

Article

Calcium Uptake Pattern and Its Transport Pathway in ‘Shixia’ Longan Fruit

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Abstract: Calcium plays an irreplaceable role as an essential mineral nutrient in plants, particularly in the formation of calcium pectinate, which is critical for cell wall construction. Fruits deficient in calcium are more susceptible to cell wall disintegration, bacterial infections, and the development of various physiological disorders and fungal diseases. Despite its importance, limited research has focused on calcium nutrition in longan, and the pathways and regulatory mechanisms underlying calcium uptake in this fruit remain unclear. In this study, we investigated calcium uptake in longan at different developmental stages, examined its variation patterns, analyzed the correlations between calcium concentrations in the pedicel and the fruit, and explored the distribution of calcium in the pedicel. We also studied the functions of xylem/apoplastic and symplastic pathways using dye tracers. Our findings contribute to a deeper understanding of calcium nutrition in longan and clarify the transportation characteristics of calcium within longan fruit.

Keywords: longan; calcium; calcium transport pathway; calcium oxalate



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1. Introduction

Calcium acts as a key messenger in signal transduction and plays a central role in regulating plant growth, development, and responses to environmental stresses [1,2]. Mineral elements in plants generally exist in four main forms: inorganic salts, complexes with organic acids, as chelates, and as polymers or other forms [3,4]. In fruit trees, calcium can be categorized into three forms: water-soluble calcium (free Ca^{2+}), non-water-soluble calcium (such as calcium pectinate bound to cell wall pectin), and calcium oxalate crystals formed with oxalic acid [5,6]. Calcium deficiency significantly reduces plant tolerance to both biotic and abiotic stresses, often leading to several physiological disorders that severely affect fruit quality [6–8], such as citrus peel pitting [9], lemon petechiae [10], tomato blossom-end rot [11], apple bitter pit [12], and lychee fruit cracking [13].

It has been well established that spraying calcium onto the fruit surface is ineffective in significantly increasing the calcium content of fruit tissues [14]. Moreover, numerous studies have demonstrated that calcium, which plays a critical role in fruit quality, is primarily absorbed and stored during the early stages of fruit growth, particularly in the young fruit stage, whereas it is rarely absorbed during the later stages [15–17]. Early research by Quinlan et al. (1969) [18] identified two stages of calcium accumulation in apple fruit: the first is during cytokinesis, where 80% to 90% of the total calcium is absorbed; the second is during the rapid fruit expansion and ripening phase, where calcium uptake slows, and accumulation becomes limited. However, the calcium uptake patterns differ among fruit species and vary at different developmental stages.

Calcium oxalate formation in the fruit pedicel does not appear to affect calcium uptake during early fruit development. However, as the fruit matures, calcium oxalate crystals accumulate in the vascular tissues, obstructing calcium transport and leading to calcium deficiency in the fruit. As the calcium supply increases, calcium oxalate content and crystal volume also increase [19–22]. Although calcium oxalate formation is generally considered irreversible due to its solubility in strong acids and insolubility in organic acids, some studies suggest that calcium oxalate formation may be reversible and could act as a temporary calcium reservoir that can be mobilized during calcium-deficient conditions [21,23,24]. Despite these findings, the mechanisms underlying calcium oxalate formation and its regulatory processes remain unclear and require further investigation.

Longan (*Dimocarpus longan* Lour.) is a subtropical fruit highly valued for its crisp texture, rich nutritional content, and high concentrations of calcium and magnesium [25,26]. In traditional Asian medicine, dried longan fruit is commonly used to treat insomnia, palpitations, and memory disorders [27–29].

2. Materials and Methods

2.1. Experimental Materials

The longan variety used in this study was ‘Shixia’, an 18-year-old cultivar grafted onto ‘Chuliang’ rootstock. The trees were cultivated at the Xili Fruit Farm in Shenzhen, Guangdong Province, China. Three healthy, vigorously growing trees were selected as testing trees. All experimental samples in this study were obtained from these three trees.

2.2. Sample Processing Method

Fruit samples were collected at the young, middle, rapid expansion and mature stages of development, namely, at 6, 14, 37, 55, 72, and 107 days after anthesis of the ‘Shixia’ from the testing tree. Each fruit was separated into pedicel, peel, aril, and seed. Freshly weighed samples were wrapped in tin foil and dried in a blast oven (DHG-9140A, Shanghai Shenxian Constant Temperature Equipment Factory, Shanghai, China) at 65 °C for a minimum of 72 h. The dry weight of each tissue was then recorded.

