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Research Article

Fruit Softening in Cherry Tomato: The Role of Harvesting Stages, Fruit Physical Parameters and Cell Wall-Modifying Enzyme

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Abstract

In cherry tomato, knowledge about cell wall breakdown at different harvesting stages due to increased polygalacturonase (PG) activity remains meager. The present investigation was undertaken to assess fruit firmness of parental lines and promising hybrids of cherry tomato grown under open field in relation to fruit physical parameters and PG activity, and to study the possible association ship between them at green, turning and ripe stage of harvest. A decreasing trend of pericarp thickness and fruit firmness, and a gradual increase of PG activity were observed from green to ripe stage of harvest, suggesting a progressive degradation of the middle lamella and primary cell wall during aging. Thinner pericarp, less fruit firmness and the enhanced activity of PG, leading to fruit softening, was found maximum at the ripe stage of cherry tomato. The hybrid 16/ToCVAR-1 × 16/ToCVAR-3 which showed comparatively less PG activity with high firmness of fruit at ripe stage could have longer shelf life and suitable for long distance transportation. PG activity was significantly and negatively correlated with pericarp thickness and fruit firmness at ripe stage. The results suggest that pectolytic enzyme activity is one of the predominant factors governing the firmness of cherry tomato fruit.

Keywords: *Cherry Tomato; Fruit Firmness; Harvesting Stage; Pericarp Thickness; Polygalacturonase Activity*

Introduction

Harvest indices such as color, firmness, removal force, size, weight, and soluble solids are traditionally used to market maturity in vegetable crops. Turning fruit vegetables into the last developmental phases involves many genetic, biochemical and physiological modifications. Tomato fruit quality diminishes as ripening reaches an advanced stage, mainly due to over-softening, increased pathogen susceptibility and the development of undesirable flavour and skin colour, leading to important economic losses during postharvest management. Thus, the rate of fruit softening determines not only the postharvest shelf life but also other economically important aspects, such as the

frequency of harvesting, the handling procedures and the distance that the fruits can be transported. Therefore, delaying fruit softening is one of the major targets of breeding programmes for most commodities.

Firmness and juiciness are the most important textural components in the case of fleshy fruits [1]. Both features are largely determined by the characteristics of parenchyma cells (shape and size, cell wall thickness and strength, cell turgor) and the extent and strength of adhesion areas between adjacent cells [2]. During ripening, parenchyma cell walls are extensively modified, altering their mechanical properties, and cell adhesion is significantly reduced as a result of middle lamella dissolution. Cell wall and middle lamella modifications leading to fruit softening result from the action of cell wall modifying enzymes (e.g. polygalacturonase, pectin methylesterase, pectate lyase, β-galactosidase, cellulase), generally encoded by ripening-related genes [3-5]. Other cell wall proteins, with no hydrolytic enzymatic activity, such as expansins, also have a role in softening [6]. In general, the cell wall disassembly process responsible for softening involves the depolymerization of matrix glycans, the solubilization and depolymerization of pectins and the loss of neutral sugars from pectin side chains [4,7]. The extension of these changes varies greatly among different species [5]. The effect of several cell wall-modifying enzymes on fruit ripening and softening has been studied in many plant species, the most intensively studied of these enzymes being polygalacturonase (PG). PG is one of the most active and paramount agents of fruit aging and cell wall softening. The normal role of PG is to hydrolyze pectins during fruit ripening, which leads to softening of the fruit [8-10]. In tomatoes, the high level of endo-PG activity detected in ripe fruits has led to the hypothesis that PG plays an important role in fruit softening [11]. The importance of enzymes in fruit softening has been investigated by many, but few reported on such activity in cherry tomato [12]. PG activity was related to the loss of firmness in tomatoes [13], green peppers [14] and cucumbers [15]. All hypothesized that the physical changes in fruit firmness, or other maturity indices, may be related to increasing activity of softening enzymes.

The tomato presents a huge fruit diversity, which makes it to be classified in commercial groups: Cherry, Grape, Santa Cruz, Italian, Round, Saladette and Industrial [16]. Among these, cherry tomatoes [*Solanum lycopersicum* var. *cerasiforme* (Dunnal) A. Gray] present small fruits and a sweeter taste in relation to other groups. The crop is thought to be the ancestor of cultivated tomato, based on its wide presence in Central America and the presence of a shorten style length in the flower [17]. However, recent genetic investigations have shown that the plants known as '*cerasiforme*' are a mixture of wild currant-type tomatoes and domesticated garden tomatoes rather than being 'ancestral' to the cultivated tomatoes [18]. Cherry tomato often called 'salad tomato' and being high content of antioxidant and phytochemical compounds, it is needless to emphasize the importance of quality parameter for fresh produce.

