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Article

Abscisic acid application regulates vascular integrity and calcium allocation within apple fruits

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Abstract

Calcium is a very poorly translocated nutrient in the flesh of apple fruits with the advancement of growth. Within the fruit, movement is further restricted toward distal portion relative to proximal. Even numerous foliar applications of calcium do not always achieve the desired effect. Thus, the objective of this study was to enhance the calcium allocation in distal parts of apple fruit in response to whole plant application of abscisic acid (ABA). Five-year-old apple plants of Super Chief Sandidge on M9 were treated with ABA at 400 ppm, calcium chloride at 0.4% and water (Control) at four stages (30, 65, 100, and 135 days after full bloom (DAFB)), and then analyzed for xylem functionality and calcium allocation in leaf and various fruit tissues at 10 days after each treatment, i.e., S1—40, S2—75, S3—110, and S4—45 DAFB. The results obtained showed that xylem functionality started impairing just after S2; consequently, the calcium allocation was also reduced in middle and calyx portions of fruit after that stage. However, xylem functionality was significantly retained (up to 30%) in ABA-treated fruits at S4 stage, which in other treatments was found to be nil at the calyx end of the fruit. This retention of xylem tissue functionality enhanced allocation of calcium from roots in middle and calyx end of the apple fruit. Leaf calcium was reduced with ABA applications. With the enhancement of calcium in the fruits, increases in soluble solid content and titratable acidity were observed at maturity.

Key words: xylem functionality, physico-chemical, Super Chief Sandidge, Streif index, bitter pit

Résumé

Le calcium est un oligoélément qui se déplace très mal dans la chair des pommes, quand elles grossissent. Ces déplacements se limitent de surcroît à la partie distale du fruit plutôt qu'à sa partie proximale. Enfin, les applications foliaires de calcium, même nombreuses, ne donnent pas toujours l'effet escompté. Les auteurs voulaient améliorer la part de calcium acheminée jusqu'aux parties distales du fruit après application d'acide abscissique (AA) à la plante entière. Pour cela, ils ont appliqué de l'AA à 400 ppm, du chlorure de calcium à 0,4 % et de l'eau (témoin) à des pommiers Super Chief Sandidge sur M9 de cinq ans, à quatre stades (30, 65, 100 et 135 jours après la floraison - JAF). Ensuite, ils ont analysé la fonctionnalité du xylème et la répartition du calcium dans les feuilles ainsi que divers tissus du fruit, dix jours après chaque traitement, soit S1-40, S2-75, S3-110 et S4-145 JAF. Les résultats obtenus indiquent que le xylème commence à perdre sa fonctionnalité immédiatement après le deuxième stade (S2), avec la diminution subséquente de la quantité de calcium acheminée au centre du fruit et au niveau du calice. Le xylème des fruits traités à l'AA avait néanmoins gardé passablement sa fonctionnalité (jusqu'à 30 %) au stade S4, alors qu'elle était inexistante au niveau du calice dans les autres cas. Le fait que le xylème demeure fonctionnel signifie une meilleure répartition du calcium venant des racines dans le cœur du fruit et au niveau du calice. L'application d'AA réduit la concentration de calcium dans les feuilles. La plus forte teneur en calcium du fruit s'accompagne d'une augmentation de la concentration de solides solubles et de l'acidité totale à maturité. [Traduit par la Rédaction]

Mots-clés : fonctionnalité du xylème, physicochimique, Super Chief Sandidge, indice de Streif, point-amer

Introduction

Calcium is one of the most important elements influencing fruit quality and marketable yield. It is essential for plant growth, cell division, and membrane stability. Calcium with two positive charges (Ca^{2+}) plays a vital role in neutralizing organic and inorganic anions in the vacuole, structuring cell wall and membranes, and acting as an intracellular messenger (Marschner 1995). Calcium-binding proteins, also known

as calcium-modulating proteins like calmodulin, calmodulin-binding protein, and calcium-dependent but calmodulin-independent protein kinases, are the targets of calcium signals in cytosol and are responsible for the activation of various enzymes like membrane-bound adenosine triphosphatase (ATPase), nicotinamide adenine dinucleotide kinase (NADK), and adenylate cyclase. Diverse effects are caused by insufficient supply of calcium to fruits and leaves. Over 35 calcium-related disorders in various economically important crops have been identified (Sidhu et al. 2020). Necrotic lesions, bitter pit, and other physiological disorders have been reported to enhance respiration rates and accelerate maturation, which increase the vulnerability to certain diseases (Levin et al. 2019). Increasing fruit calcium content leads to improved fruit firmness, delayed fruit ripening, and reduced incidence of calcium-related disorders like bitter pit in apple.

