PROMOTING FOOD SECURITY VIA CONTROLLING TOMATOES MARKETABILITY BY USING RNAi TECHNOLOGY, PECTIN AND ORGANIC CALCIUM

Maryam S. Elias[1] Kadhim D. H. Al-Jubouri[2] Khalil A. Saeed[3]
Lect. Prof. Prof.
[1,2]Dept. Hort. Landscape Gard. Coll. Agric. Engin. Sci. / University of Baghdad, Iraq
[3]Dept Plant Prod. Genet.,Coll. Agric., Khuzestan Univ. of Agric.Sci.Nat. Res., Iran
maryamsami@coagri.uobaghdad.edu.iq kadhim.aljubouri@coagri.uobaghdad.edu.iq
kh_alamisaeid@asnrukh.ac.ir

ABSTRACT

RNAi gene silencing was performed by utilizing pectate lyase, an enzyme capable of degrading pectin, in conjunction with subsequent pectin (organic waste) and organic calcium treatment, allowing for prolonged retention of tomatoes on the plant without compromising quality traits. The study was conducted at two locations using a randomized complete block design (RCBD) and consisted of nine treatments. Results showed that SlPL-RNAi plants had the most fruit firmness and Mesocarp thickness; with values of 13.1 kg/cm² and 167.5 µm, respectively, followed by the *CaP1P2* treatment (8.56 kg/cm² and 117.0 µm). SlPL-RNAi fruits also showed higher calcium and pectin contents and lower polygalacturonase (PG) activity, which were associated with firmer fruits and prolonged on-plant period (from pink stage to spoil, 33.7 days). Likewise, the CaP1P2 application increased the thickness and firmness of the tissue with a shelf life of up to 20 days. These advancements were credited to increased levels of calcium and pectin along with inhibited action of ripening- associated enzymes. This study provides a useful incorporation of RNAi and biochemical treatments as effective approaches to induce postharvest loss and promote food and environmental sustainability.

Keywords: mesocarp, exocarp, firmness, on-plant fruits longevity, Responsible consumption and production

مجلة العلوم الزراعية العراقية- 2025 :56 (4):1470-1481 الياس وآخرون

تعزيز الامن الغذائي من طريق التحكم في قابلية تسويق الطماطة بأستعمال تقنية RNAi و البكتين والكالسيوم العضوي

مريم سامي الياس[1] كاظم ديلي حسن الجبوري[2] خليل عالمي سعيد[3]
مدرس استاذ استاذ
[1،2] قسم البستنة وهندسة الحدائق – كلية علوم الهندسة الزراعية – جامعة بغداد – العراق
[3]جامعة خوزستان للعلوم الزراعية – كلية الزراعة – قسم انتاج ووراثة نبات – ايران

المستخلص

وظفت تقانه الاخماد الجيني Gene Silencing بأستعمال RNAi لانزيم Pectate lyase والمعاملة بالبكتين (المخلفات العضوية) والكالسيوم العضوي في إطالة مدة الثمار على النبات دون المساس في سمات الجودة لثمار الطماطة .أُجري البحث في موقعين باستعمال تصميم القطاعات الكاملة المعشاة وشمل 9 معاملات. أظهرت النتائج تفوق نباتات SlPL–RNAi في صلابة الثمار وتثخن طبقة Mesocarp ، حيث بلغت القيم 13.1 كغم/سم² و167.5 ميكرومتر على التوالي، تليها معاملة CaP1P2 (8.56 كغم/سم² و117.0 ميكرومتر). كما تفوقت ثمار SlPL–RNAiفي زيادة تركيز الكالسيوم والبكتين وانخفاض فعالية إنزيمPG ، مما عزز صلابتها وإطالة مدة بقائها من مرحلة Pink إلى التلف بمجموع 33.7 يومًا. في المقابل، أبدت معاملة CaP1P2 تحسنًا في صلابة الثمار وتثخن الأنسجة وزيادة مدة الإطالة إلى 20 يومًا. يعزى ذلك إلى زيادة تركيز الكالسيوم والبكتين وخفض نشاط الإنزيمات المرتبطة بالنضج. يُظهر هذا البحث فعالية استعمال RNAi والمعاملة بالكالسيوم والبكتين في تقليل الفاقد الغذائي وتعزيز الاستدامة البيئية والغذائية.

الكلمات المفتاحية: Mesocarp و Exocarp، الصلابة، عمر الثمار على النبات، الانتاج والاستهلاك المسؤولان

