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ORIGINAL ARTICLE

Cell wall metabolism and related gene expression in *Malus domestica* Borkh. during fruit growth and softening

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Abstract – Introduction. Pre-harvest fruit development of apple can have a great effect on its storable quality. However, little research has focused on fruit metabolism during growth. Our objective was to study cell wall metabolism during the various stages of fruit growth and softening to gain more insight into the nature of fruit development and ripening. **Materials and methods.** Apple varieties Fuji and Golden Delicious (GD) were used to investigate cell wall metabolism and related gene expression during fruit growth and softening. **Results and discussion.** During fruit growth, cell wall components underwent similar changes in the two cultivars although the covalent soluble pectin (CSP) content in Fuji fruit was much higher than that in GD fruit. The activities of cell wall enzymes and the related gene expression indicated that cell wall degradation was involved in fruit growth. However, these changes showed significant differences between the two cultivars during storage. The water soluble pectin (WSP) content increased more rapidly and the content of CSP and hemi-cellulose decreased more obviously, showing a more significant correlation with firmness loss in GD fruit than with Fuji. Accordingly, the activities of cell wall enzymes and the related gene expression were significantly higher in the GD fruit. **Conclusion.** Cell wall metabolism and the related gene expression showed significant differences between Fuji and GD fruit during growth and softening. Both acted on fruit growth and enlargement but revealed a significant cultivar-dependency with regards to fruit softening.

Keywords: China / apple / *Malus domestica* / fruit development / cell wall metabolism / gene expression

Résumé – Métabolisme de la paroi cellulaire et expression génique associée au cours de la croissance et du ramollissement de la pomme *Malus domestica* Borkh. Introduction. La phase de développement pré-récolte de la pomme peut avoir une influence décisive sur la qualité du fruit au stockage. Toutefois, peu de recherches ont porté sur le métabolisme des fruits au cours de leur croissance. Notre objectif était d'étudier le métabolisme de la paroi cellulaire lors des différentes étapes de la croissance et du ramollissement du fruit, afin de procurer un aperçu sur la nature du développement du fruit. **Matériel et méthodes.** Les variétés de pommes Fuji et Golden Delicious (GD) ont été utilisées pour étudier le métabolisme de la paroi cellulaire et l'expression de gènes liés à la croissance et au ramollissement des fruits. **Résultats et discussion.** Au cours de la croissance des fruits, les composants de la paroi cellulaire des deux cultivars ont présenté des changements similaires, alors que la teneur en pectine soluble covalente (CSP) des pommes Fuji était beaucoup plus élevée que celle des GD. Les activités enzymatiques de la paroi cellulaire et l'expression des gènes concernés ont été également détectées dans les deux cultivars, ce qui indique que la dégradation de la paroi cellulaire est impliquée dans la croissance du fruit. Toutefois, ces évolutions ont nettement mis en valeur des différences entre les deux cultivars au stockage. La teneur en pectine soluble dans l'eau (WSP) a augmenté plus rapidement et les teneurs en CSP et en hémicellulose ont diminué de façon plus évidente dans les pommes GD que dans celles Fuji, exprimant une corrélation significative avec la perte de fermeté des fruits. En conséquence, les activités enzymatiques de la paroi cellulaire ainsi que l'expression des gènes concernés étaient significativement plus élevées dans les pommes GD que dans les Fuji. Les activités enzymatiques de la paroi cellulaire, accompagnées d'une expression plus élevée des gènes apparentés, étaient en plus forte corrélation avec la perte de fermeté et les composants de la paroi cellulaire des pommes GD. **Conclusion.** Le métabolisme de la paroi cellulaire et l'expression des gènes liés ont montré des différences

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significatives entre pommes Fuji et GD pendant la croissance et le ramollissement, par action sur la croissance et l'élargissement du fruit. Les deux jouent un rôle dans le la croissance et le grossissement du fruit, mais révèlent une dépendance génétique relative au ramollissement du fruit.

Mots clés : Chine / pomme / *Malus domestica* / développement du fruit / métabolisme de la paroi cellulaire / expression génique

1 Introduction

Fruit development, ripening, and softening involve a series of complex physiological and biochemical processes, including changes to peel color, flesh texture, fruit aroma and much more. Flesh softening is one of the most obvious signs of fruit maturation and progresses during storage. It facilitates pathogen infection and postharvest decay, thus reducing the quality and commercial value of the organ [1]. Thus, fruit firmness is an important measure for evaluating quality at harvest and during storage [2–5]. The fruit cell skeleton, composed of cell wall materials, maintains flesh texture and firmness. Firmness will decrease and storage life will decline if there is any change in the structure and composition of cell walls [6–9]. There have been attempts to connect fruit textural attributes with changes in cell wall metabolism, but the issue is far more complex. For example in tomato, fruit cuticular structure and cell turgor pressure may play a big role as well [10, 11]. Therefore, the relationship between cell wall degradation and fruit storage characteristics has been widely studied with the aim of better understanding the mechanism of fruit softening [3, 12–15].

