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DARWIN REVIEW

Homogalacturonan-modifying enzymes: structure, expression, and roles in plants

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Abstract

Understanding the changes affecting the plant cell wall is a key element in addressing its functional role in plant growth and in the response to stress. Pectins, which are the main constituents of the primary cell wall in dicot species, play a central role in the control of cellular adhesion and thereby of the rheological properties of the wall. This is likely to be a major determinant of plant growth. How the discrete changes in pectin structure are mediated is thus a key issue in our understanding of plant development and plant responses to changes in the environment. In particular, understanding the remodelling of homogalacturonan (HG), the most abundant pectic polymer, by specific enzymes is a current challenge in addressing its fundamental role. HG, a polymer that can be methylesterified or acetylated, can be modified by HGMEs (HG-modifying enzymes) which all belong to large multigenic families in all species sequenced to date. In particular, both the degrees of substitution (methylesterification and/or acetylation) and polymerization can be controlled by specific enzymes such as pectin methylesterases (PMEs), pectin acetylesterases (PAEs), polygalacturonases (PGs), or pectate lyases-like (PLLs). Major advances in the biochemical and functional characterization of these enzymes have been made over the last 10 years. This review aims to provide a comprehensive, up to date summary of the recent data concerning the structure, regulation, and function of these fascinating enzymes in plant development and in response to biotic stresses.

Key words: Biotic stress, development, homogalacturonans, pectate lyase-like, pectin methylesterase, pectins, polygalacturonase.

Introduction

The growth of plant organs involves cell expansion and cell division. Although the regulation of cell division is relatively well understood, very little is known about the control of cell expansion. It is driven by turgor pressure and notably depends on changes in the extensibility of the primary cell wall. In dicotyledonous species, such as the model plant *Arabidopsis thaliana*, the primary cell wall consists of a hydrogen-bonded network of cellulose microfibrils and xyloglucans (XyGs) embedded in a complex pectic and protein matrix (Carpita and Gibeaut, 1993). Cell growth requires creep between cellulose and XyG, which is facilitated by the presence of specific proteins such as expansins (Rose *et al.*, 2002; Cosgrove, 2005). In addition, in dicots and gymnosperms, secondary growth and biomass production involve the synthesis of large amounts of lignified secondary cell wall.

Pectins, which control cell wall porosity and hydration level as well as cellular adhesion, are known to show structural variation during growth and in response to stresses (Willats *et al.*, 2001). Recent findings underline the importance of pectins in the control of cell expansion and differentiation, presumably through changes in the rheological properties of the cell wall. Pectins, which are complex polysaccharides rich in galacturonic acid (Gal-A), contain distinct domains—homogalacturonans (HGs), rhamnogalacturonans I (RGIs), rhamnogalacturonans II (RGIIs), and xylogalacturonans (XGs)—based on two molecular backbones and differing in the diversity of their side chains (Vincken *et al.*, 2003;

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Mohnen, 2008; Burton *et al.*, 2010). HG, one of the main pectic constituents, is a linear homopolymer of α-(1–4)-linked D-Gal-A, which can either be methylesterified at the C-6 carboxyl (typically 80%;Wolf *et al.*, 2009*b*) or carry acetyl groups at O2 or O3 (up to 10%; Gou *et al.*, 2012). HG is synthesized from nucleotide sugars in the Golgi apparatus, and then secreted in a fully methylesterified form into the cell wall where its structure can be modified by the activity of cell wall enzymes. These will subsequently be referred to as HGMEs (HG-modifying enzymes), all of which, in *A. thaliana*, belong to large multigenic families. In particular, HGs can be modified by pectin methylesterases (PMEs; EC 3.1.1.11), whose activity is regulated by endogenous PMEIs (pectin methylesterase inhibitors) and which control the degree of methylesterification (DM) of HGs (Pelloux *et al.*, 2007; Wolf *et al.*, 2009*b*). Pectin acetylesterases (PAEs; EC 3.1.1.6) play a similar role by hydrolysing the *O*-acetylated bonds. Overall, the partially demethylesterified HGs can either form Ca^{2+} bonds, which promote the development of the so-called 'egg box' structures that underlie the formation of pectin gels, or become a target for pectin-degrading enzymes such as polygalacturonases (endo-PGs, EC 3.2.1.15; and exo-PGs, EC 3.2.1.67) and pectate lyases-like (PLLs), including pectate lyases (endo-PLs, EC 4.2.2.2; and exo-PLs, EC 4.2.2.9) and pectin lyases (endo-PNLs; EC 4.2.2.10). In either case, the demethylesterification of HGs has dramatic consequences on the rheological properties of the cell wall (Peaucelle *et al.*, 2011*a*, *b*), and is predicted to affect cell growth. In addition, the hydrolysis of partially demethylesterified HGs can release oligogalacturonides (OGs) with a signalling function, for instance during plant–pathogen interactions (Lionetti *et al.*, 2007; Osorio *et al.*, 2008) or in modulating growth by inhibiting auxin-induced processes such as stem elongation, rhizogenesis, and flower and stomata formation (Ridley *et al.*, 2001; Falasca *et al.*, 2008; Camejo *et al.*, 2011). However, to date, the presence of endogenous OGs *in planta* has remained elusive.

This review describes the most recent findings concerning the biochemical characterization of HGMEs and highlights the diversity of their roles during plant development and in responses to biotic stresses.

Inventory and structure of HGMEs

All HGMEs belong to large multigenic families

An analysis of the data generated by sequencing projects shows that all HGMEs belong to rather large multigenic families in several plant species [CAZy, http://www.cazy.org/ (Cantarel *et al.*, 2009); Cell Wall Genomics, http://cellwall.genomics.purdue.edu/; TAIR, http://www.arabidopsis.org/]. For instance, in dicotyledonous species such as *A. thaliana* and poplar (*Populus trichocarpa*), 66 and 88 open reading frames (ORFs) have been annotated, respectively, as putative full-length PMEs (Geisler-Lee *et al.*, 2006; Pelloux *et al.*, 2007). In contrast, in grass species such as *Brachypodium distachyon* and rice (*Oryza sativa* japonica group), only 41 putative PME-encoding genes have been identified for both species (Table 1). This lower number of *PME* genes in grass species is likely to be related to the differences in the structure of cell wall polysaccharides between poales and dicots. In particular, HGs are known to be much less abundant and

Table 1. *Inventory of the PME, PAE, PG, and PLL isoforms in four sequenced plant species*

Species	Pectin methylesterase (EC 3.1.1.11) CE8	Pectin acetylesterase (EC 3.1.1.6) CE13	Endo-polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonosidase (EC 3.2.1.82), rhamnogalacturonase (EC 3.2.1.171), endo-xylogalacturonan hydrolase (EC 3.2.1.–), rhamnogalacturonan α-L-rhamnopyranohydrolase (EC 3.2.1.40) GH28	Endo-pectate lyase (EC 4.2.2.2), exo-pectate lyase (EC 4.2.2.9), endo-pectin lyase (EC 4.2.2.10) PL1
Arabidopsis thaliana (dicots)[a]	66	12	68	26
Populus trichocarpa (dicots)[b]	88	10	89	28
Brachypodium distachyon (monocots)[c]	41	7	41	7
Oryza sativa Japonica group (monocots)[d]	41	10	45	12

[a] Full-length *Arabidopsis* protein data were retrieved from Cell Wall Genomics (http://cellwall.genomics.purdue.edu/), CAZy (http://www.cazy.org/), TAIR (http://www.arabidopsis.org/), the PFAM database (http://pfam.sanger.ac.uk/), Tian *et al.* (2006), Gonzalez-Carranza *et al*. (2012), Sun and Van Nocker (2010), and Cao (2012).
[b] *Populus* protein information were retrieved from Geisler-Lee *et al.* (2006).
[c] *Brachypodium* protein data were retrieved from the International Brachypodium Initiative (2010), Tyler *et al.* (2010), and Davidson *et al.* (2012).
[d] Full length *Oryza* protein sequence information were retrieved from Cell Wall Genomics (http://cellwall.genomics.purdue.edu/), CAZy (http://www.cazy.org/), and Davidson *et al.* (2012).
The CAZy code for each family protein was retrieved from CAZy (http://www.cazy.org/).

less methylesterified in grass species (Carpita and Gibeaut, 1993; Vogel, 2008; Burton *et al.*, 2010). The need for HGMEs would therefore be more limited and could explain the differences in the figures observed between type I and type II cell wall species when considering other HGMEs such as PGs and PLLs (Table 1). Surprisingly, regarding the *PAE* gene family, dicotyledonous and grass species have largely the same number of isoforms, with 12, 10, 7, and 11 ORFs annotated as a putative PAE for *A. thaliana*, *P. trichocarpa*, *B. distachyon*, and *O. sativa* japonica group, respectively (Table 1). The large difference between the number of PME and PAE isoforms in dicots could be related to the occurrence of specific substrates. HGs are known to be highly methylesterified (up to 80%; Wolf *et al.*, 2009*b*) while their degree of acetylation (DA) falls within the range of 0.25–10% (Gou *et al.*, 2012) depending on the species. In addition to HG, a number of plant cell wall polysaccharides, such as RGIs, XyGs, and glucuronoarabinoxylans (GAXs), can be *O*-acetylated (Kiefer *et al.*, 1989; Ishii, 1997; Kabel *et al.*, 2003; Fry, 2004; Gibeaut *et al.*, 2005; Gille and Pauly, 2012). The large amount of GAX and the presence of *O*-acetyl-substituents observed in grass species (Carpita and Gibeaut, 1993; Vogel, 2008; Burton *et al.*, 2010) could thus constitute targets for PAE activity. The diversity of putative targets for PAEs would therefore explain the relatively similar number of isoforms between type I and type II cell wall species.

In order to act on HGs *in muro*, HGMEs, which are synthesized in the endoplasmic reticulum and post-translationally modified in the Golgi apparatus (glycosylation and other modifications), must be secreted into the cell wall by exocytosis (Wolf *et al.*, 2009*a*, *b*; Worden *et al.*, 2012). Several isoforms have been shown to be present in the cell wall proteome (Borderies *et al.*, 2003; Boudart *et al.*, 2005; Charmont *et al.*, 2005; Irshad *et al.*, 2008; Jamet *et al.*, 2009). A signal peptide and/or a transmembrane domain at the N-terminus enables their secretion. Analysis of HGME sequences in *A. thaliana* and *O. sativa* japonica group reveals that the majority of sequences (65% and 57%, respectively) show a signal peptide (SP). Many sequences also possess a transmembrane domain (TM; 24% and 22%, respectively) and some possess both (Supplementary Table S1 available at *JXB* online). Surprisingly, for each family, a number of putative soluble isoforms can be identified. The proportion of soluble protein is higher in grass species (17%) than in dicot species (8%). The targets and the functional role of these soluble isoforms remain to be determined. The biochemical characterization of a soluble AtPME suggested that it could act in defence mechanisms against pathogens (Dedeurwaerder *et al.*, 2009).

Structure of HGMEs

To date, among plant HGMEs, 3D crystallographic structures have only been resolved for carrot and tomato PMEs (Johansson *et al.*, 2002; D'Avino *et al.*, 2003; Di Matteo *et al.*, 2005). The first crystallization of bacterial PME from *Erwinia chrisanthemi* was previously performed (Jenkins *et al.* 2001), and its co-crystallization with HGs helped in determining the key amino acids involved at the catalytic site in enzyme–substrate interactions (Fries *et al.*, 2007). This study not only highlighted the hydrolysis mechanism, but also the processive action of *Erwinia* PME (Fries *et al.*, 2007). So far, no PGs and PLs from plants have been resolved at their structural level. However, 3D crystallographic structures of PLs (Pickersgill *et al.*, 1994; Yoder and Jurnak, 1995; Lietzke *et al.*, 1996; Jenkins and Pickersgill, 2001; Jenkins *et al.*, 2004; Creze *et al.*, 2008; Seyedarabi *et al.*, 2010; Zheng *et al.*, 2012) and PGs (Pickersgill *et al.*, 1998; Van Santen *et al.*, 1999; Cho *et al.*, 2001; Jenkins and Pickersgill, 2001; Van Pouderoyen *et al.*, 2003; Bonivento *et al.*, 2007) from plant pathogens are available (PDB Protein Data Bank http://www.rcsb.org/pdb/home/home.do; Punta *et al.*, 2012). Identification of structural motifs was inferred from these structures, enabling models of plant PGs and PLLs to be generated (PFAM database http://pfam.sanger.ac.uk/). The structure of plant PAEs has not yet been resolved.

In *A. thaliana*, 66 PME isoforms are divided into two groups (Pelloux *et al.*, 2007; Dedeurwaerder *et al.*, 2009). The first, called group 1 (or type II), is composed of 21 isoforms that contain a mature active part (PME catalytic domain, Pfam01095), either preceded by different targeting motifs (SP for 14 sequences, SP/TM for 1 sequence, TM for 3 sequences) or without a motif for three soluble isoforms (Fig. 1A). In previous publications (Tian *et al.*, 2006; Pelloux *et al.*, 2007), two group 2 PMEs (At3g10720 and At4g33220) were considered to be group 1 PMEs as the protein sequences used to determine domains were truncated. The second group, so-called group 2 (or type I), is composed of 45 isoforms which have, in addition to the mature part, an N-terminal extension (PRO region) showing similarities with the PMEI domain (Pfam04043). As for group 1 PMEs, different targeting motifs can be identified for the 44 sequences, one being predicted to be soluble (Fig. 1A). Figures for other species are shown in Supplementary Table S1 at *JXB* online. Among group 2 PMEs, 43 isoforms show conserved dibasic amino acid sequences (such as RRLL, RKLL, KKDL, RKLM, RRLM, RKLA, RKLK, RKLR, and RRML) upstream of the mature active part, which could constitute one or more proteolytic cleavage sites. This domain, the so-called processing motif (PM), could be targeted by subtilisin-like proteases (SBTs; 56 isoforms in *Arabidopsis*) during PME *trans*-Golgi trafficking (Wolf *et al.*, 2009*a*; Schaller *et al.*, 2012). A large number of group 2 PMEs and SBTs are co-expressed during development and in response to stresses. Processing of group 2 PMEs was shown to be a prerequisite for the secretion of active enzymes in the cell wall (Wolf *et al.*, 2009*a*) and it was suggested that the PRO region could prevent group 2 PME activity during their transport through the secretory pathway (Bosch and Hepler, 2005; Bosch *et al.*, 2005; Dorokhov *et al.*, 2006). These results are notably backed up by the fact that, in the cell wall proteome, identified PMEs often lack this domain (E. Jamet, personal communication).

When considering AtPGs, the majority of the sequences show a single glycosyl hydrolase family 28 domain (GH28; Pfam00295). This is preceded by a targeting motif at the N-terminus for 59 isoforms, while five isoforms are predicted as soluble (Fig. 1B). Among PG isoforms, there are also four

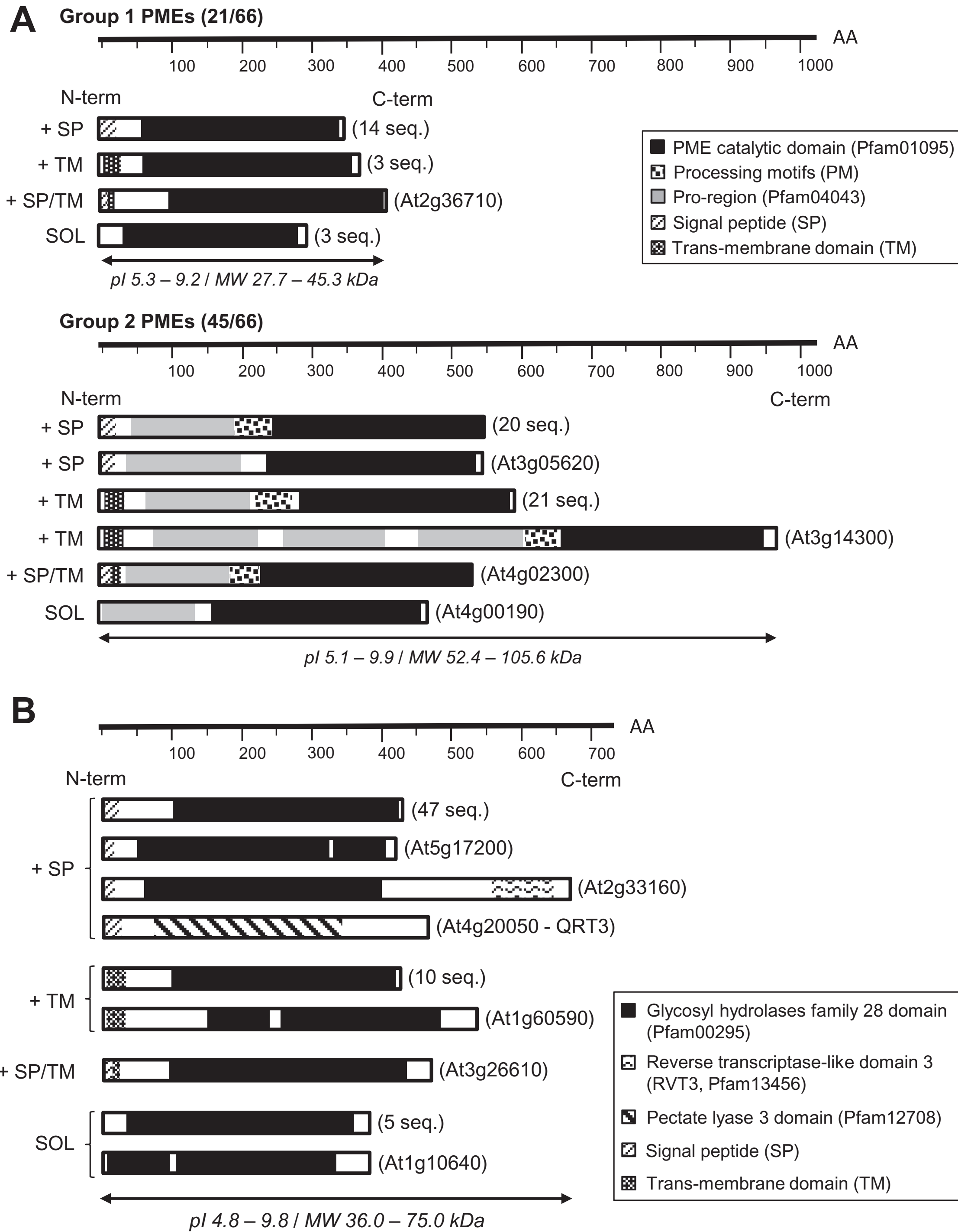

Fig. 1. *Arabidopsis* PME (A) and PG (B) structural motifs. The domains, average size of proteins in amino acid (AA), isoelectric point (pI), and molecular weight (MW) ranges of representative members are indicated according to the PFAM database (http://pfam.sanger.ac.uk/), SignalP4.0 (http://www.cbs.dtu.dk/services/SignalP/), and ExPASyProtParam Tool (http://web.expasy.org/protparam/).

atypical protein sequences, three of which harbour two GH28 domains and one with a reverse transcriptase-like 3 domain (RVT3; Pfam13456) downstream of the GH28 domain. Two asparagine and histidine residues have been identified in the GH28 domain for 53 sequences. One ORF, previously annotated as putative PG QRT3 (At4g20050; Rhee *et al.*, 2003), lacks the GH28 domain but has a pectate lyase 3 domain (Pfam12708). The number of putative PGs would therefore be 67, in line with recent published data (Cao, 2012).