2.3. Determination of the Total Calcium Content

Dried powdered samples were digested using a concentrated H_2SO_4 - H_2O_2 method. The calcium content in the tissues was analyzed using a flame atomic absorption spectrophotometer (Z-2300, Hitachi, Chiyoda, Japan). Five biological replicates were performed.

2.4. Calcium Mapping in Longan Tissues Using Electron Probe Microanalyzer (EPMA)

The methodology for calcium mapping followed that described by Song et al. [30]. Longan fruits with pedicels at different developmental stages were collected, and fresh pedicels were immediately prepared as samples. The tissue samples were analyzed in-situ using a secondary electron probe microscope (wavelength dispersive X-ray spectroscopy, WDS) (JXA-8100, JEOL, Akishima, Japan). Three replicates were included in the analysis.

2.5. Extraction of Different Forms of Calcium from Tissues

Calcium extraction was performed stepwise using distilled water, 2% acetic acid solution, and 2% HCl solution. Calcium extracted with distilled water represented water-soluble calcium, whereas calcium extracted with acetic acid represented structural calcium bound to cell walls (pectin) or membranes (phosphate groups on phospholipids) through ionic bonds. Calcium extracted with HCl primarily represented calcium oxalate. In each extract, 1 mL of 5% lanthanum chloride solution was added, and calcium concentrations were determined using atomic absorption spectroscopy (Z-5000, Hitachi, Chiyoda, Japan). Five biological replicates were performed.

2.6. Effects of Girdling on Calcium Uptake in Fruit

The girdling treatment was applied 6 days after longan anthesis by making two 5 mm wide cuts in the bark of the bearing shoots, removing the bark between the cuts. Control shoots were selected from similar positions on the canopy with equivalent fruit loads as the treatment group. Ten bearing shoots were selected for both the control and treatment groups on each tree. Fruit samples were harvested 10 days after the treatment, and fresh and dry weights of each tissue were recorded to determine calcium content and concentration in each part. Five replicates were used for each fruit unit.

2.7. Tracer Observation of Ductal and Cytoplasmic Pathway Using Flux Dyes

Dye tracer experiments were conducted at different developmental stages of the fruit. Xylem transport was traced using 1% (*w/v*) safranin O solution, and cytoplasmic transport was traced using 1 mg·mL⁻¹ of carboxyfluorescein (CF) solution. Each stemmed fruit was left with a 3–5 cm pedicel, which was diagonally cut in distilled water to prevent air embolism and inserted into a 1.5 mL centrifuge tube filled with the dye. The pedicel was regularly observed while replenishing the dye solution to avoid rapid uptake, which could lead to insufficient dye supply and severe water loss from the pedicel surface. After 24 h (at a temperature of approximately 25–28 °C and relative humidity of about 82%), the fruit was removed, cut longitudinally from the top to the base and pedicel using a single-sided blade, and photographed for observation. Fruits stained with safranin O solution were photographed outdoors under natural light, whereas fruits stained with CF were photographed in a dark room using a handheld UV lamp and a single-lens reflex camera. Five replicates were set up for each fruit unit at different developmental stages.

2.8. Determination of IAA Content

The IAA content of fruits was measured at 14, 37, and 55 days after anthesis, following the method described by Okamoto et al. [31]. The derivatization process involved adding 40 µL of 20 mg·mL⁻¹ methoxamine hydrochloride to the extract after rotary evaporation, mixing thoroughly, and shaking at 37 °C and 700 rpm for 2 h. Subsequently, 60 µL of MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) was added, followed by oscillation at 37 °C and 700 rpm for 30 min. The derivatized samples were analyzed using GC-MS (7890B-5977A, Agilent, Santa Clara, CA, USA). Five biological replicates were analyzed for each batch of mixed fruits from the same period.

2.9. Growth Hormone Analog (2,4-D) Treatment

Twenty actively growing, pest-free fruit spikes in four directions of the canopy were selected from each tree and sprayed with 10 µg·mL⁻¹ 2,4-D solution until run-off. Individual fruits were collected at 10 and 20 days after treatment to determine their calcium content and concentration. Ten replicates were set up for this experiment.

2.10. Correlation Analysis of Seed Size and Fruit Calcium Uptake Capacity

Seed fresh weight, fruit calcium concentration, calcium uptake rate, and calcium uptake activity were measured or calculated at different growth and development stages. The effect of seed weight on the calcium uptake capacity of fruits was analyzed by monitoring seed fresh weight, fruit calcium concentration, calcium uptake rate, and calcium uptake activity over the growth and development stages.

