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Genetic Determinants of Textural Modifications in Fruits and Role of Cell Wall Polysaccharides and Defense Proteins in the Protection Against Pathogens

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ABSTRACT

Plant cell wall metabolism has been suggested to play a major role in the textural changes associated with fruit ripening. The significance of cell wall degrading agents such as polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase (β-gal), endo-1,4-β-glucanase (EGase) and pectate lyase (PL) has led to important advances in our understanding of cell wall disassembly but studies looking at the way these agents may interact and work in concert as 'a cell wall disassembly line' will increase our understanding of fruit softening. In addition, the *in vivo* contribution of other cell wall degrading agents such as α-arabinofuranosidase (α-ara), rhamnogalacturonase (RGase), acetylesterase (AE) and xyloglucan transglycosylase hydrolase (XTH) to fruit softening remains to be evaluated. The role of the cell walls in the resistance against pathogens is another area of great interest from a postharvest perspective. Cell wall modifications that could reduce fruit susceptibility to decay would be of great value because of the potential to reduce pathological problems occurring during storage, handling and distribution. Interestingly it has been recently shown in *Arabidopsis* that the over-expression of a plant pectin methylesterase inhibitor can restrict fungal infection. It would be interesting to test whether or not this approach might be useful to control fruit postharvest diseases. Another aspect to explore further includes the determination of the potential applications of proteins influencing the ability of pathogen glycosidases to cleave plant cell wall polysaccharides such as polygalaturonases, pectin or pectate lyases and xyloglucanase inhibiting proteins. The present work describes some of the genetic determinants of the textural modifications in horticultural commodities and discusses the role of plant cell wall polysaccharides and defense proteins as barriers against postharvest pathogens.

Keywords: cell wall, softening, fruit, decay, resistance against pathogens
Abbreviations: α-afs, α-arabinofuranosidase; **AE**, acetyl esterase; **β-gal**, β-galactosidase; **CWDPs**, cell wall degrading proteins; **EGase**, endo-1,4-β-D-glucanase; **Exp**, expansin; **PG**, polygalacturonase; **PGIP**, polygalacturonase inhibiting protein; **PL**, pectate lyase; **PME**, pectin methyl esterase; **PR**, pathogenesis related protein; **RG I**, rhamnogalacturonan I; **RG II**, rhamnogalacturonan II; **RGase**, rhamnogalacturonase; **TILLING**, targeting induced local lesions in genomes; **XET**, xyloglucan endo-transglycosylase; **XTH**, xyloglucan transglycosylase hydrolase

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Invited Review

INTRODUCTION

Fruit quality is associated with parameters that include appearance, shape, size, color flavor, aroma, nutritional value and texture (Kader 1992) which ultimately determine acceptability to the consumer. While controlled textural modifications occurring during ripening are desired from a consumer's perspective, excessive loss of firmness is a significant problem in postharvest management of horticultural crops, since this loss limits long distance transportation and reduces shelf life (Brummell and Harpster 2001). Textural modifications in plant tissues have been associated with changes in turgor pressure, tissue structure and integrity of cell wall polysaccharides (Shackel *et al*. 1991; Sexton *et al*. 1997; Lashbrook 2005). Although the contribution of tissue architecture and cell turgor changes to fruit firmness could be substantial, research has not identified specific targets on which further studies might focus. In contrast, several research groups have carried out detailed examinations of fruit cell wall polysaccharide disassembly in attempts to identify genes whose expression is associated for this process such as polygalacturonases, pectin methylesterases, β-galactosidases, endo-1,4-β-D-glucanases, expansins and pectato lyases among others (reviewed in Brummell and Harpster 2001; Brummell 2006, Vicente *et al*. 2007b).

Besides their role in determining cell shape and tissue mechanical properties (Carpita and McCann 2000; Willats *et al*. 2001), plant cell walls also are a mechanical barrier against pathogens. It has been suggested that wall polymer disassembly events that are associated with programmed plant developmental processes could contribute to increase the susceptibility of plant tissues to pathogens (Vorwerk *et al*. 2004). Thus, in ripening fruits the necessity of maintaining a balance between softening and keeping a strong barrier for pathogen attack is a postharvest biology challenge that must be addressed. Whether it is possible to achieve the goal of generating commodities having the textural properties that consumers prefer while simultaneously maintaining cell walls as a barrier to fungal penetration and development is currently not clear. In any case, achieving this goal will require a clear understanding of the individual and collaborative roles of the cell wall degrading proteins (CWDPs) in cell wall disassembly (Fisher and Bennett 1991; Owino *et al*. 2005), as well as the recognition that fruit CWDPs action may have consequences *vis à vis* the pathogen susceptibility of ripening fruits.

In addition to the 'physical barrier' role of plant cell walls in limiting pathogen growth, there are several plant apoplast-localized proteins that could contribute to plant defenses. The best known of these are proteins that inhibit pathogen-produced cell wall degrading enzymes. Most notable are the polygalacturonase- (Albersheim and Anderson 1971; Abu-Goukh *et al*. 1983; de Lorenzo *et al*. 2001), pectin lyase- (Bugbee *et al*. 1993) and endoxylanase-inhibiting proteins (Debyser *et al*. 1997) that have been characterized in terms of their ability to provide protection against pathogens by limiting the contributions to penetration and infection of the pathogen's arsenal of CWDPs. Because the pathogen's cell wall plays crucial roles in its own vegetative development (Lorito *et al*. 1993, 1998), an additional defense strategy that targets the pathogen's extracellular matrix may have value for protection against pathogens. Along this line, plant genes encoding chitinases and β-1,3-glucanases, proteins capable of degrading fungal wall structural polymers, have been identified as pathogenesis-related (PR) proteins. In this review we describe our current understanding on the different genetic determinants of textural modifications in fruits and the role of cell wall polysaccharides and defense proteins in the protection against pathogens.

GENERAL FEATURES OF PLANT CELL WALL COMPOSITION AND STRUCTURE

Cell wall structure

Plant cells are surrounded by a complex, dynamic and organized structure composed of polysaccharides, proteins and phenolic compounds (Carpita and Gibeaut 1993). Approximately 90% of the plant cell walls on a dry weight basis are comprised by three major groups of polysaccharides: cellulose, hemicelluloses and pectins (Brett and Waldron 1996). In cereals, the pectins are a minor cell wall constituent but in fruits, pectins represent a high proportion of the wall polysaccharide complement.

Cellulose

Cellulose is a polymer of β-1,4 linked glucose (Brett and Waldron 1996). The individual glucan chains are held together by hydrogen bonds forming a supramolecular structure in which approximately 36 individual glucan chains are associated. These assemblages of glucans are the cellulose microfibrils (Carpita and McCann 2000). Cellulose organization leads to a structure that is highly resistant to enzymatic degradation. In addition cellulose microfibrils have a tensile strength that is comparable to that of steel of the same thickness (Alberts *et al*. 2002). The microfibrils play a major role in determining cell shape and providing load-bearing capacity to plant tissues (Bacic *et al*. 1988). However, as ripening-associated fruit softening occurs, there is little change in cellulosic glucan integrity or microfibrils (Brummell 2006). One exception to this is avocado in which as softening proceeds the cell walls appear to be completely disassembled (Platt-Aloia *et al*. 1980; O'Donoghue *et al*. 1994).

Hemicelluloses

Several different polymers soluble in alkalis are classified as hemicelluloses or cross-linking glycans (Brummell and Harpster 2001). The proportion of hemicellulosic compounds present in fruits usually ranges from 25 to 35% (Carpita and McCann 2000). Xyloglucan is the most abundant hemicellulosic compound in dicot species (Williats *et al*. 2001). It is composed of a backbone of β-1,4-linked glucose with lateral chains of α-1,6 linked xylose which could also be decorated with galactose, arabinose and fucose. Xyloglucan depolymerization accompanying fruit ripening has been observed in most species analyzed so far (Brummell 2006). Although a decrease in the apparent molecular weight of xyloglucan is observed as fruit ripen, relatively large xyloglucan molecules are still observed at advanced stages of development (Brummell 2006). Xylans are a second kind of hemicellulosic compounds found in plant cell walls. They have a backbone of β-1,4-linked xylose and could contain lateral chains rich in arabinose and or glucuronic acid. They are usually highly abundant cross linking-glycans in monocots species, however some studies in berry fruits suggest that these species could have a significant amount of xylose-rich polymers (Vicente *et al*. 2007a). Work in tomato (*Lycopersicon esculentum*) also showed that xyloglucomannan are abundant among hemicellulosic polysaccharides (Seymour *et al*. 1990). In banana the hemicellulosic fractions showed a high proportion of xylose (Xyl) and only traces of glucose (Glc) suggesting that xylans might be also abundant. Interestingly, increased solubilization of Xyl in the water fractions concomitant with a reduction of this sugar in the KOH-soluble fractions during ripening suggests extensive degradation of xylans (Prabha and Bhagyalakshmi 1998). Other hemicellulosic compounds usually less abundant and that have received less attention include glucomannans, galactomannans and galactoglucomannans (Carpita and McCann 2000).

