenoid analyses. Harvested fruit samples were stored at −80 °C before analysis. Leaf samples were taken above each of the two clusters at the last harvest for analysis of carotenoids and chlorophylls. 

**Fruit carotenoid tissue determination.** Carotenoids were extracted from fresh-frozen ripe fruit tissues and quantified according to the methods of Emenhiser et al. (1996) with slight modifications from Barwickman et al. (2014a). Briefly, fruit was removed from −80 °C and thawed until slightly pliable. A subsample of six ripe fruits from each experimental unit (treatment) was blended into a slurry. A 2.0-g subsample of the slurry was placed into a test tube (20×150 mm), and 5 mL of hexane and 0.8 mL of the internal standard (ethyl-β-8′-apo-carotenate; CaroteNature GmbH, Lupsingen, Switzerland) were added. Test tubes were vortexed for 1 min before addition of 5 mL of tetrahydrofuran then vortexed for 1 min before additions of 5 mL of reverse osmosis (RO) water. After vortexing for 20 s, test tubes were stored at 4 °C for 10 min to achieve aqueous-organic separations. Tubes were then centrifuged at 500 g for 10 min. The organic top layer was removed using a disposable Pasteur pipette and placed into a graduated conical test tube. The sample volume was reduced to dryness under a stream of nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, MA), and brought up to a final volume of 5 mL with MeOH. A 2-mL aliquot was filtered through a 0.2-μm PTFE filter (EconoPlus PTFE 25/20; Agilent Technologies, Santa Clara, CA) before high-performance liquid chromatography (HPLC) analysis.

An HPLC unit with a photodiode array detector (1200 series, Agilent Technologies) was used for pigment separation. Chromatographic separations were achieved using an analytical scale (250×4.6 mm i.d.) 5-μm polymeric RP-C₃₀ column (ProntoSIL; MAC-MOD Analytical Inc., Chadds Ford, PA), which allowed for effective separation of chemically similar pigment compounds. The column was equipped with a 5-μm guard cartridge (10×4.0 mm i.d.) and holder (ProntoSIL) and was maintained at 40 °C, using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 38.00% methyl tert-butyl ether (MTBE), 61.99% methanol (MeOH), and 0.01% triethylamine (TEA (v/v/v)). The flow rate was 1.0 mL·min⁻¹, with a run time of 53 min, followed by a 2-min equilibration before the next injection. Eluted compounds from a 10-μL injection were detected at 453 (carotenoids and internal standard), 652 [chlorophyll a (Chl a)], and 665 [chlorophyll b (Chl b)] nm, and data were collected, recorded, and integrated using ChemStation Software. Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards [BC, Chl a, Chl b, LUT, neoxanthin (NEO), violaxanthin (VIO), zeaxanthin (ZEA) from ChromaDex Inc., Irvine, CA].

**Soluble sugar analysis.** Tomato fruit samples were ground in a bullet grinder (Home- land Houseware, LLC) for homogenous subsamples. A 2.0-g subsample was extracted in a 15-mL test tube by adding 2 mL of RO water, vortexed, and shaken for 15 min at 72 h, rising 5 °C until 0 °C. Freeze-dried tissues were then ground in liquid nitrogen with a mortar and pestle. Pigments were extracted from freeze-dried tissues according to Kopsell et al. (2004). A 0.1 g tissue subsample was rehydrated with 0.8 mL of H₂O₂ for 20 min. After incubation, 0.8 mL of the internal standard ethyl-β-8′-apo-carotenate (Sigma Chemical Co., St. Louis, MO) was added to determine extraction efficiency. The addition of 2.5 mL of tetrahydrofuran was performed after sample hydration. The sample was then homogenized in a Potter-Elvehjem (Kontes, Vineland, NJ) tissue grinding tube using 25 % TFE plus a pestle attached to a drill press set at 13 g. During homogenization, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical centrifuge for 3 min at 500 g. The supernatant was removed, and the sample pellet was resuspended in 2-mL THF and homogenized again with the same extraction technique. The procedure was repeated for a total of four extractions to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, MA), and brought up to a final volume of 5 mL with MeOH. A 2-mL aliquot was filtered through a 0.2-μm PTFE filter using a 5-mL syringe before HPLC analysis.