For AtPLLs, all the sequences annotated as PL (26 sequences) show a Pec_lyase_C domain (Pfam00544), and only three isoforms are predicted as soluble proteins (Fig. 2A). This multigenic family also has specific features, with the presence of an N-terminal domain, called Pec_lyase_N (Pfam04431), in four sequences. Lastly, one protein (At3g54920 or PMR6), which plays a role in powdery mildew resistance, shows an additional C-terminal glycosylphosphatidylinositol (GPI) anchor site, which may be responsible

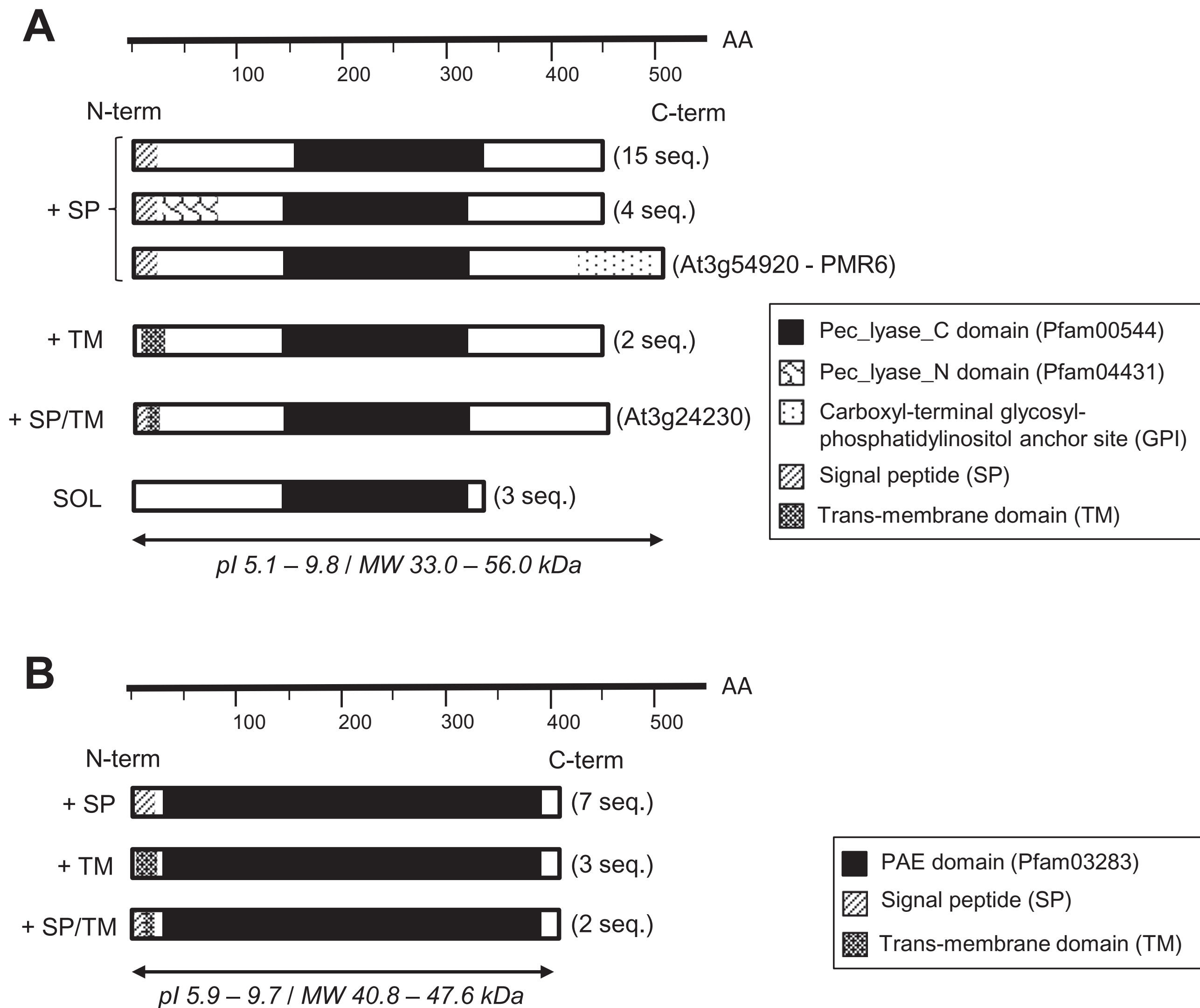

Fig. 2. *Arabidopsis* PLL (A) and PAE (B) structural motifs. The domains, average size of proteins in amino acid (AA), isoelectric point (pI), and molecular weight (MW) ranges of representative members are indicated according to the PFAM database (http://pfam.sanger.ac.uk/), SignalP4.0 (http://www.cbs.dtu.dk/services/SignalP/), and ExPASyProtParam Tool (http://web.expasy.org/protparam/).

for this resistance (Vogel *et al.*, 2002; Sun and Van Nocker, 2010).

Concerning AtPAE, all 12 annotated sequences have a PAE domain (Pfam03283) and N-terminal targeting motifs (SP, TM, or SP/TM) (Fig. 2B). No isoform is predicted to be soluble.

HGMEs display distinct patterns of expression

A number of isoforms are co-expressed

Analysis of public microarray data sets for all genes encoding HGMEs has led to the identification of several non-exhaustive co-expression clusters in *A. thaliana* (Genevestigator, http://www.genevestigator.com; Hruz *et al.*, 2008). For instance, for the six clusters reported in this review, a number of genes encoding PME, PAE, PG, and PLL are co-expressed (Fig. 3). In cluster 1, six *PME* genes, five *PAE* genes, three *PG* genes, and 11 *PLL* genes are mainly co-expressed in seedlings, leaf, and root tissues. In contrast, the majority of these genes are not expressed in seed-related tissues. In this cluster, ~42% of *PAE* and *PLL* genes are present. Cluster 2 contains only *PME* and *PG* genes specifically expressed in root tissue, while cluster 3 shows *PME*, *PAE*, *PG*, and *PLL* genes expressed in all tissues selected for the analysis apart from roots. Cluster 4 comprises *PME*, *PG*, and *PLL* genes specifically co-expressed in pollen. The expression and roles of *At1g69940*, *At2g47030*, *At2g47040*, and *At3g62170* in pollen germination and pollen tube growth have been highlighted (Jiang *et al.*, 2005; Röckel *et al.*, 2008; Wolf *et al.*, 2009*b*; Mollet *et al.*, 2013). In addition, several genes encoding PGs, PMEs, and PLLs are expressed in anthers (Gonzalez-Carranza *et al.*, 2002, 2007; Rhee *et al.*, 2003; Jiang *et al.*, 2005; Francis *et al.*, 2006; Wolf *et al.*, 2009*b*; Sun and Van Nocker, 2010) with the encoded proteins, namely AtPME At5g55590 and putative AtPG At4g20050, playing a role in pollen grain formation and tetrad separation (Rhee *et al.*, 2003; Francis *et al.*, 2006). Furthermore, transcriptomic analysis in the stamen abscission zone of *Arabidopsis* revealed a co-expression between

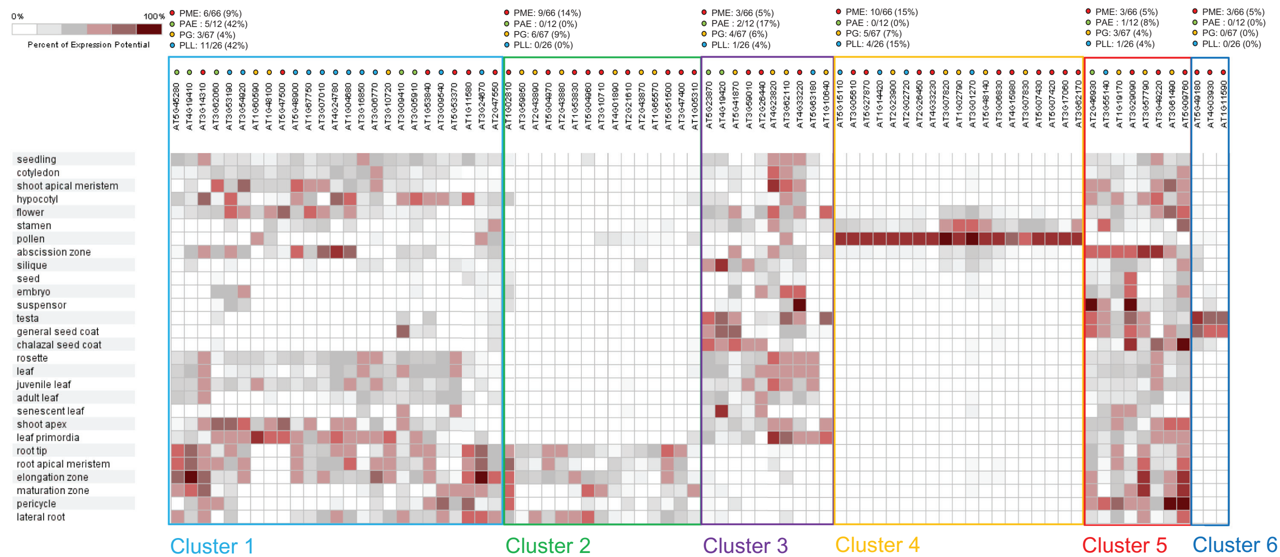

Fig. 3. Clustering analysis of *PME*, *PAE*, *PG*, and *PLL* mRNA expression during development in *A. thaliana*. PME (red circles), PAE (green circles), PG (orange circles), and PLL (blue circles) are shown together. Microarray data and cluster analysis was carried out using Genevestigator (https://www.genevestigator.com/gv/; Hruz *et al.*, 2008). Only some specific tissues were selected. Probes with a single gene and genes showing a minimal expression were used for the cluster analysis. In all clusters, for each HGME family, the proportion (%) of genes expressed among all family members is indicated.

PAE, *PME*, *PG*, and *PLL* genes (Lashbrook and Cai, 2008). In cluster 5, a few *PME*, *PAE*, *PG*, and *PLL* genes exhibit a ubiquitous expression. Interestingly, a small cluster composed of three *PME* genes only expressed in general and chalazal seed coat was also identified. It could relate to the emerging roles of PME–PMEI-mediated control of the DM of HG in mucilage structure and extrusion (Rautengarten *et al.*, 2008; Arsovski *et al.*, 2010; Saez-Aguayo *et al.*, 2013 Voiniciuc *et al.*, 2013). From this analysis, it appears that neither *PAE* nor *PLL* genes are specifically expressed in root and seed coat. In addition, no *PAE* genes appear to be solely expressed in pollen. In contrast, *PME* genes are identified in all of these clusters, potentially highlighting the major role of the control of the DM of HG in a large range of vegetative and reproductive developmental processes. Overall, this analysis shows that the control of HG structure and chemistry is likely to be a highly integrated process involving, in some developmental processes, several different HGMEs.

Regulation of HGME gene expression through hormone signalling

The gene expression of a number of HGMEs has been shown to be modulated, either directly or indirectly, through hormone signalling. A number of publications related to the analysis of phytohormone signalling, including mutants in hormone synthesis pathways and/or hormone application, report changes in the expression of several genes encoding HGMEs (Goda, 2004; Vanneste *et al.*, 2005; Che *et al.*, 2006; Laskowski *et al.*, 2006; Derbyshire *et al.*, 2007; Palusa *et al.*, 2007; Swarup *et al.*, 2008; Quesada *et al.*, 2009; Curvers *et al.*, 2010; Kanai *et al.*, 2010; Di Matteo *et al.*, 2010; Sun *et al.*, 2010; Osorio *et al.*, 2011*a*; Savatin *et al.*, 2011; Ribeiro *et al.*, 2012; Braybrook and Peaucelle, 2013). This has consequences on the structure of HGs affecting plant development. It has also been shown that local auxin accumulation at the shoot apex of *A. thaliana* leads to local demethylesterification of HG, suggesting a role for auxin in the control of PME activity (Braybrook and Peaucelle, 2013). Several expression data sets reveal that auxin specifically regulates the expression of *PME*, *PAE*, *PG*, and *PLL* genes during various developmental events, such as lateral root emergence (Vanneste *et al.*, 2005; Laskowski *et al.*, 2006; Swarup *et al.*, 2008), adventitious root formation (Savatin *et al.*, 2011), fruit development (Quesada *et al.*, 2009; Osorio *et al.*, 2011*a*), and seedling development (Goda *et al.*, 2004; Palusa *et al.*, 2007). In addition, in the latter study, *PME*, *PAE*, and *PG* genes appeared to be up-regulated by brassinosteroids (BRs). Potential regulation of *PME* expression by BRs was also observed in the transcriptome analysis of mutant lines for the *Arabidopsis* transcription factor AtBZR1, responsible for regulating the expression of specific target genes involved in developmental processes as diverse as cell elongation and root development. Based on these results, *AtPME2* and *AtPME3* were identified as putative targets of AtBZR1 (Sun *et al.*, 2010). The regulation of *AtPME41* gene expression during chilling stress in *atbzr1-1D* and *atbri1*, two mutants of the BR signalling pathway, together with the recent data obtained using a PMEI overexpressor, reinforce the hypothesis of the control of PME by BRs (Qu *et al.*, 2011; Wolf *et al.*, 2012*b*). When considering other HGMEs, it has been shown that auxin, together with cytokinins, has an effect on the expression of *PLL* and PGIP (PG inhibitor protein) genes in shoot, root, and callus development in *Arabidopsis* tissue culture (Che *et al.*, 2006). In general, it is likely that all phytohormones directly or indirectly control HGME gene expression. For instance, gibberellic acids (GAs) have an effect on the expression of genes encoding PLLs and PMEs during rosette expansion in *A. thaliana* (Ribeiro *et al.*, 2012). This is consistent with results showing a change in PME activity and in the DM of pectins in GA-deficient mutants over the course

of hypocotyl growth (Derbyshire *et al.*, 2007). Abscisic acid (ABA) can also regulate development, through the modulation of changes in pectin. For example, ABA-deficient tomato mutants cause changes in pectin composition, including levels of Gal-A (Curvers *et al.*, 2010). This could be related to changes in the expression/activity of pectin-degrading enzymes such as PLLs, known to be regulated by this phytohormone (Palusa *et al.*, 2007), or in the expression of the gene encoding PGIP, which was suggested to control PG activity during germination (Kanai *et al.*, 2010). Identification of the major involvement of HGMEs in changes in fruit texture during maturation has prompted research into the role of ethylene in regulating their gene expression. Early work on tomato (Gaffe *et al.*, 1994) and banana (*Musa acuminata*) showed that PME and PL gene expression is ethylene dependent (Dominguez-Puigjaner *et al.*, 1997) during fruit maturation. More recently, several genes encoding PME, PMEI, PG, and PLL were shown to be strongly expressed in the early stages of banana ripening, a period corresponding to the burst in ethylene production (Mbéguié-A-Mbéguié *et al.*, 2009; Srivastava *et al.*, 2012). In tomato, the expression of one *PME* and one *PG* gene was associated with inhibition of ripening, when the ethylene receptor *Never-ripe* was mutated (Osorio *et al.*, 2011*b*). Similarly, the overexpression of the *FaPE1* gene, encoding a PME from strawberry (*Fragaria vesca*), led to up-regulation of the expression of one gene involved in the ethylene response (Osorio *et al.*, 2011*a*). In distinct species, several *PG* genes showed specific changes in gene expression, which were ethylene dependent in oil palm (Roongsattham *et al.*, 2012). The role of ethylene in the control of HGME gene expression might go beyond the specific case of fleshy fruit maturation as the *Arabidopsis* transcription factor AtRAP2.6L (a member of the ethylene response factor family), which has a function in shoot regeneration, targets the *AtPG* gene *At3g15720*, which was down-regulated in the *atrap2.6l* T-DNA mutant (Che *et al.*, 2006). These regulation pathways, in particular that of auxin, may be controlled through OG-mediated negative feedback (Ferrari *et al.*, 2013). The antagonism between auxin and OGs has previously been shown during root formation in tobacco (Bellincampi *et al.*, 1993) and by using exogenous OGs in maize seedlings (Peña-Uribe *et al.*, 2012). Exogenous OGs inhibit the expression of auxin-induced genes (*IAA5*, *SAUR16*, etc.), leading to inhibition of adventitious root formation (Savatin *et al.*, 2011). Inhibition by OGs targets late rather than early auxin-responsive genes (Mauro *et al.*, 2002). In *A. thaliana*, a few *PME* and *PG* genes showed significant changes in expression in response to treatment with OGs, which could be associated with calcium signalling pathways (Moscatiello *et al.*, 2006).