Pectins

Fruit tissues are particularly rich in pectins which can account for 40% of the total cell wall polysaccharides. Particularly rich in polyuronides is the middle lamella, the region in between individual cells. Pectins include very diverse types of galacturonic acid-rich polymers; the different wall pectins contain as many as 17 different monosaccharide building blocks as constituents of polymer backbones and elaborate side chains (Ridley *et al*. 2001). The most abundant polyuronide present in plant cell walls is homogalacturonan, a polymer of α-1,4-linked galacturonic acid residues which can have different degrees of methyl esterification (Williats *et al*. 2001). In addition to the methyl esters at the C6 carboxyl group of galacturonic acid residues, acetyl esters can be found at C2 and C3 (Ishii 1997; Williats *et al*. 2001). Acetyl esterification has been also found in other cell wall components such as xylans. While methyl esters affect the capacity of pectin molecules to interact with each other by calcium bridges, acetyl esters might affect the physicochemical properties of the polymers (e.g. solubility) and could also reduce the action of pectin-digesting enzymes to cleave the polymers. Reduction in pectin polymer size during ripening has been reported in several species (reviewed in Brummell 2006). Some fruits such as avocado (*Persea americana*) show a dramatic downshift in polyuronide size (Huber and O'Donoghue 1993). On the other hand almost no pectin depolymerization is detected in peppers (*Capsicum annum*) and only moderate changes are found in banana (*Musa paradisiaca*), apple (*Malus domestica*) and blueberry (*Vaccinium corymbosum*) (Brummell 2006; Vicente *et al*. 2007a).

Other polyuronides present in plant cell walls are rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII). RGI consists of a backbone of alternating α-1,2-rhamnosyl and α-1,4-galacturonosyl residues (Willats *et al*. 2001). The rhamnosyl residues also can have lateral chains of arabinans, galactans or arabinogalactans (Carpita and McCann 2000). Homogalacturonan has also been suggested to be a side chain of RGI (Vincken *et al*. 2003). Loss in RGI arabinose and galactose by hydrolytic cleavage of the lateral chains of the polymer is a significant feature accompanying fruit ripening (Gross and Sams 1984). In some fruit, such as pears and berry fruits, arabinose is highly abundant and a marked decrease in this sugar (up to 80%) is observed as ripening proceeds. In other commodities such as apple, melon, squash, muskmelon and tomato, the reduction in cell wall galactose content (up to 70%) is a prominent feature of ripening-associated wall compositional change (Gross and Sams 1984). In many cases, these changes are associated with an increase in pectin solubility that precedes pectin depolymerization. It has been speculated that arabinose and galactose side chain removal could have an impact on overall cell wall porosity, increasing the cross-sectional area of the spaces between wall polymers and enhancing access of larger CWDPs to their wall polysaccharide substrates. However, changes in wall porosity accompanying fruit ripening have not been specifically reported although increased cell wall swelling has been described for many ripening fruits (Redgwell *et al*. 1997). Finally, a pectic polymer that has received much attention recently is rhamnogalacturonan II (RGII). RGII is the most complex polysaccharide present in the cell wall with 12 different kinds of sugars on its structure (O'Neill *et al*. 2004). Interestingly, RGII molecules can associate by borate diester bonds to form dimers (Kobayashi *et al*. 1996) and changes in the RG II monomer to dimer ratio affect cell wall mechanical properties, and plant growth (Ishii *et al*. 2001; O'Neill *et al*. 2001, 2004). For instance a reduction in RGII occurring as dimers from 90% in wild type plants to 50% in mutants defective in Arabidopsis *mur1-2* mutants decreased leaf growth five times (O'Neill *et al*. 2004). Hypocotyls from *Arabidopsis* mutants affected in RGII structure also showed reduced tissue tensyl resistance (Ryden *et al*. 2003) leading the authors to conclude that borate-complexed rhamnogalacturonan II contributes to the strength of cell walls. Whether changes in RGII structure or dimerization occur during fruit ripening and exert direct or indirect effects on wall properties (by altering cell wall pore size and mobility of cell wall degrading proteins through the apoplast) has not been characterized.

Cell wall architecture

All the polysaccharides described above together with several groups of structural proteins are responsible for the architecture of the plant cell walls. Hemicelluloses are thought to be associated with the microfibril's surface via the formation of hydrogen bonds. The resulting cellulose-hemicellulose matrix (CHM) is composed of microfibrils and hemicelluloses that both coat and cross-link them (Carpita and McCann 2000) while pectins are thought to fill the spaces between the CHM. The free carboxylic groups of unesterified pectin residues have been shown to ionically interact with calcium ions also present in the apoplast (Williats *et al*. 2001). Consequently a higher degree of methyl-esterification of pectin would decrease the leve1 of inter-pectate calcium cross bridges. Another kind of association of cell wall polymers includes the formation of RG II dimers via borate diester bonds (O'Neill *et al*. 2004). There is general acceptance of the overall structural plan of the pectin network and CHM in primary cell walls and of the kinds of polymers that these walls contain. However, more controversy has arisen in relation to the nature of the interactions between the different wall components and their distribution within the wall. Several cell wall models have been proposed to date:

Covalently-bridged matrix: Early work by the Albersheim group suggested that the primary cell wall can be considered a single macromolecule in which the different components (with the exception of the connection between xyloglucan (XyG) and cellulose) are associated by covalent bonds (Keegstra *et al*. 1973).

Sticky network model: Failure to find further evidence of the covalent associations between cell wall components, led to an alternative, the 'sticky network' model (Cosgrove 2001). In this model XyG binds to additional hemicellulose by hydrogen bonds and interconnects the cellulose microfibrils forming the CHM, while pectic polysaccharides form a second coextensive but independent network.

Multicoat model: This model suggests that hemicelluloses coating the micro-fibrils are not seen as direct bridges between microfibrils, but instead interact with other hemicelluloses and pectins in the space between them (Talbott and Ray 1992).

Stratified model: Ha *et al*. (1997) proposed a stratified model in which cellulose is proposed to be directly cross-linked by XyG, as in the sticky network model. However, in the stratified model, cross-links occur within single microfibril-XyG layers while layers of pectin polymers are located in strata that separate the distinct microfibril-XyG layers.

In the past few years there has been new evidence for covalent interactions between XyG and pectins. This was first described in rose suspension cells (Thompson and Fry 2000) and further work showed that covalent linkages between XyG and pectic polysaccharides are present in several species (Popper and Fry 2005). Recently it was also reported that pectin lateral chains also can bind to cellulose microfibrils (Zykwinska *et al*. 2005) and this interaction may prove to be also of considerable significance in the modeling of plant cell walls.

GENES CODING FOR PROTEINS INVOLVED IN CELL WALL DEGRADATION

Several cell wall degrading enzymes and proteins involved in cell wall disassembly have been characterized (Fisher and Bennett 1991; Hadfield and Bennett 1998; Brummell and Harpster 2001; Cosgrove *et al*. 2002).

Pectin degrading proteins

Polygalacturonase (PG)

PGs, poly (1→4-α-galacturonide) glycanohydrolases, are involved in the hydrolytic cleavage of homogalacturonan (Hadfield and Bennett 1998). PG has been described in several fruit species and its activity usually increases during fruit ripening. Exo-polygalacturonases (EC 3.2.1.67) cleave pectin form the non reducing end removing one sugar residue at a time while endo acting polygalacturanases (EC 3.2.1.15) can hydrolyze the polymer at internal sites. For a long time it was thought that PG played a central role in polyuronide degradation and fruit softening. However, antisense suppression of PG in tomato did not lead to fruit with dramatically reduced softening (Brummell and Harpster 2001) and transgenic lines suppressed in PG activity remained slightly firmer than the untransformed controls only late in ripening. These results suggested that tomato fruit softening could be independent from PG activity. However, it is not clear if this finding holds true for other fruits.

The interaction between the different cell wall degrading agents is not clearly understood yet, but we have recently found that the simultaneous over-expression of *PG* and *Exp1* resulted in tomato fruits that softened faster than control fruit (Vicente *et al*. unpublished results). It would be interesting to evaluate if the combined suppression of PG and other cell wall degrading proteins with potential functional redundancy, such as pectate lyase (Marín Rodríguez *et al*. 2002) also could delay fruit softening.

Pectate lyase (PL; EC 4.2.2.2)

Lyases cleave glycosidic bonds by β-elimination, giving rise to unsaturated products. Among these enzymes, pectin lyases show specificity for methyl esterified substrates (pectin), while pectate lyases catalyze the eliminative cleavage of de-esterified pectin (Medina-Escobar *et al*. 1997). Pectate lyases are widely distributed among microbial plant pathogens (Searle-van Leeuwen *et al*. 1992; Shvchik *et al*. 1997). In the case of fruits they have been identified in banana (Dominguez-Puigjaner *et al*. 1997; Pua *et al*. 2001), grape (*Vitis vinifera*) (Nunan *et al*. 2001) and strawberry (*Fragaria × anannasa*) (Medina-Escobar *et al*. 1997) and although PL activity was previously thought to be absent in tomato fruit (Besford and Hobson 1972), the unsaturated oligosaccharides diagnostic of PL action have been detected (An *et al*. 2005). Suppression of a strawberry pectate lyase (*pl*) genes resulted in significantly firmer fruit and a reduction in both ripening-related *in vitro* wall swelling and in the normal shift of covalently bound wall pectins to chelator-soluble form (Jiménez-Bermúdez *et al*. 2002), suggesting that the enzyme might have a significant role in controlling pectin disassembly and fruit softening.