It has been reported that calcium is transported within the plant exclusively through the xylem (Ho and White 2005). In the xylem vessel, leaf, and fruit, calcium uptake is driven by transpiration pull, and partitioning of calcium toward leaves and fruits depends on the rates of fruit and leaf transpiration. Xylematic water flow is much higher toward leaves as compared with fruits because of the higher rate of transpiration in leaves. Hence, more calcium ions get transported toward leaves. Recent research suggests that fruit calcium uptake can be promoted by reducing leaf transpiration and by increasing the abundance of functional xylem vessels in fruit (Gomez and Kalcsits 2020), and also by manipulating the fruit water status through the use of anti-transpirants. Therefore, treating the plants with anti-transpirants may prove to be a useful approach for specific reduction of leaf transpiration without significant changes in fruit transpiration. Several workers have indicated that the plant growth regulator abscisic acid (ABA), used as an anti-transpirant, increases calcium concentration in tomato (De Freitas et al. 2011). It is also believed that ABA could enhance the number and functionality of xylem vessels connecting the fruit to the plant and thus increase Ca^{2+} movement to the fruit (De Freitas et al. 2014). Thus keeping in view the essentiality of calcium in apple fruit and its impeded movement, the present investigation was taken with the objective to enhance the calcium allocation in distal parts of apple fruit in response to whole plant application of ABA.

Material and methods

Present study was carried out in an orchard at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar (34°08′43″ N and 74°52′46″ E). Twelve apple plants cv. Super Chief Sandidge on M-9 rootstock established on sandy loam soil (pH, 6.5; electrical conductivity, 0.17 and cation exchange capacity, 14.3) grown under standard cultural practices (Anonymous 2018), were used for present study. The trees selected for the study were five-year-old having uniform size and vigor, with the average yield of 6 kg/tree (0.4 kg cm^{-2} trunk cross sectional area). The experiment comprised three treatments: water spray (control treatment), ABA (New Sunshine–Xiangtan Agrochemicals Co. Ltd.) at 400 ppm, and $CaCl_2$ (HiMedia Laboratories Pvt. Ltd.) at 0.4% (Ernani 2008; Buran et al. 2012; Falchi et al. 2017), and each treatment was applied on four trees representing four replications of that treatment. The spraying was carried out using knapsack sprayer till slight run off of the spray liquid from the leaves and fruits. Spray solutions of ABA and calcium chloride were prepared just before being used. The stock solutions of ABA and calcium chloride were prepared by dissolving weighted quantity of these substances in 100 mL of distilled water each and then diluted with distilled water to reach the required concentrations (400 ppm and 0.4%, respectively). All treatments (water spray, ABA, and calcium chloride dehydrated) were sprayed four times, once at each growth stage. The first spray was at 30 DAFB (days after full bloom) while the second, third, and fourth sprays were done at intervals of 35 days each. All observations were recorded 10 days after each spray, i.e., S1—40, S2—75, S3—110, and S4—145 DAFB. Fruits were harvested after attaining commercial maturity, i.e., 150–155 DAFB. The experiment was conducted using a Randomized Complete Block Design (RCBD) with four replications (1 tree/replicate), with each replicate representing 12 fruits. The application of treatments were randomized within blocks and plants marked accordingly. Slope of the field was taken as a criterion for blocking.

Collection and preparation of fruit samples

Fruit samples of approximately uniform weight representing all the four directions, i.e., east, west, north, and south, were collected from middle portion of peripheral branches of the canopy. Fruits were washed with tap water and then dipped in dilute hydrochloric acid (0.1 M HCl). Later fruits were washed again repeatedly using single and double distilled water, after which the adhering moisture was wiped away with filter paper and muslin cloth. Fruits were peeled off using a peeler (1 mm thickness); the peeled fruits were cut equatorially into three discs of equal proportion (proximal, middle, and distal) and the central cores along with the seeds were removed. Samples were dried at 60–80 °C in an oven until constant weight was obtained. For subsequent chemical analysis, the samples were first crushed in a steel blender and sieved through 2 mm mesh.

Collection and preparation of leaf samples

Taking all the directions (east, west, north, and south) into consideration, current-season peripheral leaf samples from middle of the twigs were collected from the trees and washed gently under tap water and dipped in dilute HCl (0.1 M HCl). Latter samples were washed again repeatedly using single and double distilled water. The samples were air-dried on a filter paper. After air drying, samples were oven-dried at 60±5 °C until constant weight was obtained. For subsequent chemical analysis, the samples were first crushed in a steel blender and sieved through 2 mm mesh.

Calcium concentration

After collection and processing, fruit and leaf samples were digested in acid mixture (in a 9:4 ratio) of nitric acid and perchloric acid. The digest was dissolved in double distilled water, filtered in 100 mL volumetric flask, final volume made

to 100 mL, and analyzed for elemental calcium. The calcium (Ca) concentration of fruits and leaves was assessed by Flame Photometer-128 as described by Ranganna (1986).