Regulation of biochemical activity

Mode of action and regulation of plant PMEs

As shown previously, PMEs are commonly present in plants and microorganisms such as fungi and bacteria (Giovane *et al.*, 2004; Pelloux *et al.*, 2007). In plants, several neutral and basic isoforms (Micheli, 2001; Giovane *et al.*, 2004; Verlent *et al.*, 2007; Jolie *et al.*, 2010; Dixit *et al.*, 2013), as well as a few acidic isoforms (Lin *et al.*, 1989; Bordenave and Goldberg, 1994; Mareck *et al.*, 1995; Micheli *et al.*, 2000; Ding *et al.*, 2000, 2002; Thonar *et al.*, 2006), have been identified. Although all isoforms catalyse the specific hydrolysis of methylester bonds at C-6 from Gal-A residues, and the subsequent formation of free carboxyl groups, release of methanol (MeOH), and acidification of the cell wall (Micheli, 2001; Giovane *et al.*, 2004; Pelloux *et al.*, 2007; Jolie *et al.*, 2010), PME activity is dependent upon a rather large range of factors. For instance, the presence of free carboxyl groups near the active site appears to be required for enzyme action, which might explain the affinity of PME for partially demethylesterified HG (Rexova-Benkova and Markovic, 1976; Bordenave, 1996; Grasdalen *et al.*, 1996; Van Alebeek *et al.*, 2003; Fries *et al.*, 2007). The reaction mechanism of PMEs, which act as monomers according to resolved crystallographic structures (Johansson *et al.*, 2002; Di Matteo *et al.*, 2005; Fries *et al.*, 2007), consists of a nucleophilic attack and an acid/base catalysis by conserved aspartate catalytic residues on the carbonyl carbon of the C-6 methylester of Gal-A (Fries *et al.*, 2007). Co-crystallization between *Erwinia* PME and various substrates highlighted a preference towards substrates with an alternation of methylesterified and non-methylesterified Gal-A residues, corresponding to partially methylesterified HGs. Consequently, the presence of methylesterified Gal-A residues upstream and non-methylesterified Gal-A residues downstream of the catalytic site could determine the processive action of *Erwinia* PME (Fries *et al.*, 2007). Moreover, recent results using molecular dynamics approaches on the *Erwinia* PME suggest that the rotation of the substrate is necessary for access to the subsequent site and the processive demethylesterification of HG by the enzyme (Mercadante *et al.*, 2013). A similar mechanism could be hypothesized for plant PMEs that have a similar mode of action.

Several factors can affect plant PME activity, which appears to be very sensitive to changes in pH and in the concentrations of cations (Catoire *et al.*, 1998; Denès *et al.*, 2000; Ly-Nguyen *et al.*, 2004; Do Amaral *et al.*, 2005; Verlent *et al.*, 2007; Jolie *et al.*, 2009; Dixit *et al.*, 2013). The effect of pH on PME activity may be related to the pI of the isoforms, which is neutral to alkaline for most plant and bacterial PMEs and acidic to neutral for fungal PMEs. In an acidic environment, alkaline plant PMEs may be positively charged and poorly active due to a strong interaction between PMEs and the negatively charged free carboxyl groups of HG. This has consequent effects on growth (Fig. 4). In contrast, at slightly alkaline pH, basic isoforms are less positively charged and can be released from the substrate due to electrostatic repulsion between the enzyme and the free carboxyl groups (Bordenave, 1996; Jolie *et al.*, 2010). However, as some acidic plant PMEs have been identified in various species such as flax (Mareck *et al.*, 1995), mung bean (Bordenave and Goldberg, 1994), jelly fig (Lin *et al.*, 1989; Ding *et al.*, 2000, 2002), chicory (Thonar *et al.*, 2006), and aspen (Micheli *et al.*, 2000), distinct scenarios are likely to occur in the cell wall (Fig. 4). Thus, the control of the DM of pectins probably depends on the recruitment of specific PME pools, according to the DM of the substrate,

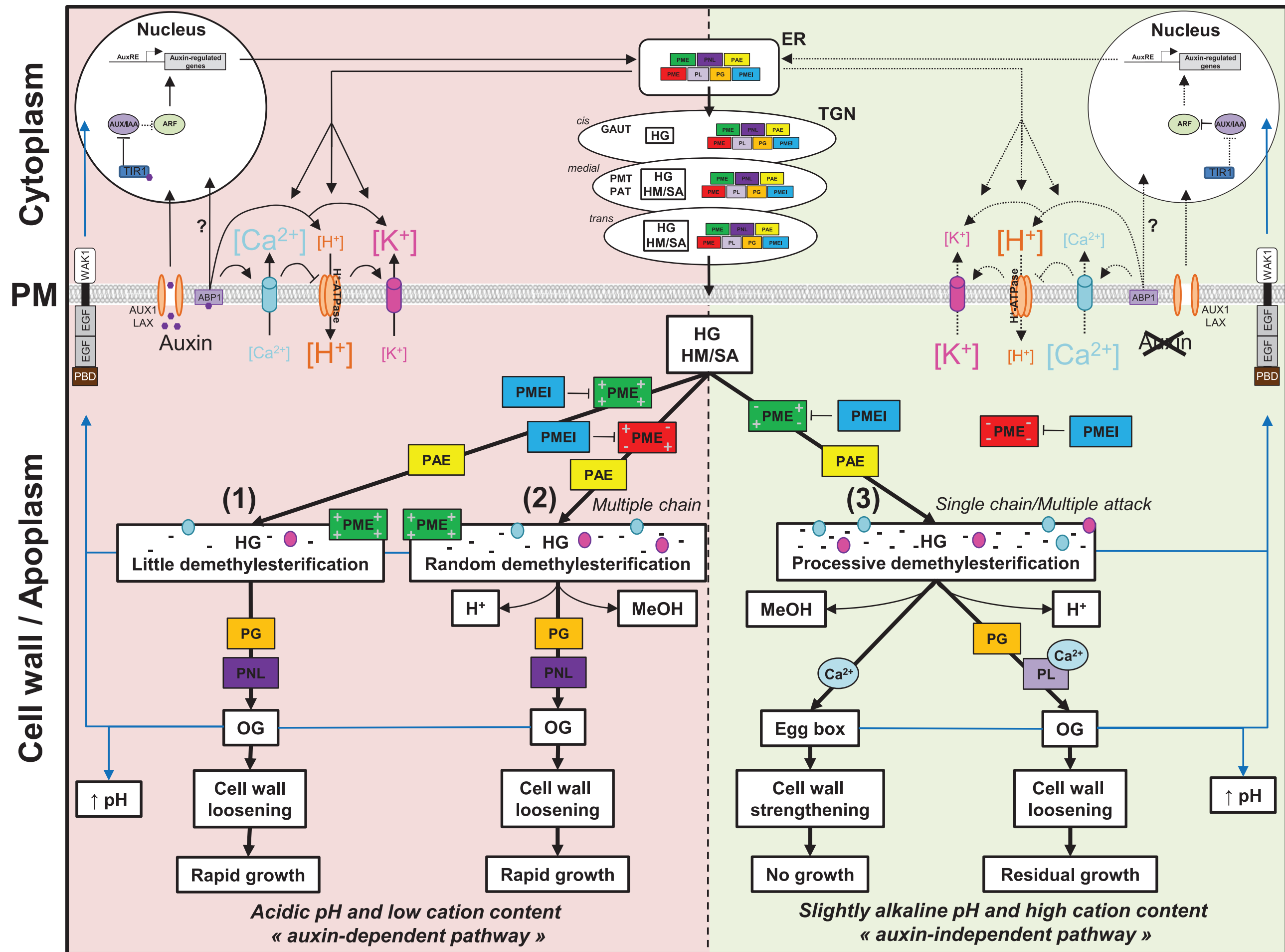

Fig. 4. Schematic diagram showing the regulation of HGME pools in distinct cell wall microenvironments and consequent effects on growth. In the presence of auxin, the auxin-binding protein 1 (ABP1) receptor, localized at the plasma membrane (PM), activates Ca^{2+} and K^+ influx into the cytoplasm (Philippar *et al.*, 1999, 2004; Shishova and Lindberg, 2010; Perrot-Rechenmann, 2010; Tromas *et al.*, 2010; Sauer and Kleine-Vehn, 2011) as well as H^+-ATPase, leading to H^+ efflux into the cell wall and acidification of the apoplasm (Perrot-Rechenmann, 2010; Tromas *et al.*, 2010; Sauer and Kleine-Vehn, 2011). In parallel, auxin imported into the cytoplasm through AUX1/LAX penetrates the nucleus, where it acts on the expression of auxin-regulated genes via the action of TIR1, AUX/IAA, and ARF proteins (Robert and Friml, 2009; Shishova and Lindberg, 2010; Hayashi, 2012). ABP1 can induce downstream responses by regulating gene expression (Rayle and Cleland, 1992; Rober-Kleber *et al.*, 2003; Perrot-Rechenmann, 2010; Monshausen *et al.*, 2011), including that of *HGME* genes (Vanneste *et al.*, 2005; Laskowski *et al.*, 2006; Swarup *et al.*, 2008). PMEs (red, green), PAEs (yellow), PNLs (dark purple), PGs (orange), and PMEIs (blue) are synthesized in the endoplasmic reticulum (ER) and matured in the *trans*-Golgi network (TGN), before secretion into the cell wall (Wolf *et al.*, 2009*b*; De Caroli *et al.*, 2011*a*, *b*). In parallel, HGs are synthesized in the *cis*-Golgi by galacturonosyltransferases (GAUTs), methylesterified and acetylated by pectin methyltransferases (PMTs) and pectin acetyltransferases (PATs), respectively, in the medial-Golgi, and secreted into the cell wall as highly methylesterified (HM) and slightly acetylated (SA) forms (Atmodjo *et al.*, 2013). In an acidic cell wall context (left), basic PMEs (in green), which are the major PME isoforms, are strongly positively charged (represented by positive symbols) and can be trapped by free carboxyl groups (represented by negative symbols) (Jolie *et al.*, 2010) (1). In parallel, acidic pH stimulates inhibition of basic PMEs by several PMEIs (Raiola *et al.*, 2004). This leads to a decrease in HG demethylesterification. HGs can become targets of PG and PNL, which depolymerize HGs, leading to the release of OGs, cell wall loosening, and rapid growth. In the same conditions, acidic PMEs (in red) are neutrally charged (represented by positive and negative symbols) and can act on HGs by multiple chain demethylesterification and/or be inhibited by PMEIs (2) (Catoire *et al.*, 1998; Denès *et al.*, 2000). Following PME action, PAEs can deacetylate HGs (Williamson, 1991; Bordenave *et al.*, 1995). Randomly demethylesterified and deacetylated HGs are depolymerized by PGs and PNLs (Pressey and Avants, 1973; Themmen *et al.*, 1982; Mayans *et al.*, 1997; Protsenko *et al.*, 2008), leading to the release of OGs, cell wall loosening, and rapid growth. OGs can increase the apoplasmic pH and control gene expression through binding to membrane receptors such as PBD-EGF-EGF-WAK1 (Wolf *et al.*, 2012*a*; Ferrari *et al.*, 2013). In the absence of auxin (right), H^+ is not imported into the cell wall, and Ca^{2+} and K^+ are not exported into the cytoplasm. Consequently, the cell wall is slightly alkaline with a high ionic concentration. Auxin-independent *HGME* genes can be expressed, generating distinct pools. In these conditions, acidic PMEs (in red) are strongly negatively charged (represented by negative symbols). By electrostatic repulsion with the free carboxyl group, acidic PMEs cannot bind to their substrate. In parallel, basic PMEs (in green) are less positively or neutrally charged (represented by positive and negative symbols) and can bind to HG HM/SA (Jolie *et al.*, 2010). Slightly alkaline pH can decrease PME–PMEI complex formation and stability, but other PMEIs showing various pH sensitivities can be stimulated and inhibit acidic and basic PMEs (Raiola *et al.*, 2004). Basic PMEs perform the demethylesterification of the HG HM/SA, by single chain or multiple attacks, leading to processively demethylesterified HG (Catoire *et al.*, 1998; Denès *et al.*, 2000; Ngouémazong *et al.*, 2012). PAEs could subsequently act on processively demethylesterified HGs (Williamson, 1991; Bordenave *et al.*, 1995). Depending on Ca^{2+} content and PG/PL presence, processively demethylesterified and deacetylated HGs can form so-called egg box structures through binding with Ca^{2+}, leading to cell wall strengthening and growth retardation (3). Several basic PGs and PLs, stimulated by Ca^{2+} (Pressey and Avants, 1973; Themmen *et al.*, 1982; Protsenko *et al.*, 2008; Chotigeat *et al.*, 2009; Wang *et al.*, 2010), can depolymerize HG to form OGs, which could increase apoplasmic pH and lead to residual growth. The egg box and OGs can regulate gene expression

the pI of the isoforms, and the pH microenvironment at the cell wall. Therefore, depending on the patterns of demethylesterification produced, the properties of pectin are likely to be modified and could differentially affect cell wall rheology and cell growth (Fig. 4).

In plants, cations are often essential for PME activity (Jolie *et al.*, 2010). Although some salt-independent plant PME isoforms have been described, PME activity normally increases up to an optimal cation concentration, above which it decreases (Do Amaral *et al.*, 2005; Verlent *et al.*, 2007; Jolie *et al.*, 2009; Videcoq *et al.*, 2011; Dixit *et al.*, 2013). This optimal concentration is highly dependent upon the type of cation. For example, some salts, such as NaCl or KCl, have a positive effect on the activity up to a certain range of concentration, while others, such as LiCl, are strong inhibitors regardless of the concentration (Do Amaral *et al.*, 2005; Verlent *et al.*, 2007; Dixit *et al.*, 2013). This latter effect is related to competition between cations and positively charged PMEs for interaction with the free carboxyl groups of HG (Fig. 4). In acidic or slightly alkaline cell wall contexts, basic PMEs, positively charged, could be more sensitive to the ionic environment than acidic PMEs.

Different modes of action of plant PMEs have been reported. Three mechanisms have been described: (i) a single-chain mechanism, where PMEs remove all contiguous methylesters from a single chain of HG before dissociating from the substrate; (ii) a multiple-attack mechanism, in which several PMEs catalyse the release of a limited number of methylesters on several chains of HG; these two modes of action produce similar processively demethylesterified HG and are often attributed to basic PMEs, in plants and in bacteria (Johansson *et al.*, 2002; Willats *et al.*, 2006; Jolie *et al.*, 2010); (iii) a multiple-chain mechanism, where PMEs remove only one methylester before dissociating from Gal-A. This mechanism is likely to be that of acidic PMEs in plants and in fungi (*Aspergillus* sp.) and allows a random demethylesterification (Micheli, 2001; Johansson *et al.*, 2002; Willats *et al.*, 2006; Jolie *et al.*, 2010). However, fungal PME from *Trichoderma reesei* shows a processive demethylesterification. Thus these different modes of action are probably dependent upon several factors including the enzyme properties characterized by the pH and ionic environment as well as the substrate specificity, rather than the origin of PMEs (Denès *et al.*, 2000; Johansson *et al.*, 2002; Videcoq *et al.*, 2011). In fact, the action of apple PME has been shown to be pH dependent, with a possible shift between a blockwise and non-blockwise mode of action (Catoire *et al.*, 1998). In addition, the activity of isoforms can vary for the same given substrate (Ly-Nguyen *et al.*, 2004; Do Amaral *et al.*, 2005; Jolie *et al.*, 2009).

Plant PMEs can also be regulated by inhibition of their activities by proteinaceous or non-proteinaceous compounds. Protein inhibitors of plant PMEs (PMEIs) can form a stoichiometric 1:1 complex with PMEs (Di Matteo *et al.*, 2005), leading to an inhibition of PME activity which is dependent on the pH and ionic environment of the cell wall (Bellincampi *et al.*, 2004; Giovane *et al.*, 2004; Raiola *et al.*, 2004). Like PMEs, PMEIs belong to large multigenic families in plants, which further questions the occurrence of specific PME–PMEI pairs in the cell wall. Non-proteinaceous compounds include a wide range of substances including OGs, iodine, detergents, tannins, phenolic acids, glycerol, some sugars, and epigallocatechingallate (Rexova-Benkova and Markovic, 1976; Lewis *et al.*, 2008).

Regulation of biochemical activity of plant PAEs

In plant cell walls, several polysaccharides such as pectins, XyGs, xylans, and mannans can be acetylated (Ishii, 1997; Ralet *et al.*, 2005; Scheller and Ulvskov, 2010; Orfila *et al.*, 2012). However, acetyl groups are not distributed homogeneously in the cell wall, and for pectins they are mainly clustered in specific regions of HG and RG-I, as shown in sugar beet (Ralet *et al.*, 2005; Orfila *et al.*, 2012). Gal-A residues from RG-I can be *O*-acetylated at the O2 and/or O3 positions (Ishii, 1997), while Gal-A from the HG backbone can be *O*-acetylated at the O2 or O3 position, but not di-acetylated (Ralet *et al.*, 2005). In the HG backbone, the simultaneous presence of acetyl and methyl groups on the same Gal-A residues is observed infrequently, but might influence either PME or PAE activities (Ralet *et al.*, 2005). Several investigations have indicated an effect of acetylation on pectin properties. The presence of acetyl groups on Gal-A of HG has an effect on cell wall viscosity (Gou *et al.*, 2012; Orfila *et al.*, 2012) and impairs the formation of Ca^{2+} bonds between HG chains (Renard and Jarvis, 1999; Turquois *et al.*, 1999). Acetylation can also hinder the degradation of HG by some endo-PGs (Bonnin *et al.*, 2003). The deacetylation of HG by enzymatic activity is thus likely to be necessary to trigger changes in the cell wall mediating plant growth.

