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Review

β-Galactosidase and α-L-Arabinofuranosidase in Cell Wall Modification Related with Fruit Development and Softening

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Fruit softening and textural changes are two important factors of fruit quality. The loss of galactosyl and arabinosyl residues from cell wall polysaccharides is observed in many fruit species during ripening. The release of neutral sugar residues could change wall polysaccharides properties of accessibility or reactivity for other cell wall hydrolases. β-Galactosidase and α-L-arabinofuranosidase contribute to the loss of neutral sugar residues. Thus, the enzymes alter polysaccharide properties and might contribute to fruit softening or textural changes during ripening. β-Galactosidase is composed of multiple isozymes and they are clearly distinguishable by their substrate specificity and expression pattern of the related gene. A transgenic experiment revealed that a β-galactosidase isozyme plays an important role in tomato fruit softening. In addition to fruit softening, the contribution of β-galactosidase to plant development is also indicated. α-L-Arabinofuranosidase genes constitute a gene family and the isozymes are expressed in various organs and stages. Moreover, several α-L-arabinofuranosidases possess β-xylosidase activity in addition to α-L-arabinofuranosidase activity, therefore, substrate specificity against native polysaccharides is very complex. Arabinose containing polysaccharides seem to contribute to cell to cell adhesion but the crucial roles of α-L-arabinofuranosidase in fruit development or softening remain unclear. Further biochemical and physiological studies of the enzyme are required.

Key Words: arabinose, galactose, ripening, texture, β-xylosidase.

Introduction

Fruit become edible during ripening when accompanied by several phenomena such as sugar accumulation, starch degradation, production of aroma, color change, and softening and textural changes. Fruit softening and textural changes are two important factors that influence fruit quality. Excessive softening limits fruit shelf life and postharvest handling. The structure of cell wall polysaccharides is very complex and their interactions/connections *in situ* have not been entirely defined. Numerous enzymes, which may contribute to modifications in their architecture, have been found in the cell walls. Moreover, many wall-modifying enzymes constitute multiple isozymes with different functions or roles and it is still difficult to understand the softening mechanism in detail. To elucidate the basic process of softening during fruit ripening, physiological studies are important and show common features of softening. On the other hand, fruit properties such as textural changes vary in fruit species, cultivars, and storage conditions; therefore, it is important to elucidate the unique contribution of each enzyme (isozyme) to the individual character of fruit formed with softening. This would be helpful to develop high quality fruit and horticultural knowledge at the field, handling, and storage levels. Moreover, the modification of cell wall architecture is involved in not only fruit softening but also plant growth, development, and shape formation. Cooperative biosynthesis and degradation of several cell wall components are necessary. Many enzymes are found in the cell wall. In this review, two glycosidases, β-galactosidase, and α-L-arabinofuranosidase are examined. The enzymatic characters of the enzymes and expression pattern of the genes are described and discussed in relation to fruit softening and other cell wall modification.

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1. Pectin degradation and the contribution of polygalacturonase

Fruit softening results from modifications of cell wall architecture caused by several cell-wall-metabolizing enzymes (Fischer and Bennett, 1991). Plant cell-wall polysaccharides are generally divided into three classes:

pectin, hemicellulose and cellulose, based on their solubility. During fruit ripening, the modification of pectin is the major phenomenon among the three. The pectic macromolecule, which consists of a polygalacturonan main chain, is degraded to a smaller size during ripening (Brummell and Labavitch, 1997; Huber and O'Donoghue, 1993). It is apparent that the decrease in mechanical strength of pectic polymers caused by the change to small molecules is responsible for fruit softening. Endo-type polygalacturonase is an enzyme which is able to hydrolyze polygalacturonan to a small size. Indeed, polygalacturonase activity is not detected in immature fruit while its activity increases with fruit ripening. Corresponding transcripts are also detected in ripening fruit. Until the 1990's, it had been considered that polygalacturonase was a key enzyme for fruit softening; however, transgenic tomato (*Solanum lycopersicum*) fruit, in which polygalacturonase activity was suppressed to 1% in the wild type, showed almost same softening pattern as wild-type fruit (Smith et al., 1988). Polygalacturonase activity was recovered up to 60% in transgenic *rin* (ripening inhibitor) fruit, but did not affect fruit firmness (Giovannoni et al., 1989). These results suggest that polygalacturonase is not the sole determinant of fruit softening. Suppression of polygalacturonase, which prevents the depolymerization of polyuronide, contributed to the improvement of fruit shelf life, decreased cracking and helped processing by increasing the viscosity of paste or juice (Kramer et al., 1992; Langley et al., 1994; Schuch et al., 1991). Therefore, depolymerization of polyuronide mediated by polygalacturonase contributes to fruit texture rather than fruit softening. It is noted that the results of transgenic experiments described above are limited to tomato fruit. Besides tomato fruit, it has also been shown that polygalacturonase expression is regulated by ethylene. Both the expression of polygalacturonase and softening of fruit are severely suppressed in ripening controlled peach (*Prunus percica*) (Hayama et al., 2006) and melon (*Cucumis melo*) (Nishiyama et al., 2007) treated with 1-methylcyclopropene. Tatsuki and Endo (2006) showed the relationship between ethylene sensitivity and apple (*Malus domestica*) fruit shelf life. Inaba (2007) summarized the necessity of ethylene for fruit softening and expression of related enzymes. Ethylene is essential for softening of several fruit species and the expression of polygalacturonase; however, direct evidence of role of polygalacturonase in fruit softening is still unclear.

2. Post polygalacturonase

Following polygalacturonase, many researchers tried to mine new candidates for fruit-softening enzymes. Other cell-wall-metabolizing enzymes or proteins, such as pectin methylesterase (Tieman et al., 1992), xyloglucan endotransglucosylase/hydrolase (XTH) (Arrowsmith and de Silva, 1995), β-1,4-glucanase (Brummell et al., 1999a), and expansin (Brummell et al., 1999b), were also examined for their contribution to fruit softening using transgenic experiments in which gene expression was suppressed; however, except for expansin, these enzymes do not contribute to the softening of tomato fruit (Brummell and Harpster, 2001). Although the changes were small, expansin-suppressed fruit showed rather limited fruit softening and over-expressed fruit showed softening progress compared to the wild type (Brummell et al., 1999b). The precise action of expansin is unclear. Its role in polysaccharides may be partial and transgenic fruit showed complex changes in cell-wall polysaccharides.

Besides studies of cell-wall-modifying enzymes, changes in cell-wall polysaccharides during fruit ripening have been often reported. Polyuronide depolymerization mediated by polygalacturonase is observed during ripening although its extent seems to vary depending on the fruit species (Brummell, 2006). Polyuronide is considerably depolymerized into small molecules in melting or highly softened stages in fruit such as avocado (*Persea americana*) (Huber and O'Donoghue, 1993; Sakurai and Nevins, 1997; Wakabayashi et al., 2000), tomato (Brummell and Labavitch, 1997; Huber and O'Donoghue, 1993) and kiwifruit (*Actinidia deliciosa*) (Redgwell et al., 1992; Terasaki et al., 2001). Extremely limited depolymerization of polyuronide during ripening was even observed in strawberry (*Fragaria* × *ananassa*) (Huber, 1984), apple (Yoshioka et al., 1992), banana (*Musa* spp.) (Wade et al., 1992), and pepper (*Capsicum annuum*) (Harpster et al., 2002). Recently, Goulao and Oliveira (2008) summarized the extent of depolymerization of pectic polysaccharides and other wall components in various fruit species during ripening. Depolymerization of polyuronide occurs at a relatively late stage of ripening; therefore, it may contribute to cell-wall breakdown with over-ripening or may alter fruit textural properties. In addition to polyuronide depolymerization during ripening, the solubilization of pectic polysaccharides is also observed (Brummell and Labavitch, 1997; Huber and O'Donoghue, 1993). 'Solubilization' can be defined as polysaccharides, which previously could not dissolve in water or a certain buffer, become soluble the solutions. It is clear that cell-wall materials (mass) decrease with fruit ripening. Increasing the solubility of wall polysaccharides seems to be a general event during fruit softening. Moreover, the solubilization of wall polysaccharides occurs without a degree of polymerization in kiwifruit and nectarine (*Prunus persica*) (Dowson et al., 1992; Redgwell et al., 1992). Corresponding to the solubilization of pectic polymer, the loss of arabinosyl and galactosyl residues from wall polysaccharides is widely observed in many kinds of fruit (Gross and Sams, 1984). Except for homogalacturonan, almost all pectic or hemicellulosic polysaccharide backbones constituting the cell wall possess branched side chains. The release of neutral sugar residues, which may be located on the

surface of wall polysaccharides, attributes to changes in the structure of side chains and the interaction of neighboring polysaccharides. The changed wall polysaccharide properties are assumed to change the sensitivity of enzymatic degradation or accessibility of other glycan hydrolases to polysaccharide substrates internally. Indeed, the release of galactosyl residues from side chains of pectic polysaccharides is at least involved in fruit softening (Smith et al., 2002, see details below). Therefore, enzymes addressing side chains, such as β-galactosidase and α-L-arabinofuranosidase, might play important roles in fruit softening and textural change during ripening. Pectic polysaccharides and related enzymes are shown schematically in Figure 1.

