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RESEARCH ARTICLE

A core of cell wall proteins functions in wall integrity responses in *Arabidopsis thaliana*

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Abstract

Cell walls surround all plant cells, and their composition and structure are tightly regulated to maintain cellular and organismal homeostasis. In response to wall damage, the cell wall integrity (CWI) system is engaged to ameliorate effects on plant growth. Despite the central role CWI plays in plant development, our current understanding of how this system functions at the molecular level is limited. Here, we investigated the transcriptomes of etiolated seedlings of mutants of *Arabidopsis thaliana* with defects in three major wall polysaccharides, pectin (*quasimodo2*), cellulose (*cellulose synthase3*^{je5}), and xyloglucan (*xyloglucan xylosyltransferase1* and *2*), to probe whether changes in the expression of cell wall-related genes occur and are similar or different when specific wall components are reduced or missing. Many changes occurred in the transcriptomes of pectin- and cellulose-deficient plants, but fewer changes occurred in the transcriptomes of xyloglucan-deficient plants. We hypothesize that this might be because pectins interact with other wall components and/or integrity sensors, whereas cellulose forms a major load-bearing component of the wall; defects in either appear to trigger the expression of structural proteins to maintain wall cohesion in the absence of a major polysaccharide. This core set of genes functioning in CWI in plants represents an attractive target for future genetic engineering of robust and resilient cell walls.

KEYWORDS
cell wall integrity, cell wall proteins, cell wall transcriptome, cellulose, pectin, plant cell wall, xyloglucan

1 | INTRODUCTION

Plant cell walls are made of complex wall polymers that function in growth, development, and adaptation to biotic and abiotic stresses. Maintenance of the functional integrity of cell walls, which in the growing cells of eudicots include pectins, cellulose, and xyloglucan, is key to preserving wall mechanical properties and increasing lignocellulose biomass and is also required to strike the correct balance between turgor pressure and wall strength that underpins cell expansion in plants (Engelsdorf & Hamann, 2014). When cell wall integrity (CWI) is compromised due to wall damage or the absence of one or more essential components, mechanical and metabolic stresses or failures can occur that can negatively impact plant growth (Vaahtera et al., 2019). A functional CWI system is therefore required to prevent mechanical distortions and defects in plant growth, involves crosstalk between different CWI signaling pathways, and results in changes

in wall composition to maintain a structurally sound cell wall (Hamann, 2015).

Although available evidence suggests analogies in the molecular components involved in CWI maintenance between plants and the yeast *Saccharomyces cerevisiae* (Wolf et al., 2012), information regarding the precise mode of action and compensatory responses involved in plant CWI maintenance is limited. Pectic polysaccharides are important in wall homeostasis as they are more sensitive to mechanical deformation than other cell wall components like cellulose (Bacete et al., 2018). Pectin fragments such as oligogalacturonides can bind to receptor-like kinases (RLKs) to initiate, integrate, and transmit signals that result in the repair of cell wall damage (Ferrari et al., 2013). Manipulation of cellulose biosynthesis by isoxaben or genetic mutation of *CELLULOSE SYNTHASE 3* (*CESA3*) results in the ectopic production of lignin and callose (Cano-Delgado et al., 2003; Caño-Delgado et al., 2000). Virus-induced gene silencing of a primary wall *CESA* in tobacco results in a compensatory increase in homogalacturonan (Burton et al., 2000). These and other data imply the existence of a coordinated signaling network that senses, integrates, and responds to defects in cellulose biosynthesis. As wall polymer characteristics are expected to change in response to cell wall damage and nearly 10% of many plant genomes encode wall-related genes (Somerville et al., 2004), we hypothesized that compensatory alterations in wall composition and assembly might be mediated in part by changes in the transcriptome. Testing this hypothesis offers an opportunity to gain additional insights into which wall-related genes are altered when one or more cell wall components (pectin, cellulose, and hemicellulose) are functionally impaired. Notably, mutants for the pectin methyltransferase *QUASIMODO2* (*QUA2*) display reduced homogalacturonan content and cell adhesion defects (Barnes et al., 2022; Du et al., 2020; Krupková et al., 2007; Mouille et al., 2007); the *cesa3*^{je5} allele displays reduced cellulose deposition (Desprez et al., 2007); and a mutant for two *XYLOGLUCAN XYLOSYLTRANSFERASES* (*xxt1 xxt2*) lacks detectable xyloglucan and displays bulging of epidermal cells in etiolated hypocotyls, among other phenotypes (Cavalier & Keegstra, 2006; Xiao et al., 2016). Interestingly, all these wall biosynthetic mutants display short hypocotyls in etiolated seedlings (Desprez et al., 2007; Du et al., 2020; Xiao et al., 2016); thus, using etiolated seedlings of wall synthesis mutants provides a comparative basis to delineate the functional compensations that occur in the wall-related transcriptome in response to wall perturbations.