Xylem functionality

To study fruit xylem functionality, vasculature was stained with dye through its path using the dye infusion technique. To avoid bubble trapping in the vasculature, fruits were cut at the base of the pedicel, instantly placed in polythene bags containing water, sealed promptly to avoid fruit transpiration, and then transferred to the laboratory. Fruits were then kept in dye (1% acid fuchsin) for 2 h at 22 °C and 65% relative humidity for drawing up the dye through the stalk. Fruits were sectioned equatorially into three equal sections (style end, middle portion, and calyx end) using a microtome blade to produce smooth cuts and to minimize tissue damage. The transverse sections with clearly visible dyed vascular bundles were scored for dye intensity under a dissecting microscope. Ten ventral, 5 dorsal, and 10 primary bundles were assayed for dye (Drazeta et al. 2004). The percentage of functionality was calculated using the following formula:

$$\text{Xylem functionality (\%)} = \frac{\text{Number of stained xylem bundles}}{\text{Total number of xylem bundles}} \times 100$$

Fruit physico-chemical properties

Fruit length from calyx to stalk end and fruit diameter at maximum circumference of the fruit were measured with a Vernier caliper. Length-to-diameter ratio (L/D) was calculated. To calculate fruit firmness, samples were punched with an Effegi penetrometer (model Ft-3–27, probe diameter 8 mm) at three different places on its surface (equatorial region) after removing 6 cm^2 of peel. Procedure was repeated for all the cases maintaining the punching positions at similar spots. Total soluble solid content (SSC) expressed in °Brix was determined with a hand refractometer (Erma, Japan). Titratable acidity in terms of malic acid was determined using the procedure reported by Ranganna (1986) and calculated as:

$$\text{Titratable acidity (\%)} = \frac{\text{Titrate value} \times \text{normality of NaOH} \times \text{vol. made up} \times 67}{\text{Weight of sample} \times \text{aliquat taken} \times 1000} \times 100$$

Streif index (Streif 1996) of fruit samples was calculated by following formula:

$$\text{Streif index} = \frac{\text{Firmness (kg/cm}^2\text{)}}{\text{SSC(°Brix)} \times \text{starch (1–8scale)}}$$

Statistical analysis

Analysis of variance (ANOVA) was performed for each variable using R software package. The data were subjected to post hoc *t* test for pairwise comparisons using Duncan's multiple range test (Gomez and Gomez 1984).

Results

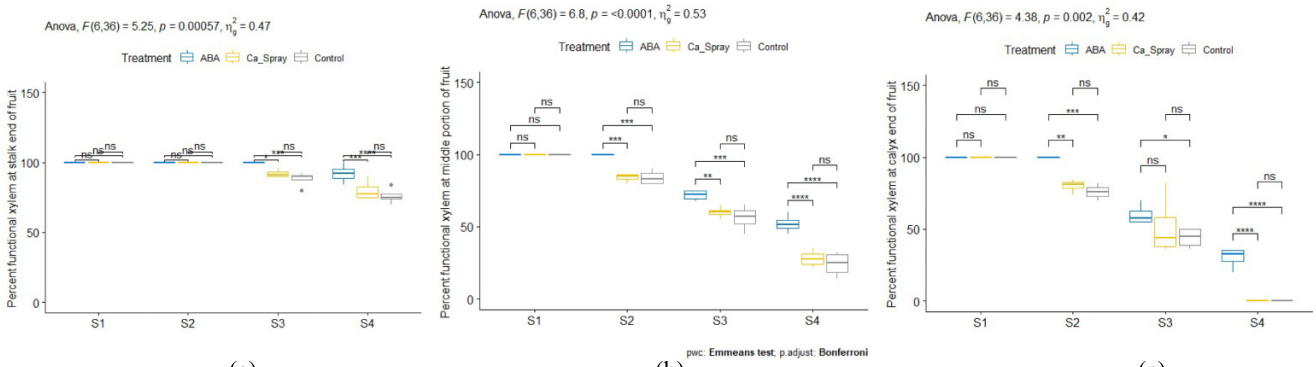
Percentage of xylem functionality was maximum at initial growth stages (Figs. 3 and 4) and decreased sharply during S3 (110 DAFB) and S4. At the stalk end, xylem functionality was found to be 100% in all treatments until fruit reached S2 (Fig. 1). But at S3 and S4, the highest levels (100% and 92%) were only maintained in ABA-treated fruits (Figs. 5 and 6). At the middle and calyx end of the fruit, some vascular bundles did not show any staining even as early as S2 stage, except in ABA-treated fruits (Fig. 4). The lowest levels of xylem functionality were observed in calcium- and control fruits at S3, which further decreased to 0% in the calyx end at S4 stage (Fig. 6). However, the ABA-treated fruits still retained 53% and 30% of xylem functionality at S4 in the middle and calyx end of the fruits, respectively.

Calcium concentration in apple fruit increased up to S2 (75 DAFB) and decreased thereafter, with a slight increase at S4 (145 DAFB). The overall fruit calcium (flesh and peel tissue) (0.06%) at S4 was found in ABA-treated-fruits, which was significantly higher than other treatments (Table 1). When stages were compared, the highest levels of calcium were found at S2 in both ABA- and calcium-treated fruits (0.07% each), and showed significantly higher values than those of control. At the stalk end of the fruit, there was no significant difference between ABA- and calcium-treated fruits throughout the various stages, but both treatments (ABA and calcium) showed higher calcium concentration than the control at each stage. At the middle and calyx end of the fruit, ABA-treated fruits showed enhanced calcium concentration of 0.06% and 0.05% at S4, respectively (Fig. 2).

