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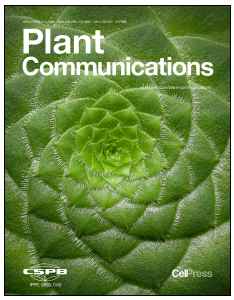

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Pectin Under Stress: Pectin Methylesterases at the Core of Cell Wall Remodeling, Integrity, and Signaling in Plants

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RUNNING TITLE

PME-dependent pectin remodeling and signaling

SHORT SUMMARY

Pectin methylesterases emerge as multifunctional regulators linking cell wall remodeling, mechanoperception, and stress signaling in plants. Through coordinated regulation by PME inhibitors, subtilases, apoplastic pH, and Ca^{2+} availability, PME activity dynamically controls pectin de-methylesterification, receptor complex organization, danger signal generation, wall mechanics, and ion buffering during biotic and abiotic stress responses.

Abstract

Plant cell walls are dynamic signaling platforms that integrate mechanical, biochemical, and immune cues during development and in response to environmental stress. Among wall polysaccharides, pectin has emerged as a central regulator of cell wall integrity and stress-responsive signaling. Here, we highlight pectin methylesterases and their regulators as a coordinated modulatory system controlling pectin remodeling in space and time during biotic and abiotic stresses. Recent discoveries on isoform-specific pectin methylesterase functions, proteolytic activation, and trafficking routes are discussed. During biotic interactions, pectin methylesterase activity promotes pectin remodeling for cell wall reinforcements. It also contributes to cell wall integrity sensing by supporting the generation of methanol and, indirectly, oligogalacturonides, and by enabling the formation of homogalacturonan structures that facilitate mechanosensor and receptor binding. Under abiotic stress, pectin methylesterase-driven de-methylesterification influences cell wall stiffness, mechanics, porosity, and ion homeostasis. This review highlights pectin methylesterases as central regulators of pectin dynamics that underlie cell wall remodeling, signaling, and stress responses, identifies key knowledge gaps and outlines future directions to enhance plant stress resilience.

Keywords: Plant stress, Pectin methylesterase, Pectin methylesterase inhibitors, Pectin remodeling, Damage associated molecular patterns, Cell wall integrity

1.INTRODUCTION

As sessile organisms, plants are continuously exposed to biotic and abiotic stresses and rely on coordinated extracellular and intracellular responses to maintain fitness (Kong and Yang, 2023; Traubenik et al., 2024). In this context, the cell wall (CW) acts as a dynamic and responsive interface that integrates environmental signals and orchestrates defense responses (Mohnen, 2008; Pogorelko et al., 2013; Molina et al., 2024; Vicré and Lionetti, 2023). The CW is a dynamic structure composed mainly of polysaccharides, including cellulose, hemicellulose, and pectin, together with phenolic compounds, proteins, ions, and water (Delmer et al., 2024). This structural complexity underpins the concept of CW integrity (CWI), defined as the functional state of the wall that ensures proper cellular homeostasis and growth. CWI is preserved through surveillance systems that sense structural perturbations and activate downstream signaling pathways, triggering compensatory responses to restore wall functionality under both developmental and stress conditions (Engelsdorf et al., 2018; Bacete and Hamann, 2020; Baez et al., 2022).

Among CW components, pectin plays a central role in CWI surveillance and maintenance (Lionetti et al., 2017; Bhandari et al., 2025). These galacturonic acid-rich polysaccharides comprise several structural domains, including homogalacturonan (HG), rhamnogalacturonans I and II and xylogalacturonan (Lionetti et al., 2007; Delmer et al., 2024). HG is a linear homopolymer of α-1,4-linked D-galacturonic acid (GalA) residues. HG biosynthesis occurs in the Golgi apparatus and contributes to CWI maintenance and immunity (Bethke et al., 2016; Du et al., 2022). The HG backbone is synthesized by galacturonosyltransferases (GAUTs), which transfer GalA from UDP-GalA (Atmodjo et al., 2011; Engle et al., 2022). HG is methylesterified at the C-6 position by HG methyltransferases (HG-MTs), which use S-adenosylmethionine (SAM) as the methyl donor, imported into the Golgi lumen by Golgi SAM transporters (GoSAMTs) (Mouille et al., 2007; Ibar and Orellana, 2007; Temple et al., 2022) (Figure 2).

These activities establish the initial degree and pattern of HG methylesterification prior to secretion thereby providing the biochemical framework for subsequent HG remodeling in the apoplast. This is driven by integrating biosynthesis and continuous post-synthetic modification of wall polymers, particularly under stress conditions (Rui and Dinneny, 2020; Swaminathan et al., 2021; Swaminathan et al., 2022). Post-synthetic remodeling is regulated by multiple factors, including pectin-modifying enzymes, notably pectin methylesterases (PMEs) (Lionetti et al., 2010; Yang et al., 2018). PMEs catalyze the hydrolysis of methyl ester groups at the C-6 position of GalA residues in HG, releasing protons and generating free carboxyl groups along the pectin backbone (Figure 1A). This activity impact on both the degree and spatial pattern of HG methylesterification (Lionetti et al., 2012; Wu et

al., 2018). PMEs belong to the carbohydrate esterase family CE8 (CAZy database) and are encoded by multigene families. They are involved in a wide range of developmental processes, including organ growth, stomatal function, and reproduction, often acting in coordination with hormonal signaling pathways (Ren and Kermode, 2000; Bosch and Hepler, 2005; Peaucelle et al., 2011; Amsbury et al., 2016). This developmental context need to be considered for better understanding PMEs functions during stress responses and their mechanistic interpretation.

PMEs play a critical role in multiple plant-pathogen interactions and abiotic stress responses (Lionetti et al., 2012; Wu et al., 2018). PME-mediated pectin remodeling can promote CW reinforcement or loosening in an isoform- and context-dependent manner (Willats et al., 2001; Del Corpo et al., 2020). PMEs can contribute to CWI sensing and surveillance both by generating HG domains that support sensor binding and by promoting the release of danger signals including oligogalacturonides (OGs) and methanol (MeOH) (Osorio et al., 2008; Komarova et al., 2014; Liu et al., 2024). In abiotic stresses, PME-mediated modulation of pectin charge and Ca^{2+}-binding capacity influences mechanical wall properties and ion homeostasis (Wu et al., 2018; Coculo and Lionetti, 2022; Jiang et al., 2025).

These multifaceted functions require tight spatial and temporal control of PME post-trafficking maturation, activity, and function, achieved through coordinated regulation by PME inhibitors (PMEIs) (Coculo and Lionetti, 2022), subtilisin-like proteases (subtilases; SBTs) (Senechal et al., 2014; Coculo et al., 2023), and the extracellular pH (Hocq et al., 2024). In this review, we highlight PMEs as central modulators of pectin dynamics linking CW remodeling, signaling, and plant responses to biotic and abiotic stresses.

2. STRUCTURAL BASIS OF PMEs TARGETING, TRAFFICKING, AND FUNCTION

PMEs structural features, together with post-translational modifications, are key determinants of intracellular trafficking, localization, and deployment under stress conditions (Mareck et al., 2012; Canut et al., 2016). Plant PMEs are classified into two main types based on their domain organization (Coculo and Lionetti, 2022) (Figure 1B). Type I PMEs (Group 2; 45 isoforms in *Arabidopsis thaliana*) includes enzymes synthesized as inactive precursors with an N-terminal pro-domain. This pro-region, which is structurally related to PMEIs, acts as an intramolecular inhibitor that prevents premature PMEs activity (Del Corpo et al., 2020). Although some PMEs can be secreted as precursors, the presence and proper processing of the pro-region in specific Type I PMEs contributes to correct targeting and secretion to the CW (Bosch and Hepler, 2005; Wolf et al., 2009; Li et al., 2022). Type II PMEs (Group 1; 21 isoforms in *A. thaliana*) comprise proteins containing only the

catalytic domain. In contrast to Type I PMEs, much less is known about the biogenesis and trafficking of Type II isoforms (Figure 2).

