EXTENDING THE MARKETABILITY OF TOMATO FRUITS AND ENHANCING THEIR QUALITY THROUGH GENE SILENCING AND ORGANIC CALCIUM-PECTIN APPLICATION

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ABSTRACT

This study aimed to investigate the impact of nine treatments on tomato plants and their planting location within a randomized complete block design. The treatments included gene silencing of the PL enzyme (RNAi-SlPL) and application of organic pectin and calcium to extend the marketability of tomato fruits and improve their quality. The RNAi-SlPL treatment outperformed others, revealed the lowest weight loss (41.4%) and highest firmness (4.89 kg·cm⁻²) after 36 days of cold storage, along with strong performance after 21 days at room storage (41.31% weight loss, 4.29 kg·cm⁻²). This was followed by CaP1P2 treatment, with 43.7% weight loss and 4.48 kg·cm⁻² firmness after 24 days of cold storage, and 45.87% weight loss with 4.15 kg·cm⁻² firmness after 14 days at room storage. Both treatments significantly boosted carotenoid (2.640 and 2.728 mg/100ml respectively) and lycopene (4.62 and 4.81 mg/100ml) levels post- cold storage. These findings highlight the effectiveness of RNAi technology and the use of organic pectin and calcium in extending the marketability of tomato fruits.

Keywords: RNAi, fruit weight loss, fruit firmness, responsible consumption, vitamin C, total acidity percentage, carotene, lycopene.

إطالة القابلية التسويقية لثمار الطماطة وتعزيز جودتها بأستعمال الاخماد الجيني و الكالسيوم البكتين العضويين

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المستخلص

درس تأثير 9 معاملات لنباتات الطماطة وموقع زراعتها ضمن تصميم القطاعات الكاملة المعشاة وتضمنت المعاملات الاخماد الجيني لانزيم PL (RNAi-SlPL) والمعاملة بالبكتين والكالسيوم العضويين في إطالة القابلية التسويقية لثمار الطماطة وتحسين جودتها . اظهرت النتائج سيادة معاملة RNAi-SlPL بأقل نسبة للفقد بالوزن واعلى صلابة للثمار بعد 36 يوم من الخزن في البراد (41.4 % و 4.89 كغم .سم$^{-2}$ بالتتابع) و بعد 21 يوم من الخزن في الغرفة (41.31 % و 4.29 كغم .سم$^{-2}$) تليها المعاملة CaP1P2 بأقل نسبة للفقد بالوزن وقيمة عالية في صلابة الثمار بعد 24 يوم من الخزن في البراد (43.7 % و 4.48 كغم .سم$^{-2}$ بالتتابع) و بعد 14 يوم من الخزن في الغرفة (45.87 % و 4.15 كغم .سم$^{-2}$) . تفوقت المعاملتين RNAi-SlPL و CaP1P2 في تركيز صبغة الكاروتين (2.640 و 2.728 ملغم/100مل بالتتابع) واللايكوبين (4.81 و 4.62 ملغم/100مل بالتتابع) بعد الخزن في البراد. يظهر هذا البحث الى كفاية استخدام تقانة RNAi والمعاملة بالبكتين والكالسيوم العضويين في إطالة القابلية التسويقية لثمار الطماطة مع تقليل الهدر الغذائي وتعزيز مقومات الاستدامة .

الكلمات المفتاحية: RNAi، الفقد بالوزن للثمار، صلابة الثمار، الاستهلاك الواعي، فيتامين C، النسبة المئوية للحموضة الكلية للثمار، كاروتين، لايكوبين