However, there is still much to learn about how cell wall metabolism is involved in the whole process of fruit growth and softening in apple. Pre-harvest fruit growth can influence post-harvest fruit quality [5, 16]. For apple fruit, there is ample research on flesh softening but little work has focused on how cell wall metabolism and the related-gene expression influence fruit growth or softening. Fuji is a crisp and juicy apple that stores well whereas Golden Delicious (GD) apple often becomes mealy after harvest. For this research we used Fuji and GD apples as our test materials to analyze alterations in cell wall components, cell wall hydrolase activities, and the related gene expression during fruit growth and softening. We hoped to gain insight into the relationship between cell wall metabolism and fruit texture development and understand the nature of fruit maturation and softening, which could provide a theoretical basis for improving fruit quality and its preservation technology.

2 Materials and methods

2.1 Plant materials and treatments

This experiment was conducted in the fruit production area of Changli County in Hebei province (China). The fruit of two apple (*Malus domestica* Borkh.) cultivars, Fuji and Golden Delicious (GD), were used for this study, with five mature-tree replications of each cultivar. During the growing season of 2013, fruits were sampled every 14 days from 14 days after bloom until maturation, involving 12 and 9 sampling dates for Fuji and GD, respectively. Each sample was taken between 8:00 to 9:00 a.m. and consisted of 10 randomly sampled fruits per tree. The samples were then immediately transferred to the laboratory. For the determination of cell wall components and measurement of enzyme activities and gene expression, samples taken during the growing season were then grouped into five development stages based on the fruit growth curve: the young fruit stage (I), fruit cell division stage (II), fruit cell enlargement stage (III), fruit dry matter accumulation stage (IV), and fruit maturation stage (V).

At proper commercial maturity for Fuji (about 160–165 days after bloom) and GD (about 140–145 days after bloom), blemish-free fruits were harvested and held at room temperature (20 ± 1 °C). The first sample for postharvest fruit analysis was taken on the day of harvest and continued at 2-week intervals until the conclusion of the experiment. For these analyses, the flesh tissue of each sample was cut into pieces, frozen in liquid nitrogen, and stored at –70 °C.

2.2 Determination of fruit firmness

Measurement of peeled fruit firmness started 42 days before harvest (cell enlargement stage of fruit), and continued until the end of storage. Using a hand-held penetrometer (model GY-B; China) equipped with a flat probe, we determined firmness at four equatorial regions on each of three apples with ten replications per sampling date.

2.3 Cell wall material extraction, separation and assay

Extraction and separation of cell wall material (CWM) were modified based on the methods described by Wei *et al.* [1]. Fruit flesh (2.0 g) was powdered and homogenized in 8 mL 80% (v/v) ethanol, stirred 20 min at 80 °C and centrifuged. The precipitate was washed with 80% (v/v) ethanol and pure acetone three times, respectively, and was sequentially immersed in 95% (v/v) dimethyl sulfoxide (DMSO) for 12 h, then centrifuged to remove starch. Finally, the residue was oven- dried at 45 °C. This dried residue was the crude CWM. Fifty mg CWM was extracted with distilled water to produce the water-soluble pectin (WSP) and sequentially extracted with 0.5 mol L^{-1} trans-1,2-diaminocyclohexane- N,N,N9,N9-tetraacetic acid (CDTA) and with 0.1 mol L^{-1} Na_2CO_3 containing 0.1% (w/v) $NaBH_4$ to produce extracts containing the ionic-soluble pectin (ISP) and the covalent soluble pectin (CSP), respectively. Deposited cell

Table I. Primers of cell wall enzyme-related genes for qRT-PCR in apple fruit.

Gene name	Sense primer sequence	Antiense primer sequence
MdPG	5′ GTAACTGCACCAGAGGACA 3′	5′ TTCTTCACCACCAAGTTATT 3′
MdPME	5′ GATGCCTTGGAGTGGAGA 3′	5′ TGCTAATGTATTGCGTTC 3′
MdGal	5′ AAGAACGGAAAGTCCCCAC 3′	5′ TCCAATGACCCATACACGG 3′
MdAf	5′ AGAAACGCCTATCCTGAC 3′	5′ CACGGCATACTCGCTCAC 3′
18S	5′ CCATTGGAGGGCAAGTCT 3′	5′ GGTTCTCACGCTACACGA 3′

wall residues were extracted with 4 mol L^{-1} KOH containing 0.1% (w/v) $NaBH_4$ to produce extracts of hemicellulose. The remaining residue was the cellulose. The pectin content was measured by the carbazole method, the hemicellulose content was determined with the anthrone colorimetric method, and determination of cellulose content was by gravimetric analysis.