Pigments were analyzed according to Kopsell et al. (2007). An Agilent 1200 series HPLC unit with a photodiode array detector was used for pigment separation. Chromatographic separations were achieved using an analytical scale (4.6 mm i.d.×250 mm) 5 μm, 200 Å polymeric RP-C₃₀ column (ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, PA), which allowed for effective separation of chemically similar pigment compounds. The column was equipped with a 5-μm guard cartridge (4.0 mm i.d.×10 mm) and holder (ProntoSIL), and was maintained at 30 °C using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 88.99% MeOH, and 0.01% TEA (v/v/v). The flow rate was 1.0 mL·min⁻¹ with a run time of 53 min, followed by a 2-min equilibration before the next injection. Eluted compounds from a 10-μL injection were detected at 453 (carotenoids and internal standard), 652 [chlorophyll a (Chl a)], and 665 [chlorophyll b (Chl b)] nm, and data were collected, recorded, and integrated using ChemStation Software. Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards [BC, Chl a, Chl b, LUT, neoxanthin (NEO), violaxanthin (VIO), zeaxanthin (ZEA) from ChromaDex Inc., Irvine, CA].
Hi-Plex H column (Agilent Technologies), which allowed for effective separation of organic acid compounds. The column was equipped with a Zorbax NH2 4.6 x 12.5 mm i.d. guard cartridge and holder, and was maintained at 50 °C using a thermostatic column compartment. All separations were achieved isocratically using a mobile phase of 100% 0.1 M H2SO4 (sulfuric acid). The flow rate was 0.6 mL·min⁻¹, with a run time of 15 min, followed by a 2-min equilibration before the next injection. Eluted compounds from a 10 μL injection loop were detected in positive detection mode, and data were collected, recorded, and integrated using ChemStation Software. Peak assignment for individual organic acids was performed by comparing retention times from the refractive index detector using external standards of malic and citric acids (Sigma-Aldrich, St. Louis, MO).

Statistical analysis. The two experiments were statistically similar. Therefore, data were pooled and analyzed together for treatment means. The experimental design was a randomized complete block in a factorial arrangement. The three Ca treatment concentrations were subdivided into four treatments: A non-ABA control treatment, A foliar spray ABA treatment, A root ABA treatment, and A foliar/root ABA treatment. Analysis of variance was used to evaluate ABA and Ca treatments on leaf chlorophylls and carotenoids, fruit carotenoids and soluble sugars using the PROC GLIMMIXED model. Statistical analysis of data was performed using SAS (Version 9.3 for Windows, SAS Institute, Cary, NC). Duncan’s multiple range test ($P \leq 0.05$) was used to differentiate between ABA and Ca application classifications when $F$ values were significant for main effects. Data are the average of four fruit, six blocks, and two replicates per treatment application. Statistical analyses indicated there were no interactions between ABA and Ca treatments. The following results are presented individually for ABA treatment effects and Ca treatment effects on leaf chlorophylls and carotenoids and fruit tissue carotenoids, soluble sugars, and organic acids.

Results

Impact of ABA on tomato leaf carotenoids and chlorophylls. Root ABA treatments significantly decreased leaf LUT concentrations in tomato plants (Table 1). LUT concentrations decreased from 9.23 to 7.91 mg/100 g FW when comparing the ABA root treatment to the ABA root treatment. Foliar spray ABA treatment significantly increased ZEA in the leaf tissue (Table 1). ZEA concentrations in tomato fruit tissue. BC concentrations decreased from 80% to 36 mg/100 g FW when comparing the ABA control treatment to the foliar spray ABA treatment. Root ABA treatments significantly decreased LYCO concentrations in tomato fruit tissue. LYCO concentrations decreased from 9.23 to 7.91 mg/100 g FW when comparing the ABA control treatment to the foliar spray ABA treatment. Ca treatments did not have a significant impact on fruit carotenoid concentrations.