3. β-Galactosidase for fruit softening

Generally limited in pectic substances galactose is composed of a side chain of pectic polysaccharides such as arabinogalactan or galactan branched from the rhamnogalacturonan backbone (Fig. 1). Galactose is also found in hemicellulosic polysaccharides. In tomato fruit, the loss of galactosyl residue during fruit ripening is observed in pectic galactan. β-Galactosidases (EC 3.2.1.23) are characterized by their ability to hydrolyze terminal, non-reducing β-D-galactosyl residues from numerous substrates. In higher plants, β-galactosidase is the only enzyme that is able to hydrolyze galactosyl residues from cell wall polysaccharides and no other enzyme capable of cleaving β-1,4-galactan in an endo fashion has been identified (Smith et al., 1998). The activity of β-galactosidase was measured in various fruit species (Table 1). The existence of multiple isoforms (isozymes) was also reported in several kinds of fruit and their different galactosyl-hydrolysing abilities were characterized using native cell-wall polysaccharides and synthetic substrate. Each isozyme discriminated at the protein level by chromatography possesses different substrate specificities and they are able to hydrolyze the galactosyl residue at different positions (linkage). For example, a ripening-related β-galactosidase isozyme is capable of hydrolysing a native substrate, β-1,4-galactan, more effectively than an artificial substrate, 4-nitrophenyl-β-D-galactopyranoside. Among three β-galactosidase isozymes (β-Gal I to III) isolated from tomato fruit, β-Gal II is capable of releasing galactosyl residues from the pectic side chain and activity increased during ripening (Carrington and Pressey, 1996; Pressey, 1983). β-Galactosidases isolated from melon fruit are able to reduce the molecular size of cell-wall polysaccharides, as observed during ripening (Ranwala et al., 1992). DeVeau et al. (1993) also indicated that β-galactosidase purified from avocado fruit is capable of depolymerizing chelator-soluble pectin of tomato. Among three avocado β-galactosidase isoforms, AV-GAL I, AV-GAL II and AV-GAL III, AV-GAL III shows the highest activity of releasing free galactosyl residue from the native cell-wall polysaccharides isolated from fruit and its activity increases with fruit softening whereas AV-GAL I can not hydrolyze native polysaccharides (Tateishi et al., 2001a). Yoshioka et al. (1995) indicated that one β-galactosidase fraction, termed GA-ase I, from apple fruit, whose activity

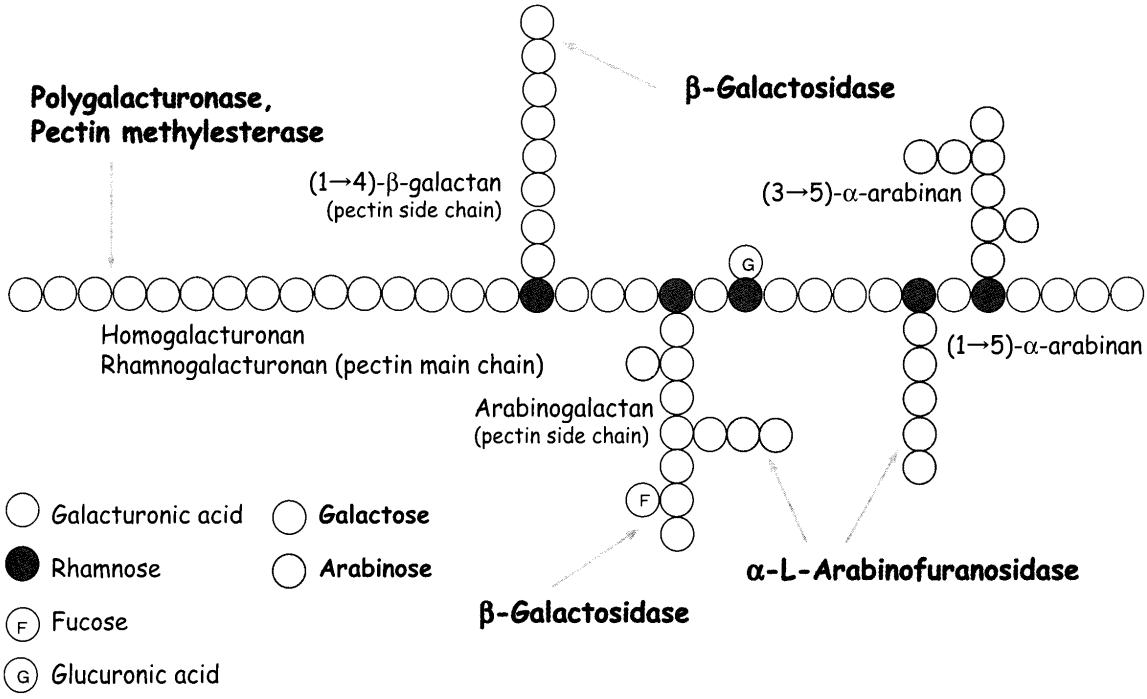

Fig. 1. Schematic model of pectic polysaccharides and related enzymes. Polygalacturonase hydrolyzes homogalacturonan, which was de-esterified previously mediated by pectin methylesterase, reducing size of pectin. Prior to depolymerization, pectic substances are solubilized and galactosyl and arabinosyl residues are released. β-galactosidase and α-L-arabinofuranosidase approach the side chains and release neutral sugar residues. Abbreviated hemicellulosic polysaccharides are also likely their substrates.

Table 1. Enzymatic activities of β-galactosidase found in various fruit species.

Fruit	References
Apple (*Malus domestica*)	Bartley, 1974; Dick et al., 1990; Ross et al., 1994; Yoshioka et al., 1995
Avocado (*Persea americana*)	DeVeau et al., 1993; Tateishi et al., 2001a
Bell pepper (*Capsicum annuum*)	Ogasawara et al., 2007
Grape (*Vitis vinifera*)	Barnavon et al., 2000; Nunan et al., 2001
Japanese pear (*Pyrus pyrifolia*)	Kitagawa et al., 1995; Mwaniki et al., 2007; Tateishi and Inoue, 2000; Tateishi et al., 2001b, 2005b
Kiwifruit (*Actinidia deliciosa*)	Bonghi et al., 1996; Wegrzyn and MacRae, 1992
Musk melon (*Cucumis melo*)	Fils-Lycaon and Buret, 1991; Ranwala et al., 1992
Papaya (*Carica papaya*)	Ali et al., 1998; Lazan et al., 1995, 2004
Peach (*Prunus persica*)	Brummell et al., 2004
Pear (*Pyrus communis*)	Ahmed and Labavitch, 1980; Mwaniki et al., 2005, 2007
Tomato (*Solanum lycopersicum*)	Carey et al., 1995; Smith and Gross, 2000
Zucchini (*Cucurbita pepo*)	Balandrán-Quintana et al., 2007

increased during storage, effectively released galactose from pectic polysaccharides but not from larch wood arabinogalactan, whereas GA-ase II, III, and IV were able to hydrolyze the arabinogalactan. Kitagawa et al. (1995) also indicated that five β-galactosidase isozymes (Gal I to V) fractionated from Japanese pear (*Pyrus pyrifolia*) fruit possessed different activities against native cell-wall polysaccharides. In this case, Gal III has the highest activity of releasing galactose from Na_2CO_3-soluble pectic polysaccharides, guanidine thiocyanate-soluble pectic polysaccharides, and hemicellulosic polysaccharides isolated from Japanese pear. Lazan et al. (2004) also fractionated β-galactosidase from papaya (*Carica papaya*) fruit into three isozymes with different substrate specificities and indicated that the enzymes were able to solubilize and depolymerize not only pectic polysaccharides but also hemicellulosic polysaccharides. Thus, β-galactosidase can be clearly separated at the protein level with different substrate specificities. This implies that each isozyme plays a different role in the modification of cell-wall architecture during fruit development and ripening. These differences may depend on fruit species with different characters. If we evaluate β-galactosidase in fruit softening, it is necessary to determine softening-related β-galactosidase at the gene level.