In this context, PAEs can act by specific hydrolysis of acetylester bonds at O2 and/or O3 from Gal-A residues, making up the linear HG and RG-I of pectins (Gou *et al.*, 2012). Indeed, partial deacetylation of HG improved the gelation properties of sugar beet (Williamson, 1991; Ralet *et al.*, 2003) and increased the enzymatic degradation of pectins (Biely *et al.*, 1986; Schols and Voragen, 1994; Chen and Mort, 1996; Benen *et al.*, 1999; Bonnin *et al.*, 2003). PAE enzymes have been identified in plants (Williamson, 1991; Bordenave *et al.*, 1995; Christensen *et al.*, 1996), bacteria (Shevchik and Hugouvieux-Cotte-Pattat, 1997; Bolvig *et al.*, 2003; Shevchik and Hugouvieux-Cotte-Pattat, 2003), and fungi (Kauppinen *et al.*, 1995; Bonnin *et al.*, 2008). Generally, they act specifically on HG and RG-I polymers and seem to be inactive against acetyl groups present in XyGs, xylans, and

through their binding to membrane receptor such as PBD-EGF-EGF-WAK1 (Wolf *et al.*, 2012*a*; Ferrari *et al.*, 2013). Otherwise, in acidic or slightly alkaline cell wall contexts, basic PMEs, positively or neutrally charged, could be more sensitive to the ionic environment than acidic PMEs. Particularly in a slightly alkaline cell wall context, basic PMEs could be in competition with cations such as K^+ (pink circle) and Ca^{2+} (light blue circle) for interaction with free carboxyl groups of HG, avoiding basic PME trapping by processively demethylesterified HG. The solid arrows correspond to the actions taking place in the presence of auxin (purple circle), whereas dotted arrows represent the lack of action when there is no auxin signal.

mannans (Bolvig *et al.*, 2003; Bonnin *et al.*, 2008). This differentiates them from rhamnogalacturonan acetylesterase from *Aspergillus aculeatus*, which is highly specific for RG-I and does not deacetylate HG (Kauppinen *et al.*, 1995). In *Arabidopsis*, the majority of PAE isoforms have a neutral to basic pI. Purified plant PAEs characterized from mung bean and orange, which are highly active against synthetic substrates such as triacetin and *p*-nitrophenyl acetate and sugar beet pectins, have an optimal activity at pH ranging from 5 to 6.5, depending on the substrates used in the assay (Williamson, 1991; Bordenave *et al.*, 1995; Christensen *et al.*, 1996). Moreover, PAE activity is increased when the substrate has previously been demethylesterified (Williamson, 1991; Bordenave *et al.*, 1995; Oosterveld *et al.*, 2000). Therefore, a synergistic effect between PME and PAE is likely to occur at the cell wall to mediate either egg box formation or degradation of HG by PGs and PLLs.

Mode of action and regulation of plant PGs

PGs belong to an enzyme family identified in plants (Pressey *et al.*, 1973, 1977; Themmen *et al.*, 1982; Nogota *et al.*, 1993; Chun and Huber, 1998; Hadfield and Bennett, 1998; Pathak and Sanwal, 1998; Pathak *et al.*, 2000; Verlent *et al.*, 2004, 2005), herbivorous insects (Shen *et al.*, 2003), and microorganisms such as bacteria, fungi, and nematodes (Pickersgill *et al.*, 1998; Jaubert *et al.*, 2002; André-Leroux *et al.*, 2005; Jayani *et al.*, 2005; Kars *et al.*, 2005; Mertens and Bowman, 2011). Whatever their origin, PGs cleave by hydrolysing the α-(1–4) bonds linking D-Gal-A residues, mainly from the HG linear homopolymer (Brummell and Harpster, 2001; Verlent *et al.*, 2005; Protsenko *et al.*, 2008). In both plants and microorganisms, PGs are constituted of two types, the endo-PGs (EC 3.2.1.15) and the exo-PGs (EC 3.2.1.67) (Brummell and Harpster, 2001; Verlent *et al.*, 2005; Protsenko *et al.*, 2008). Generally, their activities increase with the decrease in the DM of HG (Thibault and Mercier, 1979; Bonnin *et al.*, 2002). Consequently, and in agreement with previous comments, prior action of PME seems to be necessary to enable the degradation of HGs by plant PGs and, for the same DM, the pattern of demethylesterification could affect the action of plant PGs (Verlent *et al.*, 2005). For example, PG isolated from tomato prefers HG previously demethylesterified blockwise by the endogenous PME of tomato, rather than randomly demethylesterified by the exogenous fungal PME of *A. aculeatus* (Verlent *et al.*, 2005). More precisely, endo-PGs catalyse the hydrolytic cleavage of the α-(1–4) linkage between at least two demethylesterified Gal-A residues of the HG backbone with a random action pattern, leading to the formation of OGs of various degrees of polymerization (DPs; Verlent *et al.*, 2005; Ferrari *et al.*, 2013). In plants, endo-PGs particularly prefer HG with a chain region containing more than four demethylesterified Gal-A residues between methylesterified Gal-A (Clausen *et al.*, 2003; Protsenko *et al.*, 2008). Consequently, endo-PGs could act against HG demethylesterified blockwise, and the variation of the demethylesterification pattern may regulate the action of endo-PGs. In contrast, exo-PGs act only at the terminal position of the HG backbone to hydrolyse the α-(1–4) linkage, producing monogalacturonides (Verlent *et al.*, 2005; Protsenko *et al.*, 2008). In this case, they could act together against HG, which is partially, randomly, or blockwise demethylesterified at the terminal position.

In plants such as *Arabidopsis*, all isoforms have a pI varying from 4.8 to 9.8 (Cao, 2012), which could regulate their optimum activity depending on the pH and the ionic microenvironment. With regard to pH, several plant PGs have been partially purified from plants and biochemically characterized. Overall, like PGs from microorganisms such as *Botrytis cinerea*, that show optimal activities at approximately pH 4.5 (Kars *et al.*, 2005), plant PGs prefer an acidic pH from 3.3 to 6 (Pressey *et al.*, 1973, 1977; Themmen *et al.*, 1982; Nogota *et al.*, 1993; Chun and Huber, 1998; Pathak and Sanwal, 1998; Pathak *et al.*, 2000; Verlent *et al.*, 2004). Furthermore, the pH of optimal activity of plant PGs seems to be dependent on the ionic microenvironment. For example, PG purified from tomato shows an optimal activity at pH 4–4.5 with NaCl, compared with pH 5–6 in the presence of KCl (Chun and Huber, 1998). Other ions such as Cd^{2+} and Ca^{2+} could affect PG activity in *Avena sativa* (Pressey *et al.*, 1977). Regarding Ca^{2+}, an exo-PG from peach fruit is Ca^{2+} dependent (Pressey *et al.*, 1973) as is an endo-PG from strawberry fruit because with EDTA, which chelates Ca^{2+} ions, PG activity is inhibited (Nogota *et al.*, 1993). Nevertheless, not all plant PGs are Ca^{2+} dependent. For instance, among three PG isoforms isolated from banana, where two endo-PGs (PG1 and PG3) and one exo-PG (PG2) are characterized, only PG1 was shown to be activated by Ca^{2+} (Pathak *et al.*, 1998). Consequently, ion dependence does not seem to be related to the mode of action of PGs (endo- or exo-PGs).

PG activity can also be inhibited by proteinaceous compounds (Juge, 2006; Protsenko *et al.*, 2008). It has been widely demonstrated in plants that there are PGIPs directed against secreted pathogen PGs (Federici *et al.*, 2001; Di Matteo *et al.*, 2003; Ferrari *et al.*, 2006; Oelofse *et al.*, 2006; Sathiyaraj *et al.*, 2010; Benedetti *et al.*, 2011). Plant PGIPs are bound to HG substrate as a guardian, thus inhibiting pathogen PG activity by formation of a stoichiometric complex, which prevents access to the substrate (Spadoni *et al.*, 2006). More precisely, when PGIPs interact with pathogen PG, the complex formed can move the substrate away, thus preventing PG action against the HG substrate (Spadoni *et al.*, 2006). Moreover, it appears that PGIPs act mainly against endo-PGs from pathogens (Protsenko *et al.*, 2008). This is confirmed by the mode of action of PGIPs, which bind strongly to HG demethylesterified blockwise, in contrast to HG randomly demethylesterified, and prevent the action of endo-PGs (Spadoni *et al.*, 2006). Currently, limited data indicate a role for plant PGIPs in the regulation of plant PG activity and development. However, a recent publication shows changes in radicle protrusion in *pgip1* mutants and PGIP1 overexpressors, probably caused by an alteration of *Arabidopsis* PG activity (Kanai *et al.*, 2010).

Mode of action and regulation of plant PLLs

PLLs are mainly found in plants (Domingo *et al.*, 1998; Chourasia *et al.*, 2006; Chotigeat *et al.*, 2009; Wang *et al.*,

2010) and microorganisms such as bacteria, fungi, and nematodes (Popeijus *et al.*, 2000; Jayani *et al.*, 2005). They cleave, by β-elimination, the α-(1–4) bond linking methylesterified or non-methylesterified D-Gal-A units mainly from the HG backbone, giving rise to an unsaturated C4–C5 bond at the non-reducing end of the newly formed OG (Mayans *et al.*, 1997; Pilnik and Rombouts, 1981). PLLs comprise the PL and PNL family of enzymes. PLs are more specific for non-methylesterified or slightly methylesterified HG and require Ca^{2+} for their activity whose optimal pH is near 8.5. In contrast, PNLs degrade highly methylesterified HG with an optimal pH of 5.5 for their activity and do not require Ca^{2+} (Mayans *et al.*, 1997; Herron *et al.*, 2000). PLs have both endo (EC 4.2.2.2) and exo (EC 4.2.2.9) activities, while only endo (EC 4.2.2.10) activity has been discovered for PNLs (Sinitsyna *et al.*, 2007). PLs and PNLs are commonly found in microorganisms; fungi usually secrete PNLs (Sinitsyna *et al.*, 2007) while bacteria produce predominantly PLs (Payasi and Sanwal, 2003).

To date, of PNLs and PLs, only PLs have been discovered and biochemically characterized in plants. For instance, the plant PL from *Zinnia elegans* shares homology with the microbial PLs from *Bacillus subtilis* and *Pectobacterium carotovorum* (Domingo *et al.*, 1998). Moreover, the presence of PL isoforms has been demonstrated in plants such as *Hevea brasiliensis*, *Z. elegans*, and *Gossypium hirsutum*, and in the ripening fruits of banana. More precisely, coding sequences isolated from these latter and expressed in a heterologous system led to the production of *Escherichia coli* or yeast expressed recombinant enzymes showing PL activities (Domingo *et al.*, 1998; Pua *et al.*, 2001; Chotigeat *et al.*, 2009; Wang *et al.*, 2010). PLs from pathogens are rather active at alkaline pH (Jayani *et al.*, 2005); recombinant PLs from *H. brasiliensis*, *Z. elegans*, and mango fruit also show optimal activities at basic pH, namely pH 7, 10, and 8, respectively (Domingo *et al.*, 1998; Chourasia *et al.*, 2006; Chotigeat *et al.*, 2009). Moreover, as in microorganisms, PL activity from *H. brasiliensis* and mango fruit is inhibited by EDTA and stimulated by $CaCl_2$ (Mayans *et al.*, 1997; Chourasia *et al.*, 2006; Chotigeat *et al.*, 2009). A multiple sequence alignment suggests that the calcium-binding site of plant PLs could be conserved and may involve three aspartate residues (Chotigeat *et al.*, 2009). Lastly, plant PLs, like those from bacteria (*E. chrysanthemi*), seem to be more active against non-methylesterified HG because their optimal activities have often been quantified with non-methylesterified substrates (Tardy *et al.*, 1997; Chotigeat *et al.*, 2009; Wang *et al.*, 2010). To date, a PNL inhibitor protein has been found (Bugbee, 1993), but no proteic inhibitor of PL enzyme.

Roles of HG modifications in vegetative and reproductive development

Early reports showed the multiple roles of pectin modifications in the control of vegetative development (Hasunuma *et al.*, 2004; Pilling *et al.*, 2004). The current review focuses on the role(s) of HGME-mediated HG modification during cell elongation and differentiation in specific developmental processes.

Roles of HGMEs in organ growth

Understanding the role of pectin modifications in the control of growth requires the use of simple models in which developmental and cell biology, genomics, biochemistry, and biophysics can be integrated at a cellular level. The pollen tube, such as dark-grown hypocotyl, is a powerful system to analyse the roles of the cell wall in modulating cell elongation.

PMEs have been reported to play a role in pollen grain formation. In *A. thaliana*, the *atqrt1* mutant, which does not express the *AtQRT1* gene encoding a PME (At5g55590), does not show cell wall degradation and separation of the haploid spores during microsporogenesis (Francis *et al.*, 2006). Consequently, the spores remain fused and pollen grains are released as tetrads. The modifications of HGs by PMEs could play a central role in the first step of wall degradation, by creating substrates for pectin-degrading enzymes. A similar phenotype was shown for mutants in the *AtQRT3* gene encoding a putative PG (At4g20050; Rhee *et al.*, 2003). A model was proposed in which the action of PME would create specific substrates for downstream enzymes. The structure of the pollen grain might be a determinant of the subsequent capacity for pollen tube emergence. In *A. thaliana*, the *AtVGD1* gene (*At2g47040*), which encodes a PME, is involved in pollen tube elongation. The *atvgd1* knockout (KO) mutant showed a slight reduction in pollen PME activity together with a retarded pollen tube growth within the style and transmitting tract. In addition, the pollen tube had an abnormal shape with frequent tip explosions (Jiang *et al.*, 2005). The mutant had lower than wild-type levels of pollen fertility, and hence smaller siliques with fewer seeds. A similar phenotype, albeit less drastic, was observed for the *AtPPME1* KO mutant (Tian *et al.*, 2006). In other species, such as *Nicotiana tabacum*, silencing of *NtPPME1*, encoding the main tobacco PME isoforms, led to a decrease in pollen tube growth (Bosch and Hepler, 2006). Recent advances in the understanding of the role of HG modifications in pollen tube elongation include the tight spatial and temporal regulation of PMEs by PMEIs. For instance, it was shown that AtPMEI2 inactivates AtPPME1 *in vitro* and that both proteins are located in the pollen tube where they physically interact (Röckel *et al.*, 2008). More recently, *BoPMEI1*, a novel *Brassica oleracea* gene, was characterized (Zhang *et al.*, 2010). Heterologously expressed BoPMEI1 showed PMEI activity while a transgenic *Arabidopsis* plant, expressing antisense *BoPMEI1*, suppressed the expression of the orthologous gene *At1g10770* altering pollen tube growth. The fine control of PME activity modulates HG structure on the lateral sides of the pollen tube, with consequences on cell wall rheology, enabling apical growth. In contrast to PMEs, the functional characterization of the roles of PAEs, PGs, and PLLs in pollen tube growth has remained elusive. However, a recent report showed a role for the control of the DA of pectins in pollen tube growth (Gou *et al.*, 2012). In *Brassica campestris*, a putative BcPG encoded by *BcMF2* is specifically expressed in tapetum and

pollen after the tetrad stage of anther development. In a transgenic plant with reduced levels of *BcMF2* expression, mature pollen presents a distorted morphology with abnormal intine development, leading to abnormal pollen tube growth and a consequent reduction in male fertility (Huang *et al.*, 2009*a*). In the same species, *BcMF9*, encoding a distinct BcPG, was shown to play a role in intine and exine formation (Huang *et al.*, 2009*b*). It is therefore likely that PGs are involved in the changing intine cell wall structure affecting subsequent pollen tube development. Similarly, the activity of PL Cry j I, expressed in pollen of *Cryptomeria japonica*, could cause cell wall loosening during pollen development, thus improving pollen tube emergence and growth as well as its penetration within the style (Taniguchi *et al.*, 1995). Overall, recent data generated about the control of HG structure, including the role of HG acetylation (Gou *et al.*, 2012) during pollen development, have enabled new models to be established linking pollen tube growth to the spatial distribution of polysaccharides (Zonia and Munnik, 2011; Chebli *et al.*, 2012; Mollet *et al.*, 2013).

On the vegetative side, PME play a role in the early stages of radicle emergence, as shown by the faster germination rate in *A. thaliana* plants overexpressing *AtPMEI5* (Müller *et al.*, 2013). PME activity would therefore modulate the mechanical properties of the cell wall, between opposing forces of radicle elongation and resistance of the endosperm. PG activity can also be involved in radicle protrusion. For example, when the *AtPGIP1* gene, which is regulated by the transcription factor ABA insensitive 5 (ABI5), is mutated or overexpressed, PG activity is modified, leading to changes in the seed coat mucilage released and the timing of radicle protrusion (Kanai *et al.*, 2010). Seed coat mucilage production has been shown to be affected by PME (Rautengarten *et al.*, 2008; Voiniciuc *et al.*, 2013; Saez-Aguayo *et al.*, 2013).

Once germinated, dark-grown hypocotyl has a simple anatomy that elongates, in the absence of cell division, from 10 µm up to 1 mm during post-embryonic development (Gendreau *et al.*, 1997). Previous work has shown that the initial elongation rate of hypocotyl cells is developmentally controlled. At first, all cells elongate uniformly and slowly up to 48 h after germination, after which abrupt growth acceleration takes place (Refregier *et al.*, 2004). An important relationship has been demonstrated between the DM of HGs in primary cell wall and hypocotyl elongation in *A. thaliana*, as well as other species (Al-Qsous *et al.*, 2004; Paynel *et al.*, 2009). First, two mutants deficient in GA biosynthesis (*ga1-3* and *gai*) showed changes in the DM of HGs, with consequences on hypocotyl length (Derbyshire *et al.*, 2007). More recently, microarray analysis in *A. thaliana* showed that several genes, encoding PMEs, PMEIs, PGs, and PLLs, were up- or down-regulated at the growth phase transition (Pelletier *et al.*, 2010). The down-regulation of PME expression, using the *atpme3* KO mutant, had consequences on hypocotyl length (Guénin *et al.*, 2011). The overexpression of one of these genes, *AtPMEI4* (*At4g25250*), increased carboxylic ester bonds in the primary cell wall of dark-grown hypocotyls and delayed growth (Pelletier *et al.*, 2010). A similar approach showed that the overexpression of *AtPMEI5* (*At2g31430*) had dramatic effects on plant growth, including a drastic reduction in dark-grown hypocotyl length (Wolf *et al.*, 2012*b*). It was further shown that feedback signalling from the cell wall is integrated by the BR signalling module to ensure homeostasis of cell wall biosynthesis and remodelling. This adds a new component to understanding the regulation and roles of pectin modifications in the control of growth rate. BRs have been shown to modulate PME activity and the expression of specific PME genes (Qu *et al.*, 2011). Surprisingly, the overexpression of another *PMEI*, *AtPMEI-2*, did not show similar results (Lionetti *et al.*, 2010). This suggests that, depending on the PMEI used, specific PME targets could be present or absent, with consequences on the cell wall and growth phenotype. This introduces a fascinating perspective in understanding the role of the sensing of cell wall integrity and its consequences on modulating cell growth (Kohorn and Kohorn, 2012).