Reports regarding PG activity at different harvesting stages of cherry tomato are lacking. The relationship between PG and fruit firmness related traits might not only provide direct evidence for the involvement of the pectic enzymes in ripening but might also be of use in selecting firm cherry tomato lines in future breeding programme. Our objective was to measure the activity of softening enzyme (polygalacturonase) in cherry tomato at different harvesting stages and attempt to correlate such changes with physical parameters (pericarp thickness and fruit firmness) related to maturation.

Materials and Methods

Plant material and cultivation conditions

Field experiments were conducted during autumn-winter seasons (September, 2018 to February, 2019) at the research plot of AICRP on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India, situated at 23.5°N latitude and 89°E longitude at a mean sea level of 9.75 m. Six parents 2016/ToCVAR-1, 2016/ToCVAR-3, 2016/ToCVAR-4, 2016/ToCVAR-5, 2016/ToCVAR-6 and Cherry Round Yellow along with five promising hybrid combinations (2016/ToCVAR-1 × 2016/ToCVAR-3, 2016/ToCVAR-1 × 2016/ToCVAR-4, 2016/ToCVAR-1 × 2016/ToCVAR-5, 2016/ToCVAR-1 × 2016/ToCVAR-6 and 2016/ToCVAR-1 × Cherry Round Yellow) arising out from previous study [19] were used for the present study. True-selfed seeds of 6 parents and 5 F_1s were sown in nursery beds, after soil solarization with transparent polymulch of 100 μm thickness for 4 weeks, were made 20 cm tall and 1.0m wide in a sandy loam soil. Weathered cowdung manure at 2.5 kg/m^2 was mixed into the beds. Beds were drenched with chlorothalonil (0.2%) + carbendazim

(0.01%) to avoid damping off disease. Nursery beds were covered with 200 µm ultraviolet (UV)-stabilized polyethylene film supported by bamboo poles with open sides to protect seedlings from rain and direct sunlight. Seedlings were hardened by withholding water 4 days before transplanting. The experimental fields were plowed and brought to fine tilth. FYM @ 15 Mt·ha^{-1} and 120N:60P:60K kg·ha^{-1} was applied. Half N and full of P and K were applied as basal and rest of N was top dressed at 30 and 45 days after transplanting. Thirty-day old seedlings of parental lines and hybrids were transplanted separately in 2.5 × 2.5m plots spaced 50 cm in both ways in a randomized complete block design with 3 replications and maintained the crop according to Malik., *et al* [20].

Data recording

Fruits of parental lines and hybrid combinations were harvested separately at green, turning and ripe stage. Twenty randomly selected fruits of different trusses from each parental line and hybrid were brought replication wise for data recording in the laboratory of Post Harvest Technology of Horticultural Crops. The pericarp thickness of fruit (mm) was measured at different harvesting stages with the help of digital slide calipers. The firmness of the randomly selected fruits were measured at green, turning and ripe stage with fruit penetrometer and expressed in kg/cm^2. Polygalacturonase (PG) activity in cherry tomato was measured from the promising hybrids along with their parental lines at three different harvesting stages (green, turning, ripe) following the method of Lazan., *et al*. [21] with minor modifications. Enzyme extract was prepared by taking 1g of cherry tomato pulp and homogenized in 10 ml sodium acetate buffer (0.2M), (pH 6.0) with a pinch of Na_2SO_4 and polyvinylpyrrolidone (PVP) in chilled mortar. The homogenized was centrifuged at 15000 × g for 20 minute at 4°C and supernatant was used for the assay of polygalacturonase (PG) activity. PG enzyme assay mixture consisted of 0.45g of pectin and 0.1g casein were dissolved in 0.4% sodium acetate buffer (pH 3.8) and then the solution was diluted to 100 ml with 0.4% sodium acetate buffer (pH 3.8) for measuring the PG enzyme activity. 0.2 ml of enzyme extract was added to 2 ml of assay mixture and incubated at 37^{0}C for 2 hours. From this incubated mixture, 0.05 ml was added to 1 ml 5% phenol, followed by 5 ml distilled water, thoroughly mixed and cooled to room temperature. The absorbance was recorded at 490 nm in a spectrophotometer (double beam UV-VIS spectrophotometer UV5704SS). Blank was prepared by adding distilled water instead of enzyme extract in the assay mixture.