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INTRODUCTION

Enhancing the marketability of tomato fruits and minimizing post-harvest losses has become a strategic priority, particularly considering worldwide threats of food security and climate variability. Such efforts also have a direct implication of increasing the efficiency of food supply chains, and without having to grow more crops. In addition, storage methods and techniques for pre- and post-harvest management are believed to be the backbone of sustainable food security interventions. Given the significance of tomato fruits, their rapid perishability, and limitations in their marketing, the use of calcium has become increasingly recognized as an essential and necessary element in plant growth, flowering and seeds production (1), as well as in improving fruit quality and increasing their marketability (26, 40). This has positively reflected on reducing fruit waste, thereby strengthening food security within sustainability practices. This is especially important due to calcium's role in strengthening cell wall integrity by inhibiting the activity of pectinolytic enzymes, acting as a secondary messenger that enhances the transport of nutrients and hormonal signals, regulating genetic markers, and sensing environmental influences to regulate epigenetic alleles. Moreover, it plays a role in cation-anion balance and transport processes within cell membranes, as well as in cell wall stabilization (6). The fruit epidermal cells represent a multifaceted interface, crucial for preventing uncontrolled water and gas loss. The cells of the peel are a composite structure that is necessary for the regulation of uncontrolled water and gas loss. Working as an insulating cover, they protect plants against various biotic and abiotic stresses (2). Since the cell wall contains complex carbohydrates (e.g., pectins, cellulose, and hemicelluloses) and their associated proteins that have a structural and enzymatic function in the cell wall, preservation of its integrity is of high importance. Fundamental to the structure of pectins are the units of Galacturonic acid (GalA) which are linked between each other by covalent bonds, resulting in a super-molecular three-dimensional network in the cell wall. This intricate structure governs various cellular attributes, notably mechanical strength and wall porosity (28). The cross-linking between pectin and Ca^{2+} further reinforces this, contributing to cell wall stabilization and reduced water loss, while simultaneously enhancing the amount of bound water within the cells. As pectin is an indispensable component of cell growth, formation, and development, (HG) and (RG-II) constitute approximately 65% and 10%, respectively, of the total pectin content. Ca^{2+} ions form cross-linkages with the carboxyl ions of the methyl-ester-free Galacturonic acid residues in HG, and RG-II forms cross-linkages with boric acid. These interactions are vital for maintaining structural stability, reducing porosity, and enhancing the hydroscopic strength, thereby preserving turgor pressure and cell wall rigidity (14). In addition to these, the above factors illustrate how pectin is eagerly followed by researchers as a way to affect the build and ceiling of plant cell, and to promote uninterrupted, fine plant growth by increasing plant resistance against biotic and abiotic factors, thereby resulting in a higher quality and value price of crop yield. The full exploitation of the pectin potential to improve on growth resistance, fruit quality and shelf life is not only a specific scientific achievement but a conscious step towards smarter farming practice, a safer food system and a cleaner environment and production which translate to a sustainable production. The application of gene silencing represents a groundbreaking innovation with the potential to recalibrate agricultural production rhythms at a time when the world faces an almost impossible equation: growing population pressures, shrinking agricultural land, and increasing climate variability. Gene silencing, initially recognized as a natural defense mechanism against double-stranded RNA (dsRNA) (30), has been adapted as a tool for genetically suppressing specific plant genes, thereby modifying particular traits. This stands in contrast to traditional breeding programs, which typically require significant time and labor (36). It is noteworthy that the fruit wall of tomatoes is composed of fine cellulose fibers interconnected through a glycan network and pectin, providing essential structural integrity and acting as a barrier

against environmental stresses. Tomato fruit ripening is a genetically coordinated process involving various chemical and physiological changes, particularly through the activation of enzymes that lead to the breakdown of the cell wall (20). In plant breeding and molecular genetics, these epigenetic mechanisms have been harnessed under the concept of epigenetic breeding, facilitating the overcoming of challenges faced by breeders in developing or deriving new cultivars. Much like other molecular breeding techniques, epigenetic breeding has played a pivotal role in identifying transferable traits and developing new cultivars, as documented by several researchers (12). Dodgson *et al.* (9) treated tomato plants of the cultivar Micro Tom by foliar spraying with either 1 L/ha of calcium or 1 L/ha of calcium supplemented with a calcium transport enhancer (MCAS – Mid1-Complement Activity Protein Channels) at 7 and 14 days after flowering. It was found that fruits from plants treated with calcium enhanced by the activator maintained their shelf life more stably, remaining viable for up to 10 weeks when stored at room temperature. Similarly, Jhanani *et al.* (19) applied pectin extracted from zucchini squashto tomato fruits at progressive ripening stages: green, breaker, pink, and red, at concentrations of 1%, 3%, and 5% (w/v). Notably, fruits treated with 5% pectin displayed a mere 25% weight loss compared to untreated controls. Yang et al. (47) reported that modifications to histone SlH2AZ affect carotenoid biosynthesis gene expression during tomato ripening. Additionally, Lata *et al.* (24) demonstrated the use of RNA interference (RNAi) to silence the APX gene (ascorbate peroxidase), responsible for the oxidation of vitamin C in tomato fruits. Targeted silencing within mitochondria led to an increase in vitamin C concentration in the fruits. Moreover, Kim *et al.* (23) reported a reduction in fruit weight following the silencing of the SllAA9 gene compared to the control treatment. Nevertheless, silenced fruits showed enhanced levels of lycopene, total soluble solids, and total acidity. Based on these findings, the present study aims to investigate the marketability of local tomato fruits after storage under both refrigerated and ambient conditions, focusing on the effects of gene silencing, calcium, pectin treatments, and their interactions.

MATERIALS AND METHODS

The study investigated the effects of nine treatments and two cultivation sites on the postharvest marketability traits of tomato fruits. The treatments included gene-silenced plants for the enzyme Pectate lyase (SlPL-RNAi), and untreated control plants (C0), and Different combinations of pectin and organic calcium are used through irrigation or spraying at different concentrations (coded P1, P2, P3, Ca, CaP1, CaP2, and CaP1P2). (P1: 1 $g \cdot L^{-1}$ through irrigation; P2: 2 $g \cdot L^{-1}$ foliar spray; P3: combined P1+P2), calcium (Ca: 2 $ml \cdot L^{-1}$ foliar spray; CaP1: P1+Ca; CaP2: P2+Ca; CaP1P2: P1+P2+Ca), treatments applied on the vegetative stage, flowering, or fruit development stage. The experiments were conducted at two locations: Al-Jadriyah (L1) and Abu Ghraib (L2). All the treatments mentioned were applied three times: at vegetative growth (2 weeks after transplanting), at flowering and initial setting stage of fruits (4 weeks after the first application), and fruit development stage (4 weeks after the second application). Fruits from each treatment were harvested at the pink stage and transported to the laboratory for evaluation of the gene silencing effect, as well as the impact of pectin, calcium, and their interactions. Each treatment was replicated five times under two storage environments: ambient room temperature and cold storage (10 ± 2°C). A total of 90 experimental units were used for each site under each storage condition. The data were statistically analyzed using a combined analysis of sites and treatments according to a randomized complete block design (RCBD). The treatment means were compared with LSD (least significant difference) test at a 5% level of probability. The fruits were stored in storage laboratory / Department of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad. Samples were placed in refrigerators set at 10 ± 2°C, while another portion of the fruits was stored at room temperature in a separate laboratory. For each experimental unit, four fruit samples were prepared. They were placed in cardboard trays and wrapped with

