SlPG49 in Solanum lycopersicum

Subjects: Agriculture, Dairy & Animal Science
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The modification and degradation of pectin in cell walls are necessary for the fruit softening process, which involves a series of pectin-modifying enzymes. Polygalacturonases (PGs) are a major group of pectin-hydrolyzing enzymes, which participate in fruit maturation, organ shedding, pollen development, and other processes by catalyzing the degradation of polygalacturonic acid. However, their function in plants has not yet been fully elucidated. In this paper, a full-length cDNA encoding SlPG49 was cloned from a tomato. Sequence alignment and phylogenetic analysis demonstrated that SlPG49 contains four typical conserved domains and belongs to clade E in PG classification. Quantitative real-time PCR analysis showed that SlPG49 was highly expressed in fruits during the softening stage, indicating that SlPG49 may be involved in fruit softening. Subcellular localization results revealed that SlPG49 was located in the cell membrane and the cell wall. In addition, an in vitro enzymatic activity assay confirmed that SlPG49 does have the ability to catalyze the hydrolysis of polygalacturonic acid. These results indicate that SlPG49 is a newly discovered PG gene involved in tomato fruit softening, and provide an experimental basis for elucidating the biological functions of plant PGs during fruit softening.

polygalacturonases (PG) Solanum lycopersicum SlPG49 gene expression

enzymatic activity

1. Analysis on Research Results

1.1. Sequence and Phylogenetic Analysis of SlPG49

A PG gene (Solyc08g082170.2.1), named SlPG49, was isolated and cloned from Micro-Tom on the basis of the sequence available in Sol Genomics Network (solgenomics.net). SlPG49 contains seven exons and six introns (**Figure 1**A), and encodes a protein of 452 amino acid residues (49.03 kD). Transmembrane domain analysis indicated that SlPG49 contains a transmembrane domain and belongs to the category of membrane localization proteins (**Figure 1**B). To determine the evolutionary position of SlPG49, a phylogenetic tree containing SlPG49 and 37 PGs (Table S2) from other species was constructed. The results showed that SlPG49 belongs to clade E, and clustered with the other eight PGs, including AtPG5 in Arabidopsis thaliana and BcMF23 in *Brassica rapa* (**Figure 1**C). Then, sequence alignment was performed on the nine PGs from clade E and the other four PGs from different clades. As expected, the nine PGs that belong to the clade E showed higher amino acid sequence similarity. Further analysis revealed that the PGs in clade E all contained conserved domains I, II, and IV, but domain III was less conserved (**Figure 1**D).
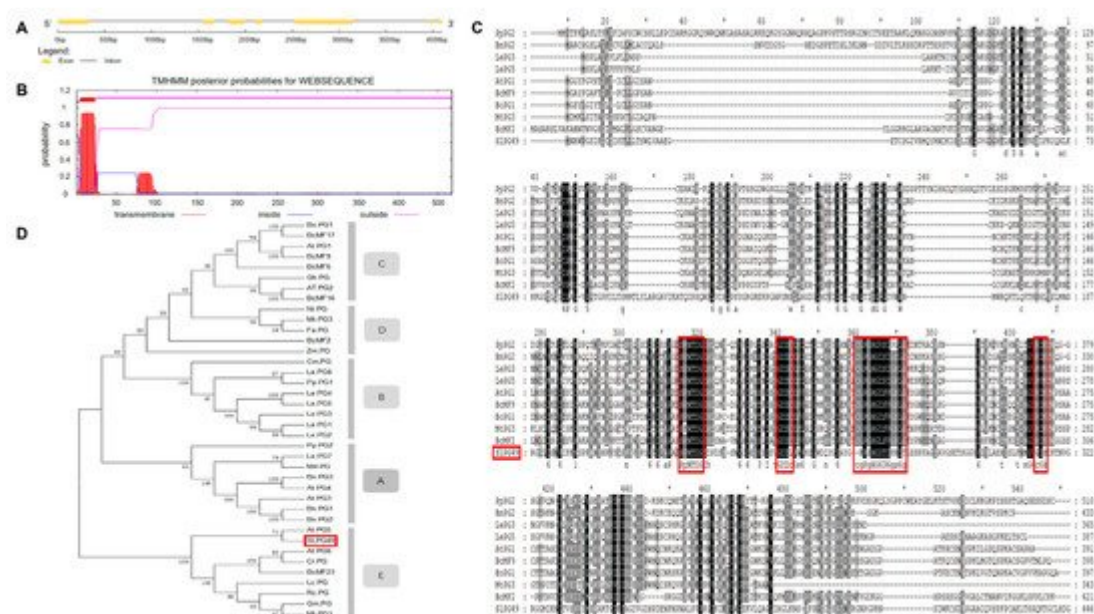
Figure 1. Gene structure, transmembrane region, multiple alignment, and phylogenetic analysis of SlPG49: (**A**) gene structure of SlPG49; (**B**) transmembrane region of SlPG49; (**C**) phylogenetic analysis of SlPG49; (**D**) multiple alignment analysis of SlPG49.