PG activity was expressed as = 288.07 × OD value µg galacturonic acid FW/g/h.

Statistical analyses

Replicated data were subjected to analysis of variance (ANOVA) for a randomized complete block design [22]. Subsequently Tukey's honest significant difference (HSD) test was computed across genotypes (parents and hybrids) as well as fruit physical parameters and PG activity over three harvesting stages to identify and evaluate the precise grouping of genotypes according to their extent of fruit softening on them. In this test, means in the same column(s) followed by the same letters are not significantly different. Correlations between variables (PG activity and fruit physical parameters) were tested for significance [22]. Data were analyzed in SAS 9.3 version.

Results and Discussion

Assessment of fruit firmness in relation to fruit softening attributes

Changes in pericarp thickness, fruit firmness and polygalacturonase (PG) activity were investigated during different harvesting stages (green, turning, ripe) of promising hybrids and their parental lines of cherry tomato to determine its association with the extent of cell wall degradation. A decreasing trend of pericarp thickness and fruit firmness was documented, and it was observed that pericarp thickness and fruit firmness decreased minutely and dramatically, respectively with the transition of fruit ripening stages (Figure 1 and 2). Parents and hybrids at the green stage showed thicker pericarp of 2.49 mm and 2.83 mm, respectively, but at the ripe stage comparatively thinner pericarp of 2.42 mm and 2.77 mm, respectively was recorded (Table 1). Thickest pericarp was recorded in parent 2016/TOCVAR-6 (2.98 mm) and in hybrid 2016/TOCVAR-1 × 2016/TOCVAR-3 (3.42 mm) at ripe stage. Percent decrease in pericarp thickness was 2.81% in parents and 2.12% in hybrids (Table 1). Similarly, fruit firmness of parents and hybrids at the green stage exhibited more

firmness of 2.69 kg/cm^2 and 3.29 kg/cm^2, respectively, but at the ripe stage comparatively lesser firmness of 0.71 kg/cm^2 and 0.66 kg/cm^2, respectively was documented. Firmest fruit was recorded in parent 2016/TOCVAR-6 (0.92 kg/cm^2) and in hybrid 2016/TOCVAR-1 × 2016/TOCVAR-3 (0.98 kg/cm^2) at ripe stage. Percent decrease in fruit firmness was 73.60% in parents and 79.93% in hybrids. Rises in the PG activity were concomitant with transition of ripening stages. The activity of PG increased exponentially with time from the green to turning stage, with a further significant increase to the ripe stage (Figure 3). Our results are well comparable with that of McColloch., *et al*. [23] who reported that the pectic enzymes were localized near the surface of ripe fruit and that a deeper red colour was associated with the higher activities of the pectic enzymes. Both parents and hybrids at the green stage showed limited PG activity of approximately 0.61 µg galactouranic acid FW/g/h and 0.57 µg galactouranic acid FW/g/h, respectively, but at the ripe stage maximum PG activity of approximately 12.86 µg galactouranic acid FW/g/h and 13.28 µg galactouranic acid FW/g/h, respectively was recorded. Comparatively less PG activity was recorded in parent 2016/TOCVAR-6 (10.22 µg galactouranic acid FW/g/h) and in hybrid 2016/TOCVAR-1 × 2016/TOCVAR-3 (9.55 µg galactouranic acid FW/g/h) at ripe stage. Mean percent increase in PG activity from green to turning stage was 11.72% in parents and 14.92% in hybrids, and from turning to ripe stage was 1.77% in parents and 1.56% in hybrids. However, percent increase in PG activity from green to ripe stage was 20.80% in parents and 23.30% in hybrids. On the other hand, 200-fold rise in PG activity from green-orange to red fruit [24] and seven-fold increase from the turning to ripe fruit [25] was reported in tomato. The present results also suggest that periodic PG activity towards ripening stages is comparatively less in cherry tomato than that of normal tomato.