perforated polyethylene films: two samples were designated for cold storage and two for room temperature storage. Each sample weighed approximately 1 kg. The first sample from each set was used to measure weight loss over specific storage periods, while the second was used for conducting additional laboratory analyses. Weight loss percentage was calculated after 12, 24, and 36 days of cold storage (when fruits reached the pink stage) and after 7, 14, and 21 days of room temperature storage. The test weight at each time point was recorded and the weight loss was determined as a percentage according to the following equation:

Percentage of weight loss = [(starting weight - weight at measurement) / starting weight] × 100

The total soluble solids (T.S.S.) percentage was measured by placing juice droplets on a portable refractometer, and readings were taken directly. Fruit firmness was assessed using a Fruit Pressure Tester ($kg \cdot cm^2$). The concentrations of beta- Beta-carotene and lycopene concentrations (mg/100g) were estimated following the method of Nagata & Yamashita (27), while vitamin C content (mg/100g f.w.) was determined using Kavanagh's (22) method. Furthermore, Total acidity percentage was estimated according to William (44). Measurements were taken for each experimental unit after the fruits reached the red stage under both cold and room temperature storage conditions, adhering to the specified timeframes.

RESULTS AND DISCUSSION

Weight Loss in Tomato Fruits: Minimizing weight loss is important to improve the storage techniques, create post-ha rvest performance enhanced varieties, assess fruit quality, predict shelf-life and time handling operations to keep consumers satisfied, minimize spoilage and ultimately reduce food waste, and enhance sustainability and food security. The results in Table (1) show significantly better performance of L1 SIPL-RNAi and L2 SIPL-RNAi treatments, showing the least percentage of weight loss after 12 days of cold storage (3.17% and 3.24%, respectively) and after 7 days of room temperature storage (7.19% and 7.25%, respectively) in comparison with the other treatments performed in this study. The L1C0 and L2C0 treatments presented the highest weight losses, which were 69.14 and 87.02%, respectively. In addition, the L2 CaP1P2 treated one showed the lowest weight loss after 12 d in cold storage (6.61%) and the L1CaP1P2 treated one showed the least weight loss after 7 d in room temperature (8.07%) compared with the L1C0 and L2C0 treatments. The SIPL-RNAi treatment consistently outperformed all other treatments with the lowest weight loss percentages during cold storage durations (3.2%, 10.5%, and 41.4%, respectively) and room temperature storage durations (7.22%, 18.48%, and 41.31%, respectively). This was particularly evident when compared to the control treatment (C0), where most fruits deteriorated after 12 days and 7 days of storage in the refrigerator and at room temperature (65.6% and 83.02%, respectively). The CaP1P2 treatment also stood out by exhibiting significantly lower weight loss percentages after 12 days and 7 days of storage in the refrigerator and at room temperature (6.7% and 8.11%, respectively). Furthermore, the P3 and CaP1P2 treatments excelled with the lowest weight loss percentages after 24 days of cold storage (43.7% and 42.1%, respectively) and after 14 days of room temperature storage (43.96% and 45.87%, respectively). The superior performance of the treatments L1 SlPL-RNAi, L2 SlPL-RNAi, and SlPL-RNAi in achieving the lowest fruit weight loss is attributed to the mechanism of gene silencing. This process involves hybridization between coding messenger RNA (mRNA) and non-coding RNA species, resulting in the production of inactive mRNA and consequently reducing the synthesis of the Pectate lyase (PL) enzyme in fruits. This reduction helps preserve pectin from degradation, thereby maintaining fruit firmness and weight by strengthening the cross-linking between microfibrillar cellulose fibers within the cell wall (43). Additionally, the synergy with proper agricultural practices and suitable growth environments enhanced photosynthesis, these treatments-maintained biomass, reduced moisture loss, and increased antioxidant activity, collectively protecting fruit cells by preventing pectin polymer release from cell walls, safeguarding covalent bonds and side chains of Rhamnogalacturonan-I

(RG-I), and preserving methyl ester groups in Homogalacturonan (HG) (38). Additionally, the availability of internal calcium capable of binding with pectin helped stabilize the cell wall and maintain nutrient reserves by reducing respiration, transpiration, metabolism rates, ethylene production, and the pace of ripening. These combined effects significantly lowered fruit weight loss. It is noteworthy that the treatments L1 CaP1P2 and CaP1P2 also showed outstanding performance by maintaining the lowest weight loss percentages even under room temperature storage conditions (4).