Pectin methylesterase (PME; EC 3.1.1.11)

Pectin degradation requires the combined action of hydrolases and lyases, which cleave the bonds between the galacturonosyl residues of homogalacturonans and methylesterases, which remove methoxyl groups from pectin (Tieman *et al*. 1992). Pectins are synthesized and secreted to the cell wall with a high degree of methyl esterification (Williats *et al*. 2001). PME-mediated methyl ester removal has several impacts on cell wall properties and metabolism. The liberation of carboxylic groups might increase the capacity of pectins to interact by calcium bridges (Tieman *et al*. 1992). However, demethylated pectin is a preferred substrate for PG. Consequently a reduction in the degree of esterification of the pectin fraction could potentially favor the hydrolysis of homogalacturonan. Tieman and Handa (1994) reported that tomato fruits from transgenic plants with down-regulated PME gene expression showed complete loss of tissue integrity during fruit senescence but this genetic manipulation had little effect on fruit firmness during ripening.

β-galactosidase (β-gal; EC 3.2.1.23)

Galactose loss from the cell wall fraction is also a common feature observed during fruit ripening in several fruits including tomato, pepper, peach, apple, melon and squash (Gross and Sams 1984). β-gal activity has been detected in several fruits. In the case of tomato, seven different β-gal-encoding genes have been identified (Smith and Gross 2000), and lines in which *β-gal4* expression was suppressed showed reduced softening (Smith *et al*. 2002). Interestingly, the effect on fruit firmness was observed in lines in which the gene was suppressed early in development suggesting that the removal of galactose from pectin side chains might be a prerequisite for late ripening softening.

α-arabinofuranosidase (α-Afs; 3.2.1.55)

α-L-arabinofuranosidases are plant enzymes capable of releasing terminal arabinofuranosyl residues from cell wall matrix polymers (Saha *et al*. 2000). Three different α-Af isoforms have been identified in tomato fruit (Sozzi *et al*. 2002). While the activity of isoforms I and II was reduced or showed no changes during ripening, isoform III markedly increased as ripening progressed, identifying it as a candidate for a role in softening-related depolymerization. However, whether or not α-Afs have an important role in fruit textural modifications during ripening has not been determined yet. Recently, a full-length cDNA clone encoding a α-Af (*PpARF2*) was cloned from pear, a fruit which shows extensive loss of arabinose during ripening (Tateishi *et al*. 2005). It would be interesting to determine the impact of suppressing *PpARF2* in pear fruit cell wall metabolism and softening.

Other pectin-degrading enzymes

Other pectin-degrading enzymes have received less attention. These include rhamnogalacturonases (RGases) and acetyl esterases (EC 3.1.1.6). RGases are involved in the cleavage of rhamnogalacturonan I backbones and were initially identified in microorganisms (Mutter *et al*. 1998). The presence of RGases in plants has been reported in some fruits (Gross *et al*. 1995). However, RGase activity in fruit extracts has not been widely tested and so the significance of these enzymes in fruit softening has not been determined. Another set of enzymes that has not received much attention includes acetyl esterases (AEs). Several cell wall components, including pectins and xylans have been shown to be acetylated (Ishii 1997; Dea and Madden 1986). For pectins, the degree of acetylation is variable depending on the species considered. Sugar beet RGI could present high degree of acetylation (DAc 60%), while other fruits such as Japanese quince the DAc is much lower (3%) (Thomas and Thibault 2002). Unfortunately the DAc in different fruit species remains uncharacterized. Despite of it could be hypothesized that acetylation of pectins would affect their physicochemical properties and reduce their susceptibility to degradation by other cell wall degrading enzymes such as PGs, PLs and/or RGases. For instance, pectate lyase secreted by *Erwinia* sp. cleaves only those galacturonic acid residues that are not acetyl-esterified (Davis *et al*. 1984; Shvchik *et al*. 1997). AEs have been identified in tomato (Savary 2001) and purified orange (Williamson 1991). It would be interesting to determine the influence of these enzymes on fruit cell wall integrity.

Glycan-degrading proteins

endo-1,4-β-glucanase (EGases; EC 3.2.1.4)

EGases hydrolyze internal linkages of (1→4)-β-D-linked glucan chains (Brummell and Harpster 2001). The enzyme's *in vivo* substrates are thought to include hemicelluloses

(xyloglucan, glucomannan) and non-crystalline cellulose. EGases have been identified in all fruit species tested (Brummell and Harpster 2001). The suppression of EGase-encoding genes in tomatoes and sweet peppers had no impact on fruit softening and the anticipated reduction of hemicellulose depolymerization in ripening peppers was not detected (Brummell *et al*. 1999a; Harpster *et al*. 2002a). Over-expression of a ripening-related EGase in transgenic tomato fruit did not result in modified fruit softening or XyG depolymerization (Harpster *et al*. 2002b). These results suggest that the enzyme is not a limiting factor for fruit softening or hemicellulose degradation, although the expression of multiple EGase isoforms in some fruits makes this conclusion somewhat uncertain. Whether this is true in other fruits is not clear yet. The interpretation of the roles of EGases in fruit ripening becomes even more complex considering that while some EGases have been shown to be involved in cell wall degradation others are thought to be primarily located in the plasma membrane and involved in polymer synthesis (Nicol *et al*. 1998).

Xyloglucan endotransglucosylase/hydrolases (XTHs; 2.4.1.207)

XTHs are identified by their *in vitro* action as transglucosylases, catalyzing the endo-cleavage of a XyG polymer backbone and subsequent transfer of the newly generated reducing end to the non-reducing terminus of an acceptor XyG. However, some proteins with *in vitro* transferase activity act preferentially as hydrolases (Rose *et al*. 2002a). XTHs have been shown to act on the XyGs attached to cellulose microfibrils (Vissenberg *et al*. 2005). Interestingly, it has been recently shown that the barley XyG xyloglucosyl transferase (HvXET5), can catalyze the formation of covalent linkages between XyGs and cellulosic substrates *in vitro* (Hrmova *et al*. 2007), suggesting a role of the enzyme in cell wall assembly, determining the interactions between different cell wall polymers. XTH expression and activity have been correlated with cell growth rate (Potter and Fry 1994; Catala *et al*. 1997). Increased XTH activity has been observed in some fruits during ripening (Redgwell and Fry 1993; Percy *et al*. 1996). Several XTHs have been isolated from ripe tomato fruit (de Silva *et al*. 1994; Saladie *et al*. 2006) and the potential role of these enzymes in fruit softening is still under study.

Expansin (Exp)

Expansins are relatively small proteins (25–27 kDa) which are able to increase the relaxation of plant cell walls (Cosgrove 2001). They were first identified due to their ability to promote the loosening of the cell wall of cucumber seedlings *in vitro* (McQueen-Mason *et al*. 1995; Cosgrove 2001). Extensive work has been done in the last 15 years to show that expansins are involved in several developmental processes such as growth, abscission, and softening (Cosgrove *et al*. 2002). Expansins have a cellulose binding domain and an endoglucanase-like domain. However, no hydrolytic activity has been demonstrated, and it has been suggested that they may act by disrupting hydrogen bonds between hemicelluloses like XyG and cellulose and consequently increase cell wall relaxation. The suppression of the fruit ripening-associated *Exp1* in tomato fruit decreased fruit softening relative to unmodified controls (Brummell *et al*. 1999b).

Other cell wall degrading enzymes such as glucosidases (3.2.1.21), xylosidases (EC 3.2.1.37-3.2.1.72) xylanases (EC, 3.2.1.8) and mannanases (3.2.1.78) have been identified in ripening fruits but have received less attention and their contribution to fruit softening and cell wall disassembly is not clear.

ROLE OF PLANT CELL WALLS AS A BARRIER AGAINST PATHOGENS

Fleshy fruit, such as peaches, apples, pears and tomatoes are particularly susceptible to rotting organisms and post-harvest diseases account for millions of dollars in losses every year (Narayanasamy 2006). Plant cell walls represent a physical barrier to pathogen infection. While some pathogens rely on natural surface breaks to penetrate host tissues, most pathogens develop within the apoplast as they colonize and infect their hosts (Vorwerk *et al*. 2004). Pathogenic fungi and bacteria utilize secreted CWDPs to digest host cell walls, a process that permits them to extend the zone of infection while they harvest sugar substrates from cell wall polysaccharides.

Among the CWDPs produced by microorganisms are endo- and exo-polygalacturonases, pectate lyases, and endo-1,4 β-glucanases (Colmer and Keen 1986), enzymes that target the key non-cellulosic networks of the host's primary cell walls. The secretion of enzymes that target plant cell walls usually characterizes necrotrophic pathogens, either fungi, such as *Botrytis* spp., or bacteria, such as *Erwinia* spp. However, utilization of CWDPs as a component of the pathogen's infection strategy is not exclusive to necrotrophs. For example, *Claviceps purpurea*, a fungal biotrophic pathogen of cereals and causal agent of the ergot disease, actively digests pectin polymers during its infection of young ovaries. The importance of CWDP production to C. *purpurea*'s pathogenicity is demonstrated by the fact that mutants lacking functional endoPGs are unable to infect compatible rye hosts (Oeser *et al*. 2002).