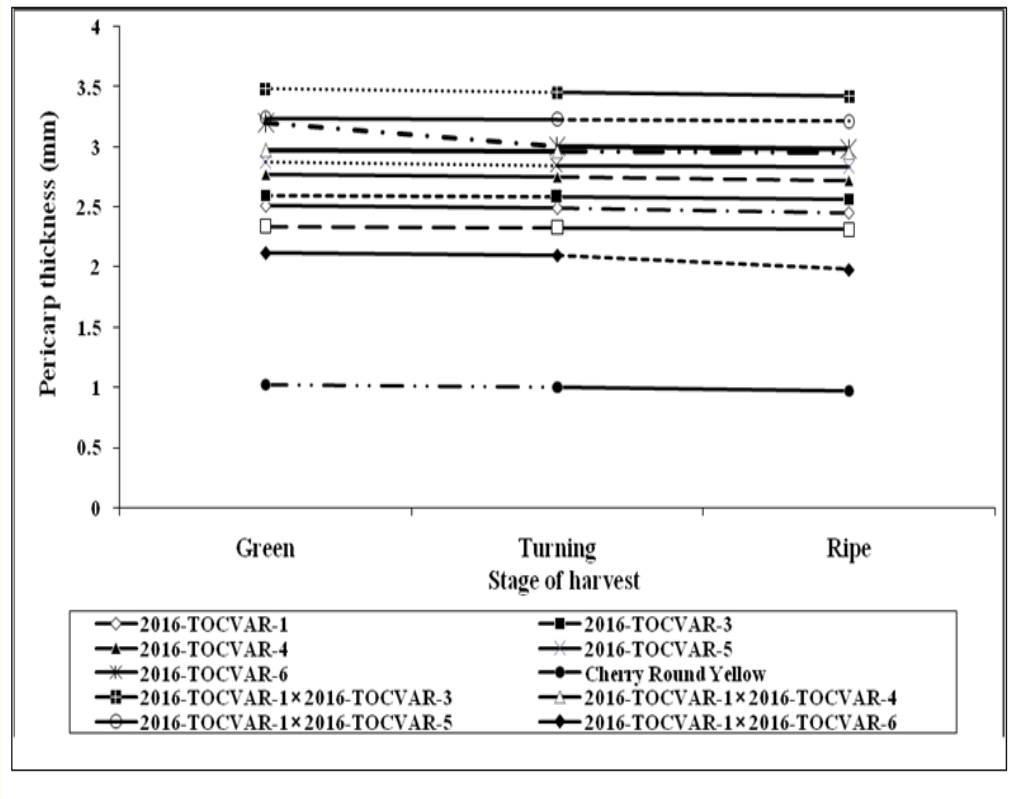

Figure 1: *Changes in pericarp thickness (mm) in parents and hybrids at different harvesting stages.*

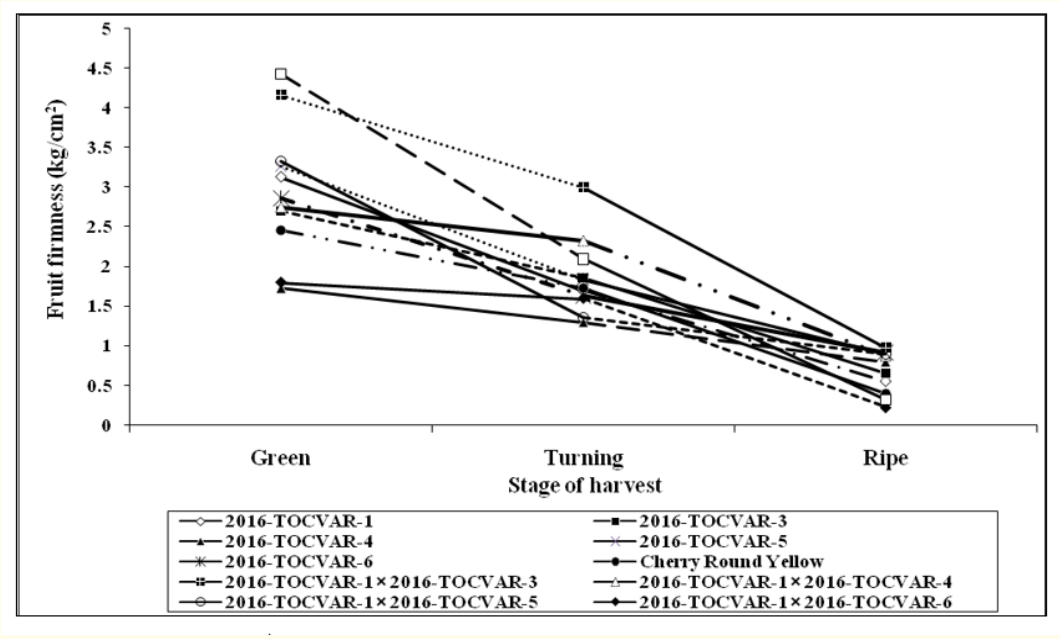

Figure 2: *Changes in fruit firmness (kg/cm²) in parents and hybrids at different harvesting stages.*