Received:3 /1/2025, Accepted:26/2/2025, Published: August 2025

INTRODUCTION

Tomato losses; particularly post-harvest losses (over 50%) pose a significant threat to food security in many countries, especially in developing regions where tomatoes are a staple and an important source of nutrients (2, 63). Extending fruits longevity on the plant while preserving their nutritional value is a smart practice to reduce food loss and mitigate environmental pollution. Helping the fruit to ripen naturally on the plant without early industrial intervention is an environmentally friendly decision. This approach contributes to minimize the use of environmentally harmful storage and packaging methods, creating an integrated cycle that enhances fruits availability and promotes food security, aligning with modern-era requirements. This is particularly relevant after harnessing the plant's defense mechanisms as a tool for silencing genes responsible for cell wall degradation, linked to pectin-degrading enzymes, by modifying specific traits such as increasing fruit firmness. This has facilitated overcoming challenges faced by plant breeders in developing or deriving new cultivars, similar to other molecular genetics technologies that have aided in identifying transferred traits and developing new varieties (11, 21). Such improvements traditionally required prolonged breeding programs using conventional methods (44), gene silencing (RNAi) has emerged as a precise molecular technique (5). It utilizes double-stranded RNA (dsRNA) to suppress the expression of specific genes by interfering with their mRNA, thereby blocking translation (22, 23). Additionally, the use of biodegradable, edible, and environmentally friendly biomaterials—naturally available and cost-effective—has gained attention (19). Plant cell walls are mainly made of complex carbohydrates like pectin, cellulose and hemicellulose along with embedded proteins which have structural and enzymatic functions (33). Pectin is a polysaccharide in the middle lamella and in the primary and secondary cell wall ls (53). The pectin is composed of five main types of polymers, Homogalacturonan (HG), Ramnoglacturonan-I (RG-I), Rhamnogalacturonan-I (RG-II), Xylogalacturonan (XGA) and Apiogalacturonan (AP) (30). These components form covalent bonds, producing a complex three-dimensional network that contributes to the strength, porosity, and rigidity of the cell wall. This structure limits the access and activity of cell wall-degrading enzymes, thereby preserving fruit firmness (45), regulating water absorption, preserving turgor pressure, enhancing adhesion, and facilitating intercellular signaling (57). Particularly, when cell wall pectin binds tightly with Ca^{2+} ions, it forms cationic bridges between pectic acids or between pectic acids and other acidic polysaccharides (3). These bridges make plant cell walls less susceptible to pectolytic enzymes and polygalacturonase, reducing moisture loss through the fruit wall and enhancing firmness (6). In addition to calcium role as an enzymatic activator and essential element in cell division, elongation, and growth; (7, 38) calcium helps maintain cell turgor and firmness, reducing physiological disorders in fruits (18). It also contributes to the expression of genes influencing reproductive organs in plant (4, 58). Tomato (*Solanum lycopersicum* L.), one of the most widely used and important vegetable crops, ranks second globally after potato (26, 34). However, its rapid spoilage has become a focus for researchers and plant breeders aiming to preserve fruit firmness without compromising quality. Ren et al. (41) found that silencing the SlPL16 gene (encoding Solyc06g083580) reduced pectate lyase (PL) activity and water-soluble sugar concentration while maintaining fruit firmness and extending its duration on the plant. Sena *et al*. (46) reported that foliar application of calcium is more effective than adding it to the soil in increasing calcium concentration in fruits. Melo et al. (27) demonstrated that applying $CaCl_2$ (0.3 g/L) every two weeks increased calcium levels in tomato fruits and enhanced their firmness. External calcium application increased homogalacturonan accumulation and strengthened the pectin network in fruit coatings (16). Additionally, calcium application reduced respiration and ethylene production, slowing ripening stages and prolonging fruit retention on the plant (49). Jhanani et al. (19) utilized pectin extracted from zucchini and treated tomatoes

during their ripening stages—starting from Mature Green, then Breaker, Pink, and Red stages—at concentrations of 1%, 3%, and 5% (w/v) of pectin. It was observed that fruits treated with 5% pectin extended their shelf life by more than 11 days, indicating the potential of using pectin to prolong fruit retention on the plant without affecting its physical and chemical properties. This research aims to extend the duration of fruit retention on the plant and control its marketing while preserving the nutritional density of the fruits through the use of gene silencing technology and treatment with pectin (organic waste) and organic calcium.

MATERIALS AND METHODS

This study was carried out during growing season 2023–2024 at two locations, Al-Jadriyah (the fields of the College of Agricultural Engineering Sciences, University of Baghdad) referred to as L1, and Abu Ghraib, (fields of the H orticulture Department, Minister of Agriculture) referred to as L2. Each site had a greenhouse prepared, and the tomato seedlings were transplanted on October 24, 2023, at the Abu Ghraib site and on October 25, 2023, at the Al-Jadriyah site. All standard agricultural practices were performed in accordance with scientific recommendations. The experiment was laid out using a randomized complete block design (RCBD) with five replicates at each location. Each replicate included nine treatments (experimental units). Data from both sites were pooled for statistical analysis, and treatment means were compared using the LSD test at a 5% probability level. The experiment included nine treatments: gene-silenced plants for the *Pectate lyase* enzyme, designated as *SlPL-RNAi*; untreated control plants, designated as *C0*; pectin irrigation at 1 $g \cdot L^{-1}$ (*P1*); pectin foliar spray at 2 $g \cdot L^{-1}$ (*P2*); a combined treatment of pectin irrigation at 1 $g \cdot L^{-1}$ and foliar spray at 2 $g \cdot L^{-1}$ (*P3*); foliar spray with organic calcium at 2 $mL \cdot L^{-1}$ (*Ca*); a combination of pectin irrigation at 1 $g \cdot L^{-1}$ and calcium spray at 2 $mL \cdot L^{-1}$ (*CaP1*); pectin spray at 2 $g \cdot L^{-1}$ with calcium spray at 2 $mL \cdot L^{-1}$ (*CaP2*); and a comprehensive treatment involving pectin irrigation (1 $g \cdot L^{-1}$), pectin spray (2 $g \cdot L^{-1}$), and calcium spray (2 $mL \cdot L^{-1}$), designated as *CaP1P2*. The calcium percentage in tomato fruits was determined using the method of William (56). Total pectin content (mg/100 g f.w.) was estimated following the procedure described by Egan et al. (10), while polygalacturonase (PG) enzyme activity (µmol/mL) was assessed according to the method of Jijan (20). Plant samples (tomato fruits at the pink stage) were collected at a rate of three fruits per experimental unit. The thickness of the Exocarp and Mesocarp layers (µm) was measured based on the procedure described by Al-Hadeethi *et al.* (1). Traits of fruit marketing control were evaluated on the plant at three distinct stages: the number of days from the pink stage to the red ripe stage (harvest maturity) while still on the plant; the number of days from the red ripe stage to fruit spoilage while still attached; and the total number of days from the pink stage to spoilage on the plant. The firmness of tomato fruits taken from the control sample of each experimental unit was measured before storage (at the pink stage).

RESULTS AND DISCUSSION

Calcium Concentration, Pectin, and PG Enzyme Activity in tomato fruits: The results in (Table 2) indicate that fruits from the CaP1P2 treatment exhibited the best values for calcium concentration, pectin content, and polygalacturonase (PG) enzyme activity, with respective values of 1.15%, 372.3 mg/100 g f.w., and 12.1 µmol/mL, compared to the control treatment. Additionally, fruits from the SlPL-RNAi treatment outperformed the control in the measured traits, recording 0.91%, 294.4 mg/100 g f.w., and 6.6 µmol/mL, respectively. Furthermore, fruits from the Al-Jadriyah site (L1) showed a higher pectin concentration (322.5 mg/100 g f.w.) compared to those from the Abu Ghraib site (L2). The superiority of the CaP1P2 treatment in the measured traits could be attributed to the role of calcium as a secondary messenger that mediates responses to environmental stimuli and hormonal signals through calcium sensors such as calmodulin. This activates Sensing Ion Selective Receptors (SISRs), which play a role in reducing and regulating cell wall-degrading enzymes. Notably, the plants were treated with calcium at an early growth stage, enhancing calcium absorption and activity, thereby improving