When considering root growth, a study showed that the *AtPME3* gene encoding one of the major PME isoforms in *A. thaliana* plays a role in controlling root elongation. The *atpme3* KO mutant, which showed decreased PME activity, had a 20% reduction in root length compared with the wild type, while *AtPME3* overexpressors showed the opposite phenotype (Hewezi *et al.*, 2008). By using a distinct KO allele, it was shown that the reduction in PME activity in the *atpme3* mutant correlated with an increased DM of HGs (Guénin *et al.*, 2011). The control of PME activity by specific PMEIs is likely to play a key role in the regulation of root growth. Transgenic plants overexpressing *AtPMEI-1* and *AtPMEI-2* showed a 50% decrease in PME activity and an increased root length compared with the wild type; this was notably related to changes in cell size in the root expansion zone (Lionetti *et al.*, 2007). These changes were associated with modifications in leaf shape/size. Recent data demonstrated a link between expression of specific PME genes and the Al-induced inhibition of root elongation in rice (Yang *et al.*, 2012). Interestingly, the number of adventitious roots was modified in PME mutants, suggesting that these enzymes influence both root elongation and root differentiation (Guénin *et al.*, 2011).

Roles of HGMEs in organ formation

As mentioned previously, the changes in the pectic network affect root emergence as shown by the modifications of the number of adventitious roots in specific *atpme* KO mutant lines (Guénin *et al.*, 2011). This could be related to OG-mediated signalling (Savatin *et al.*, 2011) and/or to phytohormone-related signalling. In this respect, auxin homeostasis is likely to be a major signal regulating the expression of HG-modifying genes, such as *PG* and *PLL* genes, during root emergence (Swarup *et al.*, 2008). Although the functional role of PLLs in root differentiation has not yet been elucidated, the increase in transcript accumulation in auxin-treated roots (Laskowski *et al.*, 2006) as well as the large number of isoforms expressed in this organ zone suggest a major involvement of these enzymes (Sun and Van Nocker, 2010). Recent data showing a role for a *Lotus japonicus* PL in the

modification of roots required for rhizobia infection support this hypothesis (Xie *et al.*, 2012).

The phyllotaxis of plant organs, which is the precise emergence of lateral organs, is controlled by a gradient of the plant hormone auxin (Rybel *et al.*, 2010; Vernoux *et al.*, 2010; Besnard *et al.*, 2011; Santuari *et al.*, 2011; Sassi *et al.*, 2012), but the chemical and mechanical status of the cell wall is also important in the formation of new organs. More particularly, the DM of HGs can affect cell wall rheology, modifying phyllotaxis. A first study has shown that the formation of flower primordia in *A. thaliana* shoot apical meristem is accompanied by a demethylesterification of HGs. In fact, the overexpression of *AtPMEI3* (*At5g20740*) and *AtPME5* (*At5g47500*), which are co-expressed in the shoot apical meristem area, alters the methylesterification status of HGs. This can lead to inhibition of either primordia formation when *AtPMEI3* is expressed or ectopic primordia formation with *AtPME5* expression (Peaucelle *et al.*, 2008). A second study reported the role of the homeodomain transcription factor BELLRINGER (AtBLR) in the establishment and maintenance of the phyllotaxis pattern in *A. thaliana* by the control of *AtPME5* expression. The study of the KO mutant *atblr-6* showed that AtBLR is required to establish normal phyllotaxis through the exclusion of *AtPME5* expression from the meristem; in contrast, phyllotaxis is maintained by the activation of *AtPME5* in the elongating stem (Peaucelle *et al.*, 2011*b*). Local accumulation of auxin in the shoot apex of *Arabidopsis* leads to local demethylesterification of HGs, suggesting a role for auxin in the control of PME activity, necessary for the decrease in tissue rigidity promoting organ formation. In an *AtPMEI3*-overexpressing line, which shows decreased demethylesterification of HGs, local accumulation of auxin did not induce organ formation, confirming that the control of the DM of HGs occurs downstream of auxin accumulation during organ formation (Braybrook and Peaucelle, 2013). The changes in HG structure underlie changes in cell wall rheology that are key elements of primordia emergence at the shoot apical meristem (Hamant *et al.*, 2011; Peaucelle *et al.*, 2011*a*). Pectins thus play a major role in controlling plant morphogenesis during development (Palin and Geitmann, 2012).

Although the parts played by other HGMEs in flower development have been less documented, some results suggest putative roles in this developmental process. For instance, a cDNA encoding a *PGIP* gene has been isolated from cotton flower. This gene, designated *GhPS1*, is specifically expressed in cotton petals and is gradually up-regulated over the course of petal development. However, its expression level declines rapidly in senesced petals after flowering, suggesting that the *GhPS1* gene may be involved in cotton petal development and senescence (Shi *et al.*, 2009).

Roles of HGMEs in the modulation of the physical properties of the end-product

Among other post-fecundation processes, the roles of pectin modifications during fleshy fruit development and maturation have been extensively studied (Brummell *et al.*, 2004; Ericksson *et al.*, 2004; Louvet *et al.*, 2011; Lunn *et al.*, 2013; Terao *et al.*, 2013). Early reports showed that, during tomato ripening, PME activity regulates MeOH and ethanol (EtOH) accumulation in the pericarp (Frenkel, 1998). Recent results showed an additional role for PME in cellular calcium distribution and blossom-end rot development in tomato fruit (De Freitas *et al.*, 2012). The link between PME levels and fruit susceptibility to pathogens was reported in the strawberry–*Botrytis* interaction (Osorio *et al.*, 2008), and the consequences of changes in PME gene expression on metabolic and signalling pathways were described (Osorio *et al.*, 2011*a*, *b*). Overall, the fine control of PME activity could be related to the interaction with specific inhibitors during fruit development, as recently shown (Reca *et al.*, 2012). The role of PME-mediated changes in the pectic network is likely to be highly conserved during fruit development, as shown by the changes in PME activity and/or transcript accumulation in various plant species (Barnavon *et al.*, 2001; Deytieux-Belleau *et al.*, 2008; Draye and Cutsem, 2008; Mbéguié-A-Mbéguié *et al.*, 2009; Cação *et al.*, 2012; Roongsattham *et al.*, 2012; Wen *et al.*, 2013). For instance, in *Musa acuminata*, PMEs are involved in cell wall modifications, responsible for softening the pedicel abscission area after induction of ripening. However, the observed effects could also be caused by cooperation of PME with other cell wall-modifying genes. In tomato, PG activity appears necessary for HG modifications, following the first stage of ripening; in transgenic plants underexpressing a PG gene, the fruit does not show any degradation in the last stage of fruit ripening (Hadfield and Bennett, 1998). The close relationship between PG activities and fruit firmness has recently been shown in apple (Atkinson *et al.*, 2012) and in strawberry (García *et al.*, 2009; Quesada *et al.*, 2009). In addition, PGs have been reported to play a central role in the modifications of the HG network associated with fruit abscission (Swain *et al.*, 2011). In banana, as in other climacteric fruits, ripening is accompanied by a high production of ethylene, which suggests a strong regulation of HGMEs by this phytohormone (Dominguez-Puigjaner *et al.*, 1997; Mbéguié-A-Mbéguié *et al.*, 2009; Srivastava *et al.*, 2012). For instance, in *M. acuminata*, the *BAN17* gene encoding PL is expressed under the control of ethylene. The role of PL in fruit softening was functionally demonstrated in strawberry (Jimenez-Bermudez *et al.*, 2002; Santiago-Doménech *et al.*, 2008). The changes in the HG network and its methylesterification status, mediated by HGMEs, are thus key elements of fruit texture that could be used for quantitative and genetic association studies (Chapman *et al.*, 2012; Lahaye *et al.*, 2012). Other post-fecundation processes appear to be under the control of HG structure. For instance, HG modification was shown to play a role in fibre elongation in cotton. In *Gossypium hirsutum*, the *GhPEL* gene, whose product was biochemically characterized as a PL, is preferentially expressed in fibres at 10 d post-anthesis. In antisense *GhPEL* transgenic cotton plants, where the expression is significantly suppressed, a reduction in PL activity was observed. This reduction led to a decreased degradation of demethylesterified HG epitopes in the primary cell wall with

consequent effects on cell wall loosening; ultimately, the elongation of the fibre was repressed (Wang *et al.*, 2010).

Recent findings also highlight emerging roles for PMEs in wood development and wood mechanical properties. In *A. thaliana*, five different *PME* genes are expressed in the xylem, one of which is more highly expressed in this tissue than in any other examined. Similarly, transcripts of a dozen *PME* genes have been found in poplar wood-forming tissues (Geisler-Lee *et al.*, 2006) and shown to be tightly regulated within the cambial meristem and during xylogenesis. In addition, several genes encoding HGMEs have been found to be regulated during wood formation in *Pinus radiata* (Li *et al.*, 2011, 2012) and *Eucalyptus* (Carvalho *et al.*, 2008; Goulao *et al.*, 2011). A role for PMEs has been demonstrated in the regulation of fibre length in poplar (Siedlecka *et al.*, 2007) as well as in the modulation of stem mechanical properties in *Arabidopsis* (Hongo *et al.*, 2012). For the latter, AtPME35 is involved in the demethylesterification of the primary cell wall, which directly regulates the mechanical strength of the supporting tissue. Mutants affected in the expression of the *AtPME35* gene showed a striking bending phenotype. Furthermore, the presence of HG and its de-esterification are likely to be essential for xylem lignification. For example, Ca^{2+}-bridged de-esterified HG is known to bind class III peroxidases that might initiate lignin polymerization (Jenkins *et al.*, 2001). In support of this hypothesis, it was shown that PME, de-esterified HGs, peroxidase, and the start of the lignification process co-localize at cell junctions in woody tissues (Wi *et al.*, 2005) and that pectins interact with lignin monomers and affect lignin polymerization *in vitro* (Lairez *et al.*, 2005; Habrant *et al.*, 2009). The control of the methylesterification status of HGs has dramatic consequences on the chemical and rheological properties of the cell wall (Wolf *et al.*, 2009*b*), and thus is likely to affect the properties of biomass-derived by-products. For instance, the PME-mediated changes in pectin structure have been shown to influence the texture of cooked potato tubers (Ross *et al.*, 2011*a*, *b*), the saccharification of plant tissues for biomass bioconversion (Lionetti *et al.*, 2010), and the solid wood properties of *Eucalyptus pilularis* (Sexton *et al.*, 2012). The differences in pectins between juvenile and adult *Eucalyptus globulus* wood, revealed by various nuclear magnetic resonance (NMR) analyses, support a major role for these enzymes during wood formation (Rencoret *et al.*, 2011).

Role of HG modifications in plant responses to biotic stress

Biotic stresses modify gene expression of HG-modifying enzymes

Plant HGMEs are involved in various biotic interactions: necrotrophic, hemibiotrophic, or biotrophic pathogens (fungi, oomycetes, bacteria, and viruses); phytophagous organisms (piercing–sucking insects, chewing insects, and nematodes); endosymbiotic microorganisms (arbuscular mycorrhizal fungi and bacteria); or plant parasites that feed and then develop on host plants. Piercing–sucking insects wound the plant by inserting their stylets (mouthpart) into tissue to suck the cell contents or sieves. This wounding stress may be mimicked using needle punctures on leaves. Plant HGME gene expression is modified by all of these stresses, but the observed patterns of expression are dependent upon the bioaggressor (Table 2). For instance, no induction of PG gene expression has yet been reported after chewing insect and plant parasite infestations. Similarly, no induction of PAE or PL gene expression was shown following infections by viruses, endosymbiotic microorganisms, and plant parasites. For each bioaggressor, the pattern of HGME expression differs depending on the plant species, ecotype (or cultivar), and plant phenology. While *A. thaliana AtPME3* (*At3g14310*) was overexpressed in the C24 ecotype (Hewezi *et al.*, 2008) after 3 d of infestation with 250 J_2 nematodes (*Heterodera schachtii*), no such effect was reported in Col-0 for a similar infestation level. In contrast, in this ecotype, the expression of two other PMEs (*At2g45220* and *At1g53830*) was down-regulated following infestation (Puthoff *et al.*, 2003). This difference could also be related to plant phenology as, although of similar age (14 d old for C24 and 12 d old for Col-0), the plants were grown under distinct photoperiods (16/8 h and 12/12 h day/night, respectively).

The analysis of available microarray data sets from *A. thaliana* Col-0 ecotype (www.genevestigator.com; Hruz *et al.*, 2008) for distinct pathogens shows that the expression of a number of HGME genes is mostly down-regulated whatever the type of biotic stress: necrotrophic (*Botrytis cinerea* and *Fusarium oxysporum*), hemibiotrophic (*Phytophthora infestans*), or biotrophic (*Pseudomonas syringae*) pathogens, phytophagous organisms (*Bemisia tabaci* and *Meloidogyne incognita*), virus (*Cabbage leaf curl virus* CalCuV), or wounding (needles, Fig. 5). In general, within the smallest multigenic families of HGMEs such as PAEs and PLs, up to 67% and 42% of genes, respectively, show distinct expression patterns in response to biotic stresses. In contrast, figures are in the range of 29% and 24% for PMEs and PGs, respectively. In more detail, when considering the number of regulated HGME genes and their expression levels (up- or down-regulation), two clusters can be distinguished. The first includes responses to *P. infestans*, *B. tabaci*, *B. cinerea*, CalCuV, and *P. syringae*, and shows an overall down-regulation of most of the genes. The second cluster, which has far fewer modifications of gene expression, comprises the responses to *M. incognita*, *F. oxysporum*, or wounding. Some genes are specifically expressed in each cluster (PL, *At5g63180*; PGs, *At4g23820*, *At3g06770*, and *At3g16850*; or PME, *At1g53830*; and PL, *At5g55720* in cluster one and two, respectively). On this basis, it would be possible to distinguish between rather ubiquitous biotic stress-related HGMEs (PME, *At2g45220*; and PL, *At3g07010*) and specific ones (PMEs *At5g04960–At3g10710* with *M. incognita*; PME *At2g47280* and PGs *At3g48950–At5g27530* with CalCuV, or PGs *At1g70500–At3g57510* with *P. syringae*). As both clusters include a necrotrophic fungus (*B. cinerea* and *F. oxysporum*), the changes in the expression of HGMEs do not appear to be dependent on the bioaggressor lifestyle *per se*. The expression of HGME genes is more likely to depend on the bioaggressor species (Fig. 5). For instance, the number

Table 2. *Gene expression variations of homogalacturonan-modifying enzymes after biotic stresses*

Pectin methylesterases, pectin acetylesterases, polygalacturonases, and pectin lyase-like involved in plant–bioaggressor interactions.

Gene name	AGI or accession	Stress	Species name(s)	Induction	References
Pectin methylesterases (PMEs)					
		Aphid	*Brevicoryne brassicae*	32 aphids/plant; 48 hpi	Kusnierczyk *et al.* (2008)
AtPME17	At2g45220			Up-regulated 48 hpi (×4.53)	
AtPMEPCRF (61)	At5g53370			Up-regulated 6, 12, 24, 48 hpi (×1.35, 1.44, 1.83, 1.85)	
Apium graveolens cv. Dulce (celery)		Aphid	*Myzus persicae*	20 (3 dpi) or 100 aphids (7 dpi)/plant	Divol *et al.* (2005)
AgPME	CN254944			Up-regulated (×3.41, 2.41)	
AgPME	CN254453			Up-regulated (×3.48, 2.19)	
		Whitefly	*Bemisia tabaci*	100 whiteflies/plant; 21 dpi	Kempema *et al.* (2007)
AtPME17	At2g45220			Up-regulated (×8.06)	
AtPMEPCRA (18)	At1g11580			Down-regulated (×–1.70)	
		Chewing insect	*Spodotera littoralis OS*	1 mm holes punctured and 1 μl of insect OS applied; 6, 24 hpi	Consales *et al.* (2011)
AtPME32	At3g43270		(oral secretion)	Up-regulated (×2.17, 1.58)	
AtPMEPCRA (18)	At1g11580			Up-regulated (×3.33, 1.77)	
		Chewing insect	*Pieris brassicae*	10–40 eggs/plant; 24, 48, 72 hpi	Little *et al.* (2007)
AtPME44	At4g33220			Down-regulated (× –1.54, –1.65, –1.39)	
Nicotiana attenuata (tobacco)		Chewing insect	*Manduca sexta* OS	Leaf wounded with a pattern wheel + 20 μl of diluted insect OS; 9 hpi	Von *et al.* (2006)
NaPME	DQ115979			Up-regulated (×2.59)	
		Nematode	*Meloidogyne javanica*	10–12 J_2 nematodes/root tip; 3 dpi	Barcala *et al.* (2010)
AtPME	At5g20860			Up-regulated (×3.05)	
AtPME	At1g11580			Up-regulated (×3.56)	
AtPME17	At2g45220			Down-regulated (× –3.54)	
		Nematode	*Heterodera schachtii*	250 J_2 nematodes/plant; 3, 8, 13 dpi	Hewezi *et al.* (2008)
AtPME3	At3g14310		*or Meloidogyne incognita*	Up-regulated (×2.0, 3.5, 3.0)	
Solanum lycopersicum (tomato)		Nematode	*Globodera rostochiensis*	10 000 J_2 nematodes/plant; 14 dpi	Uehara *et al.* (2007)
LePME	SNG-U213346			Up-regulated (×7.0)	
		Nematode	*Heterodera schachtii*	250 J_2 nematodes/plant; 3 dpi	Puthoff *et al.* (2003)
AtPME2	At1g53830			Down-regulated (× –3.8)	
AtPME17	At2g45220			Down-regulated (× –3.9)	
		Bacterium	***Pectobacterium carotovorum***	5×10^7 cfu ml^{-1}; 14 hpi	Raiola *et al.* (2011)
AtPME3	At3g14310			Up-regulated (×2.5)	
		Fungus	***Botrytis cinerea***	5×10^5 conidia ml^{-1}; 72 hpi	Raiola *et al.* (2011)
AtPME3	At3g14310			Up-regulated (×7.0)	
		Fungus	***Alternaria brassicola***	10, 24, 48 hpi	Narusaka *et al.* (2005)
AtPME3	At3g14310			Up-regulated (×7.2, 2.9, 4.1)	
		Fungus	***Alternaria alternata***	10, 24, 48 hpi	Narusaka *et al.* (2005)
AtPME3	At3g14310			Up-regulated (×11.2, 11.5, 6.8)	
Linum usitatissimum (flax)		Fungus	***Fusarium oxysporum or F. culmorum***	2 dpi (RT–PCR)	Wojtasik *et al.* (2011)
LuPME3	AF188895			Down-regulated	
LuPME5	AF355057			Down-regulated	
Medicago truncatula (barrel medic)		AM fungus	*Glomus mosseae*	28 dpi	Hohnjec *et al.* (2005)
MtPME	TC78420		or *Glomus intraradices*	Up-regulated (×2.56 GM, ×2.66 GI)	