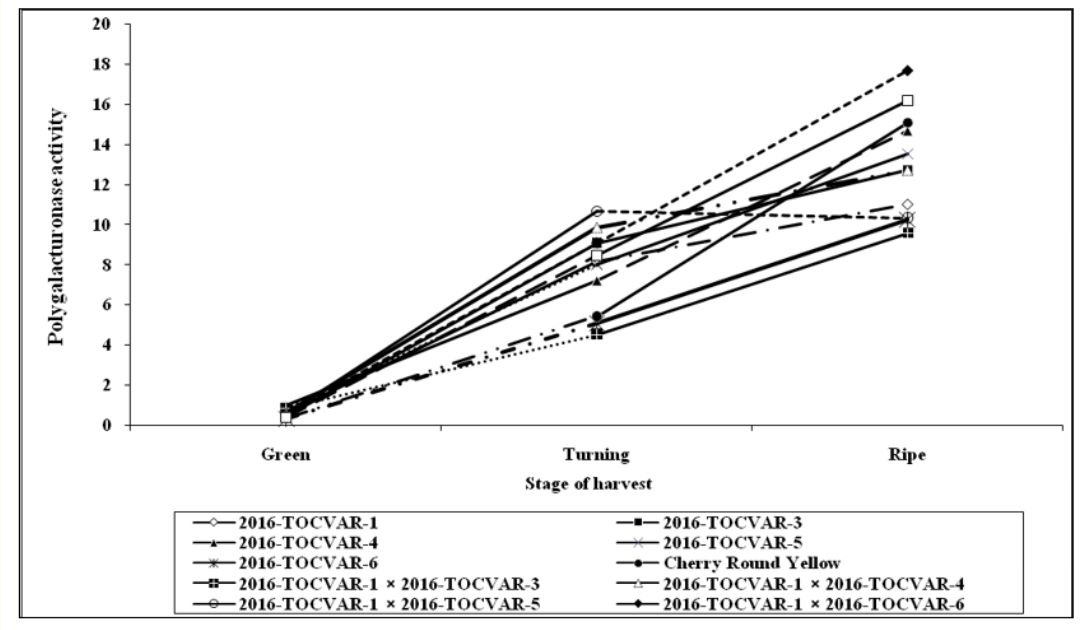

Figure 3: *Changes in polygalacturonase activity (µg galacturonic acid FW/g/h) in parents and hybrids at different harvesting stages.*

Parent/Hybrid	Pericarp thickness (mm)			Fruit firmness (kg/cm^2)			Polygalacturonase activity (µg galacturonic acid FW/g/h)		
	Green	Turning	Ripe	Green	Turning	Ripe	Green	Turning	Ripe
2016/ToCVAR-1	2.51^{bdc}	2.49^{fg}	2.45^{ef}	3.13^{cb}	1.70^{cfde}	0.56^{dc}	0.58^{c}	8.14^{ed}	11.00^{f}
2016/ToCVAR-3	2.59^{bdc}	2.58^{fe}	2.56^{ed}	2.70^{cd}	1.86^{cd}	0.66^{bc}	0.58^{c}	9.04^{c}	12.69^{e}
2016/ToCVAR-4	2.77b^{dac}	2.75^{de}	2.72^{cd}	1.73^{e}	1.30^{f}	0.80^{bac}	0.97^{a}	7.22^{f}	14.68^{c}
2016/ToCVAR-5	2.87b^{dac}	2.84^{dc}	2.83^{cb}	3.26^{cb}	1.83^{cd}	0.90^{ba}	0.86^{b}	7.98^{e}	13.49^{d}
2016/ToCVAR-6	3.20^{ba}	3.00^{bc}	2.98^{b}	2.86^{cbd}	1.63^{fde}	0.92^{a}	0.29^{e}	5.06^{g}	10.22^{g}
Cherry Round Yellow	1.02^{e}	1.00^{i}	0.97^{h}	2.46^{d}	1.73^{cde}	0.40^{ed}	0.35^{ed}	5.43^{g}	15.08^{c}
Mean	2.49	2.44	2.42	2.69	1.68	0.71	0.61	7.15	12.86
2016/ToCVAR-1 × 2016/ToCVAR-3	3.48^{a}	3.45^{a}	3.42^{a}	4.16^{a}	3.00^{a}	0.98^{a}	0.86^{b}	4.52^{h}	9.55^{g}
2016/ToCVAR-1 × 2016/ToCVAR-4	2.97b^{ac}	2.96^{dc}	2.95^{b}	2.75^{cd}	2.33^{b}	0.88^{ba}	0.60^{c}	9.84^{b}	12.69^{e}
2016/ToCVAR-1 × 2016/ToCVAR-5	3.24^{ba}	3.23^{ba}	3.21^{a}	3.33^{b}	1.36^{fe}	0.90^{ba}	0.54^{c}	10.64^{a}	10.33^{f}
2016/ToCVAR-1 × 2016/ToCVAR-6	2.12^{d}	2.10^{h}	1.98^{g}	1.80^{e}	1.60^{fde}	0.23^{e}	0.43^{d}	9.07^{c}	17.66^{a}
2016/ToCVAR-1 × Cherry Round Yellow	2.34^{dc}	2.33^{hg}	2.31^{f}	4.43^{a}	2.10^{cb}	0.33^{ed}	0.40^{d}	8.44^{d}	16.15^{b}
Mean	2.83	2.81	2.77	3.29	2.08	0.66	0.57	8.50	13.28