2.4 Enzyme extraction and analysis of enzyme activity

Cell wall enzymes were extracted according to the method described by Brummell *et al.* [17] and Wei *et al.* [4]. Frozen flesh (3.0 g) was powdered with a pestle and mortar, and stirred into 6 mL of cold 12% (w/v) polyethyleneglycol containing 0.2% (w/v) sodium bisulphite. After the homogenate was centrifuged for 10 min at 12,000 *g*, the pellet was washed with 0.2% (w/v) sodium bisulfite at 4 °C. Pellets were collected and extracted for polygalacturonase (PG) (EC3.2.1.15), pectin methylesterase (PME) (EC3.1.1.11), *α*-L-furanglycosidase (*α-L-Af*) (EC3.2.1.55) and *β*-galactosidase (*β-Gal*) (EC 3.2.1.23). The extraction conditions included 6 ml of cold extraction buffer [0.1 mol L^{-1} sodium acetate (pH 5.2), 100 mmol L^{-1} NaCl, 2% (v/v) *β*-mercaptoethanol, and 5% (w/v) polyvinylpyrrolidone (PVP)] at 4 °C for 1 h. Following centrifugation as above, the supernatant was used to analyze enzyme activity. All of these steps were performed at 4 °C.

PG activity was determined as described by Gross [18]. Enzyme extract (0.2 mL) was mixed with 0.8 mL of 0.5% polygalacturonic acid (Sigma Chemical Co., St. Louis, MO, USA) in 50 mmol L^{-1} sodium acetate buffer (pH 5.2), and incubated at 37 °C for 2 h. To measure the amount of galacturonic acid released, we added 2 mL borate buffer (0.1 mol L^{-1}, pH 9.0) and 0.3 mL cyanoacetamide to the reaction mixture. After boiling for 10 min and then cooling, absorbency was read at 276 nm. Galacturonic acid was used as the standard and the controls for the boiled extract were run in the reaction buffer. One unit of activity was defined as 1 μg of galacturonic acid released g^{-1} fresh weight (FW) min^{-1}. For PME activity [19], 1 mL crude extract was mixed with 4 mL of 1% (w/v) citrus pectin (Sigma Chemical Co., St. Louis, MO, USA) and titrated with 0.01 mol L^{-1} NaOH to maintain pH 7.4 while incubating at 37 °C for 1 h. One unit of activity was calculated as 1.0 mmol L^{-1} NaOH consumed g^{-1} FW 10 min^{-1}. Both *β*-Gal and *α-L*-Af activities were measured by using p-nitrophenyl-*β*-D-galactopyranoside and p-nitrophenyl-*α*-D-arabinofuranoside (Sigma Chemical Co., St. Louis, MO, USA) as substrates, respectively. A volume of 0.5 mL of 0.1 mol L^{-1} sodium acetate (pH 5.2) and 0.5 mL substrate were pre-incubated at 40 °C for 10 min before 0.5 mL enzyme extract was added. After incubation at 37 °C for 30 min, the reaction was stopped by adding 2.0 mL of 0.5 mol L^{-1} sodium carbonate, and the p-nitrophenol that was released was then measured spectrophotometrically at 400 nm. A calibration curve was obtained by using free p-nitrophenol (PNP) (Sigma Chemical Co., St. Louis, MO, USA) as our standard. Enzyme activity was expressed as nmol PNP g^{-1} FW min^{-1} [17]. In all assays, the boiled enzyme extract was taken as the control.

2.5 Analysis of cell wall enzyme-related gene expression by qRT-PCR assay

The expression of genes involved in cell wall metabolism, including *MdPME, MdPG, MdGal*, and *MdAf*, was determined by quantitative reverse transcription-polymerase chain reaction (QRT-PCR). Total RNA was extracted from fruit by the modified CTAB method, and DNase was used to remove DNA before reverse transcription. Gene-specific primers (*table I*) were designed (Primer5.0) from the coding sequences of apple genes. qRT-PCR was performed with a PrimeScriptTM RT Reagent Kit (TaKaRa, Japan), using oligo (dT) 20 and random primers for cDNA synthesis according to the manufacturer's protocol. The amplified PCR products were quantified on an iQ^5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), with the SYBR Premix Ex Taq kit (TaKaRa, Japan). The various cDNA samples were standardized with 18S rRNA transcripts and the qRT-PCR experiments were repeated three times with three biological replicates and the expression levels were set at 1.0 for day 0 for each gene.