Here, we performed transcriptome and gene co-expression analysis in pectin, cellulose, and xyloglucan synthesis mutants to determine whether they share similar CWI responses and what signaling cascades or changes in wall-related gene expression occur in these mutants. Fifty-one wall-related genes were differentially regulated in all three mutants, and our co-expression analysis identified hub genes that might be critical for maintaining wall integrity in the absence of key wall polymers. This study reveals new molecular components of a core wall integrity response that might serve as targets for functional analysis and in efforts to engineer plant cell walls for improved plant growth and crop yields.

2 | RESULTS AND DISCUSSION

2.1 | Cell wall synthesis mutants show transcriptome changes relative to wild type

RNA from Col-0 wild type (WT), *qua2*, *cesa3*^{je5}, and *xxt1 xxt2* mutants was extracted from 8-day-old etiolated hypocotyls and sequenced on a NextSeq 2000 P2 100-nt single-read sequencing run. After removal of adaptor sequences, duplicate sequences, and ambiguous reads and filtering low-quality reads, we obtained more than 99% clean reads, wherein the mapping ratio ranged from 94% to 99% similarity to the *Arabidopsis thaliana* TAIR10 genome for the WT, *qua2*, *cesa3*^{je5}, and *xxt1 xxt2* samples (Table S1). We defined differentially expressed genes as any gene with an adjusted p-value < .05 and $\log_2$ fold change ≥ ±1 relative to WT. The number of differentially expressed genes was highest in *qua2* (4617), followed by *cesa3*^{je5} (3014) and *xxt1 xxt2* (1938) (Figure 1). The high number of differentially expressed genes in *qua2* might reflect the ability of pectin or pectin fragments to interact with extracellular domains of CWI sensors (Vaahtera et al., 2019). In all cases, a substantial proportion of all genes (5%–14% of the transcriptome) was differentially expressed, suggesting that wall structural changes resulting from the loss of one wall component might activate CWI signaling, inducing transcriptional changes that lead to developmental defects.

Based on our initial finding of substantial transcriptomic alterations in these cell wall mutants, we next asked which wall-related genes were differentially expressed in response to all three wall defects. Out of 439 genes that were differentially expressed in all three mutants (Figure 2a), 51 genes were annotated as being cell wall related (Figure 2b). We infer that these genes, consistently altered across all