2.11. Calculation of Fruit-Related Parameters

$$\text{Total tissue calcium content (mg·Fruit}^{-1}\text{ DW)} = \text{tissue dry weight (g)} \times \text{calcium concentration (g·kg}^{-1}\text{)}$$

$$\text{Fruit growth (g)} = \text{dry weight increase of fruit during a specific period}$$

Fruit calcium uptake (mg) = increase in fruit calcium content during a specific period

Fruit growth rate ($\text{mg} \cdot \text{d}^{-1}$) = increase in fruit dry weight during a specific period (mg)/number of days in the period (d)

Fruit calcium uptake rate ($\text{mg} \cdot \text{d}^{-1}$) = increase in fruit calcium content during a specific period (mg)/number of days in the period (d)

Fruit calcium uptake relative to growth ($\text{mg} \cdot \text{g}^{-1}$) = calcium accumulation (mg) for each 1 g increase in fruit dry weight

Fruit calcium uptake activity ($\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ DW) = calcium uptake rate ($\text{mg} \cdot \text{d}^{-1}$) during a period/fruit dry weight during the period (g)

For calculating fruit calcium uptake activity during specific periods, the average dry weight of the fruit at the beginning and end of the period was used.

2.12. Statistical Analysis

The experiments were conducted using a randomized design, with the number of replicates specified in each section. Statistical analysis was performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA) for LSD multiple range tests and *t*-tests, and graphs were generated using Excel 2003 (Microsoft, Redmond, WA, USA). All data in the figures and tables are represented by mean values (M) $\pm$ standard error (SE).

3. Results

3.1. Dynamics of Calcium Accumulation in ‘Shixia’

Calcium accumulation in ‘Shixia’ longan fruit was strongly correlated with fruit growth (Figure 1A). The fruit exhibited a slow rate of dry weight accumulation up until 55 days after anthesis, after which dry weight increased linearly as the aril began to expand. The highest rate of dry weight accumulation occurred between 72 and 107 days after anthesis, accounting for approximately 57% of the total fruit weight at maturation (Figure 1B). This period corresponds to the phase of rapid calcium uptake and fruit expansion in ‘Shixia’ longan.

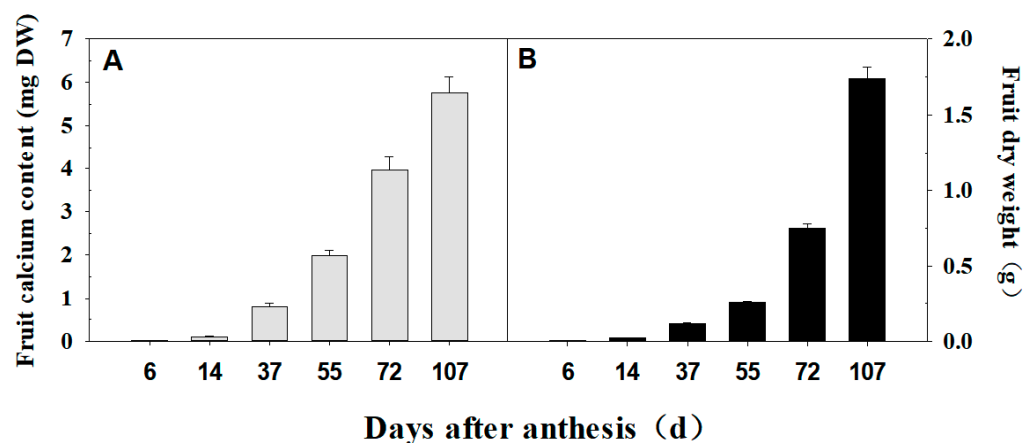


Figure 1. Fruit calcium content (A) and growth dynamics (B). The bars represent M $\pm$ SE.

3.2. Calcium Uptake Capacity of ‘Shixia’ Fruit at Various Stages

As shown in Table 1, the size and calcium uptake of fruit gradually increased with fruit growth and development. However, calcium uptake activity steadily decreased throughout the developmental stages. The most rapid fruit growth, along with the highest calcium uptake and calcium uptake rate, occurred between 55 and 72 days after anthesis. Notably, calcium uptake relative to growth was highest between 37 and 55 days after anthesis but progressively declined as the fruit expanded. The optimal periods for calcium supplementation in ‘Shixia’ longan were identified as the first week after fruit set, characterized by the

highest calcium uptake activity, and the middle stages of fruit development (37–55 days after anthesis), during which calcium uptake relative to growth was at its peak.