4. Genes for β-galactosidase

Genes for β-galactosidase have been identified in many kinds of fruit and consist of a small gene family. At least seven β-galactosidase genes are expressed during development and ripening of tomato fruit (Smith and Gross, 2000). A family of β-galactosidase was also reported in strawberry (Trainotti et al., 2001), Japanese pear (Tateishi et al., 2001b, 2005b), avocado (Tateishi et al., 2002, 2007), pear (*Pyrus communis*) (Mwaniki et al., 2005, 2007; Sekine et al., 2006), and grape (*Vitis vinifera*) (Nunan et al., 2001). Some gene expressions overlapped. According to an increase in activity during fruit ripening and the ability to hydrolyze native cell-wall polysaccharides, β-galactosidase isoforms isolated from tomato (β-Gal II, Pressey, 1983), apple (Ross et al., 1994), and Japanese pear (Gal III, Kitagawa et al., 1995) are considered as softening-related β-galactosidase. In addition, N-terminal amino acid sequences of the proteins were analyzed and the corresponding cDNA clones were revealed (Ross et al., 1994; Smith et al., 1998; Tateishi et al., 2001b). Accumulation of their mRNAs was mainly specific to ripening fruit. Transgenic experiments using tomato fruit revealed which β-galactosidase isozyme is specific to fruit softening. The significant contribution of β-Gal II to tomato fruit softening was reported using antisense suppression of *TBG4* which encodes β-Gal II (Smith et al., 2002). Tomato fruit transformed with the antisense *TBG4* gene softened with ripening, however, it was firmer than the wild type when fully ripe. Interestingly, total β-galactosidase activity and loss of galactosyl residues were not significantly affected.

Transgenic experiments also identified the roles of other β-galactosidase isozymes in the development and ripening of tomato fruit. Strong accumulation of *TBG1* mRNA of another β-galactosidase isozyme from tomato was observed at breaker and turning stages; however, down-regulated *TBG1* expression caused no effect on fruit softening and activity of β-galactosidase in fruit (Carey et al., 1995, 2001). mRNA of *TBG6*, which is also a β-galactosidase isozyme from tomato, accumulated during the fruit developmental stage, especially 20–30 days after pollination (Smith and Gross, 2000). Fruit with down-regulated *TBG6* expression showed cracking on the surface, indicating the contribution of *TBG6* to fruit development (Moctezuma et al., 2003). These observations suggest that individual β-galactosidase isozymes play a distinct role in fruit development and ripening, or more broadly, plant development. Senescence-, abscission- and sugar starvation-related β-galactosidase has also been reported in some plant species (de Alcântara et al., 2006; King et al., 1995; Lee et al., 2007; Wu and Burns, 2004).

5. Putative roles of α-L-arabinofuranosidase and arabinosyl-containing polysaccharides

In addition to the loss of galactosyl residues, the release of arabinosyl residues during fruit softening was also observed commonly during ripening in many kinds of fruit (Gross, 1984; Gross and Sams, 1984). The extent of arabinose loss during ripening varies between fruit species (Brummell, 2006). For example, extensive loss of arabinosyl residues is observed in pear and blueberry (*Vaccinium* ssp.) but is absent in watermelon (*Citrullus lanatus*), apricot (*Prunus armeniaca*) and plum (*Prunus domestica*) (Brummell, 2006; Gross, 1984; Gross and Sams, 1984). Terminal arabinosyl residues are widely distributed in pectic and hemicellulosic polysaccharides such as arabinan, arabinogalactan, arabinoxylan, arabinoxyloglucan, and glucuronoarabinoxylan (Beldman et al., 1997; Saha, 2000; Sozzi et al., 2002b). α-L-Arabinofuranosidase (α-L-arabinofuranoside arabinofuranohydrolase, EC 3.2.1.55) is an enzyme which is able to hydrolyze non-reducing arabinofuranosyl residues.

Increased α-L-arabinofuranosidase activity during ripening or storage was observed in apple (Yoshioka et al., 1995), Japanese pear fruit (Tateishi et al., 1996), avocado (Tateishi et al., 2001a), tomato (Sozzi et al., 2002a), persimmon (*Diospyros kaki*) (Xu et al., 2003), peach (*Prunus persica*) (Brummell et al., 2004a; Jin et al., 2006), and European and Chinese pear (*Pyrus bretschneideri*) (Mwaniki et al., 2007). Ethylene seems to promote an increase in the activity of climacteric fruit, although the expression pattern of an α-L-arabinofuranosidase gene did not coincide with the fruit-softening pattern in Chinese pear (Mwaniki et al., 2007). The activity did not increase in several fruit species and some tomato cultivars during ripening (Itai et al., 2003). α-L-Arabinofuranosidases in fruit have been purified from Japanese pear (Tateishi et al., 1996, 2005a) and partially purified from apple (Yoshioka et al., 1995) and tomato (Sozzi et al., 2002b) and some enzymatic properties have been characterized; however, *in vivo* substrates for α-L-arabinofuranosidases have not been clarified in detail.

The existence of arabinosyl residues consisting of cell-wall polysaccharides seems to play an important role in the adhesion of each polysaccharide or cell to cell. Iwai et al. (2001, 2002) transformed a T-DNA insertion into *Nicotiana plumbaginifolia* and obtained a non-organogenic callus with loosely attached cells (nolac). Neutral-sugar side chains, composed mainly of linear arabinan, were absent in a nolac mutant, suggesting the role of arabinan in cell adhesion. The presence of arabinan sometimes related to the mealy texture of fruit. A larger amount of tightly bound arabinosyl-containing polysaccharides was observed in softening-suppressed, colorless and non-ripening (*Cnr*) mutant tomato fruit, which has a mealy texture (Orfila et al., 2001, 2002; Thompson et al., 1999). In peach fruit, the loss of arabinosyl residues from both loosely and tightly bound matrix glycans was observed in normal ripening fruit, but not in mealy fruit, which showed a decline in the loss of arabinosyl residues containing polysaccharides firmly attached to cellulose (Brummell et al., 2004a, 2004b). In apple fruit, arabinosyl residues decreased during the over-ripening stage (Peña and Carpita, 2004) and a decrease of arabinosyl residues in cell wall polysaccharides was observed in the development of apple fruit mealiness postharvest (Nara et al., 2001). Therefore, arabinosyl residue is usually widely lost during fruit ripening; however, in mealy-textured fruit, the arabinosyl residue becomes tightly bound to matrix glycan, including cellulose, and consequently the loss of arabinosyl residue seems to be suppressed. Although direct evidence was not shown, modification of arabinosyl-containing cell-wall polymer may play an important role in the alteration of fruit texture in relation to cell-to-cell adhesion.

6. Expression pattern of α-L-arabinofuranosidases

There are relatively few reports of α-L-arabinofuranosidase isolated from fruit with biochemical characteristics compared to β-galactosidase. α-L-arabinofuranosidases were classified into five glycoside hydrolase (GH) families (family 3, 43, 51, 54, and 62), and biochemically characterized α-L-arabinofuranosidases from higher plants are found in families 3 and 51 (Fig. 2). This classification is based on the amino acid sequence rather than on substrate specificity (Coutinho and Henrissat, 1999; http://www.cazy.org/, August 10, 2008).