Table 1. Effect of gene silencing, pectin, calcium treatments, experimental site, and their interactions on fruit weight loss percentage (%) during col d and room temperature

Treatments	Fruit weight loss % / cold storage (%)			Fruit weight loss % / room temperature storage (%)		
			T			
	12 days	24 days	36 days	12 days	24 days	36 days
SIPL-	3.2	10.5	41.4	7.22	18.48	41.31
C0	65.6	100.0	100.0	83.02	100.00	100.00
P1	13.4	58.5	100.0	19.28	65.50	100.00
P2	11.8	44.8	100.0	16.61	49.65	100.00
P3	9.9	42.1	100.0	13.04	43.96	100.00
Ca	7.4	53.1	100.0	11.34	54.13	100.00
Ca P1	8.2	50.5	100.0	11.70	53.23	100.00
Ca P2	7.2	46.8	100.0	9.39	46.58	100.00
Ca P1 P2	6.7	43.7	100.0	8.11	45.87	100.00
L.S.D	1.106	5.030	1.233	2.353	4.822	1.045
			L			
L 1	15.13	49.78	93.39	19.52	52.97	93.40
L 2	14.55	50.23	93.59	20.42	53.12	93.56
L.S.D	N.S	N.S	N.S	N.S	N.S	N.S

	L X T											
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
SIPL-	3.17	3.24	10.48	10.58	40.48	42.35	7.19	7.25	18.66	18.30	40.63	42.00
C0	69.1	62.1	100.0	100.0	100.0	100.0	79.0	87.0	100.0	100.0	100.0	100.0
P1	13.0	13.8	57.94	59.07	100.0	100.0	20.0	18.5	66.61	64.39	100.0	100.0
P2	11.2	12.4	44.50	45.06	100.0	100.0	16.6	16.5	50.22	49.09	100.0	100.0
P3	9.86	9.91	40.80	43.37	100.0	100.0	12.9	13.1	43.89	44.04	100.0	100.0
Ca	7.21	7.59	52.92	53.19	100.0	100.0	11.2	11.4	53.74	54.52	100.0	100.0
Ca P1	8.47	8.00	50.65	50.35	100.0	100.0	11.1	12.2	50.10	56.35	100.0	100.0
Ca P2	7.21	7.17	46.43	47.22	100.0	100.0	9.37	9.41	47.48	45.69	100.0	100.0
Ca P1 P	6.81	6.61	44.26	43.22	100.0	100.0	8.07	8.15	46.02	45.71	100.0	100.0
L.S.D	1.564		N.S		N.S		3.328		N.S		N.S	

The combined effect of calcium and pectin integration within the fruit tissues, along with a decrease in PG enzyme activity, which improved pectin-calcium binding, may be the cause of the CaP1P2 treatment's greater performance. Cell growth and pericarp thickening—where the epidermis serves as a barrier to stop water loss and retain moisture—were favorably impacted by this interaction, which eventually decreased weight loss and increased shelf life. Low-temperature storage reduces chemical reactions in metabolic processes, such as respiration and ethylene generation, the L2CaP1P2 therapy is likely efficient in preventing weight loss. Additionally, the single-stem cultivation method at the L2 (Abu Ghraib) site enhanced leaf exposure to light, boosting photosynthesis and directing more sugars to developing fruits. Improved air circulation around fruits also reduced pathogen risks that could affect storage, indirectly limiting weight loss by minimizing damage (13,21). The P3 treatment's success appears linked to pectin's role e in forming a semi-permeable barrier that limits gas exchange (e.g., O_2 and CO_2), reducing pectinolytic enzyme activity and water vapor transfer from peel to pulp due to pectin's hydrophilic, sponge-like nature. This decreased respiration rates and moisture loss while enhancing firmness and delaying ripening stages - collectively contributing to reduced weight loss and extended storage (34). These effects may reflect epigenetic responses to environmental factors interacting with postharvest conditions. Cold storage

(refrigeration) and room temperature storage potentially induce changes in histone modifications (H3K9ac acetylation and H3K4me3 methylation), reprogramming expression of ripening-related genes to delay maturation and reduce weight loss (25,52). Alternatively, removal of acetyl groups from SlHDA7 histone proteins may inhibit ripening regulators FUL1 and RIN (which control tomato ripening and ethylene synthesis/signaling), thereby extending shelf life (51). Refrigeration temperatures may suppress SlDML2 transcription, increasing DNA methylation synergistically with reduced moisture loss to extend storage duration. Conversely, room temperature storage may elevate SlDML2 expression, inhibiting methylation while activating genes associated with fruit deterioration, moisture/weight loss, and shortened shelf life (48). These findings align with (4).

Tomato Fruit Firmness

Tomato fruit firmness serves as a critical quality and ripening indicator that plant breeders target to meet increasing global food demand while reducing refrigeration energy consumption, carbon emissions, and food waste through developing temperature-resilient varieties with extended postharvest shelf life. The results demonstrate that the SlPL-RNAi treatment achieved superior firmness values of 12.18, 9.10, and 4.89 kg·cm^{-2} after 12, 24, and 36 days of refrigerated storage respectively, along with 11.04, 8.28, and 4.29 kg·cm^{-2} after 7, 14, and 21 days at room temperature, significantly outperforming the control (C0).