Table 1. Growth and calcium uptake capacity at different fruit development stages. Data are presented as $M \pm SE$.

Stage (Day After Anthesis)	Mass Growth (g)	Fruit Growth Rate ($\text{mg} \cdot \text{d}^{-1}$)	Ca Uptake (mg)	Fruit Ca Uptake Rate ($\mu\text{g} \cdot \text{d}^{-1}$)	Ca Uptake in Relative Growth ($\text{mg} \cdot \text{g}^{-1}$)	Fruit Ca Uptake Activity ($\mu\text{g} \cdot \text{d}^{-1} \cdot \text{g}^{-1}$)
6–14	0.02 ± 0.00	1.96 ± 0.07	0.08 ± 0.01	9.83 ± 0.94	4.98 ± 0.36	792.29 ± 47.46
14–37	0.09 ± 0.00	3.91 ± 0.21	0.50 ± 0.10	21.87 ± 4.24	5.37 ± 0.82	345.02 ± 65.45
37–55	0.14 ± 0.01	7.65 ± 0.51	1.19 ± 0.12	66.31 ± 6.89	8.81 ± 0.88	360.74 ± 38.94
55–72	0.50 ± 0.03	29.15 ± 1.66	1.97 ± 0.31	115.90 ± 18.06	3.72 ± 0.58	225.70 ± 34.35
72–107	0.99 ± 0.07	28.29 ± 2.09	1.80 ± 0.43	51.29 ± 12.27	1.83 ± 0.46	42.50 ± 9.86

3.3. Variation Patterns and Correlation of Calcium Concentration in the Pedicel and the Fruit

Through fruit development, the calcium concentration in the pedicels was significantly higher than that in the fruit (Figure 2A). The fruit's calcium concentration peaked at 55 days after anthesis, whereas the calcium concentration in the pedicel remained relatively constant after this point. As the fruit matured, its calcium concentration gradually declined.

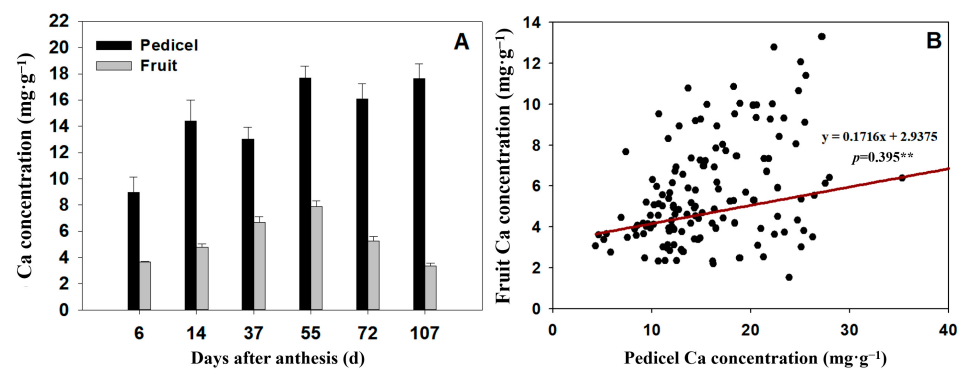


Figure 2. Calcium concentration in the fruit and the pedicel (A) and linear correlation between calcium concentration in the pedicel and the fruit (B). The bars represent $M \pm SE$. P represents the correlation coefficient between the two factors, and ** represents a highly significant correlation.

A moderate but statistically significant positive correlation was found between the calcium concentration in the pedicel and the fruit (Figure 2B), suggesting that calcium uptake by the fruit and calcium retention in the pedicel are positively correlated. This finding implies that the junction between the fruit and the pedicel may act as a 'bottleneck' for calcium accumulation in the fruit.

3.4. Calcium Distribution in the Pedicels at Different Developmental Stages

Calcium abundance in 'Shixia' longan was notably high, with the highest relative calcium content observed in the pith of the pedicel 6 days after anthesis. During this period, calcium content in the xylem exceeded that in the phloem. However, from 14 days after anthesis until fruit maturity, the relative calcium content in the phloem surpassed that in the xylem. Notably, from 55 days after the middle stage of fruit development to the rapid swelling stage, the relative calcium content in the pith increased significantly (Table 2). Calcium-rich particles were consistently distributed across all developmental stages of 'Shixia', primarily concentrated in the secondary tissues of the phloem and xylem (Figure 3).