In fruit peel, no significant difference was observed in calcium concentration of ABA- and calcium-treated fruits. However, the concentration was much higher than that of control treatment at all growth stages. Leaf calcium increased progressively throughout the growth stages and had a significantly higher proportion in calcium-treated plants at all stages with a peak of 2.26% attained at S4 (Table 1).

Fruit length at harvest varied significantly among the treatments. Maximum mean fruit length of 67.63 mm was found in calcium-treated fruits (Table 2), whereas the lowest fruit length of 63.22 mm was observed in control. There was no significant difference found in diameter of ABA- and calcium-treated fruits. However, control fruits showed significantly lower values (69.64 mm) for diameter as compared with other

Fig. 1. Xylem functionality (%) of top (*a*) stalk end, (*b*) middle, and (*c*) calyx end of "Super Chief Sandidge" apple fruit as affected by treatments throughout the growth stages (S1—40, S2—75, S3—110, and S4—145 DAFB).



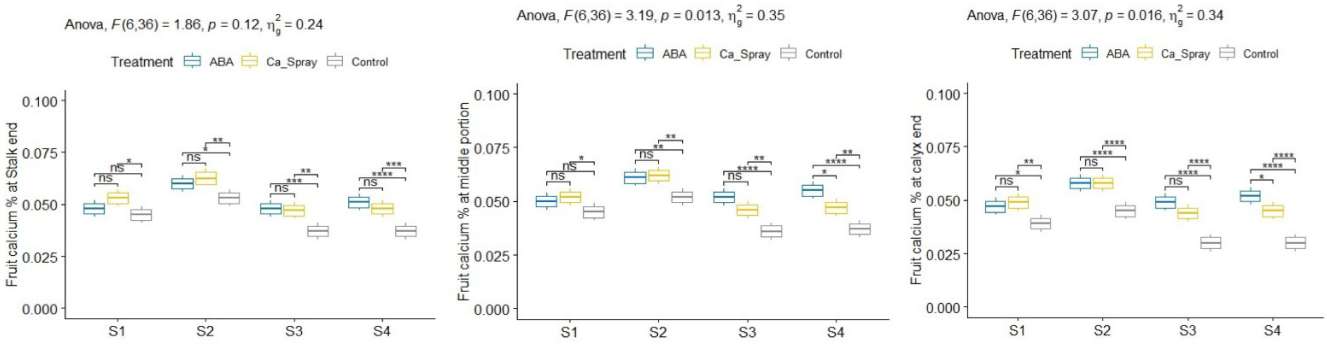
(a) (b) (c)

Table 1. Calcium concentration (%) in fruit and leaf tissues of "Super Chief Sandidge" apple at different growth stages (S1—40, S2—75, S3—110, and S4—145 DAFB, respectively).

Tissue	Treatment	Growth stages			
		S1	S2	S3	S4
Overall fruit†	ABA	0.055b	0.066b	0.057	0.060c
	Calcium sprays	0.058b	0.069b	0.055	0.056b
	Control	0.050a	0.057a	0.042	0.043a
Fruit peel	ABA	0.074ab	0.087b	0.080b	0.084b
	Calcium sprays	0.078b	0.092b	0.082b	0.084b
	Control	0.070a	0.078a	0.066a	0.068a
Leaf	ABA	0.80a	1.01a	1.27a	1.40a
	Calcium sprays	1.20c	1.50c	1.91c	2.26b
	Control	1.01b	1.25b	1.55b	1.83b

Note: Means followed by same lowercase letter within a column and tissue are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).
†Overall fruit indicates flesh and peel tissue.

Fig. 2. Top (*a*) stalk end, (*b*) middle, and (*c*) calyx end calcium concentration (%) of Super Chief Sandidge apple fruit as affected by treatments throughout the growth stages (S1—40, S2—75, S3—110, and S4—145 DAFB).



(a) (b) (c)

Table 2. Physico-chemical properties of "Super Chief Sandidge" apple fruit at harvest.

Treatment	Length (mm)	Diameter (mm)	L/D	SSC (°Brix)	Acidity (%)	SSC/acidity	Firmness (kg/cm^2)
ABA	66.45b	72.48b	0.92a	15.10ab	0.27ab	56.12a	7.58b
Calcium chloride	67.63c	72.03b	0.94b	15.50b	0.31b	50.59a	7.62b
Control	63.22a	69.64a	0.91a	14.58a	0.23a	63.57b	7.20a

Note: Means followed by same lowercase letter within a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Table 3. Effect of treatments on the ratio of calcium concentration (leaf vs overall fruit) and Streif index of "Super Chief Sandidge" apple at different growth stages (S1—40, S2—75, S3—110, and S4—145 DAFB, respectively).