Table 2. Effect of gene silencing, pectin, calcium treatments, experimental site, and their interactions on fruit firmness (kg·cm^{-2}) of fruits stored under cold and room temperature conditions

Treatments	Fruit firmness (kg·cm^{-2}) cold storage			Fruit firmness (kg·cm^{-2}) room temperature storage		
			T			
	12 days	24 days	36 days	12 days	24 days	36 days
SIPL-	12.18	9.10	4.89	11.04	8.28	4.29
C0	2.64	0.00	0.00	2.40	0.00	0.00
P1	5.32	2.79	0.00	4.37	2.54	0.00
P2	5.62	2.82	0.00	5.16	2.64	0.00
P3	5.96	3.91	0.00	5.63	3.26	0.00
Ca	5.41	2.84	0.00	5.05	2.68	0.00
Ca P1	5.72	3.00	0.00	5.64	2.75	0.00
Ca P2	6.79	3.52	0.00	6.24	3.74	0.00
Ca P1 P2	7.12	4.48	0.00	6.81	4.15	0.00
L.S.D	0.6559	0.6088	0.1807	0.7889	0.6986	0.3674
			L			
L 1	6.421	3.636	0.534	5.736	3.366	0.463
L 2	6.187	3.573	0.552	5.896	3.307	0.489
L.S.D	N.S	N.S	N.S	N.S	N.S	N.S

	L X T											
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
SIPL-	12.34	12.02	9.11	9.08	4.81	4.97	11.00	11.10	8.55	8.00	4.17	4.40
C0	2.87	2.40	0.00	0.00	0.00	0.00	2.50	2.30	0.00	0.0	0.00	0.00
P1	5.36	5.28	2.58	3.00	0.00	0.00	4.01	4.73	2.52	2.56	0.00	0.00
P2	5.68	5.56	2.89	2.74	0.00	0.00	5.14	5.18	2.58	2.70	0.00	0.00
P3	5.97	5.94	3.98	3.83	0.00	0.00	5.65	5.62	3.33	3.18	0.00	0.00
Ca	5.63	5.18	2.68	3.00	0.00	0.00	4.96	5.14	2.56	2.80	0.00	0.00
Ca P1	5.91	5.52	3.18	2.82	0.00	0.00	5.49	5.79	3.00	2.50	0.00	0.00
Ca P2	6.75	6.82	3.81	3.23	0.00	0.00	6.04	6.44	3.59	3.88	0.00	0.00
Ca P1 P	7.28	6.96	4.49	4.46	0.00	0.00	6.83	6.78	4.16	4.14	0.00	0.00
L.S.D	1.564		N.S		N.S		3.328		N.S		N.S	

The CaP1P2 treatment followed closely, recording 7.12 and 4.48 kg·cm^{-2} after 12/24 days refrigerated and 6.81/4.15 kg·cm^{-2} after 7/14 days at room temperature. The exceptional performance of SlPL-RNAi stems from pectin retention in cell walls due to PL enzyme gene silencing, which reduces pectin water solubility while modifying hormonal ripening regulation through ethylene suppression and delayed response. This

genetic intervention enhances cellulose/hemicellulose deposition and improves oxidative stress defense, collectively maintaining structural integrity (45). Meanwhile, CaP1P2's effectiveness derives from calcium bridges between pectic acids/polysaccharides that inhibit pectinases, reinforced by an enhanced pectin gel network that improves cellular adhesion. The calcium component further boosts water/nutrient uptake, stabilizes membrane lipid metabolism, and increases pathogen resistance, while pectin's selective gas exchange properties reduce respiration rates and suppress PG enzyme activity (33). The extended storage duration at low temperatures (refrigeration) compared to high temperatures (room temperature) may be attributed to the effect of low temperatures on the overexpression of the gene SlJMJ7, which increases its impact on removing the methylation of histone H3K4me3. This negatively affected the suppression of genes and transcription factors associated with ripening, such as cell wall-related genes (CEL2, XTH5, EXP1, XYL1, PG2a, TBG4, PL1/2/8) and ethylene biosynthesis-related genes (ACS2/4/8, ACO6), as well as transcription factors (RIN, NOR, CNR) (7). In contrast, high temperatures increased the overexpression of the gene SlJMJ6, which encodes an enzyme responsible for removing methylation from histone H3K27me2/3 in tomatoes. This led to the activation of genes associated with ripening, such as ethylene biosynthesis genes (ACO1, ACS4) and cell wall-related genes (PL, TBG4), along with the overexpression of the DNA demethylation gene SlDML2, accelerating the ripening process and causing early spoilage (25). These findings are consistent with those reported by Uluisik et al. (41), Dodgson et al. (9), and Zhang et al. (50).