Influence of ABA and Ca on tomato fruit soluble sugars. The application of ABA had a significant positive impact on tomato fruit soluble sugar concentrations (Table 2). Glucose increased from 13.39 to 19.19 mg/100 g FW when comparing the ABA control treatment to the ABA root treatment. Glucose increased 30.2% in the tomato fruit tissue with ABA treatment. Fructose decreased from 13.72 to 19.24 mg/100 g FW when comparing the ABA control to the root treatment. Ca treatments did not have a significant impact on fruit carbohydrate concentrations (Table 2).
Table 1. Carotenoids and chlorophyll leaf tissue pigments in 'Mountain Fresh Plus' tomato plants grown in a greenhouse and treated with exogenous applications of abscisic acid (ABA) and calcium (Ca) in the hydroponic fertilizer solution.

| Treatments       | VIO | NEO | ANTH | LUT | ZEA | BC | CHLA | CHLB |
|------------------|-----|-----|------|-----|-----|----|------|------|
| ABA              |     |     |      |     |     |    |      |      |
| Control          | 0.46a|2.46a|1.23a |9.23a|0.05b|3.56a|84.21a|31.55a|
| Foliar           | 0.42a|2.26ab|0.99b |8.76ab|0.09a|3.44ab|74.31ab|28.99a|
| Roots            | 0.42a|2.04b |1.06b |7.91b |0.09a|2.98b |69.51b |26.14b|
| Foliar/Root      | 0.44a|2.19ab|1.05b |9.01a|0.10a|3.41ab|70.22b |29.05b|
| P value*         | NS  | NS  | **   | NS  | NS  | **  | NS   | NS   |
| Ca (mg·L⁻¹)      |     |     |      |     |     |    |      |      |
| 60               | 0.40a|2.34a |1.10a |8.83a |0.09a|3.54a |77.71a |29.36a|
| 90               | 0.41a|2.21a |1.04a |8.68a |0.08a|3.21a |77.29ab|28.91a|
| 180              | 0.50a|2.17a |1.11a |8.68a |0.08a|3.30a |68.69b |25.33a|
| P value*         | NS  | NS  | **   | NS  | NS  | **  | NS   | NS   |

VIO = violaxanthin; NEO = neoxanthin; ANTH = antheraxanthin; LUT = lutein; ZEA = zeaxanthin; BC = β-carotene; CHLA = chlorophyll a; CHLB = chlorophyll b.

The standard error of the mean for ABA treatments was VIO ± 0.07; NEO ± 0.12; ANTH ± 0.14; LUT ± 0.31; ZEA ± 0.01; BC ± 0.19; CHLA ± 4.01; CHLB ± 1.08. The standard error of the mean for Ca treatments was VIO ± 0.07; NEO ± 0.11; ANTH ± 0.14; LUT ± 0.27; ZEA ± 0.01; BC ± 0.17; CHLA ± 3.57; CHLB ± 0.97. Means within a column followed by different letters denote significant differences (Duncan’s multiple range test, P ≤ 0.05).

ABA treatments control (0.0 mg·L⁻¹); spray (500 mg·L⁻¹); root (50 mg·L⁻¹); spray/root (500 mg·L⁻¹/50 mg·L⁻¹).

NS, **, ***, ***, ***Non-significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Table 2. Fruit tissue carotenoid pigments in 'Mountain Fresh Plus' tomato plants grown in a greenhouse and treated with exogenous applications of abscisic acid (ABA) and calcium (Ca) in the hydroponic fertilizer solution.