tissue stability, nutrient uptake efficiency, and the overall transport of elements, particularly calcium, to fruits (43, 51). Additionally, the presence of sodium in the pectin powder used in the experiment (Table 1) led to the substitution of sodium for some hydrogen ions bound to the carboxyl groups in pectin. This reduced repulsion between pectin molecules, stabilized the negative charge of pectin, and enhanced its binding with calcium. This positively impacted the increase in total pectin and the reduction of water-soluble pectin in tomato fruits (17). The reduction in PG enzyme activity may be attributed to external and internal factors that contributed to histone modification by suppressing the gene expression of the PcG SIMSI1 protein, which belongs to the Polycomb Group proteins, thereby reducing the genetic expression of genes controlling PG enzyme activity (28, 62). Alternatively, it may result from the epigenetic interaction between the SlLHP1b protein and H3K27me3, which influences the reduction of genetic expression for the PG enzyme (25, 55).

Table 1. Concentration of nutrients in the pectin material used in the study

	N	P	K	Ca	Mg	Na
	1.2	0.3	0.814	0.89	0.147	3.778
Element ppm		Cu	Fe	Mn	Zn	
		0.07	0.339	0.01	0.069	

Table 2. Effect of gene silencing, pectin, calcium treatments, experimental site, and their interactions on calcium and pectin concentration and PG enzyme activity in tomato fruits

Treatments	Ca %		Pectin (mg/100 g f.w.)		PG enzyme (µmol/mL)	
			T			
SIPL-RNAi	0.91		294.4		6.6	
C0	0.56		221.2		33.7	
P_1	0.74		298.4		23.5	
P_2	0.77		352.0		21.1	
P_3	0.84		356.8		19.1	
Ca	1.08		276.3		17.7	
Ca P_1	0.94		330.4		15.4	
Ca P_2	0.99		358.5		13.1	
Ca P_1 P_2	1.15		372.3		12.1	
L.S.D	0.065		8.88		2.682	
			L			
L 1	0.8846		322.5		18.12	
L 2	0.8867		313.1		17.87	
L.S.D	N.S		9.08		N.S	
			L * T			
	L1	L2	L1	L2	L1	L2
SIPL-RNAi	0.89	0.94	297.0	291.8	6.6	6.5
C0	0.59	0.53	227.2	215.3	35.1	32.3
P1	0.77	0.70	301.0	295.8	24.5	22.5
P2	0.78	0.76	354.5	349.5	20.9	21.2
P3	0.82	0.86	361.7	352.0	18.4	19.8
Ca	1.09	1.07	285.8	266.8	17.9	17.4
Ca P1	0.95	0.93	337.0	323.8	14.9	15.8
Ca P2	0.98	0.99	362.8	354.2	12.9	13.3
Ca P1 P	1.21	1.10	375.5	369.0	11.9	12.2
L.S.D	N.S		N.S		N.S	

Additionally, the superior pectin content at the Al-Jadriyah L1 site likely resulted from favorable environmental conditions (light intensity, temperature, agricultural practices) that enhanced vegetative growth, boosted photosynthesis and nutrient metabolism, leading to increased accumulation of carbohydrates, organic acids, and sugars, consequently raising pectin levels in tomato fruits, while modified metabolic pathways of pectin-degrading enzymes may have indirectly contributed to higher pectin content in the fruits (42). The superior performance of the SlPL-RNAi treatment in th e measured traits can be attributed to its preservation of pectin architecture from degradation, which positively enhanced pectin-calcium binding sites (54). Given that pectin is the main

substrate for PG enzyme interaction, any changes in the pectin composition will in turn influence the PG enzyme concentrations and activities (61). Moreover, PL silencing might have disrupted the synergy between PL and PG enzymes, contributing to a decrease in PG abundance and activity (32). These results were consistent with previous studies by Yang et al (61) and Wang et al. (54).

Tomato Fruit Firmness and Thickness of Exocarp and Mesocarp Layers: The results in Table 3 show that The L1SlPL-RNAi treatment exhibited the highest fruit firmness and Mesocarp thickness (13.1 $kg \cdot cm^{-2}$ and 167.5 µm, respectively) as compared to the control L2C0, followed by the L1CaP1P2 treatment which also showed high fruit firmness (8.56 $kg \cdot cm^{-2}$) and Mesocarp thickness (117.0 µm; Table 3). Moreover, the highest values for fruit firmness and thickness of Exocarp and Mesocarp (12.2 $kg \cdot cm^{-2}$, 65.6 µm, and 165.9 µm for the latter, respectively) were observed when the melon fruits were treated only with SlPL-RNAi, and these values were followed by CaP1P2 (8.30 $kg \cdot cm^{-2}$, 87.2 µm, and 115.4 µm), while the control treatment (C0) had the lowest values for all these traits. The fruit and Exocarp and Mesocarp thicknesses of L1 site were also significantly higher than L2 site (7.773 $kg \cdot cm^{-2}$, 61.43 µm, and 86.64 µm, respectively). The observed advantages of L1SlPL-RNAi and SlPL-RNAi in fruit firmness was because the PL gene, encoding the pectin-degrading enzyme in cell walls, was silenced, and thus the pectin cannot be solubilized in water, leading to improved fruit firmness. Also, the genetic suppression of the PL enzyme could have affected the expression of genes related to hormonal signaling pathways involved in ripening, by novel regulation mechanisms such as less ethylene production or delay in its action that modulating cell wall composition by increased cellulose and hemicellulose contents, etc. It may also have enhanced the plant's defensive response to oxidative stress. These changes contribute to improving fruit quality and firmness by modulating ripening processes at the molecular level (52; 60, 61). Particularly, the favorable environmental factors and appropriate agricultural practices at the first site (L1) further enhanced overall fruit quality and firmness (36). The hardness of the fruits of the two treatments L1CaP1P2 and CaP1P2 is due to the calcium bridges between the pectin acids or polysaccharides that hinder the activity of pectin hydrolytic enzymes. The pectin gel network strengthened the adhesion of the fruit cells to each other and preserved their architecture. Because calcium enhances the plant's ability to absorb water and nutrients from the soil, it increases the plant's ability to recover from environmental stresses, reduces membrane lipid metabolism and physiological disturbances against pathogens. Because of the role of pectin as a selective membrane permeable to gas exchange, it reduced the respiration rate and reduced the breakdown of complex carbohydrates due to oxidation. All of the above was positively reflected in their superiority in the percentage of calcium and pectin and the decrease in the activity of the PG enzyme (Table 2). This was reflected in the increased thickness of their shells (Table 3), which increased the accumulation of biomass in them and enhanced their hardness, which led to extending their survival time on the plant (Table 4)(15,37).