Table 1: *Evaluation of fruit softening attributes in cherry tomato at different harvesting stages. Means with different superscripts differ significantly (P < 0.0001, Tukey's post-hoc test).*

There were significant differences between different stages of harvest and genotypes (parents and hybrids) in terms of pericarp thickness, fruit firmness and PG activity (Table 2). Thicker pericarp occurred when parent 2016/TOCVAR-6 and hybrid 2016/TOCVAR-1 × 2016/ TOCVAR-3 interacted with all harvesting stages. Desirable firm fruit recovered when parent 2016/TOCVAR-6 and hybrid 2016/ TOCVAR-1 × 2016/TOCVAR-3 interacted with all harvesting stages. Less activity of PG occurred when parent 2016/TOCVAR-6 and hybrid 2016/TOCVAR-1 × 2016/ TOCVAR-3 interacted with all harvesting stages.

The mechanism of fruit ripening in various horticultural crops has been known for many years, and there is abundant evidence available to show that fruit ripening is a complex phenomenon. Texture is an important quality factor in both fresh and processed cherry tomato, yet the mechanism by which cherry fruit softens is not fully understood. Fruit firmness is indicative of level of softening of the fruit that can be affected by maturity stage at harvest time. Fruit firmness is related to the susceptibility of cherry tomato fruit to physical damage during harvest and storage. The softening of fruit tissues is thought to be due to the physiological changes of polysaccharide constituents including pectic polysaccharides which are the major components of the middle lamella and the primary cell walls of fruit tissues. The pectin in immature fruits is water-insoluble protopectin, which decomposes into water-soluble pectin during maturation. Therefore, pectic-hydrolyzing enzymes such as pectinesterase and polygalacturonase are assumed to contribute primarily to the softening of fruit tissues through the decomposition of pectic polysaccharides. Decrease in firmness of maturing fruits has been hypothesized to be due to alterations in both the cell wall and middle lamella [26]. Structural changes which occur in the middle lamella and primary cell wall during ripening lead to cell separation and softening of tissue [27]. Such changes are presumably the results of enzymes such as