Table 2. The relative contents of calcium in different tissues at different developmental stages. Data are presented as M ± SE. Different letters indicate significant differences as determined by one-way ANOVA (with LSD) ($p < 0.05$).

Sample Date (DAA)	Relative Content of Calcium (%)			
	Pith	Xylem	Phloem	Cortex
6	7.52 ± 3.40 a	2.73 ± 1.44 b	0.41 ± 0.41 b	1.31 ± 0.28 b
14	0.79 ± 0.79 b	0.90 ± 0.18 b	2.79 ± 1.25 a	1.62 ± 0.69 ab
37	2.01 ± 1.09 b	0.87 ± 0.36 b	4.04 ± 0.32 a	2.35 ± 0.53 b
55	7.08 ± 2.29 a	3.89 ± 0.61 b	4.35 ± 0.42 b	1.88 ± 0.09 b
72	15.22 ± 9.28 a	1.24 ± 0.16 b	3.68 ± 0.44 b	2.20 ± 0.33 b
107	5.32 ± 0.88 a	2.76 ± 0.33 c	3.90 ± 0.23 b	0.94 ± 0.24 d

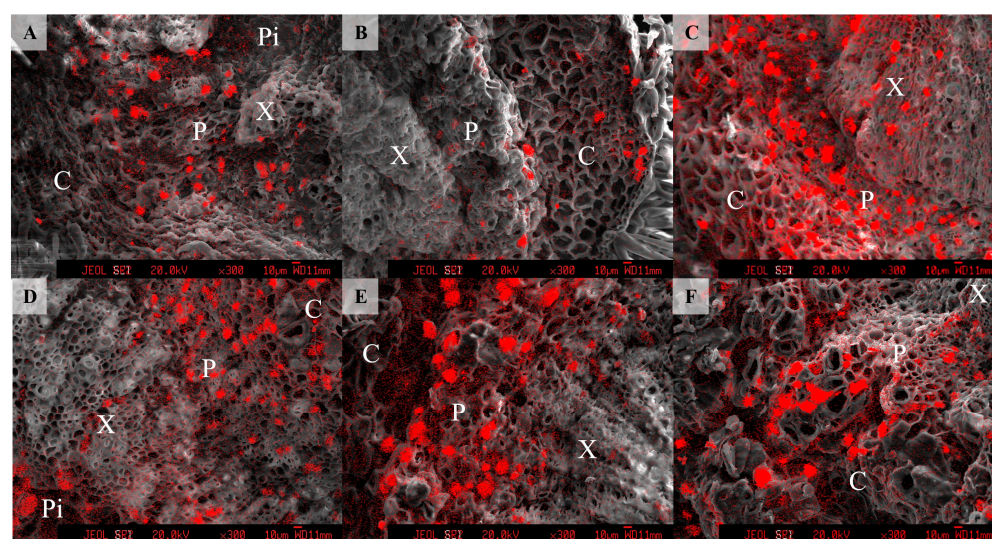


Figure 3. Calcium distribution in the pedicel at different stages of ‘Shixia’ longan. Pi, X, P and C refer to pith, xylem, phloem and cortex, respectively; (A–F) refer to 6, 14, 37, 55, 72 and 107 DAA, respectively; scale= 10 µm.

The levels of water-soluble calcium and structural calcium in ‘Shixia’ varied consistently throughout the growth and developmental stages. Structural calcium content gradually increased with fruit growth, but its relative content remained stable. The most prominent change observed was in the ratio of oxalate to water-soluble calcium, with calcium oxalate consistently being the dominant form of calcium (Figure 4).