Table 3. Effect of gene silencing, pectin, calcium treatments, experimental site, and their interactions on firmness and thickness of the Mesocarp and Exocarp layers in tomato fruits

Treatments	Fruit firmness (kg·cm^{-2}) before storage		Exocarp (µm)		Mesocarp (µm)	
			T			
SIPL-RNAi	12.2		65.6		165.9	
C0	4.71		29.8		31.2	
P_1	6.28		31.9		43.1	
P_2	6.79		34.0		55.2	
P_3	7.12		36.4		62.0	
Ca	7.40		83.4		92.1	
Ca P_1	7.58		74.4		96.6	
Ca P_2	8.04		85.4		102.8	
Ca P_1 P_2	8.30		87.2		115.4	
L.S.D	0.4212		2.568		1.798	
			L			
L 1	7.773		61.43		86.64	
L 2	7.416		55.93		83.20	
L.S.D	0.2753		1.736		1.825	
			L * T			
	L1	L2	L1	L2	L1	L2
SIPL-RNAi	13.1	11.2	67.8	63.5	167.5	164.3
C0	4.91	4.51	32.3	27.2	33.1	29.3
P1	6.12	6.45	34.4	29.4	43.4	42.7
P2	6.89	6.69	36.9	31.1	59.4	51.0
P3	7.16	7.08	39.5	33.4	63.5	60.5
Ca	7.46	7.33	86.5	80.4	96.3	88.0
Ca P1	7.62	7.54	76.6	72.2	97.7	95.4
Ca P2	8.13	7.95	88.8	82.0	101.9	103.8
Ca P1 P	8.56	8.02	90.0	84.3	117.0	113.8
L.S.D	0.5956		N.S		2.543	

The results align with findings by Uluisik et al. (52), Dodgson et al. (9), and Zhang et al. (64), where the superior performance of Al-Jadriyah site fruits (L1CaP1P2 treatment) stems from optimal environmental conditions - including light intensity enhancing photosynthetic efficiency, temperature promoting cell division/expansion, and regulated irrigation maintaining turgor pressure, coupled with calcium-pectin interactions that improved water retention and cell wall extensibility (17, 39). Mesocarp development from carpel walls post-fertilization involves environmental-hormonal crosstalk regulating floral meristem size through epigenetic mechanisms, particularly increased expression of SlWUS transcription factor (interacting with FIN, FAB2, RRA3a (59) and cell size regulator CSR locus, linked to FW11.3 gene (31, 35). The superior performance of the *CaP1P2* treatment is attributed to the binding of calcium ions with pectic carboxyl groups, which enhanced hormonal signaling by regulating internal cytokinin-related proteins. This regulation led to increased gene expression of gibberellin and auxin pathways. Additionally, the treatment slowed ethylene production and reduced respiration rates, thereby promoting enhanced cell division and expansion. These effects collectively contributed to greater pectin accumulation in the fruit *pericarp* (14, 13, 48).

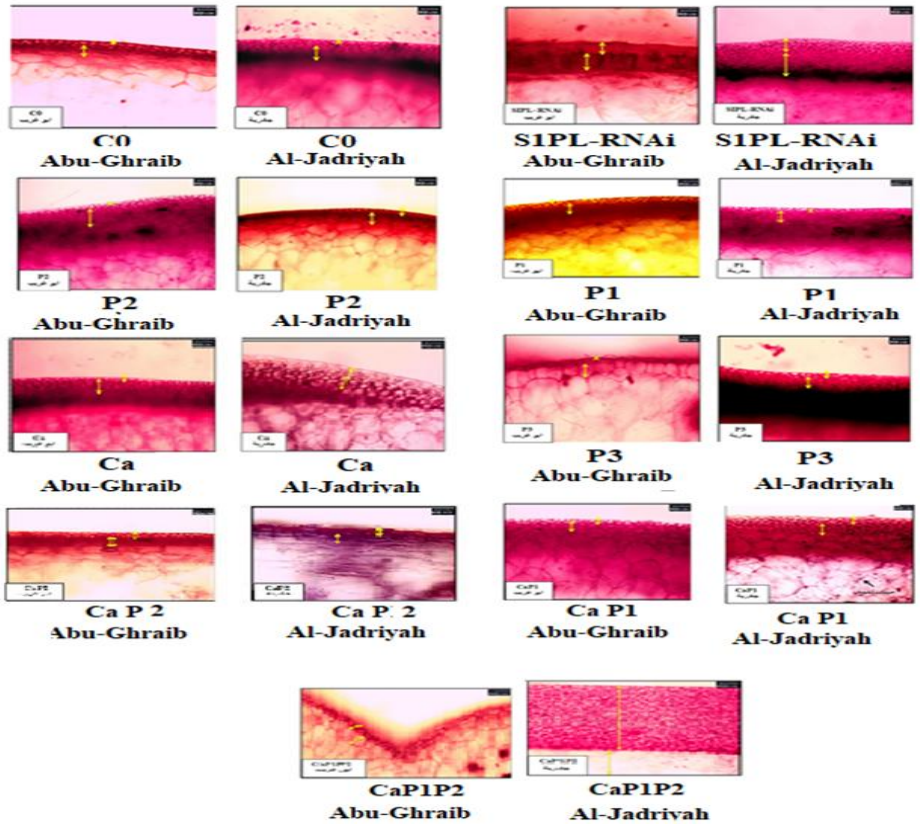

Figure 1. Thickness of the Exocarp and Mesocarp layers in tomato fruits according to the study treatments at both locations

(*Yellow arrows indicate the thickness of the Exocarp and Mesocarp layers*)

The superior anatomical performance of SlPL-RNAi-treated fruits resulted from targeted silencing of the pectin lyase gene at locus SolyC03g111690, which significantly reduced epidermal water loss and decreased water-soluble pectin content. This genetic intervention likely upregulated expression of growth-regulating proteins encoded by loci SolyC06g035940 and SolyC03g065250, while simultaneously increasing pectin density in the middle lamella and tricellular junction regions of epidermal cell walls (52). These combined effects effectively preserved pectin structural integrity, enhanced cellular density, and improved fruit tissue cohesion throughout various ripening stages, with results corroborating previous findings by Yang et al. (61) and Wang et al. (54) as illustrated in Figure 1. The treatment's success demonstrates how precise genetic modifications can optimize both the structural and functional properties of fruit tissues during postharvest phases.