Treatment	Growth stages			
	S1	S2	S3	S4
ABA	14.6a	15.2a	22.2a	23.1a
Calcium chloride	20.7b	21.8b	34.9b	40.4b
Control	20.3b	21.9b	36.8b	42.7b
	Streif index			
Treatment	S1	S2	S3	S4
ABA	1.41b	0.55b	0.23	0.06
Calcium chloride	1.34a	0.36a	0.21	0.06
Control	1.36a	0.49b	0.22	0.06

Note: Means followed by same lowercase letter within a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

treatments. L/D ratio showed significantly higher value for calcium-sprayed fruits than other treatments. Total SSC and titratable acidity of ABA-treated fruits were at par with both calcium-treated and control fruits. However, calcium-treated fruits showed significantly higher values for both SSC (15.5° Brix) and titratable acidity (0.31%) as compared to control. SSC-to-acid ratio were similar for calcium- and ABA-treated samples but differed significantly from control (63.57).

Streif index calculated showed no significant effect of the treatments on the fruits except at stages S1 and S2, where maximum values (1.41 and 0.55) were observed in ABA-treated fruits, respectively (Table 3). Streif index decreased with the maximum of 1.41 at S1, and 0.55, 0.23, and 0.06 at stages S2, S3, and S4, respectively.

Discussion

Significantly higher functionality of the xylem vessels was observed in all the three portions (stalk, middle, and calyx end) of ABA-treated fruits (Fig. 1). The higher functionality of xylem vessels observed in apple fruits treated with ABA may be due to the maintenance of the hydrostatic gradient in the apoplast, through which translocation of the dye was possible from the pedicel to the distal end region of the fruit.

Xylem functionality decreased as growth advanced, with lower drop in ABA-treated fruits (Fig. 1). High numbers of vessels were active during the early growing stages and became dysfunctional to great extent toward the end of harvest time in all the treatments except ABA-treated samples (Figs. 3–6). The xylem dysfunction may have been caused by breakage of vessels upon stretching from fruit elongation, as the compression promoted by greater elongation of parenchyma cells causes the collapse and loss of functionality in the vasculature (Miqueloto et al. 2014). Furthermore, as the xylem vessels get obstructed, hydrostatic pressure gradient is reduced between the peduncle and fruit flesh xylem vessel elements (Chatelet et al. 2008). Reduction in functional xylem bands could be the main reason for low calcium content generally observed in the distal portions of the fruit, as calcium gets transported exclusively through xylem vessels. Disappearance of existing stomata and lenticels at latter stages (after June drop) (Schlegel and Schönherr 2002) might also add to low hydrostatic pressure.

This study demonstrated that there was higher calcium concentration in flesh (stalk, middle, and calyx end) of the treated fruit tissues over control (Fig. 2). Maximum overall fruit calcium concentration was observed in the ABA-treated samples rather than calcium-treated ones, indicating that most of the sprayed calcium might have been retained in the peel of the fruit. As the abundance of functional xylem vessels increased, as evident from the staining experiment, more calcium would be expected to be translocated to the fruit tissues, which may have resulted in higher calcium concentration in the fruit. There is reduced resistance to xylemic water toward the distal tissue and this enhanced vessel functionality is what moves the calcium (De Freitas et al. 2014).

Comparing various portions of the fruit flesh (stalk, middle, and calyx), the maximum calcium concentration was observed in the proximal (stalk) end of the fruit, followed by middle and distal end. Calcium binds to the first open cation exchange sites in the cell wall as it is transported into the fruit tissue, which turns out to be proximal tissue of the fruit (Wang et al. 2021). The results evidenced that in ABA-treated fruits, there was a significantly higher proportion of calcium uptake in the two portions (calyx and middle). In the proximal portion of the fruit, the effects of ABA and calcium sprays were similar with slightly higher proportion in calcium-sprayed fruits. Also, from the results, it was evident that ABA applications significantly enhanced calcium concentration in distal tissue of the fruit till the end, and the final concentration at S4 was higher than initial (S1), which was not the case with other treatments. This would indicate that xylem functionality may be the essential factor in maintaining the calcium concentration all through the various fruit portions and developmental stages.

The foliar application of calcium chloride significantly enhanced the peel calcium concentration (11%–24%) of the apple fruit (Table 1); this was due to higher deposition and absorption of calcium into the peel of the fruits by these preharvest foliar sprays. The results obtained were in accordance with the findings of Raese and Drake (1993,2000) who reported that foliar calcium sprays increased at least 10% concentrations of this element in fruit peel and flesh over control; however, in the present study, the increase was observed only in fruit peel and not in the flesh. Second to calcium chloride spray was the ABA treatment, though not differing significantly; it also showed an increase of close to 15% in peel calcium concentration over control. The reason may be the steady uptake throughout the fruit development to the end tissues augmented by the abundance of functional xylem vessels.

Calcium uptake or concentration in fruit peel and flesh was highest at early stages and had its maximum at S2 (75 DAFB). The reason for gaining more calcium during the early developmental stages may be due to the intact xylem vessels and a higher fruit transpiration rate. Montanaro et al. (2014) have reported highest rate of transpiration at fruit set stage and also added that the rate declines to nearly one-tenth of

Fig. 3. Xylem functionality at stalk, middle, and calyx end of Super Chief Sandidge fruit at S1 (40 DAFB).