Ferré et al. (2000) purified α-L-arabinofuranosidase from a monocotyledon, barley (*Hordeum vulgare*) belonging to the GH family 51. The α-L-arabinofuranosidase is an arabinoxylan arabinofuranohydrolase which is able to release arabinose from both singly and doubly substituted xylose. The enzyme could not release arabinose from linear or branched-chain arabinan, although distinct results of the enzyme activity against arabinan were shown by Lee et al. (2001). α-L-Arabinofuranosidase isolated by two groups was able to hydrolysis arabinoxylan and produced monomeric arabinose (arabinoxylan arabinofuranohydrolase) (Ferré et al., 2000; Lee et al., 2001). Two α-L-arabinofuranosidases, ASD1 and ASD2, belonging to family 51, were found in Arabidopsis (*Arabidopsis thaliana*). ASD1 showed higher expression in cell proliferation zones, the vascular system, developing and regressing floral tissues, and floral abscission zones, while ASD2 was expressed in the vasculature of older root tissue and in some floral organs and floral abscission zones (Fulton and Cobbett, 2003). Itai et al. (2003) reported that family 51 α-L-arabinofuranosidase cloned from tomato fruit were expressed during fruit development and declined during ripening. Sekine et al. (2006) reported that family 51 α-L-arabinofuranosidase

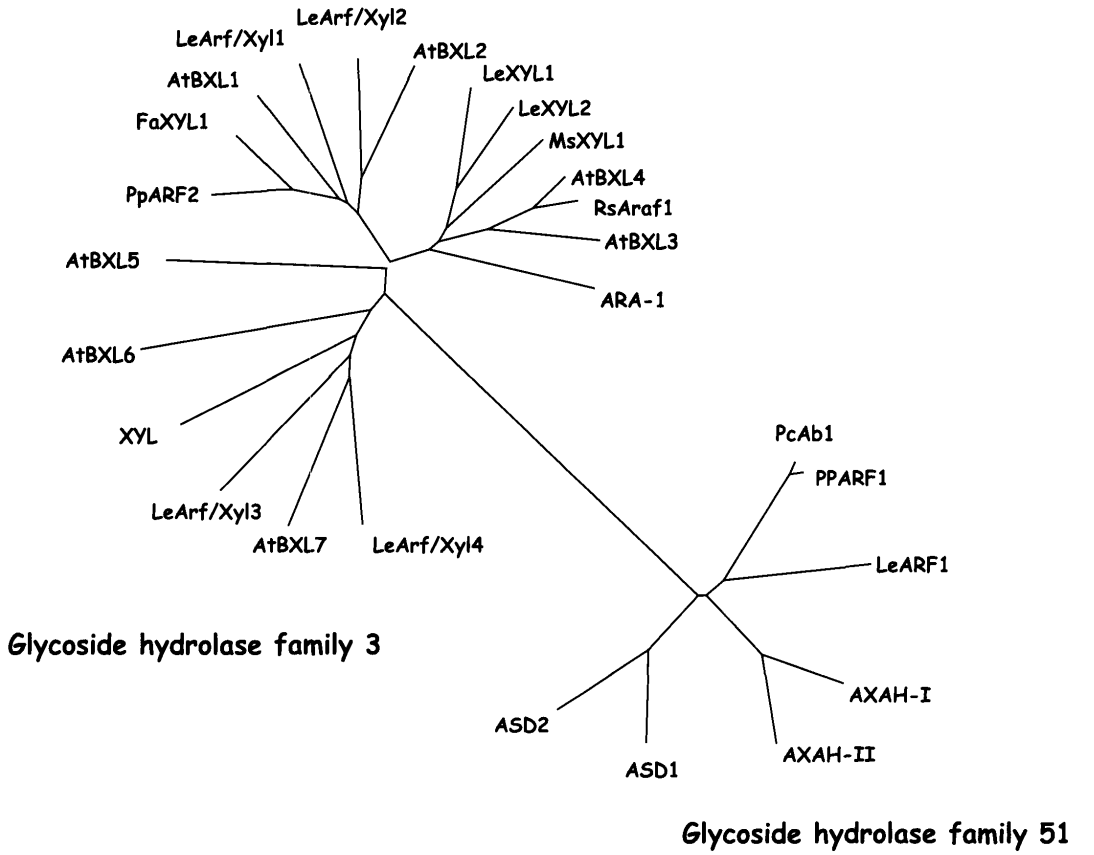

Fig. 2. Phylogenetic relationships of bi-functional α-L-arabinofuranosidase/β-xylosidases and β-xylosidases classified into GH family 3 and family 51. Amino acid sequences were aligned with the ClustalW program and phylogenetic tree was drawn. ASD1 and ASD2 from Arabidopsis (AY243509 and AY243510), PPARF1 from Japanese pear (AB073311), LeARF from tomato (AB073310), PcAb1 from pear (AB067643), AXAH-I and AXAH-II from barley (AF320324 and AF320325), XYL1 from strawberry (AY486104), ARA-1 and XYL from barley (AY029259 and AY029260), and LeXYL1 and LeXYL2 from tomato (AB041811 and AB041812) and LeArf/Xyl1 to 4 from tomato (unpublished). AtBXL1 to 7 from Arabidopsis. RsAraf1 from radish (AB234292) and MsXYL1 from alfalfa (EF569968).

cloned from pear fruit was expressed constitutively during fruit storage. From only expression analysis of family 51 α-L-arabinofuranosidase, it seems to contribute less to fruit softening, but its detailed role in physiological metabolism remains unclear.

α-L-Arabinofuranosidase is also classified into GH family 3 besides family 51, although they are clearly distinguishable based on sequence homology (Fig. 2). Lee et al. (2003) purified a new α-L-arabinofuranosidase from monocotyledonous barley and indicated the enzyme belongs to GH family 3. Following barley, family 3 α-L-arabinofuranosidase was purified from Japanese pear, a dicotyledonous plant (Tateishi et al., 2005a). These were the first reports that α-L-arabinofuranosidases isolated from higher plants belong to GH family 3. Based on amino acid sequencing similarity, β-glucosidase and β-xylosidase have been grouped into GH family 3 in higher plants; however, it had not been elucidated whether the proteins possessed β-xylosidase or β-glucosidase activity. The analysis of substrate specificity of purified enzymes revealed that the enzymes grouped into family 3 appear to be both single-functional β-xylosidase and bi-functional α-L-arabinofuranosidase/β-xylosidase for artificial substrates (Table 2). Therefore, several putative β-xylosidases in family 3, which were classified by only sequence similarities, may possess α-L-arabinofuranosidase activity in addition to β-xylosidase activity. Hence, the expression pattern of family 3 α-L-arabinofuranosidase described below was also summarized including β-xylosidase and putative α-L-arabinofuranosidase/β-xylosidase. cDNA clones of the enzymes were isolated from barley (Lee et al., 2003), tomato (Itai et al., 2003), Arabidopsis (Goujon et al., 2003; Minic et al., 2004, 2006), Japanese pear (Tateishi et al., 2005a), radish (*Raphanus sativus*) (Kotake et al., 2006), peach (Hayama et al., 2006), strawberry (Bustamante et al., 2006), and alfalfa (*Medicago sativa*) (Xiong et al., 2007). They constitute a small gene family and the expression of each isozyme was found in various organs and developmental stages. It is suggested that enzymes exhibiting hydrolysis activity of arabinosyl or xylosyl residues might act in plant development. Limited in fruit, *LeXYL1* and *LeXYL2*, which are two putative β-xylosidase cDNA clones, were isolated from tomato fruit. *LeXYL1* expression was observed during fruit ripening while *LeXYL2* was expressed in fruit development and markedly declined with fruit ripening (Itai et al., 2003). *PpARF2* from Japanese pear, which encodes family 3 α-L-arabinofuranosidase, was expressed in ripened fruit (Tateishi et al., 2005a).