Both PMEs types may contain an N-terminal signal peptide directing entry into the secretory pathway and/or a transmembrane domain mediating membrane association, although some lack one or both elements (Micheli, 2001; Bosch and Hepler, 2005) (Figure 1B). Representative PME isoforms highlight the diversity of trafficking strategies. *AtPME17* (Type I PME), a central regulator of CW-based immunity and marker of pathogenesis, contains a signal peptide but lacks a transmembrane domain (Lionetti et al., 2012; Del Corpo et al., 2020; Coculo et al., 2023). It localizes to the endoplasmic reticulum and Golgi, before secretion into the apoplast, where it likely acts as a soluble and mobile enzyme enabling rapid CW remodeling during defense activation (Figure 2). By contrast, *AtPME34* (Type I PME), highly expressed in guard cells and required for heat-stress tolerance, contains a transmembrane domain but lacks an signal peptide. It localizes to the plasma membrane facing the apoplast prior to release, suggesting that membrane anchoring spatially organize PME activity (Huang et al., 2017) (Figure 2). Differently, AtPME31 (Type I PME), predicted to lack both signal peptide and transmembrane domains, localizes to the cytosol and peroxisomes and does not traffic to the CW. Collectively, these findings indicate that signal peptide is determinant of CW targeting, whereas transmembrane domains primarily confer membrane retention and spatial control of PME activity. Additional structural determinants can modulate PME trafficking. In *Nicotiana tabacum*, N-glycosylation of NtPPME1 at N482 is required for transport from Golgi-derived vesicles to the apoplast bypassing the trans-Golgi network (Weng et al., 2025). This unconventional secretory pathway highlight an additional layer of spatial control over pectin remodeling. In addition, *AtPME35*, a Type I PME is predicted to carry a glycosylphosphatidylinositol anchor, which could enable stable attachment to the plasma membrane (Zhou, 2022; Xu et al., 2022). Overall, distinct combinations of structural features and post-translational modifications across PME isoforms direct their trafficking and targeting, thereby controlling the spatial degree and pattern of pectin methylesterification, an effective strategy in stress responses.

The resolution of three-dimensional PME structures reveals key features influencing PME substrate specificity and mode of action. Tertiary structures of *Daucus carota* and *Solanum lycopersicum* PMEs have been resolved by X-ray crystallography, providing insights into catalytic mechanism and functional specialization (Johansson et al., 2002; Di Matteo et al., 2005; Ciardiello et al., 2008). Plant PMEs share a conserved right-handed parallel β-helix fold with a surface cleft accommodating HG and highly conserved catalytic site (e.g., Q113, Q135, D136, D157, and R225 in DcPME numbering), whereas D157 act as the nucleophile and D136 in proton transfer and MeOH release (Figure 1C). Structural models of PME-pectin interaction suggest that plant PMEs operate in a processive mode

of action (Kim et al., 2018). Variations in cleft geometry and electrostatic properties, together with apoplastic pH, likely contribute to distinct modes of action of PMEs (Mercadante et al., 2014; Sénéchal et al., 2017; Hocq et al., 2024). Structures of PMEIs and PME-PMEI complexes provide insights into the molecular basis of catalysis and inhibition. AtPMEI1 adopts a four-helix bundle structure stabilized by conserved disulfide bridges and displays a dimeric arrangement (Hothorn et al., 2004a; Hothorn et al., 2004b). Complex structures reveal a 1:1 SlPME-AcPMEI interaction in which the inhibitor occludes the catalytic cleft, blocking substrate access (Di Matteo et al., 2005; Ciardiello et al., 2008) (Figure 1D). These findings support a PME-centered model of inhibition, where enzyme surface geometry determines PMEI binding. Interestingly, microbial PMEs are not inhibited by plant PMEIs. While plant PMEs are structurally conserved, bacterial PMEs display variations in substrate-binding clefts, including extended loops and altered geometry, likely affecting substrate accessibility, mode of action, and PMEI interaction (Markovic and Janecek, 2004) (Figure 1E). These differences provide a framework to engineer PME and PMEI structural features to enhance plant resistance under stress conditions. Consistently, recent studies have shown that AlphaFold-guided redesign of PMEIs can expand their inhibitory spectrum toward pathogen-derived PMEs (Xia et al., 2024).

3. FUNCTIONAL PLASTICITY OF PMEs IN BIOTIC STRESS: FROM CW REINFORCEMENT TO SIGNALING

Plant pathogens adopt distinct lifestyles that shape their interaction with the host. Necrotrophs actively kill host cells to exploit dead tissue, whereas biotrophs depend on living cells and therefore evade or suppress host immune responses; hemibiotrophs combine these strategies by transitioning from an initial biotrophic to a necrotrophic phase (Kim et al., 2022). Despite these differences, successful colonization requires the secretion of a wide range of microbial CW-degrading and modifying enzymes (Engelsdorf et al., 2018). The control of pectin integrity lies at the heart of a molecular tug-of-war between plant physiological success and the onset of disease (Bellincampi et al., 2014; Munzert and Engelsdorf, 2025). In this context, plant PMEs act as central mediators of CW remodeling and surveillance (Figure 3; Table 1).

3.1 Hormonal and Transcriptional Regulation of PMEs in Pathogen Responses

Activation of specific plant PME isoforms with distinct temporal patterns across multiple pathosystems highlights the importance of tightly regulated pectin de-methylesterification and associated signaling during pathogen attack (Lionetti et al., 2012; Bethke et al., 2014; Del Corpo et al., 2020) (Figure 3). Type I PMEs are particularly involved, and their expansion across plant lineages likely reflects evolutionary selection for tight regulatory control, allowing PME precursors to be

activated in a timely manner during pathogen challenge (Coculo and Lionetti, 2022; Coculo et al., 2023). At*PMEPCRA*, *AtPME3*, *AtPME17*, *AtPME20*, *AtPME21*, and *AtPME41* are induced at specific stages of infection, whereas *AtPME1* and *AtPME34* are repressed (Lionetti et al., 2017). These patterns suggest isoform-specific functions during pathogen colonization.

Stress-responsive PME genes, is induced by multiple defense-associated phytohormones, including abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Nafisi et al., 2015; Del Corpo et al., 2020; Sun et al., 2022) (Figure 3). Functional analyses show that loss-of-function *pme17* mutants display enhanced susceptibility to *Botrytis cinerea*, accompanied by reduced expression of the JA/ET-dependent defense gene *PLANT DEFESIN 1.2 (PDF1.2)*. Consistently, PME-mediated de-methylesterification releases MeOH, which can stimulate ET biosynthesis, thereby reinforcing JA/ET-dependent defense responses (Tran et al., 2018). Overall, PME activity emerges as mediator of JA- and ET-dependent pathways during plant-pathogen interactions (Bethke et al., 2014; Taurino et al., 2014).