Fruit Marketing Control Traits

The results in Table (4) highlight the superior performance of SlPL-RNAi treatment in extending tomato marketability on-plant, showing the longest durations from pink to harvest (26.2 days), red stage to spoilage (7.50 days), and pink to spoilage (33.7 days), followed by CaP1P2 treatment (14.9, 5.13, and 20.0 days, respectively), both significantly outperforming the control (5.30, 1.86, and 7.12 days). The effectiveness of the SlPL-RNAi treatment is attributed to gene silencing of the Pectate lyase (PL) enzyme, preventing its translation into a functional protein. This inhibited pectin degradation and enhanced its polymerization within the cell wall and middle lamellae, preserving the mechanical integrity of the cell wall and maintaining fruit stability for over 30 days under field conditions, while preserving quality traits that meet market standards and consumer preferences (60). The enhanced performance of CaP1P2 treatment could be ascribed to the synergistic action of calcium and pectin. Calcium increases the stiffness of the middle lamella by facilitating the cross-linking of polygalacturonic acid residues and forming calcium pectate complexes through interaction with pectic acid. This interaction leads to the reinforcement of the cell wall; thus, making it structurally resilient to physical and pathogenic aggressions. Moreover, calcium plays its role in decreasing respiration, respiration, and water loss by preventing the activity of cell wall degrading enzymes (12, 40). In addition, a CaM–Ca^{2+}

complex are formed by the binding of Ca^{2+} to calmodulin, which could also potentially mitigate ethylene emission in tomatoes since calmodulin has been implicated in the inhibition of ethylene biosynthesis pathways in tomato fruits (29, 47). It can be speculated that epigenetic regulation contributes as well; perhaps CaP1P2 is effective due to its suppression of ethylene biosynthesis (ACS2, ACS4, and ACO1), ethylene receptor (ETR3, ETR4, and ERF) and major ripening regulator (FUL2, FUL1, TAGL1, and RIN) genes that are likely partners in tetrameric complexes (24, 50). We have also observed the methylation of both DNA regions and histones (H3K9 and H3K27) related to the repression of ripening-related gene expression (8). The enhanced performance of both CaP1P2 and SlPL-RNAi treatments is also attributed to their high calcium and pectin content and reduced PG enzyme activity (Table 2), which resulted in thicker Exocarp and Mesocarp layers (Table 3). This anatomical reinforcement improved the fruits' resistance to mechanical and physiological damage, ultimately contributing to better on-plant fruit marketing control traits. The superior performance of the CaP1P2 treatment showed the best performance probably due to the epigenetic reprogramming caused by it that silences genes involved in ethylene biosynthesis (ACS2, ACS4, ACO1), ethylene receptors (ETR3, ETR4, ERF), and genes involved in ripening transcription factors (FUL2, FUL1, TAGL1, RIN), where they generated the tetrameric complex that regulates the synthesis of ethylene (24, 50). This repression may also be due to increased DNA methylation or histone methylation at H3K9 and H3K27, both associated with reduced gene expression upon fruit ripening (6, 9). Moreover, the enhanced performance of the CaP1P2 and SlPL-RNAi treatments can be explained by their accumulation of calcium and pectin and the reduced activity of the PG enzyme (Table 2), which contributed to the thickening of the Exocarp and Mesocarp layers (Table 3). These anatomical enhancements reinforced the fruit's protective tissues against mechanical and physiological damage, thereby significantly improving the traits of fruit marketing control while the fruits remained on the plant.

Table 4. Effect of gene silencing, pectin, calcium treatments, experimental site, and their interactions on the traits of on-plant fruit marketing control in tomato

Treatments	Extension of fruit duration on the plant from the pink stage to harvest		Extension of fruit duration on the plant from harvest to spoilage		Extension of fruit duration on the plant from the pink stage to spoilage	
			T			
SIPL-RNAi	26.2		7.50		33.7	
C0	5.3		1.86		7.12	
P_1	9.3		3.26		12.6	
P_2	11.0		3.40		14.4	
P_3	12.0		4.15		16.2	
Ca	10.2		2.88		13.0	
Ca P_1	12.7		4.47		17.2	
Ca P_2	13.9		4.68		18.5	
Ca P_1 P_2	14.9		5.13		20.0	
L.S.D	2.882		1.429		3.163	
			L			
L 1	12.6		3.99		16.62	
L 2	13.0		4.30		17.31	
L.S.D	N.S		N.S		N.S	
			L * T			
	L1	L2	L1	L2	L1	L2
SIPL-RNAi	25.8	26.5	7.0	8.0	32.8	34.5
C0	5.0	5.52	1.72	2.0	6.7	7.5
P1	9.6	9	3.11	3.4	12.7	12.4
P2	10.8	11.25	3.3	3.5	14.1	14.75
P3	11.8	12.24	4.0	4.29	15.8	16.53
Ca	9.8	10.5	2.76	3.0	12.56	13.5
Ca P1	12.4	13.0	4.4	4.54	16.8	17.54
Ca P2	13.7	14.0	4.6	4.75	18.3	18.75
Ca P1 P	14.8	15.0	5.0	5.25	19.8	20.25
L.S.D	N.S		N.S		N.S	

In conclusion, this study demonstrates that both gene silencing (SlPL-RNAi) and calcium-pectin (CaP1P2) treatments effectively enhance tomato fruit firmness, structural integrity, and on-plant shelf life without compromising quality. The SlPL-RNAi and CaP1P2 treatments outperformed others by reducing polygalacturonase activity, increasing pectin and calcium accumulation, and thickening the Exocarp and Mesocarp layers, ultimately leading to prolonged fruit retention from the pink stage to spoilage. These actions were reinforced by optimal environmental conditions and potential epigenetic changes that silenced ripening-responsive genes through hormonal control. Overall, the integrated application of molecular and organic treatments may be a potential strategy for enhanced postharvest traits in tomato, which can ultimately be translated into reduced food loss and greater sustainability in horticultural production systems.