Table 2. Substrate specificities and grouping of α-L-arabinofuranosidase and β-xylosidase.

Scientific name	Common name	Glycoside hydrolase family	Substrate specificities[z]	Note	References
Arabidopsis thaliana	Arabidopsis	51	Bifunction (4NPAf > 4NPXp), Ara from arabinan and Xyl from AX	ASD1	Fulton and Cobbett, 2003; Minic et al., 2004
		51	Unknown	ASD2	Fulton and Cobbett, 2003
		3	Bifunction (4NPAf > 4NPXp), Ara from arabinan > Xyl from AX	AtBXL1	Goujon et al., 2003; Minic et al., 2004
		3	Unknown	AtBXL2	Only database
		3	Bifunction (4NPAf > 4NPXp), Ara from arabinan > Xyl from AX	AtBXL3	Minic et al., 2006
		3	Bifunction (4NPXp > 4NPAf), Xyl from AX	AtBXL4	Minic et al., 2003
		3	Unknown	AtBXL5	Only database
		3	Unknown	AtBXL6	Only database
		3	Unknown	AtBXL7	Only database
Fragaria × ananassa	Strawberry	3	4NPXp	FaXyl1	Bustamante et al., 2006
Hordeum vulgare	Barley	51	4NPAf (No activity against 4NPXp and arabinan), Ara from AX	Ara1	Ferré et al., 2000
		51	4NPAf (No activity against 4NPXp), Ara from xylanase treated AX = arabinan > AX.	AXAH I	Lee et al., 2001
		51	4NPAf	AXAH II	Lee et al., 2001
		3	Bifunction (4NPAf > 4NPXp), Xyl from xylanase treated AX	ARA-I	Lee et al., 2003
		3	4NPXp, Xyl from xylanase treated AX	XYL	Lee et al., 2003
Medicago sativa	Alfalfa	3	Bifunction (4NPAf > 4NPXp), Ara from arabinan, Ara and Xyl from CWM and AX, Xyl from xylanase-treated xylan	MsXyl1	Xiong et al., 2007
Pyrus communis	European pear	51	Unknown	PcARF1	Sekine et al., 2006
Prunus percica	Peach	3	Unknown	PpARF/XYL	Hayama et al., 2006
Pyrus pyrifolia	Japanese pear	51	Unknown	PpARF1	Only database
		3	Bifunction (4NPAf > 4NPXp), Ara from arabinan and CWM. No activity against AX.	PpARF2	Tateishi et al., 2005a
Raphanus sativus	Radish	3	Bifunction (4NPAf > 4NPXp), Ara from arabinan and AX	RsAraf1	Kotake et al., 2006
Solanum lycopersicum	Tomato	51	Unknown	LeARF	Itai et al., 2003
		3	Unknown	LeXYL1	Itai et al., 2003
		3	Unknown	LeXYL2	Itai et al., 2003
		3	Bifunction (4NPAf = 4NPXp),	LeArf/Xyl1	Tateishi et al., (unpublished data)
		3	Bifunction (4NPAf = 4NPXp),	LeArf/Xyl2	Tateishi et al., (unpublished data)
		3	Unknown	LeArf/Xyl3	Tateishi et al., (unpublished data)
		3	Bifunction (4NPAf < 4NPXp),	LeArf/Xyl4	Tateishi et al., (unpublished data)

[z] Indicates relatively preferable substrates, although each isozyme showed broad substrate specificities. Activity against oligosaccharides is not shown. Abbreviations: 4NPAf, 4-nitrophenyl α-L-arabinofuranoside; 4NPXp, 4-nitrophenyl β-xylopyranoside; Ara, arabinose; AX, arabinoxylan; CWM, cell-wall materials; Xyl, xylose.

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PpARF/XYL, which was cloned from peach and belonged to family 3, was also expressed in softened fruit but not in stony hard peach fruit (Hayama et al., 2006). The expressions of *PpARF2* and *PpARF/XYL* were regulated by ethylene (Mwaniki et al., 2007; Hayama et al., 2006). *FaXYL1*, which exhibited only β-xylosidase activity isolated from strawberry, was expressed higher and accumulated at an earlier stage in a softer strawberry cultivar (Bustamante et al., 2006). According to expression analysis of family 3 α-L-arabinofuranosidase and β-xylosidase, they seem to play a certain role in fruit softening while they were expressed at a relatively late stage of ripening. A transgenic experiment is necessary to elucidate the role of α-L-arabinofuranosidase in fruit softening or textural changes.

7. Substrate specificity of α-L-arabinofuranosidase

Several enzymes belonging to both families 3 and 51 showed bi-functional activity against artificial substrates (4-nitrophenyl α-L-arabinofuranoside or 4-nitrophenyl β-D-xylopyranoside); therefore, the enzymes are considered as bifunctional α-L-arabinofuranosidase/β-xylosidase. It is more difficult and complex to understand substrate specificity against native substrates of the enzymes. The ability of enzymes or isozymes to release both arabinosyl and xylosyl residues from various native substrates *in vitro* is distinct (summarized in Table 2). There is no correlation between substrate specificities and the primary structure of the enzyme, suggesting the necessity of biochemical properties when using enzyme annotations in a database. Minic et al. (2004, 2006) showed that several β-xylosidase and α-L-arabinofuranosidase from Arabidopsis possessed similar substrate specificities against both arabinan and arabinoxylan and suggested that these broad substrate specificities are rather convenient for modification of the complex cell wall structure. On the other hand, α-L-arabinofuranosidase isolated from Japanese pear showed limited activity of releasing arabinose from pectic arabinan (Tateishi et al., 2005a). Radish α-L-arabinofuranosidase seems to hydrolyze only arabinosyl residues constituting arabinogalactan protein (Kotake et al., 2006). Although they showed broad substrate specificity *in vitro*, it suggests that substrates of the enzymes seem to be limited *in vivo*.

Conclusion

Cell-wall polysaccharides are composed of limited sugar residues; however, linkage diversity complicates its architecture. Moreover, many cell-wall-modifying enzymes consist of multiple isozymes found in the wall with different activities and expression patterns. Several cell-wall-modifying enzymes, such as polygalacturonase, pectin methylesterase, XTH, and β-1,4-glucanase were evaluated by a transgenic technique and showed less contribution of the enzymes to fruit softening. Among them, transgenic fruit, which suppressed the accumulation of an expansin or the activity of β-galactosidase, kept relatively higher mechanical strength than the wild type during ripening; however, fruit softening could not be prevented entirely. Consequently, limited contribution of each enzyme to fruit softening was shown. At present, it is suggested that the degradation of several cell-wall polysaccharides is necessary, and that numerous cell wall-metabolism enzymes are implicated in fruit softening. Therefore, the softening process requires the cooperative action of several enzymes (isozymes) to degrade cell-wall polysaccharides or the sequential degradation of wall polysaccharides.

On the other hand, several enzymes also contribute to the modification of cell-wall structures during plant development in addition to the softening observed during ripening. In the case of β-galactosidase, according to the expression pattern and substrate specificity, each isozyme is active against various galactosyl-containing polysaccharides and plays a role in cell-wall modification in several aspects of plant development besides softening. The roles of α-L-arabinofuranosidase in fruit ripening/softening are still unclear. It may contribute to a change in fruit texture involving softening according to the role of arabinosyl residues in wall polysaccharides, such as cell-to-cell adhesion. *In vitro* experiments revealed that α-L-arabinofuranosidases possess broad substrate specificities. The expression of α-L-arabinofuranosidase in non-fruit tissues indicates that the enzyme also plays a role in not only fruit softening but also plant development.

Although transgenic experiments are a good tool to assess whether cell-wall-modification enzymes contribute to fruit softening, this technique is not sufficient to elucidate their biochemical characteristics, such as substrate specificity. Moreover, the transgenic technique is useful for limited fruit species such as tomato. Numerous fruit species and cultivars are found with their own unique characters related to their softening pattern and textual changes; therefore, it is necessary to make genetically modified various plant species, in which the introduced individual enzyme (isozyme) gene is regulated, and to evaluate them. It is also necessary to investigate the cooperative effects or interaction of multiple enzymes on fruit softening. Transgenic plants with introduced multiple cell-wall-modifying genes or the progeny of plants crossed by a previously transformed parent may reveal the more precise mechanism of fruit softening. These experiments will also be helpful for producing high quality fruit.