Table 2. *Continued*

Gene name	AGI or accession	Stress	Species name(s)	Induction	References
MtPME	TC82059			Up-regulated (×2.33 GM, ×4.11 GI)	
Sesbania rostrata (Sesbania)		Bacterium	*Azorhizobium caulinodans*	48 hpi	Lievens *et al.* (2001)
SrPME1	Srdd18			Up-regulated (RT–PCR)	Lievens *et al.* (2002)
Solanum tuberosum cv Igor (potato)		Virus	PVY^{NTN}	0.5 hpi	Baebler *et al.* (2009)
StPME	STMHY50			Down-regulated (× –1.27)	
		Virus	TuMV	5 dpi	Yang *et al.* (2007)
AtPME3	At3g14310			Down-regulated (× –2.64)	
		Virus	CaLCuV	12 dpi	Ascencio-Ibanez *et al.* (2008)
AtPMEPCRA (18)	At1g11580			Down-regulated (× –1.14)	
		Virus		4 dpi	Whitham *et al.* (2003)
AtPMEPCRA (18)	At1g11580		CMV, ORMV, TuMV, PVX, TVCV	Up-regulated (×2.2–3.8)	
Vigna inguilata (cowpea)		Parasitic plant	*Striga gesnerioides*	6 dpi or 13 dpi	Huang *et al.* (2012)
VuPME	33686210			Up-regulated 6 dpi (×3.24) and 13 dpi (×3.29)	
Pectin acetylesterases (PAEs)					
		Whitefly	*Bemisia tabaci*	100 silverfly/plant, 21 dpi	Kempema *et al.* (2007)
AtPAE	At4g19420			Up-regulated (×2.89)	
AtPAE	At5g45280			Down-regulated (× –1.78)	
		Aphid	*Myzus persicae*	20 (3 dpi) or 100 aphids (7 dpi) /plant	Divol *et al.* (2005)
AtPAE	CN254169			Up-regulated (×3.78, 2.16)	
Malus domestica (apple tree)		Aphid	*Dysaphis plantaginea*	20 aphids/leaf, 72 hpi	Qubbaj *et al.* (2005)
MdPAE	CB035291			Up-regulated	
		Chewing insect	*Spodotera littoralis* OS	1 mm holes punctured and 1 μl of insect OS applied; 6, 24 hpi	Consales *et al.* (2011)
AtPAE	At2g46930			Up-regulated (×2.83, 2.30)	
		Nematode	*Meloidogyne incognita* or *Heterodera schachtii*	1, 2, 3, 5, 7 dpi	Vercauteren *et al.* (2002)
AtPAE	AY050847			Up-regulated	
Polygalacturonases (PGs)					
		Aphid	*Brevicoryne brassicae*	32 aphids/plant	Kusnierczyk *et al.* (2008)
AtPG	At5g49215			Down-regulated 6, 12, 24, 48 hpi (× –1.21, –1.46, –1.38, –1.52)	
AtPG	At3g62110			Down-regulated 6, 12, 24, 48 hpi (× –1.53, –1.43, –1.56, –1.55)	
AtPG	At1g60590			Down-regulated 6, 12, 24, 48 hpi (× –1.33, –1.45, –1.39, –1.51)	
AtPG	At4g23820			Down-regulated 6, 12, 24, 48 hpi (× –1.14, –1.27, –1.51, –1.62)	
AtPG	At3g06770			Down-regulated 6, 12, 24, 48 hpi (× –1.48, –1.83, –1.92, –1.83)	
AtPG	At1g10640			Down-regulated 6, 24, 48 hpi (× –1.78, –1.41, –1.88)	
		Aphid	*Myzus persicae* saliva infiltration	50 aphids/plant, 24 (OS), 48, 72 (OS+feeding) hpi	De Vos and Jander (2009)
AtPG	At1g60590			Down-regulated (× –2.69, –22.7)	
		Nematode	*Heterodera schachtii*	250 J_2 nematodes/plant, 3 dpi	Puthoff *et al.* (2003)

Table 2. *Continued*

Gene name	AGI or accession	Stress	Species name(s)	Induction	References
AtPG	At2g41850			Up-regulated (×3.7)	
AtPG	At1g05660			Down-regulated (×3.1)	
		Nematode	*Meloidogyne javanica*	10–12 J_2 nematodes/root tip; 3 dpi	Barcala *et al.* (2010)
AtPG	At4g23820			Up-regulated (×3.03)	
Medicago truncatula (barrel medic)		AM fungus	*Glomus mosseae*	28 dpi	Hohnjec *et al.* (2005)
MtPG	TC88957		or *Glomus intraradices*	Up-regulated (×2.53 GM, ×4.50 GI)	
Medicago truncatula (barrel medic)		Bacterium	*Sinorhizobium meliloti*	24, 48 dpi	Lohar *et al.* (2006
MtPG	TC78631			Up-regulated (×1.14, 1.41)	
MtPG	TC89800			Up-regulated (×1.45, 1.25)	
MtPG	TC91368			Up-regulated (×1.14, 1.12)	
MtPG	TC87651			Up-regulated (×2.88, 2.23)	
Medicago sativa (alfafa)		Bacterium	*Sinorhizobium meliloti*	24 dpi	Munoz *et al.* (1998)
MsPG	MsPG3			Up-regulated (RT–PCR)	
Solanum tuberosum cv Igor (potato)		Virus	PVY^{NTN}	0.5 hpi	Baebler *et al.* (2009)
StPG	STMIS28			Down-regulated (× –1.43)	
Pectate lyase-like proteins (PLLs)					
		Aphid	*Brevicoryne brassicae*	32 aphids/plant, 6, 12, 24, 48 hpi	Kusnierczyk *et al.* (2008)
AtPL	At1g67750			Down-regulated (× –2.00, –1.62, –1.64, –1.85)	
Glycine max (soybean)		Aphid	*Aphis glycines*	40 aphids/plant, 6 hpi	Li *et al.* (2008)
GmPL	AI748551 (At1g67750)			Down-regulated (× –1.52)	
GmPL	AW099533			Down-regulated (× –1.54)	
GmPL	AW309146 (At1g11920)			Down-regulated (× –1.62)	
		Chewing insect	*Spodotera littoralis* OS	1 mm holes punctured and 1 µl of insect OS applied; 6, 24 hpi	Consales *et al.* (2011)
AtPNL	At3g15720			Up-regulated (×5.28, 2.0)	
AtPNL	At3g61490			Up-regulated (×12.13, 2.0)	
		Chewing insect	*Pieris brassicae*	Down-regulated (× –1.48, –1.45, –1.23)	Little *et al.* (2007)
AtPNL	At5g48900			10–40 eggs/plant, 24, 48, 72 hpi	
		Nematode	*Meloidogyne javanica*	10–12 J_2 nematodes/root tip; 3 dpi	Barcala *et al.* (2010)
AtPL	At3g53190			Up-regulated (×4.88)	
Medicago truncatula (barrel medic)		AM fungus	*Glomus mosseae*	28 dpi	Hohnjec *et al.* (2005)
MtPL	TC88957		or *Glomus intraradices*	Up-regulated (×2.03 GM, ×3.00 GI)	
Medicago truncatula (barrel medic)		Bacterium	*Rhizobium meliloti*	6, 12, 24, 48 dpi	Lohar *et al.* (2006)
MtPL	TC89125			Up-regulated (×5.12, 2.37, 2.73, 1.18)	
Vigna inguilata (cowpea)		Parasitic plant	*Striga gesnerioides*	13 dpi	Huang *et al.* (2012)
VuPL	33691436			Down-regulated (× –8.86)	

Piercing–sucking insects (yellow box), chewing insects (turquoise), nematodes (blue), bacteria (black), fungi (light grey), arbuscular mycorrhizal fungus (dark grey), viruses (red). and parasitic flowering plants (green).
Species names in bold indicate necrotrophic pathogens and underlined gene names refer to characterized enzymes.

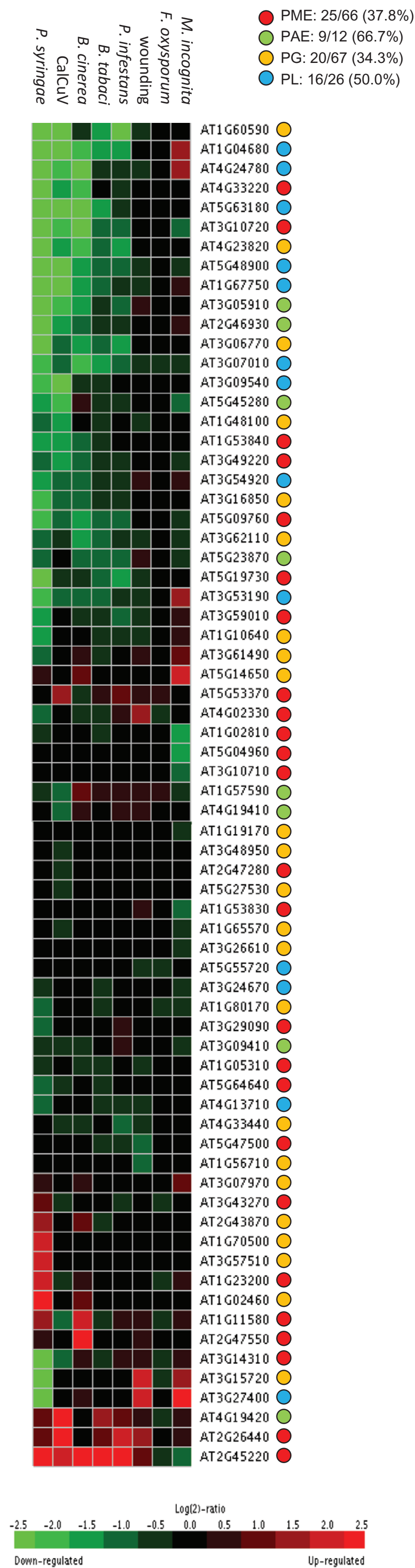

Fig. 5. Expression pattern of PME, PAE, PG and PLL mRNA in *A. thaliana* Col-0 in response to necrotrophic fungi (48 h, *Botrytis cinerea*, *Fusarium oxysporum*), hemibiotrophic oomycete (24 h, *Phytophthora infestans*), biotrophic bacterium (24 h, *Pseudomonas syringae*), herbivore insects (21 d, *Bemisia tabaci*), nematode (28 d, *Meloidogyne incognita*), virus (24 h, *Cabbage leaf curl virus* CalCuV), and leaf wounding with needles (24 h). PMEs, PAEs, PGs, and PLLs (red, green, orange, and blue circles) are shown together. Data were analysed using the Genevestigator Meta-Analyzer Tools (www.genevestigator.com/gv/; Hruz *et al.*, (2008). Only probes with a single gene and genes showing a minimal expression were used for the analysis. For each HGME family, the percentage (%) of genes expressed among all family members is indicated.

of HGME genes regulated following a 48 h infestation by *B. cinerea* was 3-fold higher than for an *F. oxysporum* infestation. This suggests distinct response pathways at the cell wall level. Interestingly, whatever the HGME family considered, *F. oxysporum* induces the fewest changes in gene expression levels. While it has been suggested that necrotrophs and hemibiotrophs can modulate the expression of a higher number of PMEs than biotrophs (Lionetti *et al.*, 2012), it seems important to consider the pathogen species and probably the duration of infection. For example, after 48 h of infestation, HGME modifications induced by the two necrotrophic fungi, *F. oxysporum* and *B. cinerea*, belong to the two different clusters defined before, while, within the same cluster, most HGME genes are more extensively modified after 24 h of infection by the biotrophic bacterium *P. syringae* than by the necrotrophic fungus *B. cinerea* (29, 24, and 42% against 23, 16, and 35% of PME, PG, and PL, respectively). Only PAEs are recruited at the same level in both infections. Changes in the expression of HGME genes were observed for the duration of an interaction that can reach several weeks in the case of piercing–sucking insects and nematodes; the time needed to complete development steps or even a whole reproduction cycle. After 21 d of continuous feeding in a single phloem sieve element by the whitefly nymph *B. tabaci*, or after 28 d of infestation by the nematode *M. incognita*, the number of genes with modified expression was almost the same as after *B. cinerea* infection. Comparing the two piercing–sucking phytophagous insects, each belonging to the two different clusters, the overall number of PMEs and PLs differentially expressed is relatively similar, but differences lie in the identity of the isoforms. The changes in gene expression are likely to concern isoforms that are organ specific; nematodes feeding on roots and whiteflies on leaves. Interestingly, 24 h after leaf wounding, the HGME pattern belongs to the same cluster as that of nematode infestation. Current challenges include the identification of the specific roles of HGME isoforms in biotic interactions (host species, bioaggressor lifestyle, plant phenology, attacked organ, and duration of infestation/infection). This might help to build general and specific models of the response of the plant at the cell wall (pectin) level.

Role of HGMEs in the establishment of feeding structures during biotic interactions

Except for chewing insects and necrotrophic pathogens, in most cases a feeding connection is established during plant–bioaggressor interactions. Most hemibiotrophic and obligate biotrophic fungi, or oomycetes use an appressorium followed by a penetration peg that goes through the plant cell wall to develop a haustorium for host cell nutrient absorption. Nutrient trafficking passes through the plant extrahaustorial membrane and matrix to the hyphal wall and membrane

(Szabo and Bushnell, 2001; Underwood, 2012), while the whole haustorium is encased. Haustoria are also described as special organs of most parasitic plants and arbuscular mycorrhizal fungi for penetration and development of vascular connections within the host plant for feeding (Leake, 1994; Lee, 2007). Cell wall remodelling, and thus cell wall-degrading enzymes (CWDEs), including HGMEs, are necessary to establish a conductive system (Deising *et al.*, 1995). CWDEs are secreted by pathogens, plant parasites, or mycorrhizal fungi to develop the haustorium and by the plant to restrict its development, especially through the cell wall apposition (papilla). Although callose and lignin have been described as the main embedded compounds for cell wall strengthening in the vicinity of the haustorium, pectate gel generated following PME activity could also play a role (Micheli, 2001) (Fig. 4). In fact, two PME genes are overexpressed in *Medicago truncatula* roots infected by the endosymbiotic fungus *Glomus* sp. (Hohnjec *et al.*, 2005). In cowpea (*Vigna unguiculata*) infected by the holoparasite *Striga gesnerioides*, in parallel with the overexpression of one PME gene, one PLL is underexpressed in roots (Huang *et al.*, 2012). Although to date no specific role has been demonstrated for plant HGMEs during haustorium differentiation, in wheat leaves infected by the pathogen *Blumeria graminis*, papilla size correlated with peroxidase activity and H_2O_2 accumulation, depending on the DA of plant pectin fragments used as elicitors (Randoux *et al.*, 2010).

HGMEs are also good candidates for modulating cell wall structure during intrusive cell growth that occurs when plants interact with biotrophic, hemibiotrophic, and arbuscular mycorrhizal fungi (Perfect and Green, 2001) and with parasitic plants (Leake, 1994). HGMEs could also modulate the interaction with aphids and nematodes (Wyss and Zunke, 1986; Tjallingii and Esch, 1993). For instance, the trophic behaviour of the aphid *Myzus persicae* was modified within 8 h of infestation in *A. thaliana* plants knocked out for the expression of the *AtPME17* gene (C. Wattier *et al.*, unpublished). Interestingly, the expression of *PME17* is markedly increased following interactions with other aphids (*Brevicoryne brassicae*) or whitefly (*B. tabaci*) (Fig. 5) (Kempema *et al.*, 2007; Kuśnierczyk *et al.*, 2008). As the salivation phases were longer in the *pme17* mutant, PME17 could have a role in facilitating the progression of the stylets in wild-type plants. Similarly, very early in root knot and cyst nematode infections (*M. incognita* and *Heterodera schachtii*, respectively), an up-regulation of one *AtPAE* was shown using *in situ* localization (Vercauteren *et al.*, 2002). The activity of this HGME could be involved in softening the plant cell wall to establish a feeding site, which is an induced multinucleate and physiologically active aggregation of fused root cells that exclusively provides nutrients during the nematode sedentary life (Szabo and Bushnell, 2001; Vercauteren *et al.*, 2002). Similarly, AtPME3 seems to be crucial in the early phase of the establishment of the syncytium, the feeding site of the cyst nematode, as it appeared to be a virulent target in the *A. thaliana–H. schachtii* interaction (Hewezi *et al.*, 2008). Using a yeast two-hybrid screen, PME3, whose gene expression was increased during infestation, was identified as interacting with a cellulose-binding protein (CBP) secreted by *H. schachtii* (Hewezi *et al.*, 2008). Results obtained using transgenic lines suggest that PME3 could be recruited by CBP to modify the plant cell wall, thus helping cyst nematode parasitism. However, this is unlikely to be the whole mechanism for parasitism success. Similarly, a so-called 'welcoming programme', characterized by huge cell reorganizations and by the formation of root hairs, has been related to the facilitation of the intercellular progression of infection threads or hyphae during the initiation of endosymbiotic interactions (van Brussel *et al.*, 1992; Bonfante, 2001; Hause and Fester, 2004). For instance, during legume–*Rhizobium* or arbuscular mycorrhizal fungus symbioses, several plant HGMEs were involved in the early stages of the interaction (Lohar *et al.*, 2006; Oldroyd *et al.*, 2011). Four PG genes and one PLL gene were overexpressed in *M. truncatula–Rhizobium meliloti* interactions, while in *Medicago sativa* one PG was specifically localized in the cell wall of nodule primordia and invasion zones from 1 h to 2 d post-inoculation (Muñoz *et al.*, 1998; Lohar *et al.*, 2006). Using mutant plants, a PL from *L. japonicus* appears essential for the proper initiation of *Rhizobium trifolii* infection (Xie *et al.*, 2012). Moreover, *in situ* localization showed that PMEs could contribute to the development of new vascular tissues during rhizobial infection (Lievens *et al.*, 2002). This is consistent with the analysis of microarray data sets, showing that *M. truncatula* PGs, PLLs, and PMEs are overexpressed 28 d after inoculation with *Glomus* sp. (Hohnjec *et al.*, 2005) and that PG activity is localized in the cell wall of lateral root primordia (Peretto *et al.*, 1995). The specific case of parasitic plants is of interest as, like host plants, they secrete their own HGMEs to facilitate their penetration into the host plant root cortex. For instance, *Orobanche* sp. secretes PMEs and their activity is correlated with the localization of low esterified HG in the cell wall and the middle lamella at the site of contact between host and parasitic cells (Ben-Hod *et al.*, 1993; Losner-Goshen *et al.*, 1998). This plant–plant interaction raises fascinating questions concerning the roles and specificity of host and parasite HGMEs. In particular, the study of the potential inhibition of parasitic plant PMEs by host plant PMEIs would be of great interest.