In addition to hormonal regulation, transcriptional control of specific PME genes also contributes to the modulation of pectin remodeling during immune responses. In *Vitis vinifera*, *VvPME10*, the ortholog of *AtPME17*, primes early defense responses against *B. cinerea* and improve resistance to the fungus (Lagrèze et al., 2025). *VvPME10* expression is activated by the defense-related transcription factor WRKY03, highlighting the importance of transcriptional control in PME-mediated immunity (Eulgem et al., 2000). However, this regulatory layer remains relatively underexplored. Insights from developmental studies may help bridge this gap, as multiple transcription factor families, including MYB, MADS-box, BEL1-like, APETALA2/ETHYLENE-RESPONSIVE FACTOR (AP2/ERF), zinc-finger, and hormone-responsive regulators, coordinate PME expression during seed development, organ growth, and fruit ripening (Ezquer et al., 2016; Xu et al., 2020; Liu et al., 2021; Wang et al., 2025b). Whether these developmental regulatory modules are also recruited during stress responses remains to be determined.

3.2 Post-Translational Processing of PME Precursors Enables Timely Generation of Apoplastic Danger Signals

The absence of the N-terminal pro-region in type II PMEs suggests that these isoforms may reach their functional sites in a readily active form. In contrast, proper processing of type I PMEs is critical for an efficient plant response to stresses (Del Corpo et al., 2024). Type I PMEs contain a conserved processing motif, typically RRLL or RKLL, which can be cleaved by SBTs (Wolf et al., 2009; Coculo et al., 2023) . In *A. thaliana* and other plant species, SBTs activate specific PME isoforms in response to developmental programs and environmental stress cues (Senechal et al., 2014; Meyer et al., 2016;

Coculo et al., 2023). In *A. thaliana*, AtSBT3.3 and AtSBT3.5 act as modulators of PME activity during *B. cinerea* infection, promoting rapid immune responses (Coculo et al., 2023). AtSBT3.3 cleaves AtPME17 precursor, enabling the mature enzyme to de-methylesterify pectin and thereby contribute to defense responses against pathogens (Del Corpo et al., 2020). Notably, AtSBT3.3 is a key regulator of immune priming, a physiological state that enables faster and more robust activation of defense responses upon subsequent pathogen challenge (Ramirez et al., 2013; Conrath et al., 2015). Although it likely acts on multiple substrates, this raises the possibility that PME activation by SBTs can contribute to priming. Given the metabolic cost of defense, which imposes a growth-defense trade-off (He et al., 2022), timely proteolytic activation of PMEs upon pathogen challenge may help mitigate this constraint. The tight regulation of this system is further highlighted by the autocatalytic activation of SBTs synthesized as pro-enzymes (Cedzich et al., 2009; Plattner et al., 2014).

Intriguingly, AtPME17 and AtSBT3.3 follow distinct yet coordinated trafficking routes to reach the apoplast. AtPME17 is delivered via the conventional secretory pathway, involving the endoplasmic reticulum and Golgi apparatus (Wang et al., 2017). Conversely, AtSBT3.3 is secreted through an unconventional secretory pathway, mediated by exocyst-positive organelles (EXPOs) (Ding et al., 2014; Coculo et al., 2023) (Figure 2). Moreover, in *N. tabacum* co-transformed cells, AtPME17 was detected in the apoplast prior to the arrival of AtSBT3.3. Such spatial and temporal segregation likely act as a regulatory safeguard, preventing premature PME activation and untimely HG de-methylesterification during the secretory process. Evidence that microbial and insect PMEs lack the pro-region further corroborates this view (Markovic and Janecek, 2004). The absence of pectin-rich in microbes and insects likely reduces the selective pressure for such regulatory control.

In addition to PME activation, *At*SBT3.5 also processes SCOOP12, a precursor of serine-rich endogenous peptides, releasing the active phytocytokine SCOOP12 and promoting immune responses (Rzemieniewski and Stegmann, 2022; Yang et al., 2023). These findings support a model in which specific SBTs act as shared proteolytic switches coordinating the activation of PMEs and phytocytokines, thereby enhancing plant immunity through both CW remodeling and peptide-mediated signaling (Del Corpo et al., 2024). How these pathways are integrated with other elicitor-triggered signaling networks remains an open question.

3.3 Dynamics of Pectin Methylesterification, Cross-Linking, and Remodeling in CWI and Protection

During multiple plant-microbe interactions, plants deploy different strategies to preserve CWI and restrict pathogen invasion (Seifert and Blaukopf, 2010; Rui and Dinneny, 2020). One important

mechanism is the synthesis and maintenance of highly methylesterified HG, which limits pectin accessibility to microbial pectinolytic enzymes, such as polygalacturonases and pectate lyases, and reduces its susceptibility to degradation (Lionetti et al., 2012) (Figure 3A). Higher HG methylesterification correlates with increased resistance to *Pectobacterium carotovorum* in potato (McMillan et al., 1993; Marty et al., 1997), *Colletotrichum lindemuthianum* in beans (Boudart et al., 1998), *Ralstonia solanacearum* in *S. lycopersicum* (Wydra and Berl, 2006) and *Fusarium graminearum* in *Triticum aestivum* (Lionetti et al., 2015; Giancaspro et al., 2018). Early studies showed that overexpression of *AtPMEI1* and *AtPMEI2* increases pectin methylesterification and enhances resistance to *B. cinerea* drew attention to PMEIs as key regulators of pectin status during plant defense (Lionetti et al., 2007; Lionetti et al., 2014b). Subsequent studies identified the PMEI isoforms induced and exploited in plant-pathogen interactions (Table 2). In particular, *AtPMEI10*, *AtPMEI11*, and *AtPMEI12* modulate PME activity during *B. cinerea* infection (Lionetti et al., 2017). These inhibitors are induced to maintain CWI, especially during the later stages of infection, by preventing excessive pectin de-methylesterification that would otherwise promote pectin degradation by microbial pectinase. Consistent roles for PMEIs have been reported in other pathosystems. *T. aestivum* plants expressing *AcPMEI* exhibit increased tolerance to *Bipolaris sorokiniana* and *F. graminearum* (Volpi et al., 2011; Tundo et al., 2016). In *Capsicum annuum*, silencing *CaPMEI1* increases susceptibility to *Xanthomonas campestris*, whereas its expression in *A. thaliana* enhances resistance to *Pseudomonas syringae* pv. *tomato* (An et al., 2008). Similarly, *GhPMEI3* in *Gossypium hirsutum* inhibits *GhPME2* and *GhPME31* restricts infection by *Verticillium dahliae* (Liu et al., 2018; Zhang et al., 2022), while *PvPMEI3* contributes to defense against *P. syringae* pv. *phaseolicola* in common bean (De La Rubia et al., 2024). PMEIs have also been implicated in defense against other types of biotic stress. For instance, *AtPMEI13* limits damages caused by green peach aphid (*Myzus persicae*), restricting both feeding and colonization (Silva-Sanzana et al., 2019). Engineering PMEI expression is a promising biotechnological targets for improving disease resistance across multiple pathosystems, without typical growth-immunity trade-offs and can even increase biomass (Ferrari et al., 2008; Lionetti et al., 2010; Francocci et al., 2013).