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Literature Cited

Ahmed, A. and J. M. Labavitch. 1980. Cell wall metabolism in ripening fruit. II. Changes in carbohydrate-degrading enzymes in ripening 'Bartlett' pears. Plant Physiol. 65: 1014–1016.

Ali, Z. M., S-Y. Ng, R. Othman, L-Y. Goh and H. Lazan. 1998. Isolation, characterization and significance of papaya β-galactanases to cell wall modification and fruit softening during ripening. Physiol. Plant. 104: 105–115.

Arrowsmith, D. A. and J. de Silva. 1995. Characterisation of two tomato fruit-expressed cDNAs encoding xyloglucan endo-transglycosylase. Plant Mol. Biol. 28: 391–403.

Balandrán-Quintana, R. R., A. M. Mendoza-Wilson, I. Vargas-Arispuro and M. A. Martínez-Téllez. 2007. Activity of β-galactosidase and polygalacturonase in zucchini squash (*Cucurbita pepo* L.) stored at low temperatures. Food Technol. Biotechnol. 45: 396–401.

Barnavon, L., T. Doco, N. Terrier, A. Ageorges, C. Tomieu and P. Pellerin. 2000. Analysis of cell wall neutral sugar composition, β-galactosidase activity and a related cDNA clone throughout the development of *Vitis vinifera* grape berries. Plant Physiol. Biochem. 38: 289–300.

Bartley, I. M. 1974. β-Galactosidase activity in ripening apples. Phytochemistry 13: 2107–2111.

Beldman, G., H. A. Schols, S. M. Pitson, M. J. F. Searle-van Leeuwen and A. G. J. Voragen. 1997. Arabinans and arabinan degrading enzymes. p. 1–64. In: R. J. Sturgeon (ed.). Advances in macromolecular carbohydrate research. Vol 1. Jai Press, Greenwich, CT.

Bonghi, C., S. Pagni, R. Vidrih, A. Ramina and P. Tonutti. 1996. Cell wall hydrolases and amylase in kiwifruit softening. Postharvest Biol. Technol. 9: 19–29.

Brummell, D. A. 2006. Cell wall disassembly in ripening fruit. Func. Plant Biol. 33: 103–119.

Brummell, D. A. and J. M. Labavitch. 1997. Effect of antisense suppression of endopolygalacturonase activity on polyuronide molecular weight in ripening tomato fruit and in fruit homogenates. Plant Physiol. 115: 717–725.

Brummell, D. A., B. D. Hall and A. B. Bennett. 1999a. Antisence suppression of tomato endo-1,4-β-glucanase Cel2 mRNA accumulation increased force required to break fruit abscission zones but does not affect fruit softening. Plant Mol. Biol. 40: 615–622.

Brummell, D. A. and M. H. Harpster. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. 47: 311–340.

Brummell, D. A., M. H. Harpster, P. M. Civello, J. M. Palys and A. B. Bennett. 1999b. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell 11: 2203–2216.

Brummell, D. A., V. D. Cin, C. H. Crisosto and J. M. Labavitch. 2004a. Cell wall metabolism during maturation, ripening and senescence of peach fruit. J. Exp. Bot. 55: 2029–2039.

Brummell, D. A., V. D. Cin, S. Lurie, C. H. Crisosto and J. M. Labavitch. 2004b. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall. J. Exp. Bot. 55: 2041–2052.

Bustamante, C. A., H. G. Roslia, M. C. Añónb, P. M. Civelloa and G. A. Martínez. 2006. β-Xylosidase in strawberry fruit: Isolation of a full-length gene and analysis of its expression and enzymatic activity in cultivars with contrasting firmness. Plant Sci. 171: 497–504.

Carey, A. T., D. L. Smith, E. Harrison, C. R. Bird, K. C. Gross, G. B. Seymour and G. A. Tucker. 2001. Down-regulation of a ripening-related β-galactosidase gene (TBG1) in transgenic tomato fruits. J. Exp. Bot. 52: 663–668.

Carey, A. T., K. Holt, S. Picard, R. Wilde, G. A. Tucker, C. R. Bird, W. Schuch and G. B. Seymour. 1995. Tomato exo-(1→4)-β-D-galactanase. Isolation, changes during ripening in normal and mutant tomato fruit, and characterization of a related clone. Plant Physiol. 108: 1099–1107.

Carrington, C. M. S. and R. Pressey. 1996. β-Galactosidase II activity in relation to changes in cell wall galactosyl composition during tomato ripening. J. Amer. Soc. Hort. Sci. 121: 132–136.

Coutinho, P. M. and B. Henrissat. 1999. Carbohydrate-active enzymes: an integrated database approach. p. 3–12. In: H. J. Gilbert, G. Davies, B. Henrissat and B. Svensson (eds.). Recent advances in carbohydrate bioengineering. The Royal Society of Chemistry, Cambridge.

Dawson, D. M., L. D. Melton and C. B. Watkins. 1992. Cell wall changes in nectarines (*Prunus persica*): solubilization and depolymerization of pectic and neutral polymers during ripening and in mealy fruit. Plant Physiol. 100: 1203–1210.

de Alcántara P. H. N., L. Martim, C. O. Silva, S. M. C. Dietrich and M. S. Buckeridge. 2006. Purification of a β-galactosidase from cotyledons of *Hymenaea courbaril* L. (Leguminosae). Enzyme properties and biological function. Plant Physiol. Biochem. 44: 619–627.

DeVeau, E. J. I., K. C. Gross, D. J. Huber and A. E. Watada. 1993. Degradation and solubilization of pectin by β-galactosidase purified from avocado mesocarp. Physiol. Plant. 87: 279–285.

Dick, A. J., A. Opoku-Gyamfua and A. C. deMarco. 1990. Glycosidases of apple fruit: a multi-functional β-galactosidase. Physiol. Plant. 80: 250–256.

Ferré, H., A. Broberg, J. Ø. Duus and K. K. Thomsen. 2000. A novel type of arabinoxylan arabinofuranohydrolase isolated from germinated barley. Eur. J. Biochem. 267: 6633–6641.

Fils-Lycaon, B. and M. Buret. 1991. Changes in glycosidase activities during development and ripening of melon. Postharvest Biol. Technol. 1: 143–151.

Fischer, R. L. and A. B. Bennett. 1991. Role of cell wall hydrolases in fruit ripening. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 675–703.

Fulton, L. M. and C. S. Cobbett. 2003. Two α-L-arabinofuranosidase genes in *Arabidopsis thaliana* are differentially expressed during vegetative growth and flower development. J. Exp. Bot. 54: 2467–2477.

Giovannoni, J. J., D. DellaPenna, A. B. Bennett and R. L. Fischer. 1989. Expression of a chimeric polygalacturonase gene in transgenic *rin* (*ripening inhibitor*) tomato fruit results in polyuronide degradation but not fruit softening. Plant Cell 1: 53–63.

Goujon, T., Z. Minic, A. E. I. Amrani, O. Lerouxel, E. Aletti, C. Lapierre, J-P. Joseleau and L. Jouanin. 2003. *AtBXL1*, a novel higher plant (*Arabidopsis thaliana*) putative beta-xylosidase gene, is involved in secondary cell wall metabolism and plant development. Plant J. 33: 677–690.

Goulao, L. F. and C. M. Oliveira. 2008. Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends Food Sci. Tech. 19: 4–25.

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiol. Plant. 62: 25–32.

Gross, K. C. and C. E. Sams. 1984. Changes in cell wall neutral sugar composition during fruit ripening: a species survey. Phytochemistry 23: 2457–2461.

Harpster, M. H., D. A. Brummell and P. Dunsmuir. 2002. Suppression of a ripening-related endo-1,4-β-glucanase in transgenic pepper fruit does not prevent depolymerization of cell wall polysaccharides during ripening. Plant Mol. Biol. 50: 345–355.