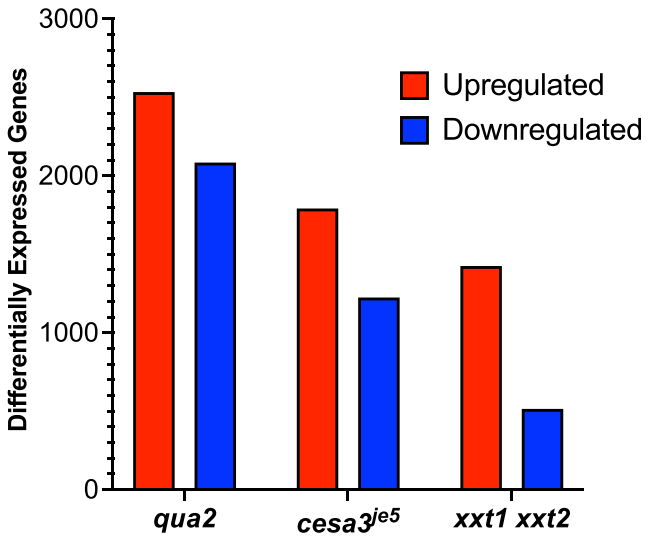

FIGURE 1 Numbers of significantly upregulated and downregulated genes for each genotype. RNA was extracted from three biological replicates each of *cesa3*^{je5}, *qua2*, and *xxt1 xxt2* 8-day-old etiolated hypocotyls. Differentially expressed genes were defined as those with an adjusted p-value < .05 and $\log_2$ fold change ≥ 1 (upregulated) or ≤ −1 (downregulated).