For many years, the ability to digest plant cell walls has been associated with the virulence of rotting organisms. For example, when one of the six endoPGs secreted by *B. cinerea* was knocked out by partial gene replacement, the mutants retained the pathogenicity on tomato leaves and on apple and tomato fruits, but showed a decrease in their ability to develop expanding macerated lesions (ten Have *et al*. 1998). Black rot symptoms on citrus fruit were also significantly reduced when an endoPG of the fungal pathogen *Alternaria citri* was mutated (Isshiki *et al*. 2001) and PG activity is required for full virulence of *Aspergillus flavus* on cotton bolls. However, probably due to the functional redundancy of the secreted CWDPs, targeted knock outs of these proteins have resulted in a decreased virulence of only a few pathogens (Rogers *et al*. 2000; Valette-Collet *et al*. 2003; Kars *et al*. 2005; Reis *et al*. 2005).

Fruit ripening is typically associated with increased susceptibility to opportunistic pathogens (Giovannoni 2004). In many cases, unripe fruit show high tolerance to fungal infection, whereas these same fruit become increasingly pathogen-susceptible as ripening occurs. Rotting organisms fail to develop on mature green tomato fruit, whereas red ripe fruit are colonized rapidly with extensive tissue maceration (Prusky 2003). Strawberries, blueberries, raspberries and blackberries suffer severe rotting caused by *B. cinerea* and *Rhizopus stolonifer* at ripe stage (Vicente *et al.* 2005). Although infection occurs at early developmental stages, only ripe peaches and grapes show high disease severity form *Monilinia fruticola* (Lee and Bostock 1996) and *B. cinerea* infections (Hill *et al.* 1981); both brown and gray rots are extremely serious diseases of these commodities. In avocado fruit, ripening is accompanied by a dramatic increased susceptibility to *Colletotrichum gloeosporioides*, the causal agent of anthracnose, a major cause of decay during storage of the fruit (Prusky *et al.* 1993).

During fruit ripening cell walls undergo extensive disassembly, a process that is particularly interesting in the context of the relationship of ripening and increased susceptibility because many of the CWDP types produced by ripening fruits are the same as the CWDPs secreted by invading pathogens. Although cell wall metabolism in the context of ripening of many edible fruit has been extensively studied, the contribution of that self-disassembly of cell wall to the susceptibility of the ripening fruit is still a

matter of debate.

The outcome of fruit interactions with necrotrophic pathogens depends on many factors that are unrelated to the ripening-associated disassembly of the host's cell walls. Several authors have reviewed aspects of the preformed factors and induced responses that are related to an effective defense (Elad 1997; Labavitch 1998; Prusky 2003). We will not comment on these aspects of pathogen defenses in this short review. Several observations support the idea that fruit wall metabolism is also important factor in determining susceptibility. For instance, calcium treatments delay cell wall disassembly and have been used to reduce the rate of softening and spoilage of fruits (Poovaiah 1986; Mignani *et al*. 1995). Tomato fruit mutations, such as the nonripening (*nor*) and the never ripe (*nr*) with reduced ripening-associated softening show decreased susceptibility to rotting (Lavy-Meir *et al*. 1989; Kramer *et al*. 1992). Furthermore, tomato fruit with reduced levels of PG (Smith *et al*. 1990; Kramer *et al*. 1992) are less susceptible to *Geotrichum candidum* and *R. stolonifer*. It is not clear whether other proteins involved in ripening-related wall change also affect a fruit's pathogen susceptibility, but this point has not been widely addressed. Suppression of the expression of the tomato fruit expansin gene *LeExp1* resulted in firmer fruit with prolonged shelf-life, but did not reduce the ripened fruit's susceptibility to *B. cinerea* and *Alternaria alternata* (Brummell *et al*. 2002). Interestingly, recombinant *nr* tomato mutants constitutively expressing a full-length *PG* cDNA are only partially more susceptible to *G. candidum* than control *nr* tomatoes (Kramer *et al*. 1992) suggesting that PG activity is not sufficient to cause all the biochemical modifications of the cell walls thought to lead to the increased susceptibility of the ripening wild-type tomato fruit. These observations suggest that the suppression of genes encoding proteins involved in cell wall disassembly during ripening may be a valid approach for developing novel horticultural commodities with reduced susceptibility to microbial spoilage. However, they also make clear that focusing on a single CWDP-encoding gene might not be sufficient to limit the increase in susceptibility that normally accompanies ripening. The simultaneous suppression of multiple fruit CWDP genes could prove to be more useful. For instance, we have recently produced transgenic tomato lines with suppressed expression of both PG and Exp1, two proteins whose independent suppression leads to reduced wall disassembly and firmer fruit (Powell *et al*. 2003). Will these lines produce ripe fruit that are less susceptible to pathogens? While such a strategy might reduce concerns about postharvest treatments of fruits with fungicidal chemicals, it is likely that public reluctance to accept genetically modified horticultural products will remain an issue, at least in the short term. This concern may be overcome using knockout lines developed through chemical mutagenesis and subsequent TILLING (targeting induced local lesions in genomes) to identify fruit lines with altered ability to disassemble wall polysaccharide networks and, potentially, enhanced pathogen tolerance (Henikoff *et al*. 2004). Such efforts would be strengthened by success in identifying which aspects of ripening-associated cell wall metabolism are linked to the increased susceptibility of ripening fruits and development of an efficient phenotyping protocol to efficiently and accurately quantify fruit susceptibility to pathogens and obtain results that are consistent and comparable between different research laboratories. The latter will require the identification of biologically relevant, standard inoculation procedures and also techniques for disease quantitation and measurement of accumulated pathogen biomass (Benito *et al*. 1998; Dewey and Yohalem 2004).

IN DEFENSE OF THE PLANT CELL WALL

Over the eons of interactions with pathogens, plants have evolved strategies to counteract pathogen virulence mechanisms. These include the secretion to the apoplast of proteins that inhibit several of the microbial CWDPs that target the plant cell wall barrier during penetration and infection. Polygalacturonase-inhibiting proteins (PGIPs) have received considerable research attention over the last 3 decades (Albersheim and Anderson 1971; Abu-Goukh *et al*. 1983; Bergmann *et al*. 1994; reviewed in de Lorenzo *et al*. 2001; Gomathi *et al*. 2006), but the involvement in defense of plant proteins that inhibit pathogen pectin lyases (Bugbee *et al*. 1993) and endoxylanases (Debyser *et al*. 1997; Rouau and Surget 1998; McLauchlan *et al*. 1999) has also been described. PGIPs have been identified in several important horticultural commodities, including tomatoes, pears, apples, raspberries and strawberries (Stotz *et al*. 1993, 1994; Yao *et al*. 1995; Mehli *et al*. 2004). PGIPs are selective inhibitors of pathogen PGs, but do not affect plant PGs. Interestingly, transcripts of the tomato fruit PGIP (*LePGIP1*) accumulate during the early stages of fruit development and decline with ripening, suggesting a role in the general resistance of green fruit to pathogen infection (Powell *et al*. 2000). Furthermore, transgenic tomato and grape tissues expressing the PGIP from pear fruit (*pPGIP*) driven by the constitutive promoter CaMV35S display reduced susceptibility to *B. cinerea* (Powell *et al*. 2000; Agüero *et al*. 2005). However, to date field tests of recombinant lines over-expressing *PGIP* gene(s) have not provided data demonstrating a PGIP role in defense. This likely supports the idea that PGIPs are only one component of a fruit's basal pathogen defenses and that, in many situations, other elements of basal defense systems play the more important roles in successful defenses (Stotz *et al*. 2004). Earlier in this review we discussed a few tests of the pathogen susceptibility of transgenic tomato fruits with suppressed expression of the ripening-associated fruit PG genes. These findings support the idea that the infection of fruit tissues by necrotrophic pathogens is assisted by the cell wall disassembly that accompanies ripening. This general idea is further supported by a recent report by Lionetti *et al*. (2007). Earlier work (Wolf *et al*. 2003; Raiola *et al*. 2004) had identified Arabidopsis genes encoding proteins that were potent inhibitors of PME. However, in this case the PME-inhibiting protein (PMEI) inhibited the PMEs of Arabidopsis, not the PMEs of potential pathogens. Transgenic Arabidopsis lines over-expressing the PMEI-encoding sequences displayed reduced endogenous PME activity and were less susceptible to infection by *B. cinerea* (Lionetti *et al*. 2007). Thus, if ripening-associated cell wall disassembly does contribute to ripening-associated increases in pathogen susceptibility, combining in a single transgenic fruit reduced capacity to disassemble cell wall networks during ripening with over-expression of PGIP or another protein inhibitor of pathogen CWDPs might lead to a substantial decrease in ripened fruit pathogen susceptibility and, consequently, substantially reduced use of fungicidal chemicals in the postharvest environment.