Besides maintaining pectin in a protective status, plants can locally and strongly reinforce HG structure, especially beneath pathogen penetration sites, by forming CW appositions (papillae). Papilla formation involves coordinated CW remodeling, including pectin modification, polysaccharide-phenolic crosslinking, callose deposition, and secretion of structural proteins (Pena et al., 2004; Rashid, 2016; Reem et al., 2016; Lionetti et al., 2017). Immunolabeling studies have detected the accumulation of low-methylesterified HG within papillae, suggesting localized PME activity (Chowdhury et al., 2014; Vogel et al., 2002). Plant PMEs, by generating contiguous blocks

of de-methylesterified GalA residues, can spatially promote Ca^{2+}-mediated crosslinking between HG chains and strengthen CW to contrast pathogen penetration (Tibbits et al., 1998; Willats et al., 2001). In the classical egg-box model, Ca^{2+} ions coordinate adjacent HG chains to form ordered ionic bridges (Grant et al., 1973) (Figure 3B), structures that have recently been observed in CWs (Temple et al., 2022). Alternative arrangements such as the zipper model have also been proposed, in which Ca^{2+} bridges form primarily between opposing carboxylate groups of adjacent HG chains (Plazinski, 2011; Obomighie et al., 2025). AtPME17-mediated blockwise HG de-methylesterification reinforces CW against *B. cinerea* (Del Corpo *et al.*, 2020). Similarly, induction of *CjPME28* in *Camellia japonica* enhances CW fortification during herbivore attack (Dao et al., 2025). Consistently, PMEs were involved in restoration of CWI in wound healing and graft union formation (Neubauer et al., 2012; Frey et al., 2024).

Apoplastic pH is dynamically modulated during plant-microbe interactions and strongly influences the outcome of PME-mediated pectin remodeling (Hua et al., 2018). Plants often trigger a transient alkalinization of the apoplast during infection (Aziz et al., 2003), creating conditions that favor Ca^{2+}-mediated pectin crosslinking. In contrast, necrotrophic fungi such as *B. cinerea* acidify host tissues through oxalic acid secretion, which chelates Ca^{2+} and destabilizes pectin networks (Manteau et al., 2003; Prusky and Yakoby, 2003). Acidic conditions may also shift PME activity from blockwise to random de-methylesterification, thereby increasing susceptibility to pectinase attack (Hocq et al., 2024). Moreover, PME activity itself releases protons, potentially reinforcing local apoplastic acidification. Together, these evidence highlight a dynamic interplay between plant- and pathogen-driven processes that compete for the control of apoplastic pH and CW stability.

3.4 Contribution of PME Activity to Danger Signal Production and CWI Sensing

In the absence of an adaptive immune system, plants rely on an innate immune network, in which membrane-resident pattern-recognition receptors detect conserved pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) and activate pattern-triggered immunity (PTI) (DeFalco and Zipfel, 2021; Zhang et al., 2024a). PTI drives local and systemic defense responses, including antimicrobial production, danger signaling and CW remodeling to maintain CWI (Pogorelko et al., 2013; Lionetti et al., 2017; Swaminathan et al., 2022).

HG methylesterification patterns may encode spatio-temporal information within the CW, with PMEs acting as gatekeepers of pectin remodeling, integrity maintenance, and signaling (Figure 3C and 3D). Increasing evidence links PME activity to receptor systems that monitor apoplastic CW status. The *A. thaliana* RESISTANCE TO FUSARIUM OXYSPORUM 1 (RFO1) acts as a sensor of HG methylesterification status to contrast *Fusarium oxysporum* (Huerta et al., 2023) (Figure 3C). PME

activity promotes the formation of negatively charged HG domains that can function as binding platforms for wall-associated remodeling proteins and wall sensors. In *A. thaliana*, de-methylesterified HG anchors the peroxidase PRX36 and Expansin-Like A1 (EXLA1), spatially modulating CW remodeling (Francoz et al., 2019; Men et al., 2026) (Figure 3D). De-methylesterified HG is a preferred substrate for the mechanosensor FERONIA (FER), a *Catharanthus rosesus* RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) receptor kinase, involved in several growth and immune signaling pathways (Guo et al., 2018; Cheung, 2024). FER can form signaling complexes with co-receptors, such as LORELEI-LIKE GPI-ANCHORED PROTEIN 1 (LLG1) and peptide ligands, including RAPID ALKALINIZATION FACTORs (RALFs) (Stegmann et al., 2017; Gronnier et al., 2020; Abarca et al., 2021) (Figure 3D). De-methylesterified HG domains contribute to the spatial organization of FER-RALF signaling complexes at the cell surface (Schoenaers et al., 2024; Rößling et al., 2024). De-methylesterified HG also acts as a ligand for Wall-Assosiacted Kinase (WAK) receptors, which function in CWI sensing and OGs perception (Decreux et al., 2006; Kohorn et al., 2014) (Figure 3D). Together, these findings propose PME-driven pectin remodeling acts as a central hub integrating CW mechanics with receptor-mediated signaling at the cell surface.

PME activity can assist the generation of OGs, key pectin-derived damage-associated molecular patterns (DAMPs). These oligosaccharides are produced during HG degradation by microbial polygalacturonases (Ferrari et al., 2013). Plants can modulate this process via controlled inhibition of polygalacturonases by polygalacturonase-inhibiting proteins, which OGs size and elicitor activity (Xiao et al., 2024). HG methylesterification determines susceptibility to enzymatic degradation (Le Cam et al., 1994; Lionetti, 2015), with fungal PMEs promoting random de-methylesterification patterns that enhance PG-mediated breakdown (Kohn et al., 1983; Limberg et al., 2000a; Limberg et al., 2000b) (Figure 3D). Some plant PMEs also generate random patterns to improve pectin accessibility to pectinase in developmental contexts, although their contribution to OGs production during infection remains unexplored (Catoire et al., 1998; Denes et al., 2000; Gallemí et al., 2025) (Figure 3). De-methylesterification of OGs by PME can also influence their elicitor activity (Osorio et al., 2008; Osorio et al., 2011). Consistently, ectopic expression of strawberry (*Fragaria* × *ananassa*) gene *FaPE1* in *Fragaria vesca* promotes the accumulation of bioactive OGs, leading to constitutive defense activation and enhanced resistance to *B. cinerea*. A complex mixture of OGs accumulates in *A. thaliana-B. cinerea* interaction, including low-methylesterified oligomers, methylesterified fragments, and oxidized OGs (Benedetti et al., 2018; Voxeur et al., 2019). Recent work shows that RALF peptides bind de-methylesterified OGs, triggering phase separation and formation of OGs-RALF-FER-LLG1 condensates, that drive receptor endocytosis and initiate cell surface responses (Moussu et al., 2023; Liu et al., 2024; Rößling et al., 2024) (Figure 3D). Closing

the loop on PME involvement in this context, recent evidence shows that the *N. tabacum* LORELEI-like protein NtLLG4 acts as a receptor for PME isoforms (Weng et al., 2025). Although the functional relevance of these condensates in plant-microbe interactions remains to be defined, they highlight a structural role for PMEs in organizing and modulating receptor signaling at the cell surface.

PME activity also generates MeOH , a volatile compound that acts as danger signal in intra- and inter-plant communication (Dorokhov et al., 2018) (Figure 3D). MeOH is rapidly released upon CW perturbation, particularly during wounding and herbivory, and modulates early signaling events including Ca^{2+} fluxes, membrane depolarization, and ethylene production (Von Dahl et al., 2006; Dorokhov et al., 2012a; Komarova et al., 2014; Hann et al., 2014). Moreover, MeOH emission from injured tissues can exert a "priming" effect on intact leaves within the same plant or neighboring plants, preparing them for potential attack. Interestingly, MeOH may act primarily as a modulator, fine-tuning immune signaling rather than functioning as a strong elicitor since it influences the amplitude of Mitogen-Activated Protein Kinase (MAPK) and Reactive Oxygen Species (ROS) responses triggered by DAMPs/MAMPs (Tran et al., 2018). Studies in *Nicotiana attenuate* challenged with *Manduca sexta* and *Succisia pratensis* with *Euphydryas aurinia* indicates that PME-mediated MeOH release contributes to early defense activation, CW maintenance and resistance to the herbivores (Peñuelas et al., 2005; Körner et al., 2009). Contrasting evidence indicates that MeOH does not always enhance plant defense but can instead suppress specific defense traits, potentially increasing herbivore performance (Von Dahl et al., 2006). Moreover, MeOH-induced changes in plasmodesmata permeability may facilitate viral spread (Dorokhov et al., 2012a), highlighting that MeOH can acts as a context-dependent regulator. Finally, MeOH signaling has been hypothesized to extend beyond plant-plant communication, potentially acting as a cross-kingdom signal influencing animal responses, although its ecological relevance remains unclear (Dorokhov et al., 2012b). The mechanisms of MeOH perception and its physiological relevance remain poorly understood.