| Treatments       | LUT | BC | LYCO | Glucose | Fructose | Malic acid | Citric acid |
|------------------|-----|----|------|---------|----------|------------|-------------|
| ABA              |     |    |      |         |          |            |             |
| Control          | 0.11b|0.29a|5.97a |13.39b  |13.72c    |2.06a       |4.07a        |
| Foliar           | 0.13b|0.36b|6.07a |18.74a  |18.32ab   |0.81b       |1.59b        |
| Roots            | 0.15a|0.31a|5.31a |19.19a  |19.24a    |0.74b       |1.34b        |
| Foliar/Roots     | 0.12b|0.30a|4.91a |18.15a  |17.76b    |0.92b       |1.83b        |
| P value*         | **  | *  | ***  | ***     | ***      | ***        | ***         |
| Ca (mg·L⁻¹)      |     |    |      |         |          |            |             |
| 60               | 0.13a|0.31a|5.49a |15.70b  |16.29b    |1.09a       |2.21ab       |
| 90               | 0.13a|0.34a|5.38a |17.10b  |16.88b    |1.03a       |1.87b        |
| 180              | 0.13a|0.31a|5.84a |19.29a  |18.62a    |1.28a       |2.54a        |
| P value*         | NS  | NS  | ***  | **      | NS       | NS         | NS          |

L–¹ = lutein; BC = β-carotene; LYCO = lycopene.

The standard error of the mean for ABA treatments was LUT ± 0.01; BC ± 0.04; LYCO ± 0.72; glucose ± 1.24; fructose ± 1.04 malic acid ± 1.24; citric acid ± 0.24. The standard error of the mean for Ca treatments was LUT ± 0.01; BC ± 0.02; LYCO ± 0.70; glucose ± 1.25; fructose ± 1.01; malic acid ± 0.16; citric acid ± 0.21. Means within a column followed by different letters denote significant differences (Duncan’s multiple range test, P ≤ 0.05).

ABA treatments control (0.0 mg·L⁻¹); spray (500 mg·L⁻¹); root (50 mg·L⁻¹); spray/root (500 mg·L⁻¹/50 mg·L⁻¹).

NS, *; **, ***, ***, ***Non-significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.

Discussion

The applications of ABA significantly increased ZEA in tomato leaf tissue. This confirms a previous report that foliar applications of ABA increased ZEA (Barickman et al., 2014), which increases under environmental stress, especially light induced stress (Depka et al., 1998; Havaux and Niyogi, 1999). This study also demonstrated that no matter the application process of ABA, ZEA will increase in the leaf tissue. Foliar spray applications are just as effective as root and the combination of foliar spray and root applications of ABA. This may be due to ABA’s effectiveness in creating a stress response no matter how it was applied. Thus, even small amounts of ABA may increase the induction of gene expression leading to the increased production of ZEA. For example, previous research demonstrated that induction of carotenoid biosynthetic genes by ABA could alter the plant’s resistance to drought and oxidative stress by modulating levels of xanthophylls, such as ZEA and LUT (Du et al., 2010).

In addition, this study found root ABA applications significantly decreased LUT in the leaf tissue. Previous research found that exogenous applications of ABA directly to the root tissue in the hydroponic solution increased carotenoid concentrations in the leaf tissue of lettuce (Lactuca sativa) and ‘Micro’ tomato. On the other hand, Balderrmann et al. (2013) demonstrated that exogenously applied ABA decreased total carotenoids in tea (Camellia sinensis) plant flowers. These results were similar to the current study. When ABA treatments were applied to tomato plant roots only, carotenoids in the leaf tissue had a general decreasing trend. Therefore, the mixed results merit an investigation on the process of how ABA is applied to plants and the response of carotenoids in different plant tissues. Further research is required to determine the best application method and concentration to positively affect carotenoid concentrations in leaf vs. fruit tissue.