THE BEST DEFENSE MAY BE A GOOD OFFENSE: HOST DEFENSES THAT TARGET THE PATHOGEN'S CELL WALLS

Fungal cells also have cell walls. Fungal cell walls are rigid multilayered structures whose principal components, at least for true fungi, are a linear polymer of β-1,4-*N*-acetylglucosamine (chitin), 1,3- and 1,6-β-linked glucans, mannan and proteins, with extensive cross-linking between the components (Adams 2004). This protective shell is fundamental for fungal viability and pathogenicity, not only determining fungal morphology and providing an osmotic protection, but also regulating material exchange with the external environment, adhesion with the substrate, host penetration and cell-to-cell communication. Because of the importance of cell wall in fungal development and pathogenicity, several drugs and fungicides have been designed to target the biogenesis of cell wall components. Polyoxins, naturally occurring molecules that inhibit fungal chitin synthase, are used to control many fungal pathogens of plants (Gooday 1989; Asano 2003). Echinocandins, synthetically modified lipo-

peptides, inhibit the biosynthesis of β-glucans of *Candida* spp. and are used to control these important human pathogens (Denning 2002). Furthermore, the antifungal proprieties of *Trichoderma* spp., which are well known mycoparasitic and antagonistic fungi that are exploited as biocontrol agents of root rotting pathogens, are largely due to their secretion of a set of fungal cell wall-degrading enzymes (Lorito *et al*. 1993). Interestingly, transgenic potato and tobacco plants over-expressing a chitinase gene from *T. harzianum* were highly tolerant to *Rhizoctonia solani*, *Alternaria alternata*, *A. solani* and *B. cinerea* (Lorito *et al*. 1998).

In their defense, plants make use of some of the strategies used by invading pathogens. Many plants produce chitinases and β-1,3-glucanases, hydrolases that can digest fungal cell wall components. These fungal wall-digesting enzymes are considered pathogenesis-related (PR) proteins because their expression often is triggered by pathogen infection. Chitinases catalyze the random hydrolysis of the 1,4-β-linkages between the N-acetyl-glusosamine residues of chitin. Plant chitinases are organized in five different classes based on DNA sequences and structures (Neuhaus *et al*. 1996). *In vitro* experiments demonstrated that some plant chitinases degrade fungal cell walls and reduce fungal growth (Schlumbaum *et al*. 1986). The over-expression of a tobacco class I endochitinase gene in *Nicotiana sylvestris* plants did not result in increased resistance to *Cercospora nicotianae* infection (Neuhaus *et al*. 1991); however there is considerable evidence supporting a role of chitinases in plant defense. Chitinase gene expression generally is induced under biotic and abiotic stresses, although a developmental accumulation of chitinases occurs also. Roby *et al*. (1990) showed the localized activation of a bean chitinase promoter around fungal infection sites upon challenge with *B. cinerea*, *R. solani* and *Sclerotium rofsii*. A delay in disease symptom development and general tolerance to *R. solani* occurred when tobacco seedlings constitutively expressed the bean chitinase gene (Broglie *et al*. 1991). Because of the composite structure of the fungal cell walls, it is likely that the single hydrolytic activity of chitinases does not have a consistent dramatic effect on fungal growth because chitin is embedded in a matrix of glucan fibers. Leah (1991) and Mauch (1988) showed that the antifungal proprieties of plant chitinases are enhanced when β-1,3-glucanases were added in combination with chitinases; the combination likely being more effective because of the added impact on the glucan polymers of fungal walls. *In planta*, susceptibility to fungal attack decreased when chitinase and glucanase genes were over-expressed in combination in transgenic tobacco plants (Zhu *et al*. 1994). Increased tolerance of tobacco plants to *R. solani* was achieved by constitutive co-expression of a class II chitinase, a β-1,3-glucanase and a ribosome inactivating protein from barley (Jach *et al*. 1995). An indirect effect of chitinases and β-1,3-glucanases is also supported by the observation that oligosaccharides, specific oligochitin and β-1,3-1,6-oligoglucan molecules released from the fungal cell wall by plant enzymes can elicit plant responses (Côté and Hahn 1994). Recently, Kaku *et al*. (2006) cloned a plasma membrane receptor of a chitin oligosaccharide elicitor. While plants have co-opted a pathogen strategy by using CWDPs against the invader, pathogens have used a plant strategy to counter this defense. Rose *et al*. (2002b) cloned *Phytophthora sojae* genes encoding proteins that inhibit the "defense" glucanases secreted by soybean.

Besides chitin and glucans, fungal cell wall mannans also appear to play an important role in fungal growth and interaction with different types of host organisms. In *C. albicans*, cell wall β-glucans are masked by mannoproteins that protect the infecting fungus from the inflammatory response of the human immune system that otherwise would be triggered by β-glucans (Wheeler and Fink 2006). Conidia of the fungal rice blast *Magnaporthe grisea* contain mannose as the only carbohydrate in their spore tip mucilage and the polysaccharide seems to be crucial for fungal adhesion to the host surface (Howard and Valent 1996). The toxicity of the PR5 protein osmotin on *Saccharomyces cerevisiae* depends on fungal wall phosphomannans (Ibeas *et al*. 2000). In spite of the evidence identifying mannans as important to the interaction of fungi with animal and plant hosts, so far there have been no studies identifying a plant defense strategy that specifically targets fungal wall mannans. Plants produce mannanases that appear to be involved in important developmental processes, such as seed germination (Nonogaki and Bradford 2000; Mo and Bewley 2002, 2003) and anther and pollen development (Filichkin *et al*. 2004). A tomato fruit mannanase activity that is localized primarily in the epidermis and in the outer pericarp cell layers increases during ripening (Bewley *et al*. 2000); intriguingly, tomato cultivars that do not produce mannanase activity ripen and soften normally, suggesting that these enzymes may not be involved in the disassembly of fruit cell wall mannans. Mannans are abundant polymers of the walls of pathogens like *B. cinerea* and it could be speculated that perhaps the mannanase expression at the fruit periphery represents another example of a plant defense strategy that targets the cell walls of potential pathogens. To date, there have been no tests of the relative pathogen susceptibility of tomato cultivars producing differing amounts of mannanase.

CONCLUSIONS

Recent work in cell wall biology has been fruitful in identifying some agents that seem to be important in determining fruit textural changes. However, understanding and controlling fruit softening seems to be harder than it had been once envisioned. Some of the factors that contribute to this complexity are: 1) the importance of changes different from cell wall polysaccharide degradation such as modifications of turgor pressure to fruit softening is still highly unknown; 2) extensive cell wall disassembly occurs in most fruits, but factors that could be limiting in determining polymer breakdown and softening in a certain species could not be as crucial in other fruits; 3) the presence of multiple forms for many enzymes might determine high redundancy and complicate genetic strategies for controlling fruit textural modifications ('many ways of going soft I'); 4) the presence of different enzymes targeting similar polysaccharides could determine functional redundancy which could also make more difficult to control softening by genetic approaches ('many ways of going soft II"); 5) we still have little understanding of the interaction among different CWDP *in vivo*.

Regarding the role of cell wall polysaccharides and defense proteins in the protection against pathogens there have been many advances such as 1) determining that the action of some but not all plant CWDP might facilitate pathogen colonization; 2) characterizing and identifying some plant secreted inhibitors which could reduce the activity of pathogen CWDP and be part of the defensive arsenal; 3) studying some plant proteins that can target fungal cell walls (chitinases and β-1,3-glucanases). Despite that, much work is still required to identify the whole repertoire of plant proteins involved in the interactions with pathogens at the apoplastic 'battle-front'. In addition while biotechnological approaches to suppress some of the factors enhancing susceptibility or over-express defense factors in the fruits seem to be a feasible, the ultimate practical applications of altering the level of these proteins still need to be determined.

In this scenario the question raised is again: will it be possible to achieve the goal of generating commodities having the textural properties that consumers prefer while simultaneously maintaining cell walls as a barrier to fungal penetration and development? This is difficult to know. In recent years several groups have identified and characterized several genetic determinants of textural modifications in fruits and proteins that could be considered in strategies aiming to increase defenses against pathogens. Research has gone far, but we it still needs to go farther.

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REFERENCES

Abu-Goukh AA, Greve LC, Labavitch JM (1983) Purification and partial characterization of "Bartlett" pear polygalacturonase inhibitors. *Physiological Plant Pathology* **23**, 111

Adams DJ (2004) Fungal cell wall chitinases and glucanases. *Microbiology* **150**, 2029-2035

Agüero CB, Uratsu SL, Greve C, Powell ALT, Labavitch JM, Meredith CP, Dandekar AM (2005) Evaluation of tolerance to Pierce's disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. *Molecular Plant Pathology* **6**, 43-51

Albersheim P, Anderson AJ (1971) Proteins from plant cell walls inhibit polygalacturonases secreted by plant pathogens. *Proceedings of the National Academy of Sciences USA* **68**, 1815-1819

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) *Molecular Biology of the Cell*, Garland Science. Taylor and Francis Group, New York, NY USA, 1294 pp

An HJ, Lurie S, Greve LC, Rosenquist D, Kirmiz C, Labavitch JM, Lebrilla C (2005) Determination of pathogen-related enzyme action by mass spectrometry analysis of pectin breakdown products of plant cell walls. *Analytical Biochemistry* **338**, 71-82

Asano N (2003) Glycosidase inhibitors: update and perspectives on practical use. *Glycobiology* **13**, 93-104

Bacic A, Harris PJ, Stone BA (1988) Structure and function of plant cell walls. In: Priess J (Ed) *The Biochemistry of Plants*, Academic Press, New York, pp 297-371

Benito EP, ten Have A, van't Klooster JW, van Kan JAL (1998) Fungal and plant gene expression during synchronized infection of tomato leaves by *Botrytis cinerea*. *European Journal of Plant Pathology* **104**, 207-220