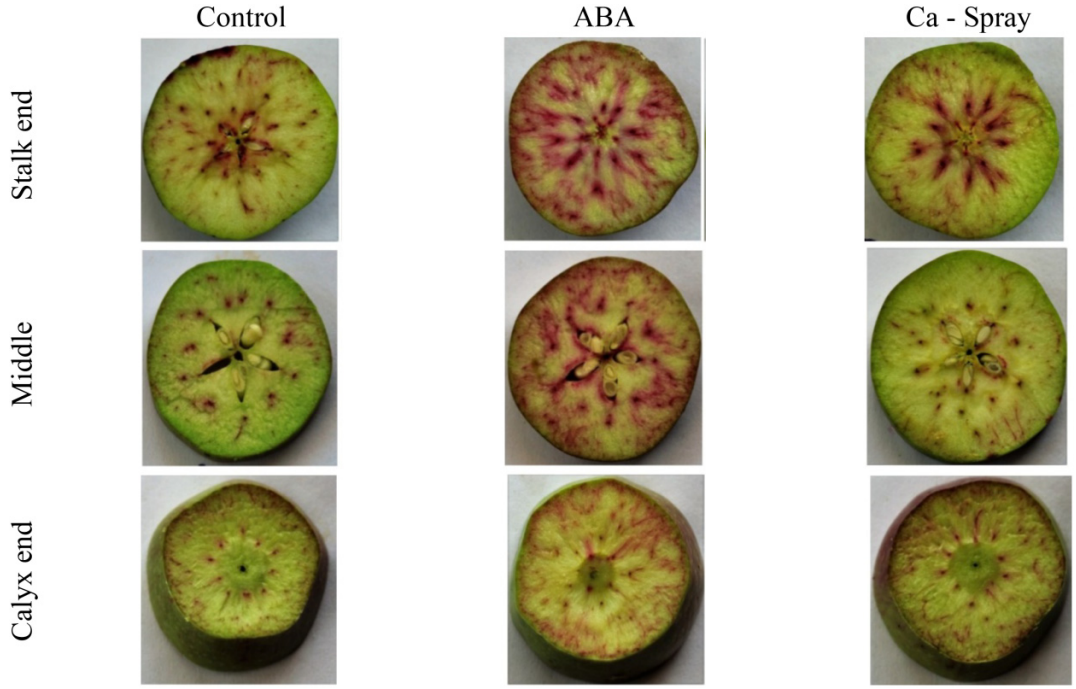
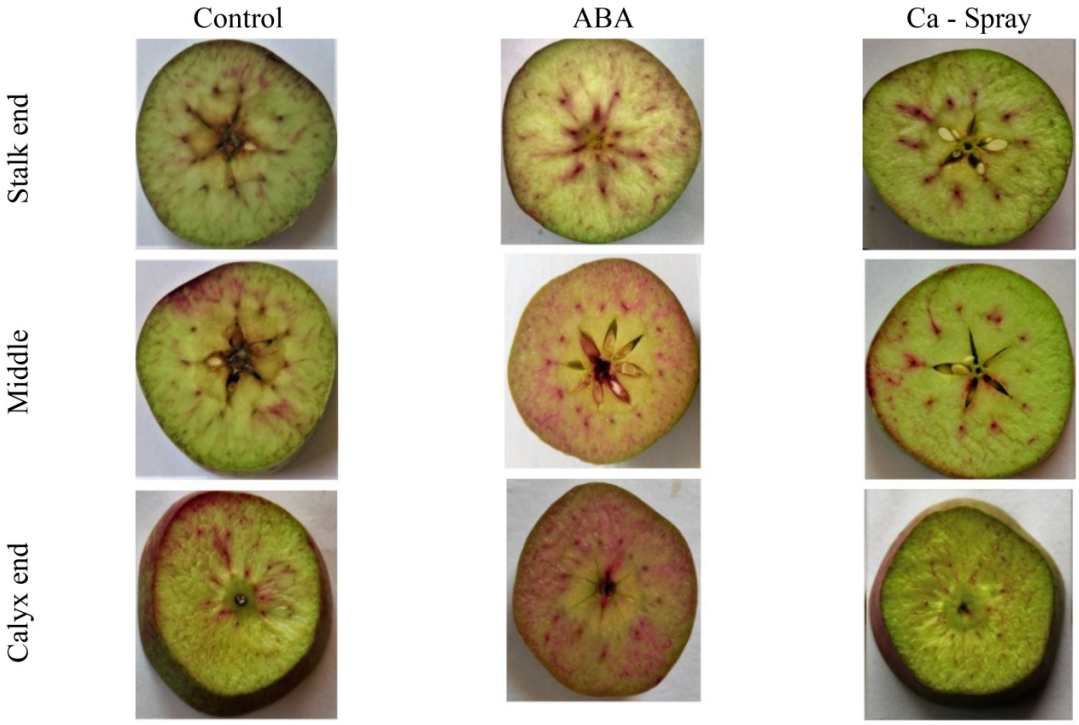


Fig. 4. Xylem functionality at stalk, middle, and calyx end of Super Chief Sandidge fruit at S2 (75 DAFB).



this value later in the development. Moreover, highest absorption of calcium sprayed onto the fruits occurred up to this stage (S2) and declined later on. Inorganic salts permeate rapidly through stomata and trichomes present at young fruitlet stage. As the development advances, the trichomes are shed, stomata lose physiological activity as it get covered by waxes, and scars are sealed with cutin and wax, thus blocking the two main pathways (Schönherr 2001). Gomez and Kalcsits (2020) also reported comparatively rapid uptake and penetration of calcium into apple fruit during the first 30–60 DAFB.