4. HIJACKING PMEs BY INTERACTING ORGANISMS TO MODULATE CW SUSCEPTIBILITY

Despite their role in CW-based defense, some PMEs can be hijacked by pathogens to promote host colonization. These PME targets are often constitutively expressed in exposed tissues, offering an immediate entry point for pathogens and mutualists without transcriptional reprogramming. Plant PMEs can be manipulated to generate de-methylesterified domains targeted by microbial pectate lyases increasing wall susceptibility and facilitating host invasion. For instance, the *Ustilago maydis* effector Nge1 targets *Zea mays* ZmPMEI45 and ZmPMEI46 as well as the pro-region of ZmPME19

and ZmPME20, thereby releasing PME activity (Bhaskar et al., 2025). Similarly, in *A. thaliana*, AtPME3 is exploited by *B. cinerea* and *P. carotovorum* to promote pectin degradation and tissue maceration (Raiola et al., 2011). AtPME3 is also targeted during nematode infection, where parasites induce extensive CW remodeling to establish feeding structures. The cyst nematode *Heterodera schachtii* secretes a cellulose-binding protein that interacts with AtPME3, likely promoting localized pectin de-methylesterification and wall loosening required for syncytium formation (Hewezi et al., 2008; Bohlmann and Sobczak, 2014; Pogorelko et al., 2019). The upregulation of *AtPMEPCRA* and *AtPME54* in *Meloidogyne javanica*-induced galls, together with the induction of soybean PME genes (*BE821923* and *AW30934*) during *Heterodera glycines* infection, suggests that this strategy is conserved across different nematode species. A similar strategy operates in other plant-pathogen interactions. AtPMEPCRA is exploited by *Plasmodiophora brassicae* to promote host cell death during clubroot disease (Stefanowicz et al., 2021). Moreover, in *G. hirsutum*, *Gh*PME36 promotes pectin de-methylesterification and increases carbohydrate availability, facilitating colonization by the leafminer *Liriomyza sativae* (Yang et al., 2024).

PMEs are also exploited by viruses for their movement. Movement proteins of several plant viruses, including *Tobacco mosaic virus (TMV)* and *Cauliflower mosaic virus (CaMV)*, interact with PMEs to facilitate cell-to-cell spread (Chen and Citovsky, 2003; Stavolone and Lionetti, 2017). Because of the restricted plasmodesmata size exclusion limit, viral movement relies on movement proteins-mediated modulation of plasmodesmata permeability and interactions with host factors (Carrington et al., 1996; Lazarowitz and Beachy, 1999; Ghoshroy et al., 1997). In this context, host PMEs contribute to plasmodesmata-mediated viral transport. A *N. tabacum* PME interacts with the *TMV* movement proteins and it is required for efficient viral spread (Chen et al., 2000). PMEs from *S. lycopersicum* and citrus interact with movement proteins of *turnip vein clearing virus (TVCV)* and *CaMV*. Consistently, antisense suppression of a phloem-specific PME isoform in *N. tabacum* reduces the systemic movement of *TMV* (Chen and Citovsky, 2003). PME-movement proteins interactions at plasmodesmata likely facilitate viral trafficking, although the underlying mechanism remains unresolved. Alternatively, movement proteins interaction may modulate PME activity, thereby altering plasmodesmata permeability (Chen et al., 2000). Consistently, overexpression of *AtPMEI2* in *A. thaliana* and *AcPMEI* in *N. tabacum* restricts both cell-to-cell and systemic spread of *tobamoviruses (ToMV)* (Lionetti et al., 2014a; Lionetti et al., 2014b). Collectively, these findings identify PMEs as a susceptibility factor recurrently targeted to reprogram host CW properties.

PME co-option is not restricted to pathogenic interactions but also occurs in beneficial associations. AtPME30 can be exploited in *A. thaliana-Bacillus subtilis* interaction to promote rhizobacteria colonization and enhance disease resistance (Lakshmanan et al., 2013). Similarly, in legume-rhizobial

and actinorhizal symbioses, coordinated regulation of PMEs, PMEIs, and pectin-degrading enzymes supports infection and tissue remodeling (Hocher et al., 2011; Su et al., 2023).

5. PMEs REWIRE CW TO TACKLE ABIOTIC STRESS

Abiotic stresses strongly impact plant growth and productivity by disrupting cellular homeostasis and impairing essential physiological processes (Jiang et al., 2025). Changes in CW mechanics and chemistry influence cell expansion, turgor maintenance, and signal transduction (Debnath et al., 2026). PMEs have emerged as key regulators of abiotic stress acclimation by modulating CW sensing and remodeling (Wu et al., 2018; Coculo and Lionetti, 2022) (Figure 4; Table 1). Compared to biotic interactions, the role of PME in CWI surveillance in abiotic stress responses remains less well defined. Nevertheless, core mechanisms related to CW sensing seems conserved. Elevated temperature and salinity promote de-methylesterified OG-RALF phase separation, recruiting FER-LLG1 into condensates and triggering receptor clustering and stress tolerance (Liu et al., 2024). Under salt stress, disruption of pectin-Ca^{2+} cross-links activates FER-dependent CWI signaling, Ca^{2+} bursts, MAPK cascades, and PME upregulation contributing to wall reinforcement and growth recovery (Feng et al., 2018). In addition, salinity rapidly induces PME activity upstream of the receptor-like kinases FER, HERCULES1 (HERK1), and THESEUS1 (THE1) (Gigli-Bisceglia et al., 2022) positioning PME-dependent pectin remodeling as an early event in salt stress perception. Receptors such as RLP44 and ERULUS, which sense pectin status and CW mechanics during development, may also mediate PME-dependent signaling under abiotic stress conditions, where CW mechanical constraints are altered (Wolf et al., 2014; Schoenaers et al., 2018). Beyond signaling, PMEs directly modulate CW physicochemical properties, including charge density, porosity, ion binding capacity, and mechanical properties such as stiffness and extensibility (Grant et al., 1973; Rondeau-Mouro et al., 2008; Caffall and Mohnen, 2009; Obomighie et al., 2025). These changes underpin key physiological processes, including stomatal dynamics, osmotic buffering, ion sequestration, and maintenance of CWI.