Conclusion

The findings of this study lead to the conclusion that the RNAi-SlPL gene silencing treatment, along with the application of pectin and organic calcium, effectively enhanced the structural integrity of the cell wall and suppressed the activity of pectinolytic enzymes This has extended its marketability on the plant. The best results showed increased fruit firmness, calcium content, and pectin concentration, decreased PG enzyme activity, and increased thickness of the outer and middle peel layers. SIPL-RNAi treatment was found to be superior in controlling fruit marketing indicators during on-plant development, extending fruit survival time to 33.7 days.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERENCES

1. Al-Hadeethi, M. A., J. K. Ali, Z. Al-Moussawi. 2020. Characters anatomy of *Corchorus olitorius* L. From malvaceae family cultivated in Iraq. International Journal of Pharmaceutical Research, 12(1), pp. 211–214. Doi: 10.31838/ijpr/2020.12.01.041

2. Aworth, O. C., Olorunda, A. O., & Akhuemonkhan, I. A. 198). Effects of post-harvest handling on quality attributes of tomatoes in the Nigerian marketing system. Food Chemistry, 10(3), 225-230. https://doi.org/10.1016/0308-8146(83)90058-4

3. AOAC .2000. Official Methods of Analysis. The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

4. Adekiya, A. O., Ogunbode, T. O., Esan, V. I., Adedokun, O., Olatubi, I. V., and Ayegboyin, M. H. 2025. Impact of Calcium Sources on Soil Chemical Properties, Tomato Growth, Yield, and Quality. The Scientific World Journal, 2025(1), 6653874. https://doi.org/10.1155/tswj/6653874 https://doi.org/10.36103/zvrre033

5. Aigner, A. 2006. Gene silencing through RNA interference (RNAi) in vivo: strategies based on the direct application of siRNAs. Journal of biotechnology, 124(1), 12-25. https://doi.org/10.1016/j.jbiotec.2005.12.003

6. Ayour, J., H. Harrak, and M. Benichou. 2024. Cell Wall Enzymatic Activity Control: A Reliable Technique in the Fruit Ripening Process. https://doi.org/10.5772/intechopen.113752

7. Caines, A. M., and C. Shennan. 1999. Interactive effects of Ca2+ and NaCl salinity on the growth of two tomato genotypes differing in Ca2+ use efficiency. Plant Physiology and Biochemistry, 37(7-8), 569-576. https://doi.org/10.1016/S0981-9428(00)80109-6

8. Delarue, M., M. Benhamed, and S. Fragkostefanakis. 2024. The role of epigenetics in tomato stress adaptation. New Crops 2: 100044 https://doi.org/10.1016/j.ncrops.2024.100044.

9. Dodgson, J., A.K. Weston, and D.J. Marks. 2023. Tomato Firmness and Shelf-Life Increased by Application of Stimulated Calcium. Crops 3(4): 251–265. https://doi.org/10.3390/crops3040023.

10. Egan, H.; R. S.Krik, and R.Sawyer, 1976. Pearson's Chemical Analysis of Foods 7th ed. chur chill living stone. NY. USA.

11. Elias, M.S., and K.D.H. Al-Jubouri. 2022. Investigate genetic relation among watermelon cultivars using molecular dna markers. Iraqi Journal of Agricultural Sciences, 53(3): 712–723. https://doi.org/10.36103/ijas.v53i3.1581.

12. Eric, A.; I. Oduro; P. Kumah. 2015. Postharvest quality response of tomato (Lycopersicon esculentum, Mill) fruits to different concentrations of calcium chloride at different dip-times. Am. J. Food Nutr. 5, 1–8.
13. Feng, D., X. Wang, J. Gao, C. Zhang, Hao Líu, et al. 2023. Exogenous calcium: Its mechanisms and research advances involved in plant stress tolerance. Frontiers in Plant Science 14.
doi: https://doi.org/10.3389/fpls.2023.1143963.
14. Gan, L., M. Song, X. Wang, N. Yang, H. Li, et al. 2022. Cytokinins is involved in regulation of tomato pericarp thickness and fruit size. Horticulture Research. https://doi.org/10.1093/hr/uhab041.
15. Garnida, Y., Y. Taufik, and Y. Yelliantty. 2022. Effect of Edible Coating Material Formulation and Storage Long on The Response of Tomato (*Solanum Lycopersicum* L.) at Cooling Temperature (Lycopersicon Esculentum Mill). Jurnal Penelitian Pendidikan IPA 8(5): 2399–2409. https://doi.org/10.29303/jppipa.v8i5.2101.
16. Huang, W., Y. Shi, H. Yan, H. Wang, D. Wu, et al. 2023. As assessment of shelf life increasing competence of pectin (Zucchini) based edible coating on tomatoes. Environmental Research, 258, 119368. https://doi.org/10.1016/j.envres.2024.119368
17. Hyodo, H., A. Terao, J. Furukawa, N. Sakamoto, H. Yurimoto, et al. 2013. Tissue Specific Localization of Pectin–Ca2+ Cross-Linkages and Pectin Methyl-Esterification during Fruit Ripening in Tomato (Solanum lycopersicum) (R. Balestrini, editor). PLoS ONE 8(11): e78949. https://doi.org/10.1371/journal.pone.0078949.
18. Jain, V., S.Chawla, , P.Choudhary, and S. Jain. 2019. Post-harvest calcium chloride treatments influence fruit firmness, cell wall components and cell wall hydrolyzing enzymes of Ber (Ziziphus mauritiana Lamk.) fruits during storage. Journal of Food Science and Technology, 56, 4535–4542. https://doi.iScience 24(8): 102926. https://doi.org/10.1016/j.isci.2021.102926.
19. Jhanani, G.K., M.S. AlSalhi, N. T, and R. Shanmuganathan. 2024. As assessment of shelf life increasing competence of pectin (Zucchini) based edible coating on tomatoes. Environmental research 258: 119368.
20. Jijan, R. A. 2012. Production, Purification, and Characterization of the Enzyme Polygalacturonase from A Local Isolate of the Mold Aspergillus Niger TP4 Using Solid-State Fermentation. Ph.D. Dissertation, Department of Food Science, College of Agriculture, University of Baghdad, Iraq.
21. Khdir, S.A., N.S., Ahmad, E.O. Hama-Ali, and Sh. M. Abdullah. 2023. Genetic diversity and population structure of common bean genotypes using morphological traits and SSR. Iraqi Journal of Agricultural Sciences, 54(3), 792–805. https://doi.org/10.36103/ijas.v54i3.1762
22. Kong, X., M. Yang, B.H. Le, W. He, and Y. Hou. 2022. The master role of siRNAs in plant immunity. 23(10): 1565–1574. https://doi.org/10.1111/mpp.13250.
23. Kryovrysanaki, N., A. James, M. Tselika, E. Bardani, and K. Kalantidis. 2022. RNA silencing pathways in plant development and defense. The International Journal of Developmental Biology.
doi: https://doi.org/10.1387/ijdb.210189kk.
24. Li, S., K. Chen, and D. Grierson. 2018. A critical evaluation of the role of ethylene and MADS transcription factors in the network controlling fleshy fruit ripening. New Phytologist 221(4): 1724–1741. https://doi.org/10.1111/nph.15545.
25. Liang, Q., H. Deng, Y. Li, Z. Liu, P. Shu, et al. 2020. Like Heterochromatin Protein 1b represses fruit ripening via regulating the H3K27me3 levels in ripening-related genes in tomato. New Phytologist 227(2): 485–497. https://doi.org/10.1111/nph.16550.
26. Masoso, F., O. A.,Zingwari, M.,Mutetwa, W.Zendera .2020. Effect of different chemical fertilizers and application rate on tomato growth and yield. International Journal of Academic Research and Development, 5 (4), 119–124.
27. Melo, R.O., H.E. Prieto Martínez, B.C.P. Rocha, and E. García Junior.2022. Production and quality of Sweet Grape tomato in response to foliar calcium fertilization. Revista Ceres, 69(1), 48–54. https://doi.org/10.1590/0034-737X202269010007.
28. Ming, Y., L. Jiang, and D. Ji. 2023. Epigenetic regulation in tomato fruit ripening. Frontiers in Plant Science 14. https://doi.org/10.3389/fpls.2023.1269090