Hayama, H., T. Shimada, H. Fujii, A. Ito and Y. Kashimura. 2006. Ethylene-regulation of fruit softening and softening-related genes in peach. J. Exp. Bot. 57: 4071–4077.

Huber, D. J. 1984. Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. J. Food Sci. 49: 1310–1315.

Huber, D. J. and E. M. O'Donoghue. 1993. Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esclentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. 102: 473–480.

Inaba, A. 2007. Studies on the internal feedback regulation of ethylene biosynthesis and signal transduction during fruit ripening, and the improvement of fruit quality. J. Japan. Soc. Hort. Sci. 76: 1–12.

Itai, A., K. Ishihara and J. D. Bewley. 2003. Characterization of expression, and cloning, of β-D-xylosidase and α-L-arabinofuranosidase in developing and ripening tomato (*Lycopersicon esculentum* Mill.) fruit. J. Exp. Bot. 54: 2615–2622.

Iwai, H., T. Ishii and S. Satoh. 2001. Absence of arabinan in the side chains of the pectic polysaccharides strongly associated with cell walls of *Nicotiana plumbaginifolia* non-organogenic callus with loosely attached constituent cells. Planta 213: 907–915.

Iwai, H., N. Masaoka, T. Ishii and S. Satoh. 2002. A pectin glucuronyltransferase gene is essential for intercellular attachment in the plant meristem. Proc. Natl. Acad. Sci. USA 99: 16319–16324.

Jin, C. H., J. Kan, Z. J. Wang, Z. X. Lu and Z. F. Yu. 2006. Activities of β-galactosidase and α-L-arabinofuranosidase, ethylene biosynthetic enzymes during peach ripening and softening. J. Food Process. Pres. 30: 515–526.

King, G. A., K. M. Davies, R. J. Stewart and W. M. Borst. 1995. Similarities in gene expression during the postharvest-induced senescence of spears and natural foliar senescence of asparagus. Plant Physiol. 108: 125–128.

Kitagawa, Y., Y. Kanayama and S. Yamaki. 1995. Isolation of β-galactosidase fractions from Japanese pear: Activity against native cell wall polysaccharides. Physiol. Plant. 93: 545–550.

Kotake, T., K. Tsuchiya, T. Aohara, T. Konishi, S. Kaneko, K. Igarashi, M. Samejima and Y. Tsumuraya. 2006. An α-L-arabinofuranosidase/β-D-xylosidase from immature seeds of radish (*Raphanus sativus* L.). J. Exp. Bot. 57: 2353–2362.

Kramer, M., R. Sanders, H. Bolkan, C. Waters, R. Sheehy and W. Hiatt. 1992. Post-harvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. Postharvest Biol. Technol. 1: 241–255.

Langley, K. R., A. Martin, R. Stenning, A. J. Murray, G. E. Hobson, W. W. Schuch and C. R. Bird. 1994. Mechanical and optical assessment of the ripening of tomato fruit with reduced polygalacturonase activity. J. Sci. Food. Agric. 66: 547–554.

Lazan, H., M. K. Selamat and Z. M. Ali. 1995. β-Galactosidase, polygalacturonase and pectinesterase in differential softening and cell wall modification during papaya fruit ripening. Physiol. Plant. 95: 106–112.

Lazan, H., S-Y. Ng, L-Y Goh and Z. M. Ali. 2004. Papaya β-galactosidase/galactanase isoforms in differential cell wall hydrolysis and fruit softening during ripening, Plant Physiol. Biochem. 42: 847–853.

Lee, E-J., Y. Matsumura, K. Soga, T. Hoson and N. Koizumi. 2007. Glycosyl hydrolases of cell wall are induced by sugar starvation in Arabidopsis. Plant Cell Physiol. 48: 405–413.

Lee, R. C., R. A. Burton, M. Hrmova and G. B. Fincher. 2001. Barley arabinoxylan arabinofuranohydrolases: purification, characterization and determination of primary structures from cDNA clones. Biochem. J. 356: 181–189.

Lee, R. C., M. Hrmova, R. A. Burton, J. Lahnstein and G. B. Fincher. 2003. Bifunctional family 3 glycoside hydrolases from barley with α-L-arabinofuranosidase and β-D-xylosidase activity. J. Biol. Chem. 278: 5377–5387.

Minic, Z., C. Rihouey, C. T. Do, P. Lerouge and L. Jouanin. 2004. Purification and characterization of enzymes exhibiting β-D-xylosidase activities in stem tissues of Arabidopsis. Plant Physiol. 135: 867–878.

Minic, Z., C. T. Do, C. Rihouey, H. Morin, P. Lerouge and L. Jouanin. 2006. Purification, functional characterization, cloning, and identification of mutants of a seed-specific arabinan hydrolase in Arabidopsis. J. Exp. Bot. 57: 2339–2351.

Moctezuma, E., D. L. Smith and K. C. Gross. 2003. Antisense suppression of a β-galactosidase gene (*TBG6*) in tomato increases fruit cracking. J. Exp. Bot. 54: 2025–2033.

Mwaniki, M. W., F. M. Mathooko, M. Matsuzaki, K. Hiwasa, A. Tateishi, K. Ushijima, R. Nakano, A. Inaba and Y. Kubo. 2005. Expression characteristics of seven members of the β-galactosidase gene family in 'La France' pear (*Pyrus communis* L.) fruit during growth and their regulation by 1-methylcyclopropene during postharvest ripening. Postharvest Biol. Technol. 36: 253–263.

Mwaniki, M. W., F. M. Mathooko, K. Hiwasa, A. Tateishi, N. Yokotani, K. Ushijima, R. Nakano, A. Inaba and Y. Kubo. 2007. β-Galactosiadse and α-L-arabinofuranosidase activities and gene expression in European and Chinese pear fruit during ripening. J. Japan. Soc. Hort. Sci. 76: 85–90.

Nara, K., Y. Kato and Y. Motomura. 2001. Involvement of terminal-arabinose and -galactose pectic compounds in mealiness of apple fruit during storage. Postharvest Biol. Tech. 22: 141–150.

Nishiyama, K., G. Monique, J. K. C. Rose, Y. Kubo, K. A. Bennett, L. Wangjin, K. Kato, K. Ushijima, R. Nakano, A. Inaba, M. Bouzayen, A. Latche, J-C. Pech and A. B. Bennett. 2007. Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J. Exp. Bot. 58: 1281–1290.

Nunan, K. J., C. Davies, S. P. Robinson and G. B. Fincher. 2001. Expression patterns of cell wall-modifying enzymes during grape berry development. Planta 214: 257–264.

Ogasawara, S., K. Abe and T. Nakajima. 2007. Pepper β-galactosidase 1 (PBG1) plays a significant role in fruit ripening in bell pepper (*Capsicum annuum*). Biosci. Biotechnol. Biochem. 71: 309–322.

Orfila, C., M. M. H. Huisman, W. G. T. Willats, G-J. W. M. van Alebeek, H. A. Schols, G. B. Seymour and J. P. Knox. 2002. Altered cell wall disassembly during ripening of *Cnr* tomato fruit: implications for cell adhesion and fruit softening. Planta 215: 440–447.

Orfila, C., G. B. Seymour, W. G. T. Willats, I. M. Huxham, M.

C. Jarvis, C. J. Dover, A. J. Thompson and J. P. Knox. 2001. Altered middle lamella homogalacturonan and disrupted deposition of (1→5)-α-L-arabinan in the pericarp of *Cnr*, a ripening mutant of tomato. Plant Physiol. 126: 210–221.

Peña, M. J. and N. C. Carpita. 2004. Loss of highly branched arabinans and debranching of rhamnogalacturonan I accompany loss of firm texture and cell separation during prolonged storage of apple. Plant Physiol. 135: 1305–1313.

Pressey, R. 1983. β-Galactosidase in ripening tomatoes. Plant Physiol. 71: 132–135.

Ranwala, A. P., C. Suematsu and H. Matsuda. 1992. The role of β-galactosidase in the modification of cell wall components during muskmelon fruit ripening. Plant Physiol. 100: 1318–1325.