In the current study the root ABA treatments decreased Chl a and Chl b in tomato plant leaf tissue. This may be because ABA links environmental stress perception with the reduction of plant growth and photosynthetic capacity (Saibo et al., 2009). Thus, ABA carries the stress signal to the stomata and acts to close them, negatively affecting plant growth and photosynthetic capacity. Apart from restricting gas exchange by stomatal closure as a short-term effect of enhanced ABA levels, long-term ABA effects on photosynthesis include the inhibition of thyloaloid formation, chlorophyll biosynthesis and Rubisco activity (Khokhlova et al., 1978; Kusnetsov et al., 1998; Lichtenhaler and Becker, 1970). Research demonstrated that exogenous applications of ABA reduce chlorophyll content and repress transcription of chloroplast genes leading to reduction in chloroplast-localized proteins that impact photosynthesis (Kusnetsov et al., 1994; Wang et al., 2010; Yambreken et al., 2013). Thus, ABA negatively impacts the production and function of proteins in the photosynthetic process. Exogenous application of ABA in the current study may indirectly affect expression of chloroplast genes and regulate their activity leading to decreases in chlorophyll concentrations in tomato leaves. Therefore, the effect of ABA may lead to an indirect down-stream signal, such as transcription factors, that regulate chloroplast genes function and, to a larger extent, photosynthesis as a response to abiotic stress.

This study found that decreasing Ca treatments increased chlorophyll concentrations while not affecting leaf carotenoid concentrations. Tomato leaf chlorophyll concentrations decreased when plants were treated with Ca treatment of 180 mg·L⁻¹. In addition, leaf carotenoid concentrations did not change from Ca treatment of 180 mg·L⁻¹ to the deficient treatments of 60 and 90 mg·L⁻¹. The effect of varying Ca supply on leaf chlorophyll and carotenoid concentrations has been investigated with mixed results. The differences in results may be due to plant species, stage of growth and environmental conditions. For example, varying Ca treatments in Arabidopsis did not impact the chlorophyll and carotenoid content in the leaves (Kaddour et al., 2012). In leaves of Cyclocarya paliurus seedlings, decreasing the Ca treatment concentration from 18 to 12 mm increased the chlorophyll concentrations (Yao and Wang, 2012). Like...
the current study, Kaddour et al. (2012) and Xu et al. (2013) recorded results for plants that were not under any environment stress. On the other hand, Ca treatments alleviated photoinhibition of the photosystem II by positively impacting the xanthophyll cycle pigment concentrations in peanut (Arachis hypogaea), a calciphilous plant species, during heat stress and high light conditions (2013). In addition, research has demonstrated that applications of CaCl
subscripts2 treatments resulted in higher concentrations of Chl a and Chl b in Zosia japonica under drought conditions (Xu et al., 2013). These studies demonstrated that Ca treatments may have a bigger impact on chlorophyll and carotenoid concentrations under environmental stress conditions. Previous research indicated that Ca has a central role in the plants’ defense mechanisms that are induced by environmental stress, and Ca signaling is required for plants’ tolerance to this stress (Cousson, 2007, 2009). In addition, Kopsell et al. (2013) demonstrated that Ca:Mg ratios significantly affect the mineral nutrient uptake and carotenoid concentrations in kale (Brassica oleracea var. Acephala). Therefore, increasing Ca treatments may be able to positively regulate chloroplast genes that help to increase leaf chlorophyll and carotenoid concentrations under environmental stress.

The application of ABA affected the concentration of different carotenoids in tomato fruit tissue in a different way. LUT increased in the fruit tissue when ABA was applied in the foliar spray or root treatments when compared with the ABA control treatment. BC increased in the fruit tissue when ABA was applied in the foliar spray treatment when compared with the ABA control treatment. However, LYCO concentrations in the foliar spray treatment did not differ from the ABA control treatment. LYCO did decrease when ABA was applied to the root tissue. Thus, the foliar spray applications of ABA had the greatest effect on carotenoids in tomato fruit tissue. Previous research supported the current study’s findings. Research has demonstrated that ABA plays an essential role in fruit ripening. For example, ABA controls ethylene production in tomato fruit (Zhang et al., 2009), leading to increases in pigmentation and carotenoid levels. Furthermore, data also indicated that ABA positively regulated the degree of pigmentation and carotenoid composition during tomato fruit ripening by acting on gene functions (Sun et al., 2012) The application of exogenous ABA to tomato plants could be a novel approach to increasing carotenoid concentrations in the fruit tissue, leading to a more nutritious tomato fruit.