29. Mishra, S., and V. Prakash. 2018. Biochemical changes in calciumchloride treated Hisar Arun (Local) and Kashi Vishesh (Hybrid) cultivars of Tomato fruit. Current Agriculture Research Journal 6(3): 395–406. https://doi.org/10.12944/carj.6.3.19.
30. Mohnen, D. 2008. Pectin structure and biosynthesis. Current Opinion in Plant Biology 11(3): 266–277. https://doi.org/10.1016/j.pbi.2008.03.006.
31. Mu, Q., Z. Huang, M. Chakrabarti, E. Berenguer, X. Liu, et al. 2017. Fruit weight is controlled by Cell Size Regulator encoding a novel protein that is expressed in maturing tomato fruits. 13(8): e1006930–e1006930. doi: https://doi.org/10.1371/journal.pgen.1006930.
32. Nie, H., Y. Shi, X. Geng, and G. Xing. 2022. CRISRP/Cas9-Mediated Targeted Mutagenesis of Tomato Polygalacturonase Gene (SlPG) Delays Fruit Softening. Frontiers in Plant Science 13. doi: https://doi.org/10.3389/fpls.2022.729128
33. Obomighie, I., I.J. Prentice, P. Lewin-Jones, F. Bachtiger, N. Ramsay, et al. 2025. Understanding pectin cross-linking in plant cell walls. Communications Biology 8(1). https://doi.org/10.1038/s42003-025-07495-0.
34. Olaniyi, J., W. Akanbi, T. Adejumo, and O. Akande. 2010. Growth, fruit yield and nutritional quality of tomato varieties. African Journal of Food Science 4(6): 398–402. https://academicjournals.org/article/article1380726269_Olaniyi%20et%20al.pdf.
35. Quinet, M., T. Angosto, F.J. Yuste Lisbona, R. Blanchard-Gros, S. Bigot, et al. 2019. Tomato Fruit Development and Metabolism.Frontiers in Plant Science 10. doi: https://doi.org/10.3389/fpls.2019.01554.
36. Patel, B., R. Sutar, S. Khanbarad, and V. Darji. 2016. Effect of different precooling techniques on the storage behavior of tomato fruits at low and ambient temperatures. Vegetable Science 43(2): 184–189.
37. Polwaththa, K.D.M., and Y.A. Amarasinghe. 2024. * Corresponding author: AA Yasarathna Amarasinghe Optimizing calcium application strategies to enhance fruit quality in tomato (Lycopersicon esculentum (L.) Mill). International Journal of Science and Research Archive 13(02): 596–602. https://doi.org/10.30574/ijsra.2024.13.2.2188.
38. Prado, R.M. 2021. 2021. Calcium. Mineral nutrition of tropical plants (pp. 149–164). Springer. Springer eBooks: 149–164. https://doi. org/10.1007/978-3-030-71262-4_8.
39. Qin, Y., A. Gong, X. Liu, N. Li, T. Ji, et al. 2024. Testing a Simulation Model for the Response of Tomato Fruit Quality Formation to Temperature and Light in Solar Greenhouses. Plants 13(12): 1662. https://doi.org/10.3390/plants13121662.
40. Ranjbar, S., M. Rahemi, and A. Ramezanian. 2018. Comparison of nano calcium and calcium chloride spray on postharvest quality and cell wall enzymes activity in apple cv. Red Delicious. Scientia Horticulturae 240: 57–64. https://doi.org/10.1016/j.scienta.2018.05.035.
41. Ren, H., Q. Zhao, Y. Feng, P. Tang, Y. Wang, et al. 2023. Gene-specific silencing of SlPL16, a pectate lyase coding gene, extends the shelf life of tomato fruit. Postharvest Biology and Technology 201: 112368–112368. https://doi.org/10.1016/j.postharvbio.2023.112368.
42. Rosales, M.A., L.M. Cervilla, J. J. Ríos, B. Blasco, E. Sánchez-Rodríguez, et al. 2010. Environmental conditions affect pectin solubilization in cherry tomato fruits grown in two experimental Mediterranean greenhouses. Environmental and Experimental Botany 67(2): 320–327. https://doi.org/10.1016/j.envexpbot.2009.07.011.
43. Santhosh, S.S., T. Chitdeshwari, and K. Chinnasamy. 2023. Calcium influences biochemical and antioxidant enzymatic activities in tomato fruits during storage. Journal of Applied Biology & Biotechnology. https://doi.org/10.7324/jabb.2023.110216.
44. Saurabh, S., A.S. Vidyarthi, and D. Prasad. 2014. RNA interference: concept to reality in crop improvement. Planta 239(3): 543–564. doi: https://doi.org/10.1007/s00425-013-2019-5
45. Savary, S., L. Willocquet, S. J. Pethybridge, P. Esker, N. McRoberts, and A.Nelson .2019 . The global burden of pathogens and pests on major food crops. Nature ecology & evolution, 3(3), 430-439..
46. Sena, J.P., S. Silva, F. Freire, G. Barbosa, and H. Lucena. 2024. Calcium fertilization strategy on mango physiological characteristics and yield. Pesquisa Agropecuária Tropical