5.1 Reprogramming CW Dynamics Under Heat, Drought, and Cold Stress

Climate change is increasing the co-occurrence of heat and drought, posing a combined threat to plant productivity and crop resilience. Although these stresses impose distinct physiological constraints, they converge on stomatal regulation as a central hub balancing water conservation and leaf cooling. During heat stress, plants must optimize stomatal aperture to sustain transpirational cooling while preventing excessive water loss. These stomatal dynamics are key determinants of thermotolerance. In addition, stomatal density contributes to longer-term acclimation (Chua and Lau, 2024). These

responses integrate ABA signaling with guard CW mechanics (Huang et al., 2016). In this context, PME-mediated pectin remodeling, together with apoplastic Ca^{2+}, controls guard CW flexibility (Wu and Jinn, 2010; Kan et al., 2023) (Figure 4A). AtPME34, expressed in guard cells, fine-tune HG methylesterification required for stomatal function and heat tolerance (Huang et al., 2017; Wu et al., 2017). Beyond stomatal dynamics, AtPME53 and AtPME12 regulate stomatal pore formation and density under heat stress, linking pectin remodeling to ABA pathways (Wu et al., 2022; Wu et al., 2025). Drought stress promotes stomatal movements to maintain water status (Osakabe et al., 2014). PME-mediated pectin remodeling contributes to drought responses (Sun et al., 2025) (Figure 4A). In *A. thaliana*, AtPME6-mediated pectin de-methylesterification supports proper stomatal function during drought stress (Amsbury et al., 2016). Overexpression of *PtoPME35* in *Populus tomentosa* and *AcPME2 in Actinidia chinensis* enhances drought tolerance by increasing de-methylesterified pectin and Ca^{2+}-pectate interactions, thereby improving stomatal control (Yang et al., 2020; Zhu et al., 2024b). Drought responses also rely on coordinated PME-PMEI activity (An et al., 2008; Cheng et al., 2022; Zhang et al., 2024b). AtPME31 and its cognate inhibitor AtPMEI18 regulate optimal guard CW mechanics in *A. thaliana* during drought conditions (Zhang et al., 2023). Together, these findings indicate that PME-dependent pectin remodeling dynamically regulates guard CW mechanics in a similar way during heat and drought stress (Figure 4A). PME activity is tightly tuned by PMEIs to maintain a highly methylesterified, elastic wall during stomatal movement. PME activation, in turn, promotes Ca^{2+}-mediated cross-linking and wall stiffening when movement ceases. This mechanism enables stomatal dynamics through stress-dependent shifts between wall flexibility and mechanical reinforcement. The biomechanical consequences of HG methylesterification cannot be generalized across different stomatal cells types. In commelinid grasses, which possess dumbbell-shaped guard cells, methylesterified HG accumulates at the polar ends of guard cells, where its polar distribution is associated with mechanically resistant wall domains that constrain stomatal opening (Zhang et al., 2026).

Cold stress primarily affects membrane stability but also induces extensive CW remodeling, including changes in wall thickness, ultrastructure, porosity, and polysaccharide composition (Campos et al., 2003; Solecka et al., 2008; Takahashi et al., 2021). Cold-acclimated tissues exhibit high PME expression and accumulate low-methylesterified HG that promotes Ca^{2+}-mediated crosslinking and formation of a semi-rigid pectate network (Baldwin et al., 2014; Lin et al., 2025) (Figure 4B). In *A. thaliana*, AtPME41 is required for cold-induced PME activity (Qu et al., 2011). *pme41* mutants show increased ion leakage without changes in membrane lipid peroxidation, indicating that cold tolerance relies on CWI rather than membrane stability. Brassinosteroid regulation of *AtPME41* further links hormonal signaling to pectin remodeling during cold acclimation. The involvement of AtPMEI13 in

freezing tolerance further highlights the importance of PME regulation under abiotic stress (Chen et al., 2018; Zhang et al., 2024b). It therefore emerges that plants express PMEs during cold stress, driving a shift toward low-methylesterified HG that promotes Ca^{2+}-mediated cross-linking and the formation of a semi-rigid pectate network, thereby reinforcing CWI and limiting ion leakage (Figure 4B). The persistence of these Ca^{2+}-mediated cross-links beyond the acclimation phase further suggests that PME-driven remodeling may establish a form of "CW structural memory", whereby enduring changes in HG organization and CW mechanics influence subsequent responses to freezing stress (Solecka et al., 2008; Shikata et al., 2026).

5.2 Tuning Ion Homeostasis through Pectin Remodeling under Salt Stress

Salt stress disrupts cellular homeostasis by combining osmotic imbalance with ionic toxicity and altered Ca^{2+} availability, ultimately destabilizing the pectin network and impairing CW mechanics. PME-mediated de-methylesterification represents a key adaptive response (Figure 4C). Genetic evidence indicates that AtPME31 and AtPME47 contribute to salt tolerance, as loss-of-function mutants display hypersensitivity to NaCl (Yan et al., 2018; Zhou et al., 2024). Intriguingly, AtPME31 shows low PME activity *in vitro* and atypical subcellular localization , suggesting that its contribution to salt tolerance may involve non-canonical functions beyond direct pectin remodeling, potentially linked to lipid metabolism (Dedeurwaerder et al., 2008; Hamade et al., 2025). Overexpression of *NtPME043* enhances root growth under high salt conditions (Sun et al., 2022). Collectively, these findings support a model in which PME activity increases availability of carboxyl groups for cation binding on HG, thereby influencing apoplastic Ca^{2+}/Na^{+} partitioning and ion buffering capacity (Pilling et al., 2000; Pilling et al., 2004). Preferential Ca^{2+} cross-linking stabilizes the pectic network, while limiting Na^{+} interference, thus preserving wall hydration and maintaining CWI under salinity. PMEI-mediated regulation further fine-tunes this balance (Jithesh et al., 2012; Chen et al., 2018) (Figure 4C).

5.3 PME-Dependent Control of Pectin Charge in Ion Homeostasis and Metal Sequestration

Beyond salinity, PME-dependent modulation of pectin charge represents a general mechanism regulating ion binding, sequestration, and distribution under diverse abiotic stresses. Heavy metal stress imposes constraints that largely converge at the CW. By increasing the density of free carboxyl groups on HG, PME-mediated de-methylesterification modulates metal binding, mobility, and toxicity. Depending on context, this remodeling can either protect the cytoplasm by immobilizing toxic ions in the CW or exacerbate toxicity by altering CW porosity, elasticity, or Ca^{2+}-mediated crosslinking. Evidence from *A. thaliana* illustrates this dual role (Figure 4D). Overexpression of *AtPME3* leads to Zn^{2+} hypersensitivity, indicating that excessive de-methylesterification enhances

metal binding and perturbs ionic homeostasis (Weber et al., 2013). Responses to aluminum (Al^{3+}) stress appear more tightly regulated: *AtPME46* limits Al^{3+} binding and alleviates root growth inhibition, whereas *AtPME17* and *AtPME54* are repressed under Al^{3+} exposure, suggesting that plants restrict excessive wall negative charge to prevent Al^{3+} over-accumulation (Geng et al., 2017). In rice, overexpression of *OsPME14* increases Al^{3+} retention and root growth inhibition, likely due to Ca^{2+} displacement within de-methylesterified pectin (Yang et al., 2013). Al-responsive *CsPME6* in *Camellia sinensis* is integrated into WRKY-dependent transcriptional networks (Huang et al., 2022; Huang et al., 2025). Consistently, increased de-methylesterification enhances binding and sequestration of other metals, including chromium (Cr^{3+}), cadmium (Cd^{2+}), and copper (Cu^{2+}) (Paynel et al., 2009; Zhang et al., 2021; Yu et al., 2023; Ullah et al., 2023). In rice, coordinated regulation of OsPME14 and its inhibitor OsPMEI6 modulates Cu-binding capacity to pectin (Wang et al., 2025a).

Beyond detoxification, PME-dependent control of pectin charge also regulates nutrient availability. In the *S. lycopersicum Colorless nonripening* (*Cnr*) mutant, reduced pectin methylesterification enhances iron (Fe) binding and immobilization triggering deficiency responses despite sufficient external supply (Zhu et al., 2024a). Similarly, under phosphorus (P) deficiency, increased de-methylesterification promotes both P remobilization and Cd^{2+} association with the wall (Qi et al., 2022; Li et al., 2024). PMEs also influence post-harvest physiology, where tissues experience mechanical injury, oxidative bursts, and dehydration. In *Manihot esculenta*, *MePME1* induction correlates with susceptibility to postharvest deterioration, whereas limited induction in resistant varieties helps maintain CWI and delays tissue collapse (Wang et al., 2023).