2.6 Experimental design and statistical analysis

Experiments were performed according to a completely randomized design. Numerical data were examined by analysis of variance (ANOVA), and significance was detected with Duncan's multiple range tests at a level of 1% or 5%.

3 Results and discussion

3.1 Changes of flesh firmness during fruit maturation and softening

Fruit firmness in both two apple cultivars decreased with fruit maturity and gradually changed from hard (unpalatable) to crisp (palatable) (*figure 1*). However, significant differences in fruit firmness were evident between the two cultivars with firmness loss during fruit maturation and ripening being slower in Fuji. For Fuji fruit, the flesh firmness decreased 36.3 N from

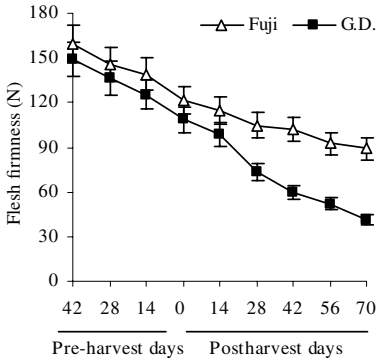

Figure 1. Changes of flesh firmness during apple fruit maturation and softening. The first determination of fruit firmness was at 42 days before harvest (cell enlargement stage of fruit) and subsequently sampled at 14 days interval until the end of storage. Values are means of 10 replicates ± S.E.

maturation (159.1 N) to harvest (121.5 N) and was 89.2 N after storage for 70 days. However, with GD fruit flesh firmness lost 50.0 N from 149.0 N at 42 days before harvest to 99.0 N at harvest and more rapidly decreased after harvest. Firmness decreased to 54.9 N after 42 days and to 41.2 N after storage of 70 days. These fruit displayed loose texture, less juice, and overall poor quality.

3.2 Changes in cell wall material and its components

In agreement with others, we observed clear changes in the content of CWM and its components during fruit development and maturation, leading to reduced fruit firmness [5, 16, 20]. As shown in *figure 2A*, the CWM content of both cultivars was high and increased during the early stage of fruit development. It then decreased rapidly after the peak (28–42 days after bloom). The turning point in the change of CWM content occurred at around the fruit enlargement stage in both Fuji and GD fruits. This agrees with results reported by Bouranis and Niavis [21], who concluded that the decrease in cell wall content per unit of tissue weight was due to the increase in cell volume (*i.e.*, fewer cells per sample). After harvest, the CWM content decreased slowly but more rapidly with GD, indicating the different softening characteristics between the two cultivars.

The content of cellulose was always higher in Fuji and its peak was later in GD, but it showed identical degradation rates in the two cultivars (*figure 2C*). In Fuji, hemi-cellulose content increased quickly during early growth, decreased rapidly after its peak, but maintained a lower level during storage. In GD, the hemi-cellulose content was clearly higher than that of Fuji at harvest but decreased remarkably during subsequent softening (*figure 2B*). Others have shown that hemi-cellulose and cellulose are important contributors to fruit texture because they are major components of the cell wall skeleton [5, 6]. Not surprising then, we found that the changes of cellulose and hemi-cellulose content were significantly related to firmness loss in both cultivars during maturation and softening (*tables II* and *III*).

Among the three kinds of pectin measured (*figures 2D, 2E, 2F*), CSP content was the highest in Fuji, which was far higher than in GD, WSP content of Fuji was the lowest, and Fuji ISP