Total Soluble Solids (T.S.S.) Percentage, Vitamin C Concentration, and Total Acidity Percentage of Tomato Fruits: The percent of TSS, total acidity and Vitamin C content in tomato fruits are important quality parameter which add to superior qualities of nutritional value. Reducing food waste due to consumer food refusal of low-grade fruits and, hence, reducing losses is essential for efficient use of resources, including water, fertilizers, and energy, and is beneficial for sustainable agricultural processes. This also extends their storage potential without compromising nutritional quality, lessening the reliance on immediate tomato availability and ensuring stable food abundance and local food sovereignty. The results in Table (3) demonstrate the superiority of the CaP1P2 treatment over the gene silencing treatment and certain other study treatments regarding the percentage of Total Soluble Solids (T.S.S.) in the fruits, registering 6.86% after refrigerated storage. Conversely, the gene silencing treatment exhibited a lower T.S.S. percentage (4.98%). This difference can be attributed to pectin's complex composition of polysaccharides and organic acids. The gene silencing-mediated inhibition of pectin degradation, synergizing with low-temperature storage, allows for a gradual, albeit slower and lesser, accumulation of sugars and soluble solids. This explains the lower T.S.S. percentage in the gene silencing treatment compared to other study treatments and the control (32). These findings are consistent with reported Zhang et al., (50). The results in Table (3) demonstrate the superiority of CaP1 in vitamin C concentration after storage in both the refrigerator and at room temperature (16.3 and 14.0 mg/100g f.w. respectively), followed by SlPL-RNAi (15.1 and 13.6 mg/100g f.w. respectively), compared to the control treatment (C0). However, C0 outperformed all other treatments in total acidity percentage after storage in both the refrigerator and at room temperature (0.63% and 0.48%, respectively). The lower total acidity percentage in SlPL-RNAi compared to the control treatment (C0) can be attributed to the reduced release of galacturonic acid molecules resulting from the degradation of pectin, which has a dynamic structure responsive to internal and environmental stimuli, particularly when conditions are favorable. As a component of the cell wall, pectin contributes to maintaining the structural integrity of fruit coatings. Gene silencing may have suppressed the synergistic interaction between pectin-degrading enzymes, leading to a reduction in total acidity (39). The increase in vitamin C levels in SlPL-RNAi fruits compared to the control treatment (C0) is

likely due to the direct impact of gene silencing on cellular cohesion and its indirect effects on changes in gene expression, hormonal signaling, metabolic flux, and biosynthetic pathways, all of which enhanced the accumulation of vitamin C—a known antioxidant in plants that increases in gene-silenced fruits (46). The decreases in total acidity percentage in CaP1P2 is attributed to calcium's role in strengthening cell walls and stabilizing membranes by inhibiting the activity of pectin-degrading enzymes, thereby reducing pectin degradation and lowering total acidity (33). Despite pectin being a polymer rich in galacturonic acid (GalA)—the substrate for pectin-degrading enzyme activity—its addition led to a significant increase in substrate levels, delaying the enzymatic activity of pectin-degrading enzymes and consequently reducing total acidity (15). The superiority of CaP1 in vitamin C concentration in tomato fruits can be attributed to the role of calcium, in synergy with pectin, in slowing metabolic processes by reducing respiration rates and ethylene production, positively impacting the increase in vitamin C levels (19). Pectin could be involved in Vitamin C synthesis through an enhancement of the genetic expression of pectin-degrading enzymes. In conjunction with environmental stimulation, such as temperature and light, hormonal stimulation, and oxidative stress, these enzymes oxidize monosaccharide D-galactose to ascorbic acid precursor L-galactono-1,4-lactone, catalyzed by L-galactose dehydrogenase. This suggests a complex interplay of pectin metabolism in bolstering Vitamin C levels (5). Furthermore, pectin contains several elements, including potassium, which activates metabolic processes (such as carbon and nitrogen metabolism), influences fundamental processes (protein synthesis), and plays a role in nutrient translocation from leaves to other plant parts. This enhanced transport of synthesized compounds can elevate Vitamin C levels, consequently contributing to a decrease in total acidity (37). Fruit ripening is a form of programmed cell death, titratable acidity values typically decline as ripening progresses due to increased activity of citric acid glycoxylase, particularly under elevated storage temperatures. During respiration at higher temperatures, organic acids are converted into sugars utilized in metabolic processes during storage (35). Despite its antioxidant properties, Vitamin C levels can decrease with increasing storage temperatures because higher temperatures exacerbate oxidative stress alongside heightened metabolic activity, accelerating redox reactions that deplete Vitamin C (17). Conversely, refrigerated storage temperatures (10 ± 2 °C) maintained more favorable ascorbic acid levels. This is likely due to the influence of low temperatures on upregulating the gene expression of enzymes involved in ascorbic acid biosynthesis and reducing the activity of redox reactions, leading to better Vitamin C retention compared to fruits stored at room temperature (10). Although Vitamin C levels may be decreases in plants as ripening advances, its role in protecting against oxidative stress or environmental pressures is evident through the enzymatic conversion of H_2O_2 to H_2O, with ascorbate (Vitamin C) acting as an electron donor (8,25). Notably, the underlying mechanisms of ripening rely on a coordinated interplay between environmental factors, plant hormones, epigenetic alleles, and ripening-related transcription factors. The possible cause of decrease in Vitamin C was that the under expression of SlAPX4 gene (which is up-regulated in the fruit ripening and responding to oxidative stress, resulting in Vitamin C catabolism) might be regulated by the epigenetics (like nucleic acid methylation and modification of histone) over the promoter region of SlAPX4. Likewise, the reduction in acidity of fruit during the ripening period could be attributed to the effect of epigenetic alleles on master transcription factors RIN, TAGL1, FUL1 and FUL2 that compose a network controlling the expression of ethylene biosynthesis genes.