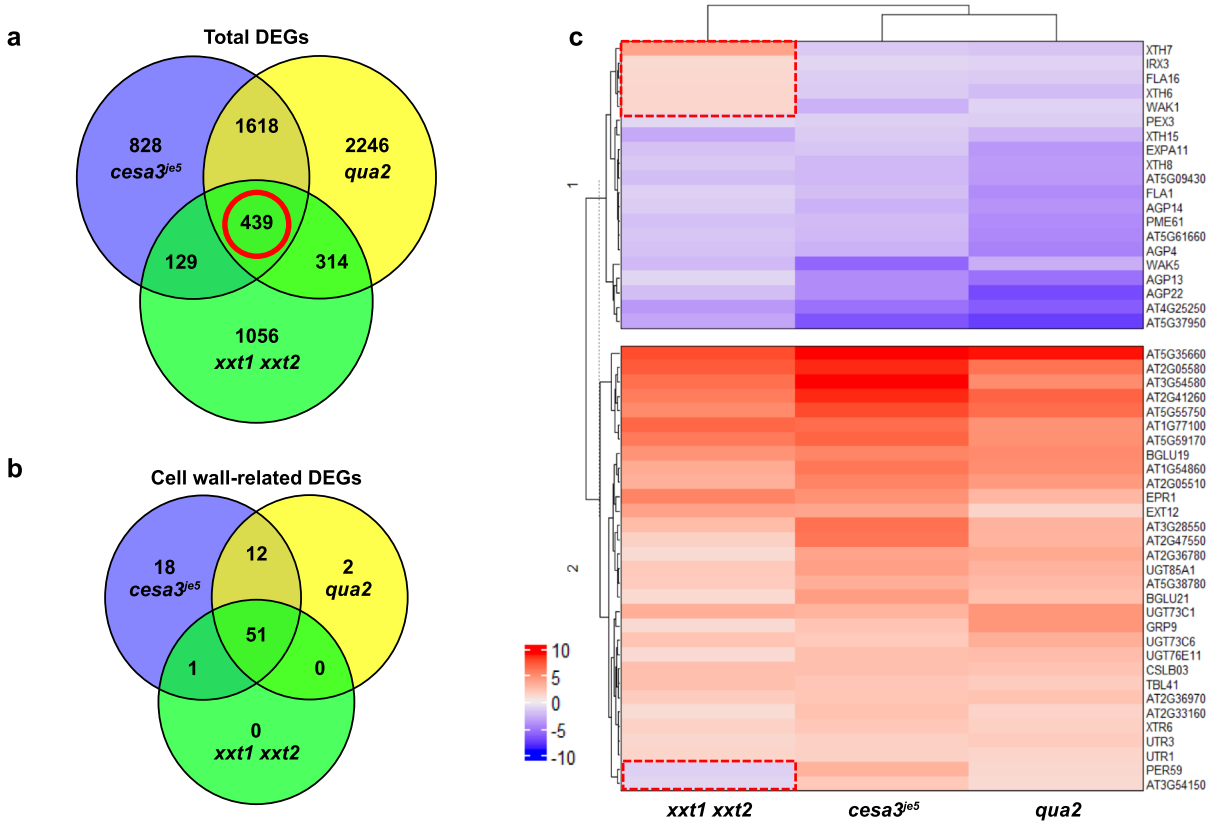

FIGURE 2 Venn diagram and heat map enumerating differentially expressed genes (DEGs). (a) Venn diagram of DEGs for *cesa3*^{je5}, *qua2*, and *xxt1 xxt2*. The red circle indicates the number of genes differentially expressed across all three mutants. (b) Venn diagram display of the DEGs related to the cell wall for *cesa3*^{je5}, *qua2*, and *xxt1 xxt2*. (c) Heat map of the 51 cell wall genes differentially expressed in all three mutants (as shown in b). Dashed boxes indicate genes in *xxt1 xxt2* that were expressed differently from *cesa3*^{je5} and *qua2*.

three genotypes, might play active roles in the CWI response. Notably, all 51 wall-related genes were consistently upregulated or downregulated in both *qua2* and *cesa3*^{je5} mutants in comparison with WT, indicating that a common adaptive response pathway might be present in mutants defective in either pectin or cellulose synthesis genes. However, seven genes in the *xxt1 xxt2* mutants behaved differently from the *qua2* and *cesa3*^{je5} mutants (Figure 2b, dashed boxes). Overall, we observed that pectin and cellulose defects induce a common CWI response that differs from the response to xyloglucan defects.

2.2 | Subsets of wall biosynthetic genes are differentially expressed in wall synthesis mutants

We next asked whether compensatory responses in the expression of genes related to other wall components could be detected based on changes in the mutant transcriptomes. Whereas primary cell wall *CESAs* were essentially unchanged in expression in all three mutant genotypes (*cesa3*^{je5} is a hypomorphic allele and would be expected to show transcription), the majority of secondary wall *CESAs*, as well as genes involved in xylan metabolism, xyloglucan biosynthesis, and pectin modification, were significantly downregulated in *qua2* and *cesa3*^{je5} mutants, whereas *CESA7* and *IRX15-L* were slightly upregulated in the *xxt1 xxt2* mutant (Table S2). The downregulation of genes encoding wall biosynthetic enzymes suggests a mechanism to reduce metabolic cost in *cesa3*^{je5} and *qua2* mutants but might also compromise the CWI response, leading to mechanical defects in the wall that are related to growth defects. Given that the three main *CESA* genes (*CESA1*, *CESA3*, and *CESA6*/*CESA6*-like) involved in primary wall assembly are co-expressed (Desprez et al., 2007), the significant downregulation of secondary cell wall *CESA* genes might represent a feedback response to defects in primary cell wall deposition that alter the expression of secondary cell wall *CESAs*. Although increased expression of wall synthesis genes might enable compensatory deposition of wall materials in the absence of one wall component, this was not observed at the transcriptome level in *qua2* and *cesa3*^{je5} mutants. This might explain why more severe growth phenotypes were observed in *qua2* and *cesa3*^{je5} etiolated seedlings than in *xxt1 xxt2* seedlings (Desprez et al., 2007; Du et al., 2020; Xiao et al., 2016). *COBRA-like* (*COBL*) genes function in cell wall expansion and are co-expressed with *CESA* genes (Persson et al., 2005), and the significant upregulation of *COBL* genes in *qua2* and *cesa3*^{je5} mutants implies that they might help mitigate cell wall damage at the transcriptional level (Table S2). In addition, previous work showed that Arabidopsis plants lacking xyloglucan undergo relatively normal development (Kim et al., 2020; Zabotina et al., 2012), possibly due to functional redundancy by the hemicellulose β-galactoglucomannan (Yu

et al., 2022). The relatively normal development of *xxt1 xxt2* mutants might align with our observation of fewer transcriptome changes in *xxt1 xxt2* mutants compared with *qua2* and *cesa3*^{je5} mutants (Figure 1). Based on these data, it appears that the loss of xyloglucan induces fewer CWI responses than