Leaf calcium concentrations were enhanced by foliar application of calcium chloride (Table 1). Kadir (2005) reported increases in leaf calcium concentration in response to frequent

Fig. 5. Xylem functionality at stalk, middle, and calyx end of Super Chief Sandidge fruit at S3 (110 DAFB).

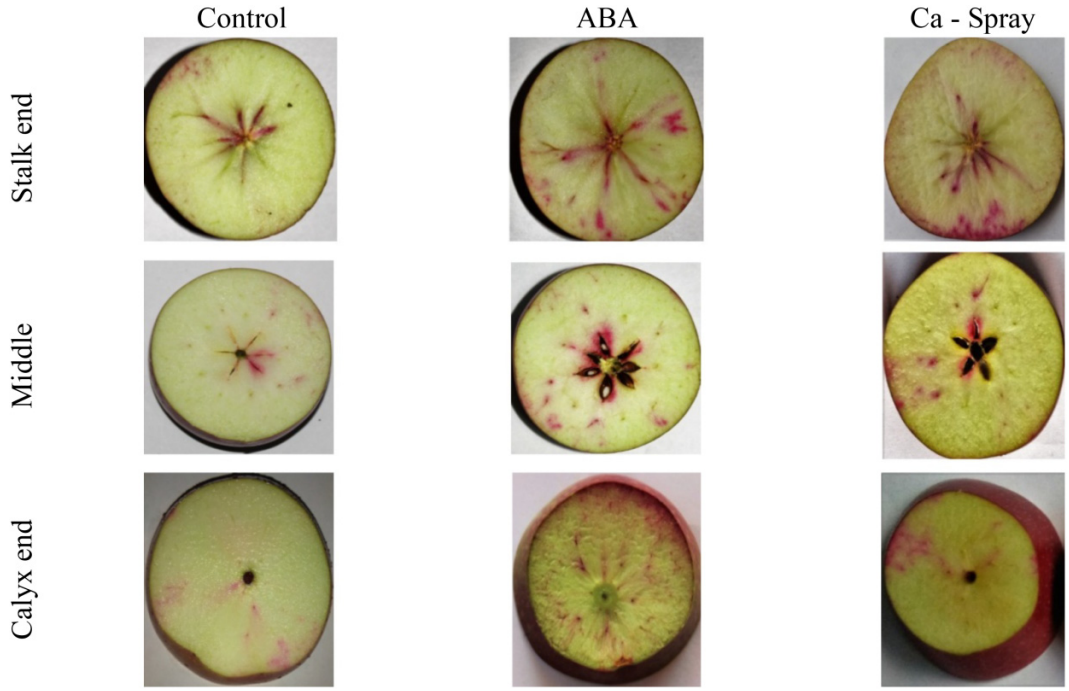
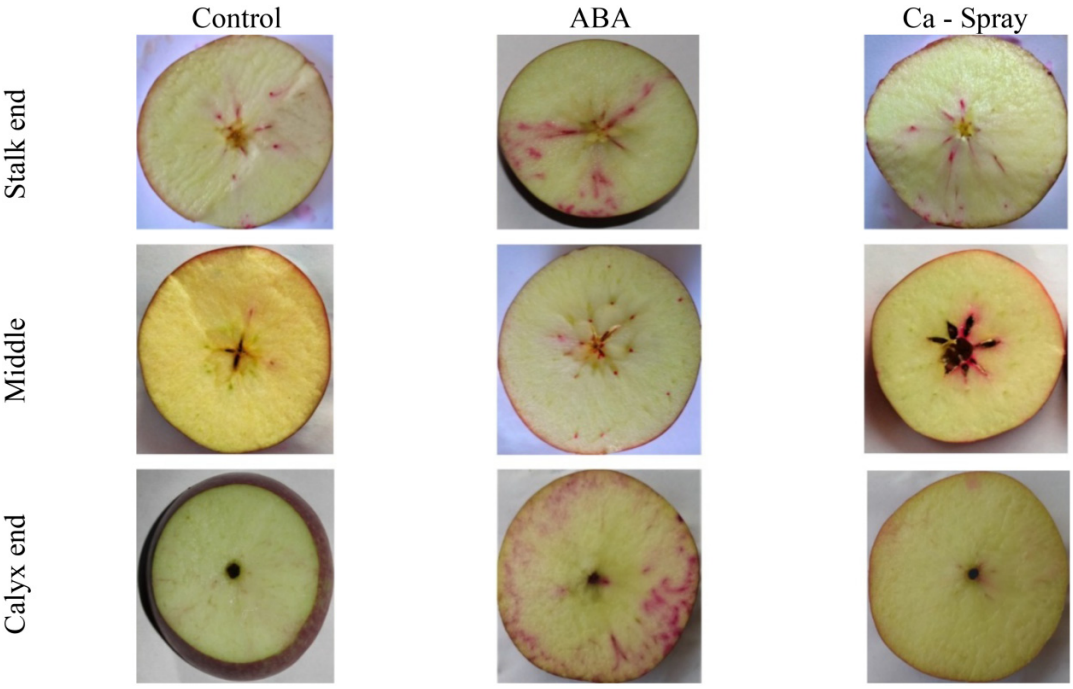