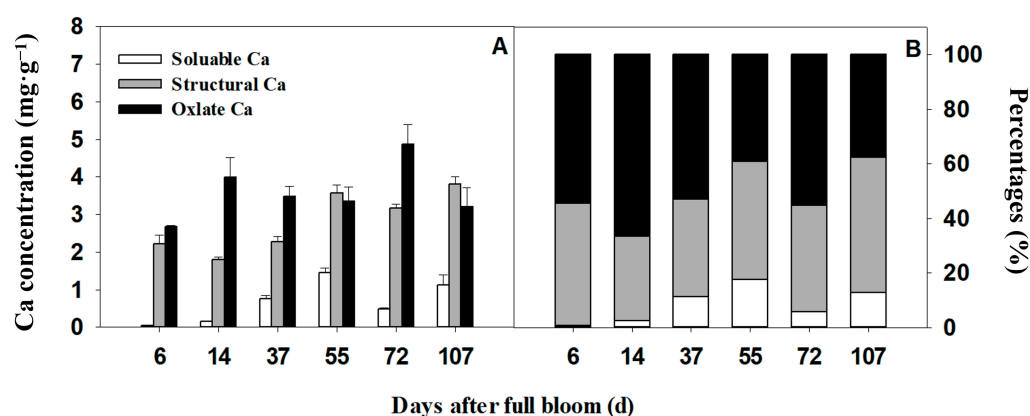


Figure 4. The absolute (A) and relative (B) contents of different forms of Ca in pedicel. The bars represent M ± SE.

In conclusion, calcium oxalate accounted for a significant proportion of calcium retained in the pedicel of ‘Shixia’ longan and was the primary form of calcium present. During fruit development, calcium oxalate content and its relative proportion fluctuated considerably, indicating that calcium oxalate formation is reversible and that dynamic transformations occur among the three calcium forms.

3.5. Changes in Fruit Calcium Content After Girdling Treatment in ‘Shixia’

Compared to the control, significant reductions were observed in both fruit weight (from 0.33 g to 0.17 g) and the fruit calcium uptake rate (from 103.45 $\mu\text{g}\cdot\text{d}^{-1}$ to 60.11 $\mu\text{g}\cdot\text{d}^{-1}$) within 10 days after girdling treatment (Table 3). These findings indicate that the phloem symplastic transport pathway plays a critical role in calcium uptake in the arillate fruit of longan, accounting for nearly half of the total calcium absorbed by the fruit.

Table 3. Effect of girdling on fruit calcium uptake within 10 days after treatment. Data are presented as $M \pm SE$. The asterisk indicates significant differences as determined by one-way ANOVA (with LSD) ($p < 0.05$).

Treatment	Fruit Weight (g)	Ca Uptake (mg)	Fruit Ca Uptake Rate ($\mu\text{g}\cdot\text{d}^{-1}$)	Ca Uptake in Relative Growth ($\text{mg}\cdot\text{g}^{-1}$)	Fruit Ca Uptake Activity ($\mu\text{g}\cdot\text{d}^{-1}\cdot\text{g}^{-1}$)
Control	0.33 ± 0.01	1.03 ± 0.07	103.45 ± 6.76	6.11 ± 0.44	988.35 ± 62.88
Girdling	0.17 ± 0.01 *	0.60 ± 0.12	60.11 ± 12.18 *	8.25 ± 1.81	1063.01 ± 220.17

3.6. Dye Tracing of the Xylem/Apoplastic and Phloem/Symplastic Pathways

Dye tracing with saffron dye in the arillate fruit of ‘Shixia’ longan revealed that xylem ducts remained well-ventilated throughout all developmental stages. However, the dye did not penetrate the aril or seeds and was primarily distributed in the pericarp tissue (Figure 5). Examination of dye distribution in the pericarp showed that the entire vascular network within the pericarp was stained, indicating that the xylem vessels were well-developed and functionally active. The symplastic pathway in ‘Shixia’ remained consistently functional throughout fruit growth and development, with continuous fluorescence observed.

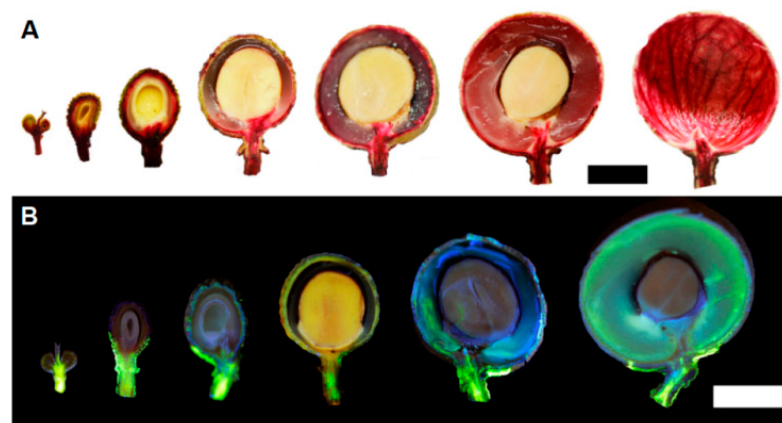


Figure 5. Functionality observations with dye tracing of xylem vessels (A) and symplasm (B). (A,B): The fruit of 6, 14, 37, 55, 72, 107 days after anthesis successively from left to right; scale = 1 cm.