Bergmann CW, Ito Y, Singer D, Albersheim P, Darvill AG, Benhamou N, Nuss L, Salvi G, Cervone F, de Lorenzo G (1994) Polygalacturonase-inhibiting protein accumulates in *Phaseolus vulgaris* L. in response to wounding, elicitors and fungal infection. *The Plant Journal* **5**, 625-634

Besford RT, Hobson GE (1972) Pectic enzymes associated with softening of tomato fruit. *Phytochemistry* **11**, 873-881

Bewley JD, Banik M, Bourgault R, Feurtado JA, Toorop P, Hilhorst HWM (2000) Endo-β-mannanase activity increases in the skin and outer pericarp of tomato fruits during ripening. *Journal of Experimental Botany* **51**, 529-538

Brett CT, Waldron KW (1996) Physiology and biochemistry of plant cell walls. In: Black M, Charlwood B (Eds) 1. *Topics in Plant Functional Biology*, Chapman and Hall, London, UK, pp 4-43

Broglie K, Chet I, Holliday M, Cressman R, Biddle P, Knowlton S, Mauvais CJ, Broglie R (1991) Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. *Science* **254**, 1194-1197

Brummell DA, Hall BD, Bennett AB (1999a) Antisense suppression of tomato endo-1,4-β-glucanase *Cel2* mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Molecular Biology* **40**, 615-622

Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, Dunsmuir P (1999b) Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *The Plant Cell* **11**, 2203-2216

Brummell DA, Harpster MH (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology* **47**, 311-339

Brummell DA, Howie WJ, Ma C, Dunsmuir P (2002) Postharvest fruit quality of transgenic tomatoes suppressed in expression of a ripening-related expansin. *Postharvest Biology and Technology* **25**, 209-220

Brummell DA (2006) Cell wall disassembly in ripening fruit. *Functional Plant Biology* **33**, 103-119

Bugbee WM (1993) A pectin lyase inhibitor protein from cell walls of sugar beet. *Phytopathology* **83**, 63-68

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal* **3**, 1-30

Carpita N, McCann M (2000) The plant cell wall. In: Buchanan B, Gruissem W, Jones R (Eds) *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists, pp 52-108

Catala C, Rose JK, Bennett AB (1997) Auxin regulation and spatial localization of an endo-1,4-β-D-glucanase and a xyloglucan endotransglycosylase in expanding tomato hypocotyls. *The Plant Journal* **12**, 417-426

Colmer A, Keen NT (1986) The Role of pectic enzymes in plant pathogenesis. *Annual Review of Phytopathology* **24**, 383-409

Cosgrove DJ (2001) Wall structure and wall loosening. A look backwards and forwards. *Plant Physiology* **125**, 131-134

Cosgrove DJ, Li LC, Cho HT, Hoffmann-Benning S, Moore RC, Blecker D (2002) The growing world of expansins *Plant and Cell Physiology* **43**, 1436-1444

Côté F, Hahn MG (1994) Oligosaccharins: Structure and signal trasduction. *Plant Molecular Biology* **26**, 1379-1411

Davis KR, Lyon GD, Darvill AG, Albersheim P (1984) Host pathogen interactions. XXV. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. *Plant Physiology* **74**, 52-60

Dea ICM, Madden JK (1986) Acetylated pectic polysaccharides of sugar beet. *Food Hydrocolloids* **1**, 71-88

Debyser W, Derdelinckx G, Delcour JA (1997) Arabinoxylan solubilization and inhibition of the barley malt xylanolytic system by wheat during mashing with wheat wholemeal adjunct: Evidence for a new class of enzyme inhibitors in wheat. *Journal of the American Society of Brewing Chemists* **55**, 153-156

de Lorenzo G, D'Ovidio R, Cervone F (2001) The role of polygalacturonase-inhibiting proteins (PGIPs) in defense against pathogenic fungi. *Annual Review of Phytopathology* **39**, 313-335

Denning D (2002) Echinocandins: a new class of antifungal. *Journal of Antimicrobial Chemotherapy* **49**, 889-891

de Silva J, Arrowsmith DA, Whiteman S, Robinson S (1994) Xyloglucan endotransglycosylase and plant growth. *Journal of Experimental Botany* **45**, 1693-1701

Dewey FM, Yohalem D (2004) Detection, quantification and immunolocalisation of *Botrytis* species, In: Elad Y, Williamson B, Tudzynski P, Delen N (Eds) *Botrytis: Biology, Pathology and Control*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 181-194

Dominguez-Puigjaner E, Llop I, Vendrell M, Prat S (1997) A cDNA clone highly expressed in ripe banana fruit shows homology to pectate lyases. *Plant Physiology* **114**, 1071-1076

Elad Y (1997) Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. *Biological Reviews* **72**, 381-422

Filichkin SA, Leonard JM, Monteros A, Liu P-P, Nonogaki H (2004) A novel endo-β-mannanase gene in tomato *LeMAN5* is associated with anther and pollen development. *Plant Physiology* **134**, 1080-1087

Fischer RL, Bennett AB (1991) Role of cell wall hydrolases in fruit ripening. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 675-703

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. *The Plant Cell* **16**, S170-S180

Gomathi V, Gayathri S, Anupama B, Teixeira da Silva JA, Gnanamanickam SS (2006) Molecular aspects of polygalacturonase-inhibiting proteins (PGIPs) in plant defense. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol III), Global Science Books, Isleworth, pp 373-379

Gooday GW (1989) Chitin metabolism as a target for antifungal and antiparasitic drugs and agrochemicals. *Progress in Industrial Microbiology* **27**, 139-150

Gross KC, Sams CE (1984) Changes in cell wall neutral sugar composition during fruit ripening: a species survey. *Phytochemistry* **23**, 2457-2461

Gross KC, Starrett DA, Chen HJ (1995) Rhamnogalacturonase, α-galactosidase, and β-galactosidase: potential roles in fruit softening. *Acta Horticulturae* **398**, 121-129

Ha M-A, Apperley DC, Jarvis MC (1997) Molecular rigidity in dry and hydrated onion cell walls. *Plant Physiology* **115**, 593-598

Hadfield KA, Bennett AB (1998) Polygalacturonases: many genes in search of a function. *Plant Physiology* **117**, 337-343

Harpster MH, Brummell DA, Dunsmuir P (2002a) 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 Molecular Biology* **33**, 47-59

Harpster MH, Dawson DM, Nevins DJ, Dunsmuir P, Brummell DA (2002b) Constitutive overexpression of a ripening-related pepper endo-1,4-β-glucanase in transgenic tomato fruit does not increase xyloglucan depolymerization or fruit softening. *Plant Molecular Biology* **50**, 357-369

Henikoff S, Till BJ, Comai L (2004) TILLING. Traditional mutagenesis meets functional genomics. *Plant Physiology* **135**, 630-636

Hill GK, Stellwaag-Kittler F, Huth G, Schlösser E (1981) Resistance of grapes in different development stage. *Journal of Phytopathology-Berlin* **102**, 328-338

Howard RJ, Valent B (1996) Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. *Annual Review of Microbiology* **50**, 491-512

Hrmova M, Farkas V, Lahnstein J, Fincher GB (2007) A barley xyloglucan xyloglucosyl transferase covalently links xyloglucan, cellulosic substrates, and (1,3;1,4)-β-D-glucans. *The Journal of Biological Chemistry* **282**, 12951-12962

Huber DJ, O'Donoghue EM (1993) Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. *Plant Physiology* **102**, 473-480

Ibeas JI, Lee H, Damsz B, Prasad DT, Pardo JM, Hasegawa PM, Bressan

RA, Narasimhan ML (2000) Fungal cell wall phosphomannans facilitate the toxic activity of a plant PR-5 protein. *The Plant Journal* **23**, 375-383

Ishii T (1997) *O*-Acetylated oligosaccharides from pectins of potato tuber cell walls. *Plant Physiology* **113**, 1265-1272

Ishii T, Matsunaga T, Hayashi N (2001) Formation of rhamnogalacturonan II-borate dimer in pectin determines cell wall thickness of pumpkin tissue. *Plant Physiology* **126**, 1698-1705

Isshiki A, Akimitsu K, Yamamoto M, Yamamoto H (2002) Endopolygalacturonase is essential for citrus black rot caused by *Alternaria citri* but not brown spot caused by *Alternaria alternata*. *Molecular Plant-Microbe Interactions* **14**, 749-757

Jach G, Gornhardt B, Mundy J, Logemann J, Pinsdorf E, Leah R, Schell J, Maas C (1995) Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *The Plant Journal* **8**, 97-109

Jiménez-Bermúdez S, Redondo-Nevado J, Muñoz-Blanco J, Caballero JL, López-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. *Plant Physiology* **128**, 751-759

Kader AA (1992) *Postharvest Technology of Horticultural Crops*, University of California, Division of Agriculture and Natural Resources, Publication 3311, California, USA, 296 pp

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proceedings of the National Academy of Sciences USA* **103**, 11086-11091

Kars I, McCalman M, Wagemarkers L, van Kan J (2005) Functional analysis of *Botrytis cinerea* pectin methylesterase genes by PCR-based targeted mutagenesis: *Bcpme1* and *Bcpme2* are dispensable for virulence of strain B05.10. *Molecular Plant Pathology* **6**, 641-652