The application of Ca treatments to tomato plants did not affect the carotenoid content in the fruit tissue. However, research demonstrated that excess Ca concentrations in the fruit tissue could affect the carotenoid concentrations. For example, Paiva et al. (1998) demonstrated that an increase in Ca concentration in the nutrient solution results in a decrease in LYCO content due to the antagonism between Ca and potassium. Increases in Ca content in plant tissue have an antagonistic effect on K and can reduce its absorption. Lack of K absorption into the fruit tissue can have a negative effect on the production of carotenoids. Research demonstrated that increases in K improve the quality of tomato fruit by positively influencing carotenoid biosynthesis (Ramirez et al., 2009). Therefore, in the current study, increasing the Ca treatment concentrations to 180 mg L
superscript–1 does not affect the K (data not shown) absorption into tomato fruit tissue and negatively influence carotenoid concentration.

Exogenous applications of ABA positively influence soluble sugar concentrations in the fruit tissue. Previous research found similar results. Bastias et al. (2011) found that over expressing key ABA regulated genes increases soluble sugar concentrations in tomato fruit. Thus, under stressful conditions ABA increases, which in turn increases sugar accumulation by activating signals associated with stress responses (Saito et al., 2008). Therefore, not only will ABA increase soluble sugar accumulation under normal ripening conditions, it will also stimulate soluble sugar accumulation under stress conditions.

This study found decreases in soluble sugar concentrations in tomato fruit tissue when Ca treatments were decreased from 180 mg L
superscript–1 to Ca deficit levels of 60 and 90 mg L
superscript–1. Thus, plants treated with Ca concentration treatments had the highest levels of soluble sugars in the tomato fruit tissue. These findings correspond to previous research, which indicated that the addition of Ca as a preharvest treatment increased total soluble solids, total soluble sugars, and Ca in pear (P. communis) fruit tissue (Omaima et al., 2010). However, in the current study there were no interactions when analyzing ABA and Ca treatments together. The increased levels of soluble sugars in the fruit tissue treated by ABA and Ca were similar, indicating that applications of either ABA or Ca alone would yield the same results. Applying ABA and Ca treatments together did not result in greater soluble sugar concentrations because, at low Ca treatments, ABA did not increase the soluble sugar concentrations in tomato fruit tissue.

Tomato plants treated with exogenous applications of ABA had decreased organic acid concentrations in the fruit tissue. Specifically, malic and citric acid decreased in tomato fruit tissue in all treatment applications of ABA when compared with the ABA control treatment. Previous research supported data that exogenous applications of ABA accelerate ethylene production leading to fruit quality changes in mango (Mangifera indica) fruit (Zaharah and Singh, 2012). In addition, Zaharah et al. (2013) found that exogenous applications of ABA promoted the activities of ethylene biosynthesis enzymes leading to fruit softening, increases in soluble sugars, and degradation of total organic acid. These experiments support the current study, which demonstrated that exogenous applications of ABA lead to an increase in carotenoids and soluble sugars and decreases in organic acids. These data indicated that ABA can improve tomato fruit quality by positively influencing the sugar to acid ratio, thereby improving the flavor and increasing the nutritional value by increasing carotenoid concentrations.

This study demonstrated that ABA can improve tomato fruit quality, specifically pertaining to carotenoids, soluble sugar, and organic acid concentrations. However, ABA applications in conjunction with low Ca treatments did not prove to be more effective in improving tomato fruit quality than each ABA or Ca treatment alone. Tomato plants still need the adequate concentration of Ca in the fertilizer solution in order for ABA to improve fruit carotenoids, soluble sugars, and decrease organic acid concentrations. The application of ABA could be a novel application of a PGR to improve overall fruit quality with adequate fertilization of the plant. The efficacy of ABA may be more an issue of application process to improve tomato fruit quality. Thus, this study indicates that foliar spray applications of ABA are a more viable choice to improve carotenoid and soluble sugar and decrease the organic acid concentrations in tomato fruit. However, further research is needed to investigate the optimum concentrations and frequency of ABA applications to the root and leaf to fine tune the impact on fruit quality.

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