54. https://doi.org/10.1590/1983-40632024v5476919.
47. Shehata, S.A., S.Z. Abdelrahman, M.M.A. Megahed, E.A. Abdeldaym, M.M. El-Mogy, et al. 2021. Extending Shelf Life and Maintaining Quality of Tomato Fruit by Calcium Chloride, Hydrogen Peroxide, Chitosan, and Ozonated Water. Horticulturae 7(9): 309. https://doi.org/10.3390/horticulturae7090309.
48. Shi, Y., B.-J. Li, D. Grierson, and K.-S. Chen. 2023b. Insights into cell wall changes during fruit softening from transgenic and naturally occurring mutants. Plant physiology 192(3): 1671–1683. https://doi.org/10.1093/plphys/kiad128.
49. Souza, J.M.A., S. Leonel, M. Leonel, E.L. Garcia, L.R. Ribeiro, et al. 2023. Calcium Nutrition in Fig Orchards Enhance Fruit Quality at Harvest and Storage. Horticulturae 9(1): 123. https://doi.org/10.3390/horticulturae9010123.
50. Tang, D., P. Gallusci, and Z. Lang. 2020. Fruit development and epigenetic modifications. New Phytologist 228(3): 839–844. doi: https://doi.org/10.1111/nph.16724.
51. Thor, K. 2019. Calcium—Nutrient and Messenger. Frontiers in Plant Science 10. https://doi.org/10.3389/fpls.2019.00440
52. Uluisik, S., N.H. Chapman, R. Smith, M. Poole, G. Adams, et al. 2016. Genetic improvement of tomato by targeted control of fruit softening. Nature Biotechnology 34(9): 950–952. https://doi.org/10.1038/nbt.3602.
53. Voragen, A.G.J., G.-J. Coenen, R.P. Verhoef, and H.A. Schols. 2009 . Pectin, a versatile polysaccharide present in plant cell walls. Structural Chemistry 20(2): 263–275. https://doi.org/10.1007/s11224-009-9442-z.
54. Wang, D., N.H. Samsulrizal, C. Yan, N.S. Allcock, J. Craigon, et al. 2019. Characterization of CRISPR Mutants Targeting Genes Modulating Pectin Degradation in Ripening Tomato. Plant Physiology 179(2): 544–557. https://doi.org/10.1104/pp.18.01187.
55. Wang, D., and G. B. Seymour. 2022. Molecular and biochemical basis of softening in tomato. Molecular Horticulture 2(1). https://doi.org/10.1186/s43897-022-00026-z.
56. William, H. 1980. Official methods of analysis of the Association of Official Analytical Chemists. 13th ed.
57. Wormit, A., and B. Usadel. 2018. The Multifaceted Role of Pectin Methylesterase Inhibitors (PMEIs). International Journal of Molecular Sciences 19(10): 2878. https://doi.org/10.3390/ijms19102878.
58. Wu, Y., X. Yang, X. Wang, L. Yan, X. Hu, et al. 2023. Effect of Foliar Calcium Fertilization on Fruit Quality, Cell Wall Enzyme Activity and Expression of Key Genes in Chinese Cherry. International Journal of Fruit Science 23(1): 200–216. doi: https://doi.org/10.1080/15538362.2023.2265656.
59. Xu, C., K.L. Liberatore, C.A. MacAlister, Z. Huang, Y.-H. Chu, et al. 2015. A cascade of arabinosyltransferases controls shoot meristem size in tomato. Nature Genetics 47(7): 784–792. https://doi.org/10.1038/ng.3309
60. Xu, Z., J. Dai, T. Kang, K. Shah, Q. Li, et al. 2022. PpePL1 and PpePL15 Are the Core Members of the Pectate Lyase Gene Family Involved in Peach Fruit Ripening and Softening. Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.844055.
61. Yang, L., W. Huang, F. Xiong, Z. Xian, D. Su, et al. 2017. Silencing of SlPL, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life and reduced susceptibility to grey mould. Plant Biotechnology Journal 15(12): 1544–1555. https://doi.org/10.1111/pbi.12737.
62. Yang, Y., K. Tang, T.U. Datsenka, W. Liu, S. Lv, et al. 2019. Critical function of DNA methyltransferase 1 in tomato development and regulation of the DNA methylome and transcriptome. Journal of Integrative Plant Biology 61(12): 1224–1242. doi: https://doi.org/10.1111/jipb.12778.
63. Zhong W, M Zhao, B Liu, C Zou, Y Sun, G Wu, Q Zhang, G Jin, Z Jin, D Chadwick, and X Chen. 2020. Integrated systematic approach increase greenhouse tomato yield and reduce environmental Journal of Environmental Management, 266, 110569. https://doi.org/10.1016/j.jenvman.2020.110569
64. Zhang, J., M. Du, G. Liu, F. Ma, and Z. Bao. 2024. Lignin Sulfonate-Chelated Calcium Improves Tomato Plant Development and Fruit Quality by Promoting Ca^{2+} Uptake and Transport. Horticulturae 10(12): 1328–1328. https: //doi.org/10.3390/horticulturae10121328