Redgwell, R. J., L. D. Melton and D. J. Brasch. 1992. Cell wall dissolution in ripening kiwifruit (*Actinidia deliciosa*). Plant Physiol. 98: 71–81.

Ross, G. S., T. Wegrzyn, E. A. MacRae and R. J. Redgwell. 1994. Apple β-galactosidase. Activity against cell wall polysaccharides and characterization of a related cDNA clone. Plant Physiol. 106: 521–528.

Saha, B. C. 2000. α-L-Arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. Biotechnol Adv 18: 403–423.

Sakurai, N. and D. J. Nevins. 1997. Relationship between fruit softening and wall polysaccharides in avocado (*Persea americana* Mill) mesocarp tissues. Plant Cell Physiol. 38: 603–610.

Sekine, D., I. Munemura, M. Gao, W. Mitsuhashi, T. Toyomasu and H. Murayama. 2006. Cloning of cDNAs encoding cell-wall hydrolases from pear (*Pyrus communis*) fruit and their involvement in fruit softening and development of melting texture. Physiol. Plant. 126: 163–174.

Schuch, W., J. Kanczler, D. Robertson, G. Hobson, G. Tucker, D. Grierson, S. W. J. Bright and C. Bird. 1991. Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. HortScience 26: 1517–1520.

Smith, C. J. S., C. F. S. Watson, J. Ray, C. R. Bird, P. C. Morris, W. Schuch and D. Grierson. 1988. Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature 334: 724–726.

Smith, D. L., D. A. Starrett and K. C. Gross. 1998. A gene coding tomato fruit β-galactosidase II is expressed during fruit ripening. Plant Physiol. 117: 417–423.

Smith, D. L. and K. C. Gross. 2000. A family of at least seven β-galactosidase genes is expressed during tomato fruit development. Plant Physiol. 123: 1173–1183.

Smith, D. L., J. A. Abbott and K. C. Gross. 2002. Down-regulation of tomato β-galactosidase 4 results in decreased fruit softening. Plant Physiol. 129: 1755–1762.

Sozzi, G. O., A. A. Fraschina, A. A. Navarro, O. Cascone, L. C. Greve and J. M. Labavitch. 2002a. α-L-Arabinofuranosidase activity during development and ripening of normal and ACC synthase antisense tomato fruit. HortScience 37: 564–566.

Sozzi, G. O., L. C. Greve, G. A. Prody and J. M. Labavitch. 2002b. Gibberellic acid, synthetic auxins, and ethylene differentially modulate α-L-arabinofuranosidase activities in antisense 1-aminocyclopropane-1-carboxylic acid synthase tomato pericarp discs. Plant. Physiol. 129: 1330–1340.

Tateishi, A. and H. Inoue. 2000. Purification and characterization of α-L-arabinofuranosidase from Japanese pear fruit. Acta Hortic. 517: 397–403.

Tateishi, A., H. Inoue and S. Yamaki. 2001a. Fluctuations in activities of three β-galactosidase isoforms from ripening avocado (*Persea americana*) fruit and their different activities against its cell wall polysaccharides as substrates. J. Japan. Soc. Hort. Sci. 70: 586–592.

Tateishi, A., H. Inoue and S. Yamaki. 2002. Cloning and expression of β-galactosidase cDNA related to softening avocado (*Persea americana*) fruit. J. Japan. Soc. Hort. Sci. 71: 48–55.

Tateishi, A., H. Inoue, H. Shiba and S. Yamaki. 2001b. Molecular cloning of β-galactosidase from Japanese pear (*Pyrus pyrifolia*) and its gene expression with fruit ripening. Plant Cell Physiol. 42: 492–498.

Tateishi, A., H. Mori, J. Watari, K. Nagashima, S. Yamaki and H. Inoue. 2005a. Isolation, characterization, and cloning of α-L-arabinofuranosidase expressed during fruit ripening of Japanese pear. Plant Physiol. 138: 1653–1664.

Tateishi, A., H. Shiba, J. Ogihara, K. Isobe, K. Nomura, K. Watanabe and H. Inoue. 2007. Differential expression and ethylene regulation of β-galactosidase genes and isozymes isolated from avocado (*Persea americana* Mill.) fruit. Postharvest Physiol. Biol. 45: 56–65.

Tateishi, A., K. Nagashima, F. M. Mathooko, M. W. Mwaniki, Y. Kubo, A. Inaba, S. Yamaki and H. Inoue. 2005b. Differential expression of members of the β-galactosidase gene family during Japanese pear (*Pyrus pyrifolia*) fruit growth and on-tree ripening. J. Amer. Soc. Hort. Sci. 130: 819–829.

Tateishi, A., Y. Kanayama and S. Yamaki. 1996. α-L-Arabinofuranosidase from cell walls of Japanese pear fruits. Phytochemistry 42: 295–299.

Tatsuki, M. and A. Endo. 2006. Analyses of expression patterns of ethylene receptor genes in apple (*Malus domestica* Borkh.) fruits treated with or without 1-methylcyclopropene (1-MCP). J. Japan. Soc. Hort. Sci. 75: 481–487.

Terasaki, S., N. Sakurai, R. Yamamoto, N. Wada and D. J. Nevins. 2001. Changes in cell wall polysaccharides of kiwifruit and the visco-elastic properties detected by a laser Doppler method. J. Japan. Soc. Hort. Sci. 70: 572–580.

Thompson, A. J., M. Tor, C. S. Barry, J. Vrebalov, C. Orfila, M. C. Jarvis, J. J. Giovannoni, D. Grierson and G. B. Seymour. 1999. Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. Plant Physiol. 120: 383–389.

Tieman, D. M., R. W. Harriman, G. Ramamohan and A. K. Handa. 1992. An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. Plant Cell 4: 667–679.

Trainotti, L., R. Spinello, A. Piovan, S. Spolaore and G. Casadoro. 2001. β-Galactosidase with a lectin-like domain are expressed in strawberry. J. Exp. Bot. 52: 1635–1645.

Wade, N. L., E. E. Kavanagh, D. G. Hockley and C. J. Brady. 1992. Relationship between softening and the polyuronides in ripening banana fruit. J. Sci. Food Agriculture 60: 61–68.

Wakabayashi, K., J-P. Chun and D. J. Huber. 2000. Extensive solubilization and depolymerization of cell wall polysaccharides during avocado (*Persea americana*) ripening involves concerted action of polygalacturonase and pectinmethylesterase. Physiol. Plant. 108: 345–352.

Wegrzyn, T. F. and E. A. MacRae. 1992. Pectinesterase, polygalacturonase, and β-galactosidase during softening of ethylene-treated kiwifruit. HortScience 27: 900–902.

Wu, Z. and J. K. Burns. 2004. A β-galactosidase gene is expressed during mature fruit abscission of 'Valencia' orange (*Citrus sinensis*). J. Exp. Bot. 55: 1483–1490.

Xiong, J-S., M. Balland-Vanney, Z-P. Xie, M. Schultze, A. Kondorosi, E. Kondorosi and C. Staehelin. 2007. Molecular cloning of a bifunctional β-xylosidase/α-L-arabinosidase

from alfalfa roots: heterologous expression in *Medicago truncatula* and substrate specificity of the purified enzyme. J. Exp. Bot. 58: 2799–2810.

Xu, C. G., A. Nakatsuka, H. Kano and H. Itamura. 2003 Changes in ethylene production and activities of cell wall degrading enzymes during rapid fruit softening of Japanese persimmon 'Saijo'. J. Japan. Soc. Hort. Sci. 72: 460–462.

Yoshioka, H., K. Aoba and Y. Kashimura. 1992. Molecular weight and degree of methoxylation in cell wall polyuronide during softening in pear and apple fruit. J. Amer. Soc. Hort. Sci. 117: 600–606.

Yoshioka, H., Y. Kashimura and K. Kaneko. 1995. β-D-galactosidase and α-L-arabinofuranosidase activities during the softening of apples. J. Japan. Soc. Hort. Sci. 63: 871–878.