6. TRACKING PME ACTIVITY IN PLANT TISSUES: FROM BIOCHEMICAL ASSAYS TO IMAGING

A wide range of analytical approaches has been developed to detect and quantify PME activity, largely based on changes in pectin charge or the release of reaction products. The PECTOPLATE assay is widely used to monitor PME activity under stress conditions (Lionetti, 2015). This approach combines a ruthenium red-based gel readout, which selectively detects de-methylesterified pectin, with high-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD), enabling simultaneous assessment of PME and pectinase activities and OGs release. Additional colorimetric methods rely on pH changes or titration of carboxyl groups released during de-methylesterification, providing simple and low-cost approaches although with limited sensitivity and potential interference from organic acids.

Quantification of PME activity can also be indirectly achieved by measuring MeOH. Alcohol oxidase-based enzymatic assays coupled to colorimetric detection have largely replaced earlier

chemical methods, offering improved specificity, sensitivity, and compatibility with high-throughput formats (Anthon and Barrett, 2004). Complementary approaches such as headspace Gas Chromatography (GC) or GC-MS enable accurate quantification of MeOH emissions from plant tissues, allowing *in vivo* assessment of PME activity, while Proton-Transfer-Reaction (PTR)-MS allows real-time monitoring of MeOH release dynamics (Nemecekmarshall et al., 1995; Fall and Benson, 1996; Dorokhov et al., 2012a).

Complementary structural techniques provide detailed information on the pectin methylesterification status. Fourier-transform infrared (FT-IR) spectroscopy is widely used due to minimal sample preparation and its ability to rapidly estimate the degree of esterification without chemical pretreatment (Manrique and Lajolo, 2002). Chromatographic approaches such as High Performance Liquid Chromatography (HPLC) and GC enable quantitative determination of the degree of methyl- and acetyl-esterification following pectin saponification and release of MeOH and acetic acid (Levigne et al., 2002; Savary and Nuñez, 2003). Proton-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy enables simultaneous and high-resolution assessment of methyl- and acetyl-esterification (Grasdalen et al., 1988). Mass spectrometry-based approaches provide high-resolution insights into pectin structure and remodeling. Oligosaccharide Mass Profiling (OLIMP) coupled with Matrix-Assisted Laser Desorption/Ionization Time-Off Flight (MALDI-TOF) MS has proven particularly powerful for detecting pathogen-induced changes in pectin methylesterification and for linking alterations in esterification patterns to modifications in CW architecture (Lionetti et al., 2007).

Recent advances in molecular recognition and imaging approaches have expanded the analytical toolbox for probing pectin methylesterification. Immunological methods based on monoclonal antibodies (e.g., JIM5, JIM7, LM series), combined with platforms such as Comprehensive Microarray Polymer Profiling (CoMPP), enable *in situ* and semi-quantitative profiling of HG epitopes with defined methylesterification patterns (Pattathil et al., 2010; Pattathil et al., 2012). (Moller et al., 2008; Kračun et al., 2017). It has been used to visualize pathogen-induced shifts in pectin structure, including changes associated with *B. cinerea* infection (Lionetti et al., 2017). Complementary low-molecular-weight probes, including propidium iodide, Coriphosphine O, chitosan oligosaccharides (COS488, COS647), and OG-based probes (OG488), allow *in situ* detection of de-esterified HG domains with improved tissue penetration and compatibility with live imaging (Harenčár et al., 2025). A super-resolution approach, 3D direct stochastic optical reconstruction microscopy (3D-dSTORM), enables nanoscale *in muro* visualization of pectin nanostructure, spatial organization of methylesterified and de-methylesterified HG domains, and their association with CW-associated proteins (Moussu et al., 2023; Jia et al., 2026).

In summary, PME activity dynamically integrates HG remodeling with CW-associated signaling across diverse environmental conditions in a spatially and temporally controlled manner, under the tight regulation of PMEIs and SBTs. PME activity can establish a functional interface that integrates mechanical reinforcement, receptor organization, and danger signal generation. Rather than a linear process, PME activity operates as a context-dependent switch, where Ca^{2+} availability, apoplastic pH, and de-methylesterification patterns collectively determine the balance between wall stiffening and loosening, as well as between resistance and susceptibility. Ultimately, PME function should be viewed as part of a dynamic and competitive process at the plant surface, where plant and microbe-derived activities converge to shape pectin structure and downstream signaling outputs. PMEs emerge as key determinants of abiotic stress adaptation enabling plants to fine-tune CW mechanics along a flexibility-rigidity continuum, while simultaneously coordinating signaling pathways required for stress resilience. Despite these advances, significant knowledge gaps persist. Although the expansion of PME gene families, the *in vivo* roles and functional specialization of individual isoforms remain largely unresolved. Dissecting the relative roles of Type I and Type II PMEs will be essential to determine how distinct regulatory architectures translate into specific patterns of pectin remodeling across developmental and stress contexts. In parallel, pectin remodeling during biotic interactions results from dynamic and potentially competitive activities of both plant- and microbe-derived PMEs, although their interplay in shaping HG structure remains poorly understood.

Acting in a highly context-dependent manner, PMEs generate distinct de-methylesterification patterns. However, understanding how these patterns arise and are precisely controlled in space and time, through secretion, proteolytic activation, and CW physicochemical properties, remains a major challenge, particularly in the context of stress responses. Importantly, the enzymatic activity of many PME isoforms has yet to be experimentally validated, limiting functional interpretation. Another unresolved aspect concerns substrate specificity. Although rhamnogalacturonan II backbone can also be methylesterified (O'Neill et al., 2020), direct evidence for PME isoforms specific for the de-methylesterification of rhamnogalacturonan is still lacking.

How PME-driven changes in wall stiffness, charge density, and Ca^{2+}-pectate organization are integrated with diverse stress signals remains to be clarified. Likewise, the extent to which de-methylesterified HG and/or OGs control the assembly, dynamics, and endocytosis of receptor complexes such as FER-RALF is only partially understood. More broadly, how specific methylesterification patterns are decoded into distinct cellular signaling outputs remains a central open question.

From an applied perspective, PME-mediated responses represent a promising target to enhance crop stress resilience. This will require the development of genetic and biotechnological strategies to

achieve spatiotemporal and isoform-specific control of PME activity, including its targeted localization at sites of CW remodeling such as pathogen penetration zones. These approaches include the overexpression of PMEIs, targeted manipulation of stress-responsive transcription factors, and structure-guided engineering of PME-PMEI and PME-SBT interactions, enabling precise modulation of pectin methylesterification, optimization of CW mechanics, and tuning of CWI sensing under stress conditions.

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No conflict of interest is declared.