Keegstra K, Talmadge KW, Bauer WD, Albersheim P (1973) The structure of plant cell walls III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Physiology* **51**, 188-197

Kobayashi M, Matoh T, Azuma J (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiology* **110**, 1017-1020

Kramer M, Sanders R, Bolkan H, Waters C, Sheehy RE, Hiatt WR (1992) Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. *Postharvest Biology and Technology* **1**, 241-255

Labavitch JM (1998) Fruit fripening and defence against pathogens – Loss of resistance or gain of susceptibility? In: Johnson GI, Highly E, Joyce DC (Eds) *Disease Resistance in Fruit*, Australian Centre for International Agricultural Research, Canberra, ACT, Australia, pp 53-59

Lashbrook CC (2005) New insights into cell wall disassembly during fruit ripening. *Stewart Postharvest Review* **1**, 1-18

Lavy-Meir G, Barkai-Golan R, Kopeliovitch E (1989) Resistance of tomato ripening mutants and their hybrids. *Plant Disease* **73**, 976-978

Lee M-H, Bostock RM (2006) Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fruticola* **96**, 1072-1080

Leah R, Tommerup H, Svendsen I, Mundy J (1991) Biochemical and molecular characterization of three barley seed proteins with antifungal properties. *The Journal of Biological Chemistry* **266**, 1564-1573

Lionetti V, Raiola A, Camardella L, Giovane A, Obel N, Pauly M, Favaron F, Cervone F, Bellincampi D (2007) Overexpression of pectin methylesterase inhibitors in Arabidopsis restricts fungal infection by *Botrytis cinerea* *Plant Physiology* **143**, 1871-1880

Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL, Di Pietro A (1993) Chitinolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology* **83**, 302-307

Lorito M, Woo SL, Fernandez IG, Colucci G, Harman GE, Pintor-Toro JA, Filippone E, Muccifora S, Lawrence CB, Zoina A, Tuzun S, Scala F (1998) Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. *Proceedings of the National Academy of Sciences USA* **95**, 7860-7865

Marín-Rodríguez MC, Orchard J, Seymour GB (2002) Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany* **53**, 2115-2119

Mauch F, Mauch-Mani B, Boller T (1988) Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase. *Plant Physiology* **88**, 936-942

McLauchlan WR, Garcia-Coneas MT, Williamson G, Rza M, Ravenstein P, Maat J (1999) A novel class of protein from wheat which inhibits xylanases. *The Biochemistry Journal* **338**, 441-446

McQueen-Mason SJ (1995) Expansins and cell wall expansion. *Journal of Experimental Botany* **46**, 1639-1650

Medina-Escobar N, Cardenas, J, Moyano E, Caballero JL, Muñoz-Blanco J (1997) Cloning, molecular characterization and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants. *Plant Molecular Biology* **34**, 867-877

Mehli L, Schaart JG, Kjellsen TD, Tran DH, Salentijn EMJ, Schouten HJ, Iversen T-H (2004) A gene encoding a polygalacturonase-inhibiting protein (PGIP) shows developmental regulation and pathogen-induced expression in strawberry. *New Phytologist* **163**, 99-110

Mignani I, Greve LC, Ben-Arie R, Stotz HU, Li C, Shackel K, Labavitch JM (1995) The effects of GA3 and divalent cations on aspects of pectin metabolism and tissue softening in ripening tomato pericarp. *Physiologia Plantarum* **93**, 108-115

Mo B, Bewley JD (2002) Beta-mannosidase (EC 3.2.1.25) activity during and following germination of tomato (*Lycopersicon esculentum* Mill.) seeds. Purification, cloning and characterization. *Planta* **215**, 141-152

Mo B, Bewley JD (2003) The relationship between beta-mannosidase and endo-beta-mannanase activities in tomato seeds during and following germination: a comparison of seed populations and individual seeds. *Journal of Experimental Botany* **54**, 2503-2510

Mutter M, Beldman G, Pitson SM, Schols HA, Voragen AGJ (1998) Rhamnogalacturonan-galactopyranosyluronohydrolase. An enzyme that specifically removes the terminal nonreducing galacturonosyl residue in rhamnogalacturonan regions of pectin. *Plant Physiology* **117**, 153-163

Narayanasamy P (2006) *Postharvest Pathogens and Disease Management*, John Wiley and Sons Inc., NJ, USA, 578 pp

Neuhaus JM, Ahl-Goy P, Hinz U, Flores S, Meins FJ (1991) High-level expression of a tobacco chitinase gene in *Nicotiana sylvestris*. Susceptibility of transgenic plants to *Cercospora nicotianae* infection. *Plant Molecular Biology* **16**, 141-151

Neuhaus JM, Ahl-Goy P, Linhorst HJM, Meins FJ (1996) A revised nomenclature of chitinase genes. *Plant Molecular Biology Reporter* **14**, 102-104

Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H (1998) A plasma membrane-bound putative endo-1,4-β-Dglucanase is required for normal wall assembly and cell elongation in Arabidopsis *The EMBO Journal* **17**, 5563-5576

Nonogaki H, Gee OH, Bradford KJ (2000) A germination-specific endo-beta-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. *Plant Physiology* **123**, 1235-1246

Nunan KJ, Davies C, Robinson SP, Fincher GB (2001) Expression patterns of cell wall-modifying enzymes during grape berry development. *Planta* **214**, 257-264

O'Donoghue EM, Huber DJ, Timpa JD, Erdos GW, Brecht JK (1994) Influence of avocado (*Persea americana*) Cx-cellulase on the structural features of avocado cellulose. *Planta* **194**, 573-584

Oeser B, Heidrich PM, Muller U, Tudzynski P, Tenberge KB (2002) Polygalacturonase is a pathogenicity factor in the *Claviceps purpurea*/rye interaction. *Fungal Genetics and Biology* **36**, 176-186

O'Neill MA, Eberhard S, Albersheim P, Darvill AG (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth. *Science* **294**, 846-849

O'Neill MA, Ishii T, Albersheim P, Darvill AG (2004) Rhamnogalacturonan II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Review of Plant Biology* **55**, 109-139

Owino WO, Ambuko JL, Mathooko FM (2005) Molecular basis of cell wall degradation during fruit ripening and senescence. *Stewart Postharvest Review* **1**, 1-10

Percy AE, O'Brien IEW, Jameson PE, Melton LD, MacRae EA, Redgwell RJ (1996) Xyloglucan endotransglycosylase activity during fruit development and ripening of apple and kiwifruit. *Physiologia Plantarum* **96**, 43-50

Platt-Aloia KA, Thomson WH, Young RE (1980) Ultrastructural changes in the walls of ripening avocados: Transmission, scanning and freeze-fracture microscopy. *Botanical Gazette* **141**, 366-373

Poovaiah BW (1986) Role of calcium in prolonging storage life of fruits and vegetables. *Food Technology* **40**, 86-89

Popper ZA, Fry SC (2005) Widespread occurrence of a covalent linkage between xyloglucan and acidic polysaccharides in suspension-cultured angiosperm cells. *Annals of Botany* **96**, 91-99

Potter I, Fry SC (1994) Changes in xyloglucan endotransglycosylase (XET) activity during hormone-induced growth in lettuce and cucumber hypocotyls and spinach cell suspension cultures. *Journal of Experimental Botany* **45**, 1703-1710

Powell ALT, van Kan J, ten Have A, Visser J, Greve LC, Bennett AB, Labavitch JM (2000) Transgenic expression of pear PGIP in tomato limits fungal colonization. *Molecular Plant-Microbe Interactions* **13**, 942-950

Powell ALT, Kalamaki MS, Kurien PA, Gurrieri S, Bennett AB (2003) Simultaneous transgenic suppression of LePG and LeExp1 influences fruit texture and juice viscosity in a fresh-market tomato variety. *Journal of Agricultural and Food Chemistry* **51**, 7450-7455

Prabha TN, Bhagyalakshmi N (1998) Carbohydrate metabolism in ripening banana fruit. *Phytochemistry* **48**, 915-919

Prusky D (2003) Mechanism of resistance of fruits and vegetables to postharvest diseases. In: Bartz JE, Brecht JK (Eds) *Postharvest Physiology and Pathology of Vegetables*, Marcel Dekker, Inc., NY, USA, pp 581-593

Prusky D, Keen NT (1993) Involvement of preformed antifungal compounds in the resistance of subtropical fruit to fungal decay. *Plant Disease* **77**, 114-119

Pua EC, Ong CK, Liu P, Liu JZ (2001) Isolation and expression of two pectate lyase genes during fruit ripening of banana (*Musa acuminata*). *Physiolo-*

gia Plantarum **113**, 92-99

Raiola A, Camardella L, Giovane A, Mattei B, de Lorenzo G, Cervone F, Bellincampi D (2004) Two *Arabidopsis thaliana* genes encode functional pectin methylesterase inhibitors. *FEBS Letters* **557**, 199-203

Redgwell RJ, Fry SC (1993) Xyloglucan endotransglycosylase activity increases during kiwifruit (*Actinidia deliciosa*) ripening: Implications for fruit softening. *Plant Physiology* **103**, 1399-1406

Redgwell RJ, MacRae E, Hallett I, Fisher M, Perry J, Harker R (1997) *In vivo* and *in vitro* swelling of cell walls during fruit ripening. *Planta* **203**, 162-173