Host plant HGMEs are thus largely involved during the establishment of the feeding structures of bioaggressors, both to facilitate their intrusion and to restrict their excessive spreading. Their role in the fine-tuning of cell wall remodelling in favour of parasitic success at an early stage suggests that HGME activity has been diverted during a co-evolution process. Virulence factors of bioaggressors that target plant protein involved in defence responses, and the binding of exogeneous protein to some plant PMEs, appear as examples. Reported for plant–nematode interactions, this targeting has previously been highlighted for plant–virus interactions. The movement protein (MP) of *Tobacco mosaic virus* (TMV), which can be transmitted by piercing–sucking insects, interacts with a PME purified from tobacco leaves and this interaction is required for TMV cell to cell movement in the host plant (Dorokhov *et al.*, 1999; Chen *et al.*, 2000). The reduction of total PME activity, using antisense suppression of the

expression of one PME or the overexpression of a characterized PMEI, led to the delayed systemic movement of TMV (Chen and Citovsky, 2003). In the meantime, using transgenic plants overexpressing PME, an inverse correlation between PME activity and TMV lesion sizes has been demonstrated (Gasanova *et al.*, 2008). In this respect, the common hypothesis that plant HGMEs play a role in plant resistance by mediating cell wall strengthening or producing endogenous elicitors is probably too simplistic.

Roles of HGMEs in structural resistance

The cell wall represents an impenetrable physical barrier with constitutive rigidity to fend off bioaggressor attacks (Vallarino and Osorio, 2012). A high DM of HG has been correlated to genotype resistance to the aphid *Schizaphis graminum*, the biotrophic fungus *Colletotrichum lindemuthianum*, and the necrotrophic bacterium *Ralstonia solanacearum* (Table 3) (Dreyer and Campbell, 1984; Boudart *et al.*, 1998; Wydra and Beri, 2006). This could be related to changes in cell wall elasticity and mechanics. As shown during organ initiation (Peaucelle *et al.*, 2011*a*), the lowest wall elasticity is correlated to the highest DM. Furthermore, a high constitutive DM of pectin was structurally related to the borate–RGII cross-link in regulating cell wall stiffness (Ishii and Matsunaga, 2001). As such, plant resistance to the necrotrophic bacterium *Pectobacterium carotovorum* is associated with a high DM of pectin in wild potato plants (McMillan *et al.*, 1993; Marty *et al.*, 1997) and in the *pme3* mutant of *A. thaliana* (Raiola *et al.*, 2011). The enhanced DM level obtained by mutation of *PME* genes or by overexpression of *PMEI* genes *in planta* increased the resistance of dicotyledonous and monocotyledonous species to biotrophic or necrotrophic pathogens, but not to the full range of pathogens for each plant (Table 3) (Lionetti *et al.*, 2007; An *et al.*, 2008; Raïola *et al.*, 2011; Volpi *et al.*, 2011). Furthermore, *in vitro*, the hydrolysis of plant pectin by endo-PGs from several necrotrophic pathogens is decreased when pectins of high DM are used as carbon sources (Bonnin *et al.*, 2002). The resistance observed could therefore be related to a decrease in the number of specific substrates for endogenous PGs, as well as the physical or chemical properties of pectins, such as the isoform pattern of HGME activity or the amount of branched pectins. Using antisense tomato plants, the decrease in plant PG activity reduced, as expected, fruit softening and ripening, but also increased tomato resistance against biotrophic or necrotrophic pathogens (Damasceno *et al.*, 2011). Nevertheless, enhancing resistance through the modulation of the DM of HG is unlikely to be an easy strategy as it appears to be pathogen specific. For instance, increased PME activity and the associated lower DM of pectins, in transgenic strawberry overexpressing one PME, enhanced fruit resistance to the necrotrophic fungus *B. cinerea* (Osorio *et al.*, 2008). Understanding this opposite effect is complex as PME activity on HG can have two distinct consequences. On one hand, the linear activity of PME can give rise to blocks of free carboxyl groups that non-covalently interact with Ca^{2+} ions, conferring a gel-like structure and cell wall strengthening (Morris *et al.*, 1982; Micheli, 2001). On the other hand, random PME activity promotes the action of pectin depolymerases (endo-PG or lyase activities) increasing both cell wall loosening and porosity and producing OGs that elicit plant defence responses (Baron-Epel *et al.*, 1988; Ehwald *et al.*, 1992). Thus, in transgenic plants with modified HGME activity, either the direct or the indirect effect on HG structure could play a role in plant resistance. For example, constitutive gene expression of antimicrobial proteins (*PR5*) in transgenic strawberry overexpressing one PME enhanced basal resistance to *B. cinerea*, while a decrease in their level (PR1 and PR10) in *PMEI1*-silenced pepper conferred decreased basal resistance to the biotrophic bacterium *Xanthomonas campestris* (An *et al.*, 2008; Osorio *et al.*, 2008). Among PMEs, some (At1g11580) that have a ribosome-inactivating protein (RIP) activity might be considered antimicrobial proteins themselves. RIPs are known to be involved in plant defence against viruses (De-la-Peña *et al.*, 2008). Resistance to the fungus *Puccinia graminis* was associated with a random distribution of the methylesters of HGs in the near-isogenic resistant line as compared with a more blockwise distribution in the susceptible cultivar (Wiethölter *et al.*, 2003). The DA of pectins is also likely to play an important role in plant resistance. While the *Arabidopsis* mutant *reduced cell wall acetylation rwa2* was more resistant against the necrotrophic fungus *B. cinerea*, it was susceptible to the biotrophic fungus *Golovinomyces cichoracearum*. The 'antagonistic responses' to these pathogens are consistent with the two distinct plant defence pathways induced [jasmonic acid (JA)/ethylene (ET), versus salicylic acid (SA)]. All these results suggest an indirect link between cell wall-related basal structural resistance and inducible plant defences. The mutation of the plant putative pectate lyase PMR6 (*POWDERY MILDEW RESISTANT 6*), required for the virulence of *Erysiphe* sp., increased the content and DM of pectin as well as plant resistance, but surprisingly did not change either fungus penetration success or SA- and JA/ET-dependent defence responses. Similar results were obtained using the *pmr5* mutant; *PMR5* encodes a protein of unknown function required for pectin production and is likely to be targeted to the endoplasmic reticulum/secretory pathway. These results suggest non-elicitor OG production, highlighting the dual role of HGMEs in the control of cell wall stiffness during plant bioaggressor interactions. Finally, since HGME activities result in direct (strengthening) or indirect (OG elicitor production) resistance, they appear good candidates for virulent factor targeting.

HGMEs are involved in induced resistance against biotic stresses

The role of HGMEs in the production of endogenous elicitors during plant–bioaggressor interactions has been indirectly shown through the induction of all the main known plant defence responses following application of purified OGs, end-products of HGMEs. Indeed, within early signalling events, OGs induce H^+ and Ca^{2+} influx, K^+ efflux, membrane depolarization, extracellular medium alkalinization (Mathieu *et al.*, 1991), protein phosphorylation/dephosphorylation,

Table 3. *Biochemical implication of HG-modifying enzymes (PMEs, PAEs, and PLLs) and their inhibitor proteins (PMEIs, PGIPs, and PNLIP) in plant resistance against bioaggressors*

Gene name	Utilization	Stress		Induction	References
Pectin methylation and PME activity					
Sorghum bicolor (sorgho)	DM of pectin	Aphid	*Schzaphis graminum*	Resistant variety has higher methylated pectins than the suceptible	Dreyer and Campbell (1984)
Phaseolus vulgaris (bean)		Fungus	*Collectotrichum lindemuthianum*	Resistant line has higher methylated pectins than the suceptible	Boudart *et al.* (1998)
DM of pectin	Near-isogenic lines				
Solanum tuberosum cv Bintje or ADG (potato) DM of pectin		Bacterium	***Pectobacterium carotovorum***	Resistant genotype (ADG) has higher methylated pectins than the susceptible genotype (Bintje)	Marty *et al.* (1997)
Potato DM of pectin	Somatic hybrid of 3 cv (Record, Estima, Katahdin)	Bacterium	***Pectobacterium carotovorum***	Resistant genotype has higher methylated pectins than the susceptible genotype	McMillan et al (1993)
Solanum lycopersicum (tomato)	DM of pectin	Bacterium	***Ralstonia solanacearum***	Resistant genotype (Hawaii7996) has higher methylated pectins than the susceptible genotype (Wva700)	Wydra and Beri (2006)
	(Hawaii7996, Wva700)				
Nicotiana attenuata (tobacco)	PME activity	Chewing insect	*Manduca sexta* OS	Leaf wounded with a pattern wheel; 20 µl of diluted insect OS applied; PME activity increased (29%) 30 min after OS applied	Von Dahl *et al.* (2006)
	Wild type				
Nicotiana tabacum (tobacco)	PME	Virus	TMV	PME specifically recognized the TMV MP (movement protein)	Dorokhov *et al.* (1999)
Nicotiana tabacum cv. Turk (tobacco)	PME	Virus	TMV	Mutant TMV without MP proteins cannot link to tobacco PME (no lesions on leaves after TMV mutant infection)	Chen *et al.* (2000)
Nicotiana tabacum cv. Samsun (tobacco)		Virus	TMV	PME activity increased >resistance increased (size of leaf necrosis and short- and long-distance transport decreased)	Gasanova *et al.* (2008)
PME activity	ProPME				
Nicotiana tabacum cv. Turk (tobacco)		Virus	TMV	PME activity decreased >symptome appearence delayed (5–12 times slower in the antisense line than in the wild type)	Chen and Citovsky (2003)
PME activity	Antisense suppression				
	pme3 KO	Fungus	***Botrytis cinerea***	PME activity decreased >DM decreased>resistance decreased	Raiola *et al.* (2011)
AtPME3	At3g14310				
AtPME3	*pme3* KO At3g14310	Bacterium	***Pectobacterium carotovorum***	PME activity decreased >DM decreased >resistance decreased	Raiola *et al.* (2011)
Fragaria vesca (wild strawberry)		Fungus	***Botrytis cinerea***	OGA with low DM >resist-ance increased	Osorio *et al.* (2008)

Table 3. *Continued*

Gene name	Utilization	Stress		Induction	References
PME activity	Overexpression line (FaPE1)				
Pectin acetylation					
	Mutant *Atrwa2*	Fungus	***Botrytis cinerea***	Pectin acetylation decreased >resistance increased	Manabe *et al.* (2011)
DA of pectin	(with 20% decreased acetylester content)				
Triticum aestivum (wheat)		Fungus	*Blumeria graminis*	OGA with high DA >resistance increased	Randoux *et al.* (2010)
DA of pectin	Chemical acetylation				
Pectate lyase-like (PLL)					
		Fungus	*Erysiphe cichoracearum*	10^8 cfu ml^{-1}; 1, 2, 4 dpi confers resitance to *E. cichoracearum*	Vogel *et al.* (2002)
AtPMR6	At3g54920				
Pectin methyl esterase inhibitors (PMEIs)					
	overexpression lines	Fungus	***Botrytis cinerea***	ATPMEI increased>PME activity decreased>DM increased>resistance increased	Lionetti *et al.* (2007)
AtPMEI1	At1g48020				
ATPMEI2	At3g17220				
Capsicum annuum (pepper)		Bacterium	*Xanthomonas campestris* pv. *vesicatoria*	CaPMEI inhibited >susceptibility increased	An *et al.* (2008)
CaPMEI1	Transgenic pepper silences CaPMEI1				
Capsicum annuum (pepper)		Bacterium	*Pseudomonas syringae* pv. *tomato*	CaPMEI overexpressed >resistance increased, but no resistance to the biotrophic fungus *Hyaloperonospora parasitica*	An *et al.* (2008)
CaPMEI1	Transgenic *A. thaliana* overexpresses CaPMEI1				
Actinidia chinensis (kiwi)		Fungus	***Fusarium graminearum***	AcPMEI expression >PME activity decreased >DM increased >resistance increased	Volpi *et al.* (2011)
AcPMEI	Transgenic wheat expresses AcPMEI				
Actinidia chinensis (Kiwi)		Fungus	***Bipolaris sorokiniana***	AcPMEI expression >PME activity decreased >DM increased >resistance increased	Volpi *et al.* (2011)
AcPMEI	Transgenic wheat expresses AcPMEI				
Polygalactuonase inhibitor proteins (PGIPs)					
		Fungus	***Botrytis cinerea***	AtPGIP1 increased >resistance increased	Ferrari *et al.* (2006)
AtPGIP1	Antisense suppression				
Brassica rapa (Chinese cabbage)		Bacterium	***Pectobacterium carotovorum***	BrPGIP2 overexpressed > resistance increased	Hwang *et al.* 2010
PGIP	Overexpression lines				
Solanum lycopersicum (tomato)		Fungus	***Botrytis cinerea***	pPGIP increased >resistance increased (*B. cinerea* endo-PGs inhibited)	Powell *et al.* (2000)
pPGIP	Expression of a pear PGIP				
Vitis vinifera (grape)		Fungus	***Botrytis cinerea***	VvPGIP1 increased >resistance increased (BcPG1 inhibited)	Joubert *et al.* (2006)

Table 3. *Continued*

Gene name	Utilization	Stress		Induction	References
VvPGIP1	Overexpression lines				
Vitis vinifera (grape)		Fungus	***Botrytis cinerea***	pPGIP increased >resistance increased	Agüero *et al.* (2005)
pPGIP	Expression of a pear PGIP				
		Fungus	***Botrytis cinerea***	AtPGIPs increased >resistance increased	Ferrari *et al.* (2003)
AtPGIP1, AtPGIP2	Overexpression lines				
Phaseolus vulgaris (bean)		Fungus	***Botrytis cinerea***	PvPGIP2 increased >resistance increased (BcPG1 inhibited)	Manfredini *et al.* (2005)
PvPGIP2	Overexpression lines				
Phaseolus vulgaris (bean)		Fungus	***Fusarium moniliforme***	PvPGIP2 inhibits FmPG	Federeci *et al.* (2001)
PvPGIP2					
Phaseolus vulgaris (bean)		Fungus	*Aspergillus niger*	PvPGIP inhibits AnPG (endoPGII)	King *et al.* (2002)
PvPGIP					
Phaseolus vulgaris (bean)		Fungus	***Botrytis cinerea***	PvPGIP2 increased >resistance increased (BcPG1 inhibited)	Sicilia *et al.* (2005)
PvPGIP2					
Pectin lyase inhibitor protein (PNLIP)					
Beta vulgaris (sugar beet)		Fungus	***Rhizoctonia solani***	Barley-grain inoculum applied for 2 weeks at 25 °C	Bugbee (1993)
PNLIP	PNLIP			PNLIP activity higher in rotted tissues than in healthy	

Piercing–sucking insects (yellow box), chewing insects (turquoise), bacteria (black), fungi (light grey), and viruses (red).
Species names in bold indicate necrotrophic pathogens.