3.7. Role of Endogenous Indole-3-Acetic Acid (IAA) and Effect of 2,4-D on Calcium Uptake in Fruit

The IAA content showed a positive correlation with both calcium content and concentration in the fruit (Table 4). Spraying exogenous 2,4-D significantly increased the calcium concentration in the pedicel 10 days after treatment. The effect of 2,4-D on increasing fruit calcium content and concentration was observed at both 10 and 20 days after treatment (Table 5). Additionally, 2,4-D treatment enhanced the calcium uptake rate and calcium

uptake activity (Table 6), although the increase was not statistically significant. These results suggest that auxins play a role in facilitating calcium uptake in ‘Shixia’ longan fruit.

Table 4. The relationship between IAA content and calcium uptake. Data are presented as $M \pm SE$. Different letters indicate significant differences as determined by one-way ANOVA (with LSD) ($p < 0.05$).

Days After Full Bloom (Days)	IAA Content (ng·Fruit ^{−1})	Fruit Ca Concentration (mg·g ^{−1})	Fruit Total Ca (mg·Fruit ^{−1})
14	1.64 ± 0.23 a	4.77 ± 0.26 b	0.10 ± 0.01 c
37	2.65 ± 0.35 a	6.67 ± 0.45 ab	0.80 ± 0.07 bc
55	3.21 ± 0.45 a	7.87 ± 0.45 a	1.99 ± 0.12 a

Table 5. Effect of 2,4-D treatment on calcium uptake of fruit. Data are presented as $M \pm SE$. The asterisk indicates significant differences as determined by one-way ANOVA (with LSD test) ($p < 0.05$).

Days After Treatment (Days)	Treatment	Pedicle Ca Concentration (mg·g ^{−1})	Fruit Weight (g)	Fruit Ca Concentration (mg·g ^{−1})	Fruit Total Ca (mg·Fruit ^{−1})
10	Control	4.85 ± 0.79	0.22 ± 0.01	3.85 ± 0.88	0.35 ± 0.09
	2,4-D	7.94 ± 1.08 *	0.22 ± 0.03	5.21 ± 0.81	0.46 ± 0.10
20	Control	7.83 ± 0.77	0.39 ± 0.03	4.99 ± 0.27	0.73 ± 0.03
	2,4-D	7.88 ± 1.61	0.48 ± 0.05	6.46 ± 1.33	1.07 ± 0.19

Table 6. Effect of 2,4-D treatment on fruit growth and calcium uptake capacity between 10 and 20 days after treatment.

Treatment	Fruit Dry Weight (g)	Fruit Ca Content (mg)	Fruit Ca Uptake Rate (μg·d ^{−1})	Fruit Ca Uptake Activity (μg·d ^{−1} ·g ^{−1})
Control	0.06 ± 0.01	0.36 ± 0.17	36.08 ± 16.65	313.32 ± 150.38
2,4-D	0.09 ± 0.02	0.66 ± 0.31	66.45 ± 30.59	585.32 ± 288.83

3.8. Seed Weight of ‘Shixia’ in Relation to Fruit Calcium Uptake

As shown in Figure 6, seed size in ‘Shixia’ exhibited a strong and statistically significant positive correlation with calcium content. Larger seeds were associated with higher calcium content in the fruit, suggesting that seed development may play an influential role in determining calcium uptake and accumulation in fruit.

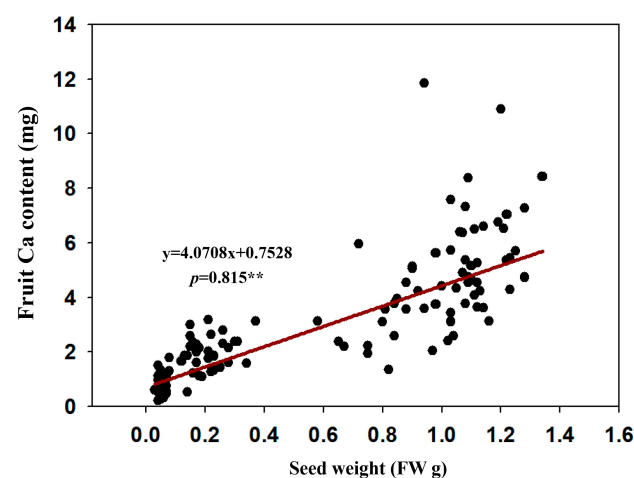


Figure 6. Correlation between seed weight and fruit calcium content. The straight line represents the fitting line of seed weight and fruit calcium content, and the equation is the line fitting equation. P represents the correlation coefficient between the two factors, and ** represents a highly significant correlation.