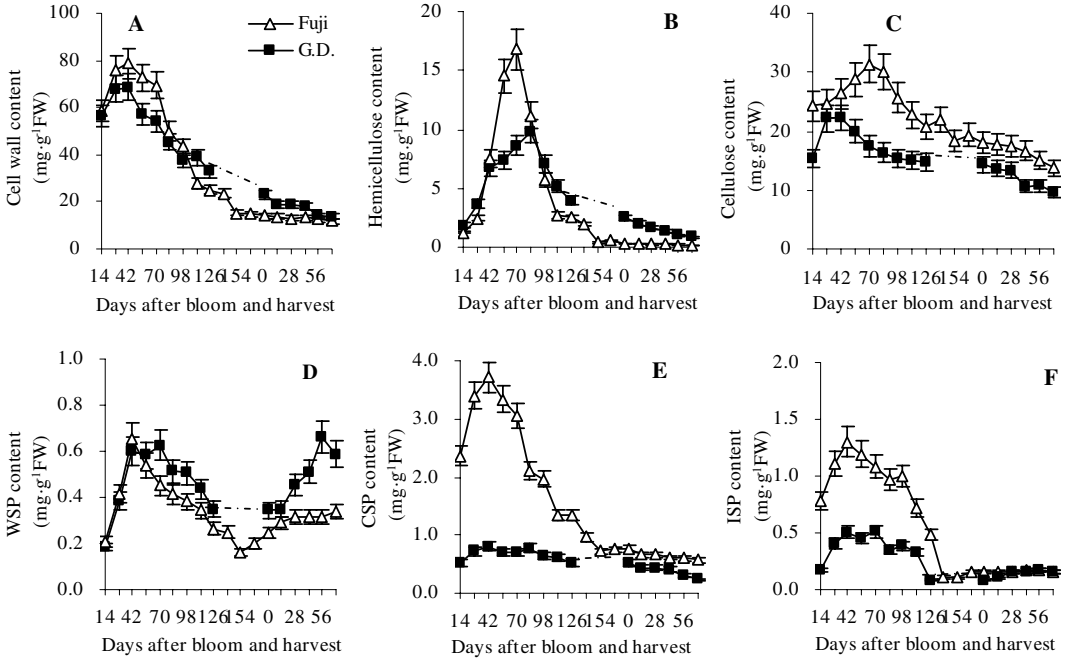

Figure 2. Changes of cell wall material and its contents during fruit growth and softening. WSP, CSP and ISP in *figures 2D, 2E, 2F* are the abbreviations of water soluble pectin, covalent soluble pectin and ionic-soluble pectin. The abscissa numbers of 14–168 identified the sampling dates at 14 days interval for Fuji, and the abscissa numbers of 14–126 identified the sampling dates at 14 days interval for Golden Delicious (GD) during fruit growth. The other abscissa numbers of 0–70 identified the sampling dates at 14 days interval for both cultivars during fruit storage. Values are means of 3 replicates ± S.E.

Table II. Correlation analysis among cell wall material components, enzyme activity and firmness in Fuji apple.

Cell wall composition	Stage	Firmness	PG	PME	β-Gal	α-L-Af
Firmness	Maturation	–	0.960	–0.854**	–0.122	–0.931**
	Softening	–	–0.412	–0.596	–0.973**	–0.745**
WSP	Maturation	0.553	0.641	–0.423	0.373	–0.569
	Softening	–0.681*	0.737*	–0.697	0.754*	0.972**
CSP	Maturation	0.942**	0.880	–0.724*	0.231	–0.743*
	Softening	0.931**	–0.937**	0.812	–0.837**	–0.991**
ISP	Maturation	0.948	0.841	–0.710*	0.360	–0.602
	Softening	–0.222	–0.524	0.842	–0.882**	–0.994**
Hemi-cellulose	Maturation	0.851**	0.978	–0.851**	0.020	–0.741*
	Softening	0.920**	–0.750*	–0.372	–0.979**	–0.954**
Cellulose	Maturation	0.957**	0.946	–0.783*	0.102	–0.730*
	Softening	0.951**	–0.540	0.491	–0.994**	–0.834**

* And ** indicate significant linear correlation at $P < 0.05$ and 0.01 levels, respectively.

Table III. Correlation analysis among cell wall material components, enzymes activity and firmness in Golden Delicious apple.

Cell wall composition	Stage	Firmness	PG	PME	β-Gal	α-L-Af
Firmness	Maturation	–	–0.614	–0.094	–0.347	–0.732*
	Softening	–	–0.807*	–0.412	–0.983**	–0.961**
WSP	Maturation	0.328	–0.819	0.152	–0.084	–0.331
	Softening	–0.880**	0.821*	0.303	0.886**	0.729*
CSP	Maturation	0.772*	–0.737*	0.115	–0.166	–0.745*
	Softening	0.885**	0.101	0.129	–0.949**	–0.994**
ISP	Maturation	0.623	–0.706	0.264	–0.085	–0.800**
	Softening	–0.865**	0.799	0.649	–0.436	–0.242
Hemi-cellulose	Maturation	0.892**	–0.813	0.091	–0.123	–0.937**
	Softening	0.923**	–0.224	–0.077	–0.797*	–0.904**
Cellulose	Maturation	0.717**	–0.932**	0.345	0.183	–0.831*
	Softening	0.934**	0.690	–0.287	–0.942**	–0.853**