1.2. Expression Pattern Analysis of SlPG49

To further understand the role of *SlPG49*, qRT-PCR was first conducted to study the expression pattern of *SlPG49* in tomato plants. The results showed that *SlPG49* transcripts were detectable in all organs examined except roots. *SlPG49* was highly expressed both in fruits of red ripening stage and leaves, but moderately accumulated in flowers, and weakly expressed in mature green fruits, breaker fruits, and stems (**Figure 2**). These findings suggested that *SlPG49* may be crucial for tomato fruit softening, as well as leaf and flower development.
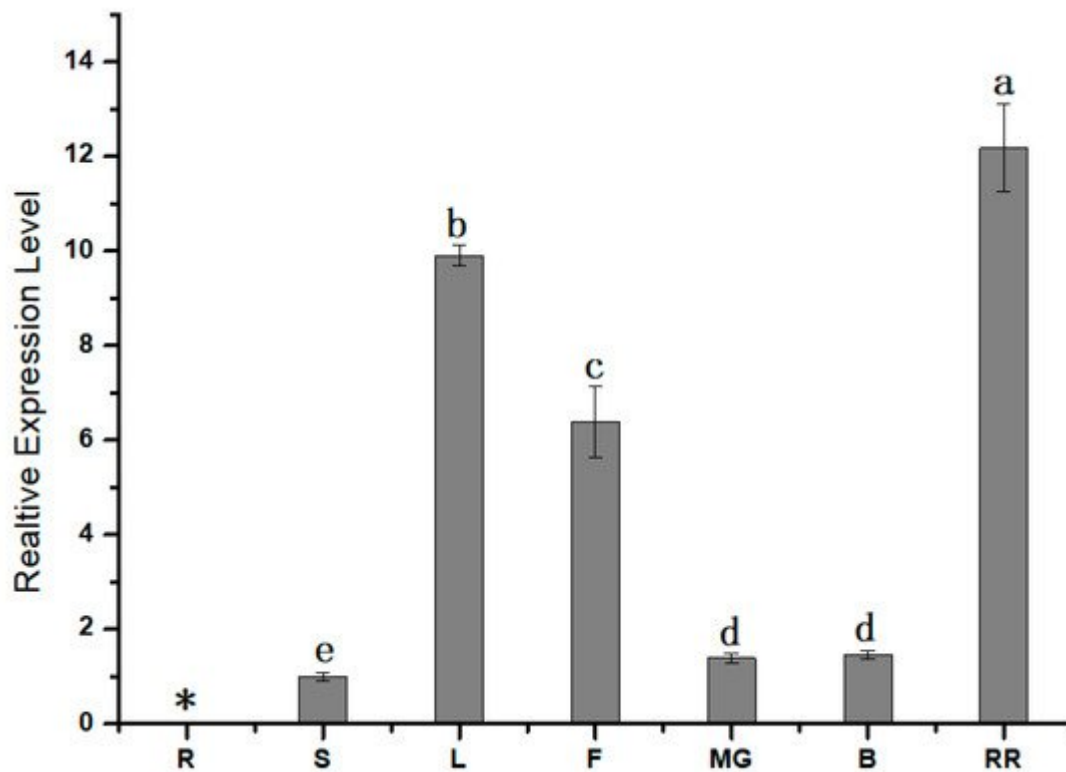
Figure 2. Expression pattern analysis of SlPG49 by qRT-PCR. R: roots; S: stems; L: leaves; F: flowers; MG: mature green fruit; B: breaker fruit; RR: red ripening fruit. Data are presented as mean ± SE with at least three biological replicates. Significant differences are indicated by different letters ($p < 0.05$). * indicates undetected expression level.

1.3. Subcellular Localization of SlPG49

Onion epidermal cells are a good material for the transient expression of exogenous genes because of their clear structure, lack of chloroplasts, and convenient materials. A transient expression assay in onion epidermal cells with *CaMV35S::SlPG49-GFP plasmids* was conducted using particle bombardment to examine the subcellular localization of SlPG49. The results showed that green fluorescence signals were widely distributed in onion epidermal cells transformed with SlPG49-GFP (**Figure 3**A–C), and the fusion protein signals of SlPG49-GFP could be detected in the cell membrane, cell wall, and extracellular space in the plasma wall-isolated onion epidermal cells (**Figure 3**D,F). These results indicate that SlPG49 is a secreted protein, and localizes in the cell membrane and the cell wall.
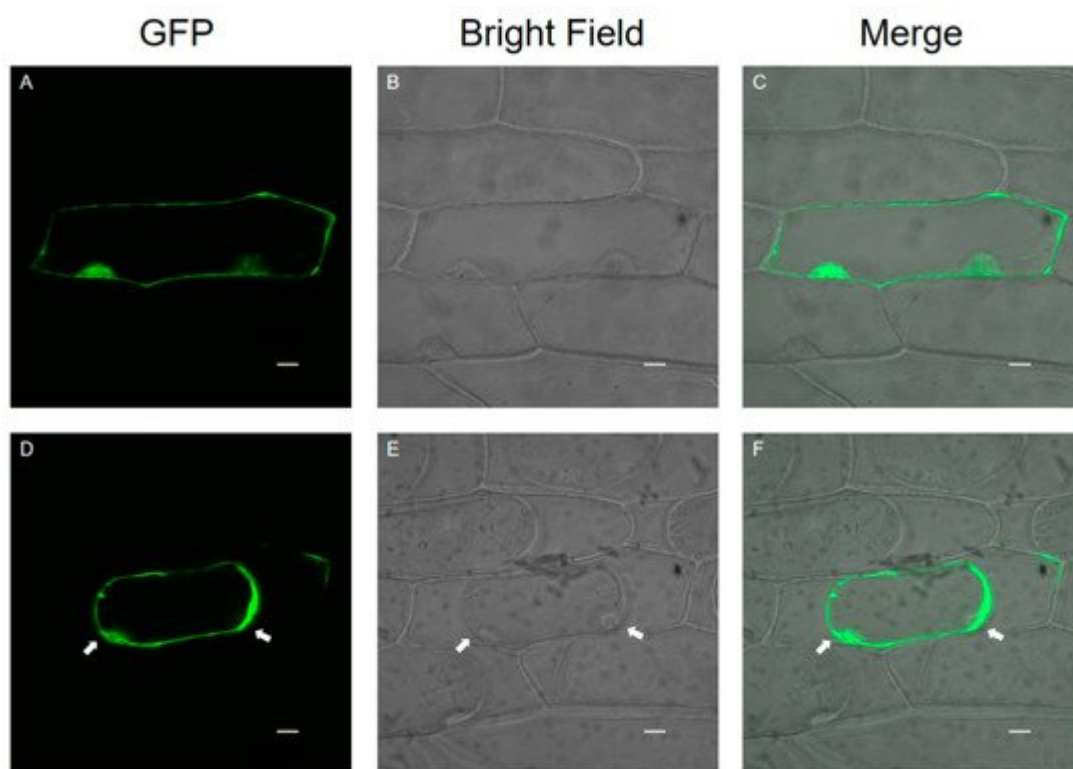
Figure 3. Subcellular localization of SlPG49-GFP fusion protein in living onion epidermal cells: (**A**–**C**) localization of SlPG49-GFP; (**D**–**F**) localization of SlPG49-GFP in plasmolyzed onion epidermal cells; cells were plasmolyzed by treatment with 1 mol·L^{-1} mannitol for 5 min. White arrowheads show the membranes separated from the cell wall. Bars = 20 µm.

1.4. Enzymatic Activity of SlPG49 In Vitro

SlPG49 was heterologously expressed, and its activity was tested to characterize whether it encoded an active PG. SlPG49 was predicted to contain a signal peptide (**Figure 4**A). A truncated CDS, including sequences encoding GH28 domain, was cloned into the pMAL-c2x vector (**Figure 4**B) to improve protein solubility, using pMAL-MBP as a negative control. The predicted sizes of SlPG49 and MBP tag protein were 49.03 kDa and 43 kDa, respectively. The results of SDS-PAGE gels and Western blot illustrated that the sizes of the tag protein band and fusion protein band were consistent with the prediction (**Figure 4**C,D), indicating that SlPG49 protein was successfully isolated and purified. The purified proteins were incubated with polygalacturonic acid to analyze its hydrolytic activity. The results demonstrated that SlPG49 showed high enzymatic activity (**Figure 4**E), indicating that SlPG49 encodes a bona fide PG.
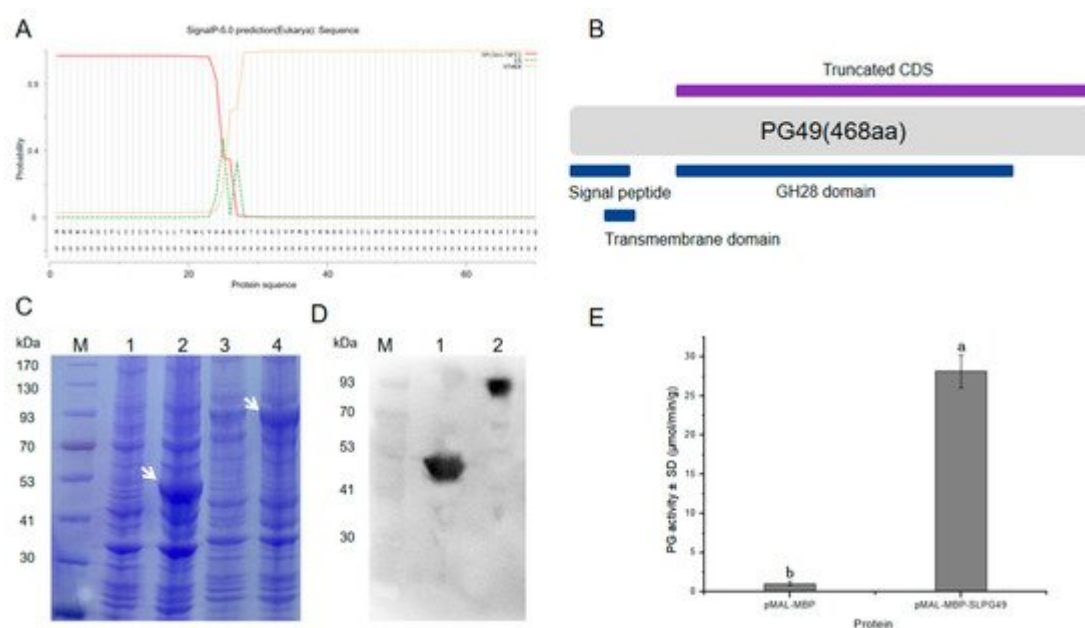
Figure 4. SlPG49 displays PG activity in vitro: (**A**,**B**) signal peptide analysis and schematic protein structure of SlPG49; (**C**) SDS-PAGE analysis; M, protein maker; 1, not induced pMAL-MBP; 2, induced pMAL-MBP; 3, not induced pMAL-MBP-SlPG49; 4, induced pMAL-MBP-SlPG49. White arrowheads show the bands of pMAL-MBP-SlPG49 and pMAL-MBP; (**D**) western blot analysis; 1: purified pMAL-MBP; 2: purified pMAL-MBP-SlPG49; (**E**) purified pMAL-MBP-SlPG49 shows PG activity in vitro. Data are presented as mean ± SE with at least three biological replicates. Significant differences are indicated by different letters ($p < 0.05$).

2. Current Insights

According to the latest classification system, PGs can be divided into seven clades (A–G), and most members of clade E can be detected at a high overall expression in all tissues [1]. In the present work, phylogenetic analysis showed that *SlPG49* was located in clade E and closed to *ATPG5* (**Figure 1**D). Interestingly, the expression of *ATPG5* was significantly increased when an *Arabidopsis* endo-PG (*AtPGLR*) was knocked out, indicating that *ATPG5* has the function of replenishing *AtPGLR*, which may also be an endo-PG [2]. Therefore, in view of the close evolutionary relationship between SlPG49 and ATPG5, we speculated that *SlPG49* was probably an endo-PG. As mentioned earlier, PG has four conserved domains (Ⅰ–Ⅳ). Generally, genes encoding the proteins of these four domains are defined as PG genes. Notably, domain Ⅲ is relatively poorly conserved [3]. The results of multiple sequence alignments show that the conservation of domain III is indeed lower than that of domains Ⅰ, Ⅱ, and Ⅳ (**Figure 1**D). Although they are different from the typical domain Ⅲ, the nine PGs from clade E (including SlPG49) are highly conserved in domain Ⅲ. It has been reported that the positively charged domain Ⅳ may participate in an ionic interaction with the carboxyl terminal of the substrate [4]. Here, although R (Arginine) in the domain Ⅳ of SlPG49 is replaced by H (Histidine), it is still positively charged. Therefore, we speculated that the structure and function of SlPG49 may not have been changed.

A previous study showed that the enzymatic activity of PG increased during the ripening period of the tomato fruit, suggesting that these enzymes may be critical for initiating the ripening process [5]. In our previous research, *SlPG49* was predicted to have a high expression in red ripening fruits [6]. In this work, qPCR analyses confirmed that *SlPG49* is extremely highly expressed in fruits at the red ripening stage (**Figure 2**), demonstrating that *SlPG49* may be involved in tomato fruit ripening and softening.

Pectin is synthesized in the Golgi apparatus and secreted into the cell wall in the state of methyl esterification and acetylation, and can be modified and degraded in the cell wall [7]. PGs are synthesized in the endoplasmic reticulum and secreted into the cell wall after maturation in the trans-Golgi network [8][9]. In accordance with their function, it is predicted that PGs exhibit extracellular localization. Previous studies have demonstrated that a PG protein in *Brassica campestris*, BcMF23, was a secreted protein and exhibited extracellular localization by subcellular localization of onion epidermis transient expression [10]. In transgenic Arabidopsis, *ATPGX3* and *AtPG45* were located in the cell wall [11][12]. The present transient expression result in the onion epidermis confirmed that SlPG49 was a secreted protein and may play a great role in the cell wall or extracellular matrix (**Figure 3**), similar to PGs in *A. thaliana*. To date, only nine PG genes have been identified in *A. thaliana*, with five that have been proven to show PG activity in vitro [11][13][14][15]. However, there is no report of in vitro activity of PGs in tomatoes. In this work, SlPG49 was successfully induced and purified by prokaryotic expression, and it was proved that SlPG49 has the activity of degrading polygalacturonic acid in vitro (**Figure 4**E), demonstrating that SlPG49 is another bonafide PG. Studies have shown that the increase in PG activity promotes the degradation of pectin in the cell walls of strawberries, apples, bananas, and other fruits, and accelerates fruit ripening and softening [16][17][18]. This study shows that the expression of SlPG49 increases significantly during the softening stage, and it has the activity of degrading one of the main components of pectin (polygalacturonic acid), indicating that it may play an important role in tomato fruit softening.

In summary, a new PG gene, *SlPG49*, has been identified in *S. lycopersicum*. The sequence analysis suggested that SlPG49 possesses the basic characteristics of PG family members. The results of expression pattern, subcellular localization, and enzymatic activity analysis indicated that SlPG49 may function in the degradation of cell wall polygalacturonic acid during fruit softening. Further studies will focus on elucidating the precise biological function and regulation of *SlPG49* in fruit ripening.

References

1. Liang, Y.; Yu, Y.; Cui, J.; Lyu, M.; Xu, L.; Cao, J. A comparative analysis of the evolution, expression, and cis-regulatory element of polygalacturonase genes in grasses and dicots. Funct. Integr. Genom. 2016, 16, 641–656.

2. Hocq, L.; Guinand, S.; Habrylo, O.; Voxeur, A.; Tabi, W.; Safran, J.; Fournet, F.; Domon, J.M.; Mollet, J.C.; Pilard, S.; et al. The exogenous application of AtPGLR, an endo-polygalacturonase, triggers pollen tube burst and repair. Plant J. 2020, 103, 617–633.

3. Park, K.C.; Kwon, S.J.; Kim, P.H.; Bureau, T.; Kim, N.S. Gene structure dynamics and divergence of the polygalacturonase gene family of plants and fungus. Genome 2008, 51, 30–40.

4. Bussink, H.J.D.; Buxton, F.P.; Visse, J. Expression and sequence comparison of the Aspergillus-niger and Aspergillus-tubigensis genes encoding polygalacturonase-II. Curr. Genet. 1991, 19, 467–474.

5. Prasanna, V.; Prabha, T.N.; Tharanathan, R.N. Fruit Ripening Phenomena-An Overview. Crit. Rev. Food Sci. 2007, 47, 1–19.

6. Ke, X.; Wang, H.; Li, Y.; Zhu, B.; Zang, Y.; He, Y.; Cao, J.; Zhu, Z.; Yu, Y. Genome-Wide identification and analysis of polygalacturonase genes in Solanum lycopersicum. Int. J. Mol. Sci. 2018, 19, 2290.

7. Asl, L.K.; Dhondt, S.; Boudolf, V.; Beemster, G.T.S.; Beeckman, T.; Inzé, D.; Govaerts, W.; De Veylder, L. Model-based analysis of Arabidopsis leaf epidermal cells reveals distinct division and expansion patterns for pavement and guard cell. Plant Physiol. 2011, 156, 2172–2183.

8. Caroli, M.D.; Lenucci, M.S.; Sansebastiano, G.P.D.; Dalessandro, G.; Lorenzo, G.D.; Piro, G. Protein trafficking to the cell wall occurs through mechanisms distinguishable from default sorting in tobacco. Plant J. 2011, 65, 295–308.

9. Caroli, M.D.; Lenucci, M.S.; Sansebastiano, G.P.D.; Dalessandro, G.; Lorenzo, G.D.; Piro, G. Dynamic protein trafficking to the cell wall. Plant Signal. Behav. 2011, 6, 1012–1015.

10. Lin, S.; Huang, L.; Yu, X.; Xiong, X.; Yue, X.; Liu, T.; Liang, Y.; Lv, M.; Cao, J. Characterization of BcMF23a and BcMF23b, two putative pectin methylesterase genes related to pollen development in Brassica campestris ssp. chinensis. Mol. Biol. Rep. 2017, 44, 139–148.

11. Yang, Y.; Anderson, C.T.; Cao, J. Polygalacturonase45 cleaves pectin and links cell proliferation and morphogenesis to leaf curvature in Arabidopsis thaliana. Plant J. 2021, 106, 1493–1508.

12. Rui, Y.; Xiao, C.; Yi, H.; Kandemir, B.; Wang, J.Z.; Puri, V.M.; Anderson, C.T. POLYGALACTURONASE INVOLVED IN EXPANSION3 functions in seedling development, rosette growth, and stomatal dynamics in Arabidopsis thaliana. Plant Cell 2017, 29, 2413–2432.

13. Babu, Y.; Musielak, T.; Henschen, A.; Bayer, M. Suspensor length determines developmental progression of the embryo in Arabidopsis. Plant Physiol. 2013, 162, 1448–1458.

14. Rhee, S.Y.; Osborne, E.; Poindexter, P.D.; Somerville, C.R. Microspore separation in the quartet 3 mutants of Arabidopsis is impaired by a defect in a developmentally regulated polygalacturonase required for pollen mother cell wall degradation. Plant Physiol. 2003, 133, 1170–1180.

15. Ogawa, M.; Kay, P.; Wilson, S.; Swain, S.M. Arabidopsis dehiscence zone polygalacturonase1 (ADPG1), ADPG2, and QUARTET2 are polygalacturonases required for cell separation during reproductive development in Arabidopsis. Plant Cell 2009, 21, 216–233.

16. Posé, S.; Kirby, A.R.; Paniagua, C.; Waldron, K.W.; Morris, V.J.; Quesada, M.A.; Mercado, J.A. The nanostructural characterization of strawberry pectins in pectate lyase or polygalacturonase silenced fruits elucidates their role in softening. Carbohydr. Polym. 2015, 132, 134–145.

17. Atkinson, R.G.; Sutherland, P.W.; Johnston, S.L.; Gunaseelan, K.; Hallett, I.C.; Mitra, D.; Brummell, D.A.; Schröder, R.; Johnston, J.W.; Schaffer, R.J. Down-regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water loss in apple (Malus × domestica) fruit. BMC Plant Biol. 2012, 12, 129.

18. Asif, M.H.; Nath, P. Expression of multiple forms of polygalacturonase gene during ripening in banana fruit. Plant Physiol. Biochem. 2005, 43, 177–184.