Interaction effects	Pericarp thickness (mm)	Fruit firmness (kg/cm^2)	Polygalacturonase activity (µg galacturonic acid FW/g/h)
Parents × Stage of harvest			
P1S1**	2.51 ± 0.47	3.13 ± 0.06	0.58 ± 0.03
P1S2	2.49 ± 0.03	1.70 ± 0.20	11.47 ± 5.59
P1S3	2.45 ± 0.04	0.56 ± 0.03	11.00 ± 0.20
P2S1	2.59 ± 0.35	2.70 ± 0.20	0.58 ± 0.02
P2S2	2.58 ± 0.08	1.86 ± 0.12	9.04 ± 0.20
P2S3	2.56 ± 0.03	0.66 ± 0.12	12.69 ± 0.16
P3S1	2.77 ± 0.50	1.73 ± 0.06	0.97 ± 0.04
P3S2	2.75 ± 0.05	1.30 ± 0.10	7.22 ± 0.06
P3S3	2.72 ± 0.03	0.80 ± 0.10	14.68 ± 0.09
P4S1	2.87 ± 0.30	3.26 ± 0.16	0.29 ± 0.02
P4S2	2.84 ± 0.03	1.86 ± 0.06	5.06 ± 0.08
P4S3	2.83 ± 0.03	0.90 ± 0.06	13.49 ± 0.13
P5S1	3.20 ± 0.36	2.86 ± 0.05	0.86 ± 0.04
P5S2	3.00 ± 0.03	1.63 ± 0.11	7.98 ± 0.12
P5S3	2.98 ± 0.06	0.92 ± 0.07	9.22 ± 0.19
P6S1	1.02 ± 0.02	2.46 ±0.15	0.34 ± 0.03
P6S2	1.00 ± 0.07	1.73 ± 0.11	5.43 ± 0.13
P6S3	0.97 ± 0.05	0.40 ± 0.12	15.08 ± 0.10
Hybrids × Stage of harvest			
H1S1	3.48 ± 0.23	4.16 ± 0.14	0.86 ± 0.05
H1S2	3.45 ± 0.13	3.00 ± 0.10	4.52 ± 0.08
H1S3	3.42 ± 0.06	0.98 ± 0.05	9.55 ± 0.49
H2S1	2.97 ± 0.37	2.75 ± 0.13	0.60 ± 0.04
H2S2	2.96 ± 0.12	2.33 ± 0.11	9.84 ± 0.06
H2S3	2.95 ± 0.07	0.88 ± 0.02	12.69 ± 0.27
H3S1	3.24 ± 0.21	3.33 ± 0.20	0.54 ± 0.04
H3S2	3.23 ± 0.11	1.36 ± 0.14	10.64 ± 0.14
H3S3	3.21 ± 0.10	0.90 ± 0.11	10.33 ± 0.32
H4S1	2.12 ± 0.15	1.80 ± 0.44	0.43 ± 0.04
H4S2	2.10 ± 0.07	1.60 ± 0.10	9.07 ± 0.07
H4S3	1.98 ± 0.17	0.23 ± 0.04	17.66 ± 0.19
H5S1	2.34 ± 0.14	4.43 ± 0.11	0.41 ± 0.03
H5S2	2.33 ± 0.06	2.10 ± 0.26	8.42 ± 0.08
H5S3	2.31 ± 0.14	0.33 ± 0.14	16.15 ± 0.22

Table 2: *Interaction effects* between genotypes (parents and hybrids) and stage of harvest for fruit softening attributes in cherry tomato.*

**Data in the interaction analyzed with Least Squares Means and means separated with Least Significant Differences.*

***P1 = 2016/ToCVAR-1; P2 = 2016/ToCVAR-3; P3 = 2016/ToCVAR-4; P4 = 2016/ToCVAR-5; P5 = 2016/ToCVAR-6; P6 = Cherry Round Yellow; H1 = 2016/ToCVAR-1 × 2016/ToCVAR-3; H2 = 2016/ToCVAR-1 × 2016/ToCVAR-4; H3 = 2016/ToCVAR-1 × 2016/ToCVAR-5; H4 = 2016/ToCVAR-1 × 2016/ToCVAR-6; H5 = 2016/ToCVAR-1 × Cherry Round Yellow; S1 = Green stage; S2 = Turning stage; S3 = Ripe stage.*

polygalacturonase, pectin methyl esterase, galactosidase, cellulase and others. Plant cell walls consist of cellulose microfibrils embedded in a complex matrix of pectic substances and hemicelluloses. These polysaccharides form the network of the cell wall and depolymerize to some extent during ripening [26,28]. The middle lamella (area between primary cell walls of adjoining cells) forms a continuous intercellular matrix. This layer is high in pectic substances and its solubilization has been correlated with fruit softening during ripening [28].