* And ** indicate significant linear correlation at $P < 0.05$ and 0.01 levels, respectively.

content was in the middle during growth. After harvest, WSP content increased rapidly and CSP content decreased in GD, while WSP content slightly increased with unchanged content of CSP in Fuji. There were no obvious changes in ISP content in the fruit of the two cultivars. *Table II* shows that Fuji fruit firmness was positively correlated with CSP content at maturation stage. When entering into storage, firmness was negatively correlated with WSP, was still positively correlated with CSP, but was not significantly correlated with ISP (*table II*). For GD, fruit firmness was significantly correlated with CSP during fruit maturation, and was significantly correlated with both WSP and ISP during storage (*table III*). The above results indicate that both CSP and WSP were closely related to apple fruit storability. This agrees with previous research that showed that firmness loss is generally accompanied by a decrease in insoluble pectin content and an increase in soluble pectin [3, 16]. Furthermore, similar relationships between pectin degradation and textural properties have been revealed in various species [15–17, 22].

3.3 Pectin methylesterase (PME) activity and related gene expression

PME activity rose slowly during fruit growth and softening in both cultivars but was always higher in GD (*figure 3A*). Expression of *MdPME* in Fuji increased slightly during growth, was lower at the time of harvest, and increased between 28 and 70 days of storage (*figure 3B*). With GD, this expression showed very high levels at stage I and stage II then decreased until harvest day. During storage it first increased and then declined. In navel orange [23] and Feicheng peach [24] PME was also found to be active during fruit growth which presumably promoted cell autolysis and facilitated fruit enlargement. PME activity in Fuji was negatively correlated with firmness, hemi-cellulose, CSP, ISP and cellulose during fruit maturation but these correlations dramatically weakened after harvest (*table II*). However, there was no significant correlation between PME activity and the changes of firmness and cell wall components during fruit maturation and softening in GD (*table III*). These results indicate that PME activity is involved in cell wall metabolism but exhibited differences between the two cultivars during fruit growth and softening.

3.4 Polygalacturonase (PG) activity and related gene expression

PG activity in Fuji increased gradually during fruit growth, plateaued during the early postharvest period, and then peaked after 70 days of storage (*figure 4A*). This activity was

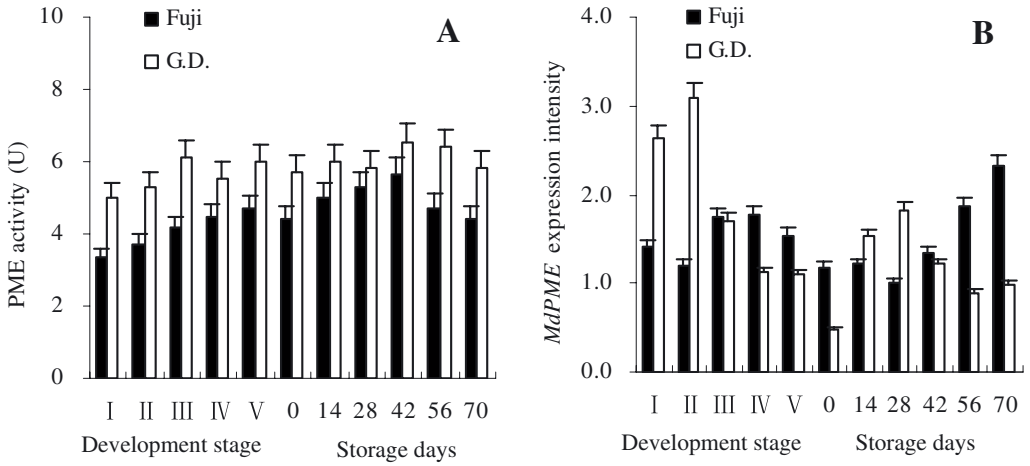

Figure 3. Changes of pectin methylesterase (PME) activity and its gene expression (*MdPME*) during Fuji and Golden Delicious (GD) fruit growth and softening. The abscissa marks of I, II, III, IV and V identified the sampling dates at the young fruit stage, the fruit cell division stage, the fruit cell enlargement stage, the fruit dry material accumulation stage and the fruit maturation stage, respectively. The other numbers in abscissa are the sampling dates during storage. The *MdPME* expression levels were set at 1.0 for day 0. Values are means of 3 replicates ± S.E.