4. Discussion

Numerous studies indicate that the early stages of fruit growth are the primary period for calcium accumulation, with limited uptake occurring during the later stages [15–17]. However, in this study, ‘Shixia’ longan exhibited its highest calcium uptake rate during the middle stages of fruit development. This finding contrasts with typical patterns in other fruit species, where calcium uptake tends to peak early in development.

Interestingly, calcium uptake activity was highest during the initial stages of fruit development and gradually decreased as the fruit matured across all fruit tree varieties evaluated. This suggests that young fruit tissues exhibit the greatest capacity for calcium uptake. Previous research has attributed this early uptake capacity to the high transpiration rate of young fruits [32] or the effective functioning of xylem during the early stages of growth [33]. Nevertheless, further research is needed to explore whether genetic or metabolic factors influence calcium uptake during fruit development.

Calcium uptake relative to fruit growth followed a similar trend to calcium uptake activity, with both peaking in the early stages before declining with fruit development. This suggests that as fruit matures, the disparity between calcium supply and fruit growth intensifies, potentially leading to physiological disorders.

Girdling treatment has proven to be an effective method for examining phloem transport [34]. Although it is generally assumed that girdling does not interfere with xylem transport, it may impact the movement and accumulation of mineral elements [35]. Studies have shown that girdling disrupts the phloem/symplastic transport pathway, leading to reduced calcium uptake in shoots or fruit [36–38]. In the present study, girdling treatment significantly decreased calcium uptake and slowed fruit growth in ‘Shixia’ longan, highlighting the importance of the phloem/bast transport pathway for fruit calcium uptake.

The determination of IAA content in longan using gas chromatography-mass spectrometry revealed a trend consistent with fruit calcium accumulation, with IAA levels increasing alongside fruit growth and development. Previous studies have shown that calcium ion treatments can influence the expression of IAA-related genes in tomatoes [39–42]. In ‘Shixia’, the application of exogenous 2,4-D auxin further demonstrated its role in promoting both fruit growth and calcium uptake, suggesting that auxin hormones facilitate calcium absorption in fruit. Additionally, studies have implicated ethylene and abscisic acid in calcium-induced processes, such as adventitious root formation under salt stress [43] and stomatal closure [44].

Seeds play a crucial role in endogenous indole-3-acetic acid (IAA) synthesis, which is linked to fruit growth potential through polar IAA transport [45]. This suggests that the developmental status of seeds influences calcium uptake in the fruit. Supporting this, Singh et al. [46] demonstrated a positive correlation between calcium content in nutrient solutions and the accumulation of calcium oxalate and calcium phytate in seeds. Furthermore, seeds contain abundant calcium channels and Ca^{2+} -ATPase that facilitate calcium transport [47]. Calcium also regulates IAA content via the CaM signaling pathway, controlling tissue healing and lateral root formation by destabilizing the IAA-ARF interaction.

5. Conclusions

Our study provides new insights into calcium transport mechanisms in ‘Shixia’ longan fruit, demonstrating that calcium in the pedicel can be transported to the fruit via the phloem for several reasons:

- (1) Calcium distribution in the phloem: microscopic observations revealed an increased distribution of calcium in the phloem, particularly as calcium oxalate crystals.
- (2) Symplastic pathway continuity: the symplastic transport pathway remained functional throughout fruit development, with calcium concentrations in the pedicel being consistently higher than those in the fruit.
- (3) Impact of girdling: girdling treatments significantly affected both fruit enlargement and calcium uptake, highlighting the importance of the phloem in calcium transport.

- (4) Correlation with seed size: seed size exhibited a significant positive correlation with fruit calcium content, whereas IAA levels closely matched calcium accumulation patterns.
- (5) Effect of 2,4-D auxin: exogenous application of 2,4-D increased calcium concentration in the pedicel and enhanced the fruit's calcium uptake rate within a week, indicating a synergistic effect between auxin and calcium.

These findings suggest that both internal and external factors, including hormones like auxin, contribute to the regulation of calcium transport and accumulation in longan fruit.

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Data Availability Statement: The data that support this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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