The importance of enzymes in fruit softening has been investigated by many, but few reported on such activity in cherry tomato [12]. Polygalacturonase (PG) activity was related to the loss of firmness in tomatoes [13], green peppers [14] and cucumbers [15]. Two types of PG have been identified (endo and exo) [29]. Endo-PG randomly hydrolyzes glycosidic bonds while exo-PG hydrolyzes bonds only on the terminal end of the pectin molecule. The activity of both generally increases during ripening, when pectic material in cell walls and middle lamella are hydrolyzed. Our findings of PG activity, mainly responsible for fruit cell wall degradation and the transition of maturity stages, are in agreement with the findings of Arancibia and Motsenbocker [30] in Tabasco pepper. Pectins are increasingly solubilized, and thus may contribute to their loss from the cell walls, resulting in softening during ripening [26,31]. This suggests that mature fruit may have greater activity of softening enzymes such as PG, contributing to the degradation of pectin cell walls [32,33]. The highest PG activity in cherry tomato occurred during the period when fruits gained the fully ripened stage and coincided with the rapid loss of fruit firmness. The results are similar to the findings of scientists who worked on the role of PG during fruit development in various kinds of vegetables and found a correlation, as PG activity and softening occurs simultaneously in pepper [30] and tomato [8,34]. Inari., *et al*. [12] recorded the polygalacturonase and pectinesterase activities of cherry tomato fruits during the three ripening stages and the activities of both the pectin-hydrolyzing enzymes increased. Brady., *et al.* [35] measured the activities of pectinesterase and polygalacturonase and the content of water-soluble uronide in tomato fruits at different stages of ripening. They indicated that pectinesterase did not vary with maturity although the activities of polygalacturonase increased and the contents of water-soluble uronide were similar to polygalacturonase. Inari., *et al*. [12] finally concluded that that the softening of cherry tomato fruits during the ripening may depend on degradation and depolymerization of pectic polysaccharides by the pectin-hydrolyzing enzymes.

At green stage, PG activity was comparatively lower in magnitude as compared to other two ripening stages. PG activity at this stage may not influence the translucent matrix of the middle lamella and early damage to the cell wall. Similar results were observed by Crookes and Grierson [36] in tomato cell wall. Softening of the tomato fruit during ripening has been studied extensively over the last 20 years, and a large body of evidence has accumulated, suggesting that the cell wall enzyme PG plays an inevitable role in this process [37]. PG activity greatly influenced the translucent matrix of the cell wall, middle lamella, and other cellular organelles, causing rapid disruption at the later part of ripening stage [38].

Correlation study

The knowledge of interrelationships between cell wall-modifying enzyme and fruit physical parameters is essential through which selection programme could be logically devised in cherry tomato breeding programme. Therefore, a simple correlation between polygalacturonase and two fruit physical parameters (pericarp thickness and fruit firmness) has been worked out at different harvesting stages (green, turning and ripe) and depicted in table 3. A positive correlation established between PG activity and pericarp thickness of fruit in different genotypes (parents and hybrids) at green and turning stages. However, a significant negative association between them was recorded at ripe stage. On the contrary, fruit firmness was negatively correlated with PG activity at all harvesting stages. Thus, the two important ripening characteristics of loss of firmness and increased polygalacturonase activity are positively associated. These results strongly suggest that pectolytic enzyme activity is one of the predominant factors governing the firmness of different genotypes of cherry tomato fruit. Our results corroborated with the earlier findings of Arancibia and Motsenbocker [30] in pepper, Fenwick., *et al*. [8] in tomato and Inari., *et al*. [12] in cherry tomato. Scanty reports are available regarding solubilisation of the cell wall due to the action of cell wall hydrolytic enzymes like PG in cherry tomato at different harvesting stages *vis-à-vis* at molecular and ultra structural levels.

Characters	Polygalacturonase activity (µg galacturonic acid FW/g/h)		
	Green	Turning	Ripe
Pericarp thickness (mm)	0.420 ^{ns}	0.115^{ns}	-0.635*
Fruit firmness (kg/cm^2)	-0.007 ^{ns}	-0.317 ^{ns}	-0.749**

Table 3: *Correlation between fruit physical characters and polygalacturonase activity in cherry tomato.*
**: Significant at P < 0.05; **: Significant at P < 0.01; ^{ns}: Not significant.*

Conclusion

The changes of fruit physical parameters and PG activity occurring during fruit development gave prominence to three harvesting stages and the results enable us to make an assumption of a progressive degradation of the middle lamella and primary cell wall during ripening. There were significant differences between different stages of harvest and genotypes (parents and hybrids) in terms of fruit physical parameters and PG activity. PG activity was found maximum in parents and hybrids occurred during the period when fruits gained the fully ripened stage and coincided with thinner pericarp and the rapid loss of fruit firmness, resulting solubilization and damage of cell wall. We could able to isolate a hybrid 2016/ToCVAR-1 × 2016/ToCVAR-3 suitable for long distance transportation. The present investigations into the relation between fruit softening attributes not only provide direct evidence for the involvement of the pectic enzymes in ripening but also are of use in selecting firm cherry tomato lines in future breeding. Characterization of the solubilized polyuronides present during fruit aging and cell wall autolysis is needed in future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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