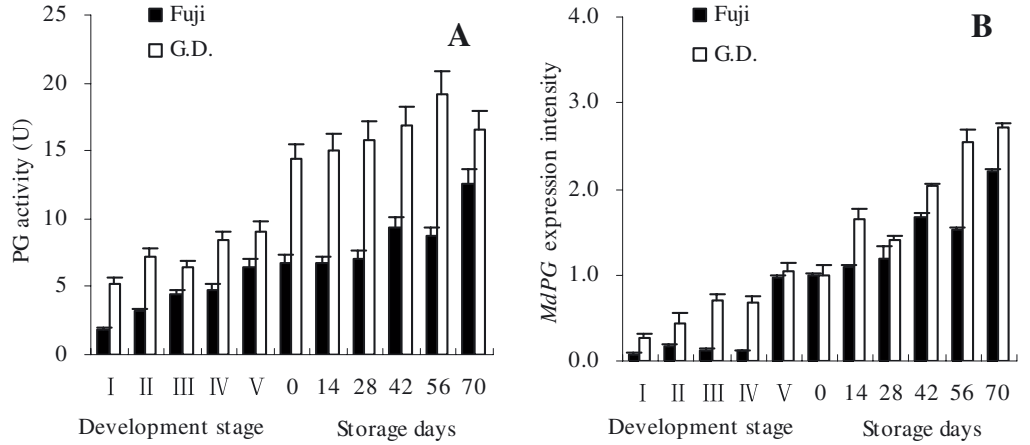

Figure 4. Changes of polygalacturonase (PG) activity and its gene expression (*MdPG*) during Fuji and Golden Delicious (GD) fruit growth and softening. Same abscissa marks as Figures 3. The *MdPG* expression levels were set at 1.0 for day 0. Values are means of 3 replicates ± S.E.

significantly correlated with CSP, WSP and hemi-cellulose during fruit storage (*table II*). With GD, PG activity was always higher than in Fuji but differed by increasing markedly after harvest (*figure 4A*). With this cultivar, PG activity was significantly correlated with CSP and cellulose during maturation and with firmness and WSP during fruit storage (*table III*). The activity of PG increased quickly and the differences between two cultivars were mainly at the softening stage, indicating that PG activity is closely related to fruit storability and, as previously reported [4, 25], may play important roles in the late stages of fruit softening. *MdPG* expression was also at very low levels and consistent with PG activity in both Fuji and GD fruits during development but was especially low in Fuji. During fruit storage, *MdPG* expression increased in both cultivars but tended to be higher in GD after 14 days of storage (*figure 4B*), further revealing that PG plays an important role in fruit softening.

3.5 β-Galactosidase (β-Gal) activity and related gene expression

Activity of β-Gal during fruit development showed its highest level at development stage I and then decreased rapidly in both cultivars. As harvest time approached this activity began to increase. but showed generally higher levels in GD with this difference being significant after 28 days of storage (*figure 5A*). As shown in *figure 5B*, *MdGal* expression levels were significantly different between the two cultivars at five stages of fruit development with higher expression levels in GD fruit. However, while *MdGal* expression increased during storage for both cultivars, it was consistently higher in GD after 14 days of storage. β-Gal activity during fruit development was not significantly correlated with firmness or any cell wall component, but during storage it was correlated with firmness and levels of all cell wall components except

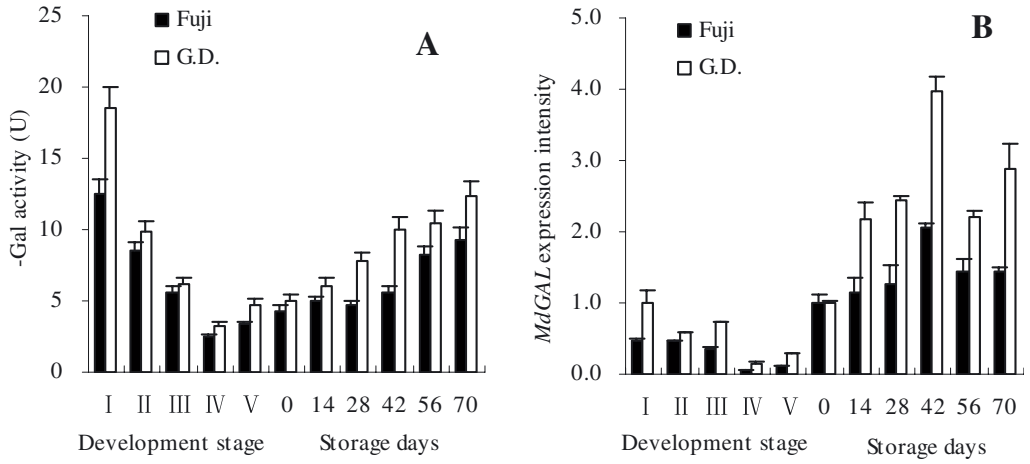

Figure 5. Changes of beta-Galactosidase (β-Gal) activity and its gene expression (*MdGal*) during Fuji and Golden Delicious (GD) fruit growth and softening. Same abscissa marks as Figures 3. The *MdGal* expression levels were set at 1.0 for day 0. Values are means of 3 replicates ± S.E.