Table 3. Effect of gene silencing, pectin, calcium treatments, experimental site, and their interactions on total soluble solids percentage (T.S.S %), vitamin C concentration (mg·100 g^{-1} f.w.), and total acidity percentage (%) in tomato fruits stored under cold and room temperature conditions

Treatments	Total soluble solids (T.S.S %)		Vitamin C concentration (mg·100 g^{-1} f.w.)		Total acidity percentage (%)	
			T			
	Stored in cold storage	Stored at room temperature	Stored in cold storage	Stored at room temperature	Stored in cold storage	Stored at room temperature
SIPL-	4.98	5.94	15.1	13.6	0.51	0.44
C0	5.10	6.06	14.3	12.4	0.63	0.48
P1	5.19	6.23	14.6	12.5	0.52	0.41
P2	5.49	6.17	13.1	10.9	0.46	0.41
P3	5.57	6.30	14.9	12.9	0.46	0.39
Ca	5.84	6.61	16.0	13.7	0.45	0.38
Ca P1	6.19	6.81	16.3	14.0	0.43	0.38
Ca P2	6.35	6.86	16.0	12.8	0.45	0.37
Ca P1 P2	6.86	7.08	15.6	13.8	0.42	0.35
L.S.D	0.5120	N.S	1.773	1.813	0.0380	0.0396
			L			
L 1	5.778	6.48	15.02	12.93	0.4791	0.3956
L 2	5.682	6.42	15.09	12.91	0.4821	0.4053
L.S.D	N.S	N.S	N.S	N.S	N.S	N.S

	L X T											
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
SIPL-	5.04	4.92	6.00	5.88	15.0	15.2	13.6	13.6	0.50	0.52	0.42	0.46
C0	5.20	5.00	6.10	6.02	14.2	14.3	12.4	12.3	0.64	0.62	0.50	0.46
P1	5.30	5.08	6.30	6.16	14.6	14.5	12.5	12.4	0.53	0.51	0.42	0.40
P2	5.60	5.38	6.20	6.14	13.0	13.1	10.9	10.9	0.47	0.45	0.40	0.42
P3	5.50	5.64	6.30	6.30	14.7	15.0	13.0	12.8	0.46	0.45	0.39	0.39
Ca	5.90	5.78	6.74	6.48	15.9	16.1	13.6	13.7	0.44	0.46	0.38	0.39
Ca P1	6.26	6.12	6.80	6.82	16.3	16.2	14.1	13.8	0.42	0.44	0.37	0.38
Ca P2	6.40	6.30	6.78	6.94	16.0	15.9	12.8	12.7	0.44	0.45	0.37	0.36
Ca P1 P	6.80	6.92	7.14	7.02	15.5	15.6	13.7	13.9	0.40	0.43	0.35	0.36
L.S.D	n.s		n.s		n.s		n.s		n.s		n.s	

The plant's perception of even minute amounts of ethylene triggers ethylene-responsive proteins that promote fruit softening, volatile compou nd release, and decreased acidity (11). This reduction in acidity can also be attributed to the gradual decrease in DNA methylation in promoter regions, allowing the expression of ripening-associated genes, potentially including those involved in organic acid metabolism (25). These findings are consistent with the results of Jhanani et al., (19) & Duret et al., (10).

Carotene and Lycopene Pigment Concentration (mg/100 mL) in Tomato Fruits: Pigments represent crucial nutritional compounds that enhance tomato fruit quality, making them both nutritionally valuable and visually appealing in dietary systems, particularly given rising consumer health awareness. The results presented in Table 4 indicate the superior carotene pigment concentration in tomato fruits of the L1SlPL-RNAi and SlPL-RNAi treatments after storage at room temperature (1.59 and 1.49 mg/100 mL, respectively). Furthermore, SlPL-RNAi exhibited higher carotene concentration after refrigerated storage (2.64 mg/100 mL) and superior lycopene concentration after both refrigerated (4.81 mg/100 mL) and room temperature (3.43 mg/100 mL) storage compared to L2C0 and C0, respectively. Notably, the L1CaP1P2 treatment showed elevated carotene concentration in fruits after room temperature storage (2.37 mg/100 mL), while CaP1P2 demonstrated superior carotene concentration after complete refrigerated (2.728 mg/100 mL) and room temperature (2.22 mg/100 mL) storage, and also higher lycopene concentration after both refrigerated and room temperature storage (4.62 and 3.48 mg/100 mL, respectively) compared to L2C0 and C0. The data in Table (4) also reveal the overall superior performance of the Al-Jadriyah (L1) location compared to the Abu-

Ghraib (L2) location across the measured traits after both refrigerated and room temperature storage. The enhanced pigment concentration observed in the gene silencing treatments (SlPL-RNAi) could be attributed to the reduced levels of water-soluble pectin, which strengthens the cell wall integrity. This preservation of cell wall structure likely minimizes the degradation of carotene and lycopene. Moreover, genetically silenced fruits often exhibit elevated levels of antioxidant enzymes. This increases in antioxidant activity could be protect carotene and lycopene from oxidative breakdown, leading to their stability and increased concentration (42). The elevated carotene levels observed in the CaP1P2 and L1CaP1P2 treatments could be attributed to calcium's involvement in the hormonal signaling pathways of ethylene, auxin, and ABA, as well as its role in regulating chlorophyll degradation. Furthermore, calcium influences physiological processes and enhances the fruit's capacity to manage oxidative stresses, thereby maintaining cell wall integrity and improving nutrient allocation towards fruit growth and pigmentation pathways, ultimately promoting the synthesis of both carotene and lycopene (16).