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Locus name	PME name	Type	Signal peptide	Transmembrane Domain	Interacting organisms	References
Biotic stress						
At1g11580	PMEPCRA (or PME18)	I	-	+	*P. brassicae*	(Stefanowicz et al., 2021)
At2g45220	PME 17	I	+	+	*B. cinerea*	(Del Corpo et al., 2020)
At3g14310	PME 3	I	-	+	*P. carotovorum*, *B.cinerea*, *H. schachtii*	(Hewezi et al., 2008; Raiola et al., 2011; Bohlmann and Sobczak, 2014)
At3g27980	PME 30	I	+	-	*B. subtilis*	(Lakshmanan *et al.*, 2013)
Abiotic stress					Type of Abiotic stress	
At1g23200	PME 6	I	-	-	Drought	(Amsbury et al., 2016)
At2g26440	PME 12	I	+	-	Heat	(Wu et al., 2025)
At2g45220	PME 17	I	+	+	Al	(Geng et al., 2017)
At3g14310	PME 3	I	-	+	Zn	(Weber et al., 2013)
At3g29090	PME 31	II	-	-	Salt and Drought	(Yan et al., 2018; Zhang et al., 2023)
At3g49220	PME 34	I	-	+	Heat	(Huang et al., 2017)
At4g02330	PME 41	I	+	-	Cold	(Qu et al., 2011)
At5g04960	PME 46	I	-	+	Al	(Geng et al., 2017)
At5g04970	PME 47	I	+	-	Salt	(Yan et al., 2018; Zhou et al., 2024)
At5g19730	PME 53	II	+	-	Heat	(Wu et al., 2022)
At5g20860	PME 54	I	+	-	Al	(Geng et al., 2017)

Table 2. ***Arabidopsis thaliana*** **PMEI isoforms mainly involved in biotic and abiotic stresses**

Locus name	PMEI name	Signal peptide	Transmembrane Domain	Specific stress	References
Biotic stress					
At1g48020	PMEI 1	+	-	*B. cinerea*	(Lionetti et al., 2007)
At1g62760	PMEI 10	+	-	*B. cinerea*	(Lionetti et al., 2017)
At3g17220	PMEI 2	+	-	*B. cinerea* and *tobamoviruses*	(Lionetti et al., 2007; Lionetti et al., 2014a; Lionetti et al., 2014b)
At3g47380	PMEI 11	+	+	*B. cinerea*	(Lionetti et al., 2017)
At5g46960	PMEI 12	+	-	*B. cinerea*	(Lionetti et al., 2017)
At5g62360	PMEI 13	+	-	*M. persicae*	(Silva-Sanzana et al., 2019)
Abiotic stress				Type of Abiotic stress	
At3g62820	PMEI 18	+	-	Drought	(Zhang et al., 2023)
At5g62360	PMEI 13	+	-	Cold	(Chen et al., 2018)

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Figure legends

Figure 1. The activity and structure of plant PMEs (**A**) Schematic representation of the reaction catalyzed by PMEs showing the hydrolysis of methyl ester groups in HG, leading to the formation of free carboxyl groups and the release of methanol and H^+. (**B**) Domain organization of plant PME isoforms. Type I PMEs contain an N-terminal pro-domain followed by the catalytic domain, whereas Type II PMEs consist solely of the catalytic domain. The presence of a SP, TM, and GPI anchor is indicated; numbers flanking each class denote the number of isoforms identified in *Arabidopsis thaliana*. (**C**) Three-dimensional structure of *Daucus carota PME (*DcPME), displaying the characteristic right-handed parallel β-helix fold and highlighting the substrate-binding cleft accommodating HG. (**D**) Structure of the PME-PMEI complex: *Actinidia chinensis* AcPMEI (magenta) in complex with *Solanum lycopersicum* SlPME (yellow). (**E**) Structural comparison of *Solanum lycopersicum* SlPME (yellow) and *Erwinia chrysanthemi* EchPME (orange), showing differences in surface loops indicated by black arrows. PME: Pectin methylesterase; HG: homogalacturonan; SP: signal peptide; TM: transmembrane domain; GPI: glycosylphosphatidylinositol.

Figure 2. Intracellular trafficking, processing, and activation of PMEs. Signal peptide and TM contribute to the intracellular targeting and secretion of PMEs. PME17 precursor, containing a signal peptide but lacking a TM domain, follows the conventional endoplasmic reticulum-Golgi pathway and is secreted into the apoplast as an inactive precursor, where it is activated by the subtilase SBT3.3. In contrast, PME34 precursor lacks a signal peptide but contains a TM domain, resulting in transient plasma membrane localization prior to release. SBT3.3 precursor is secreted via an unconventional pathway involving EXPOs, enabling temporal control of PME activation. The secretion pathway of Type II PMEs remains to be characterized. The right panel shows HG biosynthesis in the Golgi by GAUTs using UDP-GalA, followed by methyl-modifications mediated by HG-MTs. Methyl-esterification requires SAM, imported by GoSAMTs, generating HG polymers with variable degrees of methylesterification. Dashed arrows and question marks indicate putative or unresolved trafficking routes. PMEs: Pectin methylesterase; TM: transmembrane domain; EXPOs: Exocyst-Positive Organelles; HG: homogalacturonan; GAUTs: galacturonosyltransferases; GalA: D-galacturonic acid; HG-MTs: HG methyltransferases; SAM: S-adenosylmethionine; GoSAMTs: Golgi SAM transporters.

Figure 3. Mechanisms of PME-mediated defense responses during biotic stress. (**A**) Protection of HG by maintaining high levels of methylesterification, ensured by the balanced activity of PMEs and PMEIs, thus limiting the accessibility of microbial pectinolytic enzymes (e.g., PGs). (**B**) Localized CW reinforcement through HG de-methylesterification and Ca^{2+}-mediated cross-linking between adjacent HG chains. (**C**) HG de-methylesterification is perceived by specific sensors. (**D**) On the left, de-methylesterified HG acts as a scaffold for the anchoring of CW-associated enzymes and surface receptors, thereby contributing to the spatial organization of CW remodeling and signaling. On the right, PME activity promotes the generation of signaling molecules, such as OGs and MeOH, and the activation of receptor-mediated signaling pathways. These perception events activate hormonal signaling pathways, which in turn coordinate the induction of PTI-related genes as well as CW remodeling genes. Question marks indicate putative or not yet fully resolved sensing mechanisms. PMEs: Pectin methylesterase; PMEIs: PME inhibitors; HG: homogalacturonan; PG: polygalacturonases; PGIP: polygalacturonase-inhibiting proteins; OGs: oligogalacturonides; MeOH: methanol; RFO1: RESISTANCE TO FUSARIUM OXYSPORUM 1; PRXs: peroxidases; EXLAs: expansin-like A proteins; ROS: Reactive Oxygen Species; RALF: RAPID ALKALINIZATION FACTOR; FER: FERONIA; LLG1: LORELEI-LIKE GPI-ANCHORED PROTEIN 1; WAK1: WALL-ASSOCIATED KINASE 1; ET: Ethylene; JA: Jasmonic Acid; SA: Salicylic Acid; PTI: Pattern-Triggered Immunity.

Figure 4. PME-mediated regulation of cell wall dynamics and ion homeostasis under abiotic stress. (**A**) Under heat and drought stress, the balanced activity of PMEs and PMEIs, modulated by abscisic acid signaling, fine-tunes guard CW flexibility, thereby supporting efficient stomatal dynamics. (**B**) Under cold stress, PME activity, regulated by PMEIs and brassinosteroid signaling, promotes Ca^{2+}-mediated cross-linking, increasing CW stiffness and reducing porosity and ion leakage. (**C**) During salt stress, the PME–PMEI module balances the availability of free carboxyl groups on HG, promoting preferential Ca^{2+} cross-linking that strengthens the CW and reduces Na^{+} toxicity. **(D)** Under heavy metal stress, PME activity increases the density of carboxyl groups on HG, enabling the sequestration of toxic ions (Zn^{2+}, Al^{3+}, Cr^{3+}, Cd^{2+}, Cu^{2+}) within the CW. Colored spheres indicate diffusible ions (e.g., K^{+}, Cl^{-}, Mg^{2+}). PMEs: pectin methylesterases; PMEIs: PME inhibitors; HG: homogalacturonan.