mitogen-actived protein kinase (MAPK) cascades, GTP-binding protein, and reactive oxygen species (ROS) production (H_2O_2, O_2^-) (Shibuya and Minami, 2001; Vallarino and Osorio, 2012). They also induce the expression of defence genes encoding proteins involved in (i) defence protein accumulation such as protease inhibitors, pathogenesis-related proteins (PRs), or PGIPs; (ii) SA and JA/ET biosynthesis and signalling; (iii) biosynthesis of defensive secondary metabolites such as phytoalexins; or (iv) plant cell wall reinforcement (Shibuya and Minami, 2001) (Fig. 6; Supplementary Table S2 at *JXB* online). Some cell wall proteins were reported to be involved in cell wall strengthening and papilla formation [peroxidase and hydroxyproline-rich glycoprotein (HRGP)]. Among HGMEs, only PG and PL were identified following elicitation by OGs. Wound-inducible PG activity was correlated to H_2O_2 production in most plants belonging to different families (Orozco-Cardenas and Ryan, 1999). Both responses were induced by OG treatment in tomato leaves (Orozco-Cardenas and Ryan, 1999), where *PG* gene expression and its corresponding activity were transiently increased (Bergey *et al.*, 1999). In contrast, in *Arabidopsis* seedlings, a mixture of OGs (DP 9–16) did not change overall *PG* gene expression but repressed *PL-PMR6* expression (Denoux *et al.*, 2008). These differences are probably related to the plant species as well as the type and concentrations of OGs used. In fact, several studies have shown that the ability of OGs to act as elicitors is dependent on their chemical structure (DP, DM, pattern of methylesterification, and DA) (Ochoa-Villarreal *et al.*, 2012). OGs with DP ranging from 1 to 20 are efficient elicitors (Côté and Hahn, 1994) (Fig. 6). The treatment by flagellin, a microbial-associated molecular pattern (MAMP) from bacteria, is mimicked by OGs in a range of DP from 9 to 16 to induce plant defences including cell wall reinforcement (Denoux *et al.*, 2008). The critical DP triggering plant elicitation is dependent on both the type of defence responses measured for a given plant and the type of plant species (Côté and Hahn, 1994). OGs with a high DA led to wheat resistance against the necrotrophic fungus *B. graminis* (Randoux *et al.*, 2010), while fully methylated OGs failed to induce defence signalling in soybean (Navazio *et al.*, 2002). This variability highlights the difficulty in unravelling the relationship between the structure and function of OGs. Furthermore, in adequate ionic conditions, the Ca^{2+}-egg box conformation of OGs improves their biological activity (Fig. 6). For example, dimeric and trimeric association of OGs induced a higher level of early and late defence responses in carrot (Messiaen *et al.*, 1993). The ability of multimeric forms of OGs to elicit plant responses appears to depend on their maturation, which is likely to be necessary for their fixation on OG receptors (Cabrera *et al.*, 2008). To date, wall-associated kinase 1 (WAK1), belonging to the WAK gene family (five in *Arabidopsis*), is the only receptor characterized for OG recognition inducing the defence signalling cascade (Wolf *et al.*, 2012*a*) (Fig. 6). Using chimeric proteins, the binding

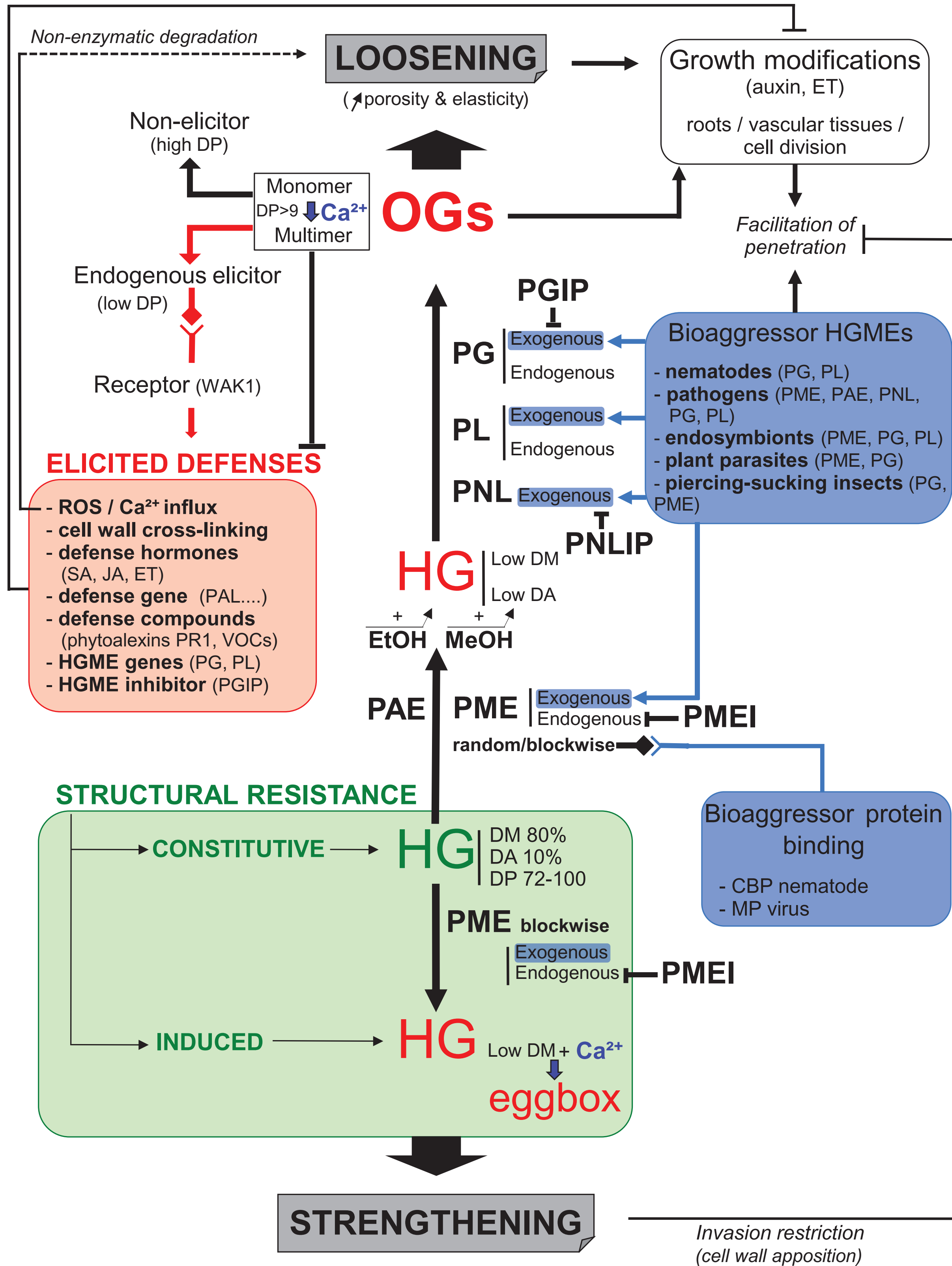

Fig. 6. Involvement of HGMEs in plant defence responses to biotic stresses. Homogalacturonans (HGs) embedded in the cell wall after their synthesis in the Golgi apparatus with a high degree of polymerization (DP, minimum 72–100 galacturonic acid residues; Thibault *et al*., 1993) are *O*-acetylated [with a degree of acetylation (DA) of ~10%; Ralet *et al*. (2005)], and highly methylated [with a degree of methylesterification (DM) of ~80%; O'Neill and Albersheim (1990)]. Depending on the stress and growth states, these HGs will be demethylesterified by pectin methylesterases (PMEs) and/or deacetylated by pectin acetylesterases (PAEs), both enzyme activities being associated with the release of the volatile compounds methanol and ethanol, respectively. Depending on the cell wall properties, PMEs can act linearly, giving rise to blocks of free carboxyl groups that interact with bivalent ions (Ca^{2+}) and contributing to cell wall strengthening (green frame), or can act randomly, promoting the action of downstream cell wall hydrolases [polygalacturonases (PGs) and pectate lyases (PLs)] and then contributing to cell wall loosening (Micheli, 2001). Cell wall strengthening restricts bioaggressor progression,

of OGs on its ectodomain was shown to activate specific plant defences following recognition by a leucine-rich repeat (LRR)-receptor kinase (EFR). Conversely, its intracellular kinase domain induced OG-specific plant defences after treatment by the EFR-specific elicitor (Brutus *et al.*, 2010). Interestingly, OG monomers, dimers, and trimers have been reported to be inhibitors of disease resistance reactions independently of the way they are produced (plant cell wall autolysis or pathogen CWDE digestion) (Messiaen *et al.*, 1993; Moerschbacher *et al.*, 1999) (Fig. 6). As monomeric OGs inhibit phytoalexin accumulation [i.e. phenylalanine ammonia lyase (PAL) activity] in carrot in contrast to the dimeric OG (Messiaen *et al.*, 1993), HGME activity associated with ionic cell status may be a way to regulate plant responses by modifying OG structure. In non-challenged plants, the difference in the rate of activity between intrinsic plant exo-PG and endo-PG that give rise to different OG structures (elicitors or not) supports a regulation of the balance between active/non-active OG forms by plant HGMEs during stress. Nevertheless, in plant–bioaggressor interactions, although the endogenous OG production is mostly described as being in favour of the plant, it may also benefit the bioaggressor. The combination of the action of both virulent microbes and plant HGMEs might suppress OGs with an eliciting function. PG, PL, and PME are found in plants and in secretions of most bioaggressors (microbes, nematodes, and insects) (Shen *et al.*, 2003, 2005; Harmel *et al.*, 2010; Sharma *et al.*, 2013) (Fig. 6). The types and distribution of HGMEs vary depending on the bioaggressor considered. For instance, among phytophagous insects, no HGME activity has been measured in chewing insects. In aphididae, PME activity has been detected in the saliva of all tested aphids except for *Sitobion avenae*, while they all possess PG activity (Harmel *et al.*, 2010). The differences in HGME content of bioaggressors, together with the activity of plant enzymes, may have an effect on the quantity and structure of OGs released, thus enhancing or inhibiting specific MAMPs or HAMPs (herbivore-associated molecular patterns) plant defence responses (Felton and Tumlinson, 2008). While an oxidative burst can be induced by OGs, ROS themselves (H_2O_2) can give rise to a non-enzymatic OG production by oxidative breakdown of pectins (Miller, 1986; Fry, 1998) (Fig. 6).

The aphid *Diuraphis noxia* induced massive H_2O_2 accumulation in resistance of wheat, while this effect was not detectable in the *Brevicoryne brassicae–Arabidopsis* interaction (Moloi and van der Westhuizen, 2006; Kuśnierczyk *et al.*, 2008). The observation of H_2O_2 accumulation in both compatible and incompatible interactions between the aphid *Macrosiphum euphorbiae* and tomatoes suggested that they might not have a preponderant role as enhancers of endogenous elicitor production (De Ilarduya *et al.*, 2003). If the production of effective OGs is considered to be related to the balance between endogenous and exogenous HGME activities, specific regulators of HGMEs (PMEI, PGIP, and PNLIP) have to be taken into account (Table 3; Fig. 6). Among these protein inhibitors, at least one PGIP appears specifically directed to exogenous HGMEs and is used as a marker of the plant defence response. Plant PGIPs interact with PGs from various bioaggressors such as bacteria, fungi, and phytophagous insects (Albersheim and Anderson, 1971; D'Ovidio *et al.*, 2004; Schacht *et al.*, 2011) but are unlikely to target plant PGs (Cervone *et al.*, 1990; Federici *et al.*, 2001). PGIPs from different plant species can reduce PG activity from distinct pathogen species (Table 3), and one single PGIP can inhibit different fungal PGs; the PGIP2 of common bean inhibits *Fusarium moniliforme*, *Aspergillus niger*, and *B. cinerea* PGs (Federici *et al.*, 2001; King *et al.*, 2002; Sicilia *et al.*, 2005). The expression of a pea PGIP gene was induced during pea defence against the cyst nematode *Heterodera goettingiana*; none of the PGs from the nematode was shown to interact with the plant PGIP (Veronico *et al.*, 2011). Results concerning potential pectin lyase inhibitor protein (PNLIP) are rather scarce. A PNLIP from sugar beet (*Beta vulgaris*) was shown to inhibit fungal pectin lyases from *Rhizoctonia solani*, *Phoma betae*, and *Aspergillus japonicus*, but information about the inhibitor structure and regulation in plants is so far lacking (Table 3) (Bugbee, 1993; Juge, 2006). Plant PMEIs, which only target plant PMEs, could, however, play a role in resistance to pathogens by targeting specific plant PME isoforms, thus modulating the structure of pectins (Lionetti *et al.*, 2007).

Plant HGMEs are involved in the emission of two volatile organic compounds (VOCs), MeOH and EtOH (Yadav *et al.*, 2009), released by PME and PAE activity, respectively (Fig. 6). PME-mediated pectin remodelling appears to be the main MeOH producer, while EtOH is mostly attributed to fermentive reactions of glucose (Fall, 1999; Seco *et al.*, 2007). Among the huge range of VOCs produced during plant defence responses, an increase in MeOH emissions has been measured after wounding (Dorokhov *et al.*, 2012*a*) and feeding by herbivore caterpillars (Peñuelas *et al.*, 2005; Körner *et al.*, 2009). In tobacco leaves, rapid and sustained emission of MeOH was observed after *Manduca sexta* wounding, and was enhanced in the presence of caterpillar oral secretions, due to both up-regulation of gene expression and activity of plant PMEs, and a decrease in the DM of pectins (Von Dahl

while cell wall loosening may facilitate their penetration. The various bioaggressors (blue frames) that attempt to breach the plant cell wall release their own HGMEs (exogenous), including pectin lyases (PNLs), that could amplify or compete with the hydrolysis by plant HGMEs (endogenous). Consequently, following the activity of plant and bioaggressor hydrolases, small HG fragments called oligogalacturonides (OGs) are released. Most of these enzymes (plant PMEs or bioaggressor PGs, PNLs) could be modulated by PME, PG, or PNL inhibitors (PMEIs, PGIPs, and PNLIPs). Depending on their DP and the amount of free Ca^{2+}, the OG monomers obtained can form multimers with an egg box conformation (Cabrera *et al.*, 2008) and/or can act as endogenous elicitors. After OG recognition by a specific receptor [wall-associated kinase 1 (WAK1); Brutus *et al.* (2010)], various defence responses (red frame) are induced against the bioaggressor (Ridley *et al.*, 2001) and could interact with growth modifications. Among them, ROS production (H_2O_2) can contribute to cell wall loosening with a non-enzymatic degradation of cell wall polysaccharides including HG (OG production; Miller, 1986; Fry, 1998). Plant PMEs also appear to act more directly during an interaction with some bioaggressors as they (bottom blue frame) specifically bind to a virus movement protein (MP; Chen *et al.*, 2000) or a nematode cellulose-binding protein (CBP; Hewezi *et al.*, 2008).

et al., 2006). The role of MeOH in induced plant resistance appears dual. It may either diffuse as a signal or be catabolized into compounds that might be used in plant defence. First converted by a putative methanol oxidase into formaldehyde, a lethal compound, it is quickly bound to a nucleophile such as glutathione (*S*-formylglutathione), turned into formate, and used as a carbon source incorporated into plant one-carbon metabolism (C1 folate pool) or the Calvin–Benson cycle (CO_2) (Gout *et al.*, 2000; Achkor *et al.*, 2003; Wojtasik *et al.*, 2011). Ethanol may also be oxidized into acetyl-coenzyme A via alcohol dehydrogenases involved in primary metabolism (Leblová *et al.*, 1977). In *Arabidopsis*, glutathione-dependent formaldehyde dehydrogenase, known as *S*-nitrosoglutathione reductase (GSNOR), plays a key role in regulating nitric oxide and *S*-nitrosoglutathione levels as well as being a signal in systemic resistance against pathogens (Martínez *et al.*, 1996; Rustérucci *et al.*, 2007). As nitric oxide and *S*-nitrosothiols are signalling molecules that regulate immunity, MeOH release by PMEs seems to act as a quantitative signal during plant–herbivore interactions. Silencing the endogenous PME gene suppressed MeOH release and led to reduced accumulation of PGIP involved in tobacco leaf resistance against *M. sexta* (Körner *et al.*, 2009). While wounding regulated the GSNOR gene, the direct effect of MeOH on its regulation is still not clear (Downie *et al.*, 2004). For instance, several MeOH-inducible genes were identified, some of which are known to encode proteins involved in plant resistance, especially in antibacterial resistance, virus spreading (Dorokhov *et al.*, 2012*a*), or anthocyanin and flavonoid content (Downie *et al.*, 2004). PME-mediated MeOH production was recently shown to act as a cross-kingdom signal (Dorokhov *et al.*, 2012*b*). Indeed, in mice, some MeOH-inducible genes are involved in their preference for MeOH sources such as wounded leaves. As caterpillar oral secretions increased VOC emission, which is known to attract predators or parasitoids against insects or nematodes (Kahl *et al.*, 2000; Heil, 2008), as well as MeOH (Von Dahl *et al.*, 2006), PME appears to play a role in both indirect (VOCs) and direct plant defences (signal/elicitor producer).

Concluding remarks and perspectives

The contribution of the changes in the pectic network to the changes in cell wall rheology, enabling anisotropic growth or response to biotic stress, has been well documented over recent years. However, how the changes in HG-type pectins are spatially and temporally mediated, through the specific action of HGMEs, remains a central issue in our understanding of plant development. Until now, major advances in understanding the contribution of these enzymes to changes in development have mainly concerned PMEs. This notably includes their roles in mediating discrete changes in HG structure during the interaction with pathogens, the regulation of primordia emergence, pollen tube and hypocotyl elongation, as well as the identification of novel post-translational control of their activity through the processing of the proteins by serine proteases and/or their interaction with specific inhibitors (PMEIs). Although much progress has been made when considering PMEs, many challenges remain; for instance, the identification of specific PME–SBT and PME–PMEI pairs in the cell wall, the understanding of the potential polarity of the trafficking of PMEs to the cell wall, and its role in generating specific localized demethylesterification patterns through interaction of the enzymes with pH and ion microdomains. In addition, given the recent discovery of the interplay between hormonal signalling and PMEs, further research could include the determination of the upstream regulators of PME transcription, including transcription factors and hormone levels, and possible feedback loops.

Other classes of plant HGMEs, including PGs, PLLs, and PAEs, have received surprisingly little attention over the last few years, which probably does not reflect the importance of these enzymes in mediating changes in HG structure. As for PMEs, this could be related to the difficulties in determining strong phenotypes in KO mutants, with the occurrence of compensation mechanisms. When considering these multigenic families, the identification of compensation isoforms using dedicated tools, at both the transcript and protein levels, will help to provide a comprehensive overview of the underlying changes in cell wall structure. In particular, the roles of pectic fragments in generating plant responses to stress and in mediating changes in development will need further investigation. This will involve the identification of potential receptors of cell wall fragments, and of their specificity with regard to DP, DM, or DA. In parallel, the biochemical characterization of PMEs, PGs, PLLs, and PAEs will enable their substrate specificity and pH preference to be determined. In particular, how the PME-mediated changes in HG structure can influence the activity of PGs, PLLs, and PAEs will be a key issue in our understanding of the possible interplay of these enzymes *in muro*. This could be used to implement the current models illustrating the interaction between HGs and HGMEs in the cell wall environment (Figs 4, 6).

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Comparative inventory of the structural motifs of PME, PAE, PG, and PLL isoforms between dicot and monocot species.

Table S2. Gene expression variations of HG-modifying enzyme inhibitor proteins (PMEIs and PGIPs) after biotic stresses.

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