Table 4. Effect of gene silencing, pectin, calciumt reatments, experimental site, and their interactions on carotene and lycopene concentrations (mg/100 mL) in tomato fruits stored in cold and room temperature conditions

Treatments	Carotene concentration in tomato fruits (mg/100 mL)		Lycopene concentration in tomato fruits (mg/100 mL)	
	T			
	Stored in cold storage	Stored at room temperature	Stored in cold storage	Stored at room temperature
SIPL-RNAi	2.640	1.49	4.81	3.43
C0	1.423	1.26	2.93	2.25
P1	2.105	1.57	3.61	2.34
P2	2.381	1.98	3.95	2.64
P3	2.159	1.83	3.96	3.14
Ca	2.355	1.05	4.26	2.57
Ca P1	2.497	1.23	4.60	3.47
Ca P2	2.252	1.32	4.24	2.76
Ca P1 P2	2.728	2.22	4.62	3.48
L.S.D	0.3749	0.1280	0.1535	0.2810
	L			
L 1	2.378	1.696	4.247	2.996
L 2	2.187	1.403	3.970	2.797
L.S.D	0.1178	0.0785	0.0546	0.1227
	LXT			
	Stored in cold storage	Stored at room temperature	Stored in cold storage	Stored at room temperature
	L1 L2	L1 L2	L1 L2	L1 L2
SIPL-RNAi	2.73 2.55	1.59 1.39	4.83 4.79	3.53 3.33
C0	1.59 1.25	1.36 1.16	3.05 2.82	2.35 2.15
P1	2.12 2.09	1.67 1.47	3.85 3.37	2.44 2.24
P2	2.42 2.34	2.26 1.70	4.10 3.80	2.74 2.54
P3	2.21 2.11	2.17 1.50	4.09 3.83	3.24 3.04
Ca	2.54 2.17	1.10 1.00	4.40 4.11	2.67 2.47
Ca P1	2.60 2.40	1.33 1.13	4.75 4.45	3.55 3.40
Ca P2	2.35 2.15	1.42 1.22	4.39 4.09	2.86 2.66
Ca P1 P2	2.84 2.62	2.37 2.06	4.77 4.47	3.60 3.35
L.S.D	N.S	0.1810	N.S	N.S

The calcium-pectin complex further stabilizes cellular structures and activates carotenoid biosynthesis enzymes (49), leading to increased lycopene and β-carotene levels during tomato ripening (3). During tomato ripening dynamics, ethylene production triggers the conversion of cyclic hydrocarbon carotenoids (β-carotene, α-carotene) to linear lycopene, particularly under favorable refrigerated storage conditions that promote

chloroplast-to-chromoplast transformation (18) The observed β-carotene reduction may result from its conversion to flavor/aroma compounds or lycopene (31), Elevated temperatures, however, accelerate the degradation and loss of β-carotene, and with prolonged storage, lycopene also degrades and begins to decline as the fruit reaches its full maturity (18). The superior performance of the Al-Jadiriya site (L1) over Abu Ghraib (L2) likely stems from favorable environmental conditions that enhanced phytoene synthase (PSY) and lycopene cyclase activity, increasing carotenoid and lycopene accumulation (29,35). Environmental factors, along with the availability of soil nutrients, play a role in stimulating epigenetic changes that influence the pathways for synthesizing linear and cyclic carotenoids, leading to variations in their concentrations depending on growth conditions. Increased activity or suppression of RIN, a key regulator, not only affects ethylene biosynthesis but also plays a critical role in carotenoid synthesis. n particular, RIN-mediated associations with other transcription factors and their effects on a set of genes involved in the response to the surrounding environment (carotenoid accumulation) have been highlighted. Removal of histone trimthylation is also connected to carotenoid pathway-related genes, leading to carotenoid accumulation during the ripening process of tomato fruit. Changes in DNA methylation patterns during fruit ripening can result in differential expression (i.e., functionally similar coding regions containing multiple promoters), which stimulates genes such as Lycopene cyclase and Phytoene synthase (PSY) , both of which are essential for carotenoid accumulation. Furthermore, accumulation levels are linked to variations in genome methylation (25). These findings align with reported by researcher (16) in tomato fruits.

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 resulted in a prolonged storage life of tomato fruits under both refrigerated (10 ± 2 °C) and room temperature conditions. Notably, the RNAi-SlPL treatment exhibited the lowest percentage of weight loss while maintaining fruit firmness after 12, 24, and 36 days of refrigerated storage and 7, 14, and 21 days of room temperature storage. A decrease in Total Soluble Solids (T.S.S.) was observed in both the RNAi-SlPL and CaP1P2 treatments. Furthermore, Vitamin C concentration increased in fruits stored under both refrigerated and room temperature conditions, accompanied by a reduction in total acidity. This lower acidity is likely due to the diminished release of Galacturonic acid molecules resulting from pectin degradation, attributed to the genetic silencing of the PL enzyme. The availability of calcium for binding with pectin further contributed to maintaining cell wall stability and stored food reserves, alongside regulating physiological processes and improving the fruits' ability to manage oxidative stresses, in conjunction with responsive epigenetic modifications. These combined effects ultimately contributed to the extended storage life observed under both refrigerated and room temperature conditions

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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