Reis H, Pfiffi S, Hahn M (2005) Molecular and functional characterization of a secreted lipase from *Botrytis cinerea*. *Molecular Plant Pathology* **6**, 257-267

Ridley BL, O'Neill MA, Mohnen D (2001) Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry* **57**, 929-967

Roby D, Broglie K, Cressman R, Biddle P, Chet IL, Broglie R (1990) Activation of a bean chitinase promoter in transgenic tobacco plants by phytopathogenic fungi. *The Plant Cell* **2**, 999-1007

Rogers LM, Kim Y-K, Guo W, Gonzalez-Candelas L, Li D, Kolattukudy PE (2000) Requirement for either a host- or pectin-induced pectate lyase for infection of *Pisum sativum* by *Nectria hematococca*. *Proceedings of the National Academy of Sciences USA* **97**, 9813-9818

Rose JKC, Braam J, Fry SC, Nishitani K (2002a) The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolysis: Current perspectives and a new unifying nomenclature. *Plant and Cell Physiology* **43**, 1421-1435

Rose JKC, Ham K-S, Darvill AG, Albersheim P (2002b) Cloning and characterization of glucanase inhibitor proteins: Coevolution of a counter-defense mechanism by plant pathogens. *Plant Cell* **14**, 1329-1345

Rouau X, Surget A (1998) Evidence for the presence of a pentosanase inhibitor in wheat flours. *Journal of Cereal Science* **28**, 63-70

Ryden P, Sugimoto-Shirasu K, Smith AC, Findlay K, Dieter Reiter W, McCann MC (2003) Tensile properties of *Arabidopsis* cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-borate complexes. *Plant Physiology* **132**, 1033-1040

Saha BC (2000) α-L-Arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. *Biotechnological Advances* **18**, 403-423

Saladie M, Rose JKC, Cosgrove DJ, Catala C (2006) Characterization of a new xyloglucan endotransglucosylase/hydrolase (XTH) from ripening tomato fruit and implications for the diverse modes of enzymic action. *The Plant Journal* **47**, 282-295

Savary BJ (2001) Perfusion chromatography separation of the tomato fruit-specific pectin methylesterase from a semipurified commercial enzyme preparation. *Preparative Biochemistry and Biotechnology* **31**, 241-258

Schlumbaum A, Mauch F, Vogeli U, Boller T (1986) Plant chitinases are potent inhibitors of fungal growth. *Nature* **324**, 365-367

Searle-van Leeuwen MJF, van den Broek LAM, Schols HA, Beldman G, Voragen AGJ (1992) Rhamnogalacturonan acetylesterase: a novel enzyme from *Aspergillus aculeatus*, specific for the deacetylation of hairy (ramified) regions of pectins. *Applied Microbiology and Biotechnology* **38**, 343-349

Sexton R, Palmer JM, Whyte NA, Littlejohns S (1997) Cellulase, fruit softening and abscission in red raspberry *Rubus idaeus* L. cv. Glen Clova. *Annals of Botany* **80**, 371-376

Seymour GB, Colquhoun IJ, Dupont MS, Parsley KR, Selvendran RR (1990) Composition and structural features of cell wall polysaccharides from tomato fruits. *Phytochemistry* **29**, 725-731

Shackel KA, Greve C, Labavitch JM, Ahmadi H (1991) Cell turgor changes associated with ripening in tomato. *Plant Physiology* **97**, 814-816

Shvchik VE, Beaudouy JR, Cotte-Pattat NH (1997) Pectate lyase *PelI* of *Erwinia chrysanthemi* 3937 belongs to a new family *Journal of Bacteriology* **179**, 7321-7330

Smith CJS, Watson CF, Morris PC, Bird CR, Seymour GB, Grey JE, Arnold C, Tucker GA, Schuch W, Harding S, Grierson D (1990) Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. *Plant Molecular Biology* **14**, 369-379

Smith DL, Gross KC (2000) A family of at least seven β-galactosidase genes is expressed during tomato fruit development. *Plant Physiology* **123**, 1173-1183

Smith DL, Abbott JA, Gross KC (2002) Down-regulation of tomato β-galactosidase 4 results in decreased fruit softening. *Plant Physiology* **129**, 1755-1762

Sozzi GO, Greve LC, Prody GA, Labavitch JM (2002) Gibberellic acid, synthetic auxins, and ethylene differentially modulate α-L-arabinofuranosidase activities in antisense 1-aminocyclopropane-1-carboxylic acid synthase tomato pericarp discs. *Plant Physiology* **129**, 1330-1340

Stotz HU, Powell ALT, Damon SE, Greve LC, Bennett AB, Labavitch JM (1993) Molecular characterization of a polygalacturonase inhibitor from *Pyrus communis* L. cv. Bartlett. *Plant Physiology* **102**, 133-138

Stotz HU, Contos JA, Powell ALT, Bennett AB, Labavitch JM (1994) Structure and function of an inhibitor of fungal polygalacturonase from tomato. *Plant Molecular Biology* **25**, 607-616

Stotz HU, Elad Y, Powell ALT, Labavitch JM (2004) Innovative approaches to *Botrytis* suppression. In: Elad Y, Williamson B, Tudzynski P, Delen N (Eds) *Botrytis: Biology, Pathology and Control*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 369-392

Talbott LD, Ray PM (1992) Changes in molecular size of previously deposited and newly synthesized pea cell wall matrix polysaccharides. Effects of auxin and turgor. *Plant Physiology* **98**, 369-379

Tateishi A, Mori H, Watari K, Nagashima K, Yamaki Y, Inoue H (2005) Isolation, characterization, and cloning of α-L-arabinofuranosidase expressed during fruit ripening of Japanese pear. *Plant Physiology* **138**, 1653-1664

ten Have A, Mulder W, Visser J, van Kan JAL (1998) The endopolygalacturonase gene *Bcpg1* is required for full virulence of *Botrytis cinerea*. *Molecular Plant-Microbe Interactions* **11**, 1009-1016

Thomas M, Thibault JF (2002) Cell wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*): Extraction and preliminary characterization. *Carbohydrate polymers* **49**, 345-355

Thompson JE, Fry SC (2000) Evidence for covalent linkage between xyloglucan and acidic pectins in suspension-cultured rose cells. *Planta* **211**, 275-286

Tieman DM, Harriman RW, Ramamohan G, Handa AK (1992) An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. *The Plant Cell* **4**, 667-679

Tieman DM, Handa AK (1994) Reduction in pectin methylesterase activity modifies tissue integrity and cation levels in ripening tomato (*Lycopersicon esculentum* Mill.) fruits. *Plant Physiology* **106**, 429-436

Valette-Collet O, Cimerman A, Reignault P, Levis C, Boccara M (2003) Disruption of *Botrytis cinerea* pectin methylesterase gene *Bcpme1* reduces virulence on several host plants. *Molecular Plant-Microbe Interactions* **16**, 360-367

Vicente AR, Civello PM, Martínez GA, Powell ALT, Labavitch JM, Chaves AR (2005) Control of postharvest spoilage in soft fruit. *Stewart Postharvest Review* **4**, 1-11

Vicente AR, Ortugno C, Rosli H, Powell ALT, Greve C, Labavitch JML (2007a) Temporal sequence of cell wall disassembly events in developing fruits. 2. Analysis of blueberry (*Vaccinium* Species). *Journal of Agricultural and Food Chemistry* **55**, 4119-4124

Vicente AR, Saladié M, Rose JKC, Labavitch JM (2007b) The linkage between cell wall metabolism and fruit softening: looking to the future. *Journal of the Science of Food and Agriculture* **87**, 1435-1448

Vincken J-P, Schols HA, Oomen RJFJ, McCannMC, Ulvskov P, Voragen AJG, Visser RGF (2003) If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiology* **132**, 1781-1789

Vissenberg K, Fry SC, Pauly M, Höfte H, Verbelen JP (2005) XTH acts at the microfibril–matrix interface during cell elongation. *Journal of Experimental Botany* **56**, 673-683

Vorwerk S, Somerville S, Somerville C (2004) The role of plant cell wall polysaccharide composition in disease resistance. *Trends in Plant Science* **9**, 203-209

Wheeler RT, Fink GR (2006) A Drug-Sensitive Genetic Network Masks Fungi from the Immune System. *PLoS Pathogens* **2**, e35

Williamson G (1991) Purification and characterization of pectin acetylesterase from orange peel. *Phytochemistry* **30**, 445-449

Willats WGT, McCartney L, Mackie W, Knox JP (2001) Pectin: cell biology and prospects for functional analysis. *Plant Molecular Biology* **47**, 9-27

Wolf S, Grsch-Rausch S, Rausch T, Greiner S (2003) Identification of pollen-expressed pectin methylesterase inhibitors in Arabidopsis. *FEBS Letters* **555**, 551-555

Yao C, Sams CE (1995) Purification and characterization of a polygalacturonase-inhibiting protein from apple fruit. *Phytopathology* **85**, 1373-1377

Zhu Q, Maher EA, Masoud S, Dixon RA, Lamb CJ (1994) Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase genes in transgenic tobacco. *Nature Biotechnology* **12**, 807-812

Zykwinska AW, Ralet MCJ, Garnier CD, Thibault JFJ (2005) Evidence for *in vitro* binding of pectin side chains to cellulose. *Plant Physiology* **139**, 397-407