Fig. 6. Xylem functionality at stalk, middle, and calyx end of Super Chief Sandidge fruit at S4 (145 DAFB).



calcium chloride application in apple. Our study also revealed that the calcium concentration of the leaves was reduced and found least in ABA application as compared with other treatments (Table 3). ABA treatment lessened leaf calcium uptake proportional to the reduction in leaf conductance, as it decreased stomatal conductance. With the closure of stomata, the portion of the calcium that was initially directed toward the leaf tissue gets diverted to the fruit tissue due to the water potential difference between these two tissues. Furthermore, reports indicate that ABA-treated plants have higher stem water potential and lower water losses on whole-plant basis (De Freitas et al. 2011). Hence with the application of ABA, calcium distribution into the leaves is reduced and flow into the fruit increases (Barickman et al. 2014). Also, the leaf calcium concentration increased with the advancement of season and was found maximum at S4 stage. The increase in leaf

calcium concentration along with the growth stages can be explained by its accumulation in the tissues (Nachtigall and Dechen 2006).

Improvement in fruit size parameters (length, diameter, and L/D ratio) were noticed in calcium-treated and ABA-treated fruits. Calcium-treated or ABA-treated fruits with maximum calcium showed higher values for size parameters than control. These results agree with an earlier report (Kadir 2005), where calcium sprays resulted in enhanced fruit growth. But at the same time within ABA- or calcium-treated fruits, ABA-treated fruits having higher calcium content than calcium-sprayed ones showed lower values for these size parameters. Thus, the variation in size would be due to the greater proportion of growth-promoting hormones released by the excess seed count noticed in calcium-treated samples (average seed count for control—7, ABA treated—5.5, and calcium sprayed—7.5).

Firmness of both calcium-treated and ABA-treated fruits were found to be equivalent. Calcium applied before harvest would enhance fruit firmness by increasing the levels of calcium bound to the cell wall. Most of the calcium that penetrates into the fruit tissue seems to accumulate in the middle lamella of the cell wall where it binds to the pectin of the cell wall, thereby maintaining its structural integrity (Ramezanian et al. 2018), and as a consequence enhances fruit firmness (Kadir 2005; Fallahi et al. 2006). Moreover, calcium can affect some hydrolytic enzymes (pectin methyl esterase) of the cell wall and possibly reduce the activity of softening-inducing polygalacturonase (Serrano et al. 2004).

SSC and titratable acidity of ABA- or calcium-sprayed fruit samples were statistically equivalent. Higher SSC might be attributed to lower utilization of sugar in metabolic processes as an outcome of retarded respiration due to calcium intake. Increase in SSC content with calcium chloride sprays has also been reported by Kadir (2005) in Jonathan apple. This reduced respiration rate also might have resulted in maintaining acidity over a longer period, with the result leading to more accumulation of organic acids due to slow rate of oxidation and conversion of some of these acids into sugars as depicted by Raese and Drake (1993). Increase in acidity as a result of calcium or ABA treatments may also be due to retained H^+ ions in the cytosol as a result of controlled membrane permeability and maintained cellular integrity.

Streif index decreased through the growth stages (Table 3), demonstrating the advancement of ripening. No significant effect of calcium concentration in fruits was evident at harvest time. Treatment effects varied significantly only at S1 and S2, when it was higher in ABA-treated fruits, which could be attributed to high starch content of the samples.

Conclusion

Reduced fruit calcium levels, responsible for calcium-related disorders in apple fruit "Super Chief Sandidge" can be attributed to increased xylem dysfunction with the progression of fruit development. Reduced levels of calcium at distal regions (calyx end) of the fruit make it more susceptible to localized calcium-related disorders. However, this research has shown that vascular integrity of apple fruits can be maintained vis-à-vis the nutrient allocation (especially calcium) to the distal portions of the fruit through transpirational stream, with whole plant application of ABA. But at the same time, application of ABA induced drooping of leaves for certain period of time, which might reduce overall photosynthetic efficiency of the plant and result in reduction of yield. Also, ABA being costlier than other calcium-supplementing alternatives, its applicability to enhance fruit calcium concentration at commercial scale can be ascertained only after working out the economics, which was not calculated in this case.

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