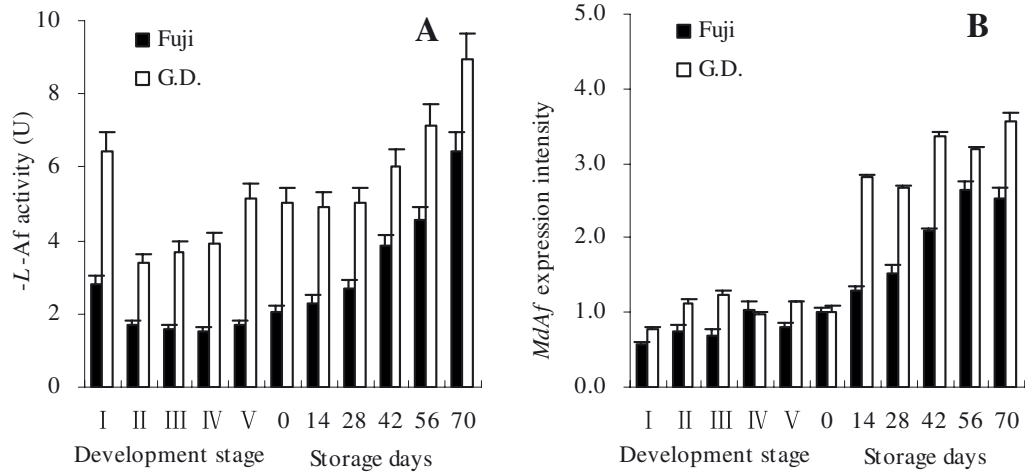

Figure 6. Changes of Alpha-*L*-Arabinofuranosidase (α-L-Af) activity and its gene expression (*MdAf*) during Fuji and Golden Delicious (GD) fruit growth and softening. Same abscissa marks as Figures 3. The *MdAf* expression levels were set at 1.0 for day 0. Values are means of 3 replicates ± S.E.

ISP (*table III*). This supports work of others showing the relationship between β-Gal and fruit softening and showing that it involves cell wall modifications [7, 26, 27]. Given that this gene expression increased immediately upon harvest supports the suggestion [14], that it can be considered as an initiator of the ripening related physiological process.

3.6 Alpha-*L*-Arabinofuranosidase (α-L-Af) activity and related gene expression

In many fruit species, α-L-Af activity, which releases arabinosyl residues from the pectic fraction, increases during fruit ripening with its mRNA being highly expressed only in ripening fruit [17, 25, 28–31]. In our trials with Fuji, α-L-Af activity hardly changed during growth, increased gradually after harvest, and showed a rapid rise only after 28 days of fruit storage. However, in GD, α-L-Af activity was very high at stage I, decreased at stage II, increased gradually until harvest, and then increased rapidly after 28 days of storage (*figure 6A*). Moreover, α-L-Af activity in GD was always higher than that in Fuji during the whole period of fruit growth and storage. *MdAf* maintained a stable expression level during fruit growth and increased after harvest in both cultivars (*figure 6B*). At stages I, II and III of GD fruit, *MdAf* expression was slightly higher than that in Fuji fruit but showed a similar expression level before harvest in both cultivars. During storage, *MdAf* expression showed an increasing trend in both cultivars, but the expression level of *MdAf* in GD after 14 days of storage was nearly 3 times that shown at harvest and significantly exceeded that of Fuji. α-L-Af activity in Fuji was negatively correlated with firmness loss, cellulose and hemi-cellulose during maturation; and with firmess loss and all five cell wall components during ripening (*table II*). Golden Delicious showed a similar pattern of significant correlations although the relationship with ISP during ripening did not prove to be significant (*table III*).

4 Conclusion

The present study revealed significant differences between Fuji and GD apple fruits in cell wall metabolism and related gene expression. During fruit growth, the changes in cell wall components and the activity of degrading enzymes indicated that these changes contribute to fruit growth by acting on cell wall materials. However, during fruit storage, Fuji showed greater retention of firmness, changed little in WSP, CSP and hemi-cellulose contents, and displayed lower cell wall-degrading enzyme activities and related gene expressions. By comparison, GD fruit showed more rapid quality losses associated with softening and all indicators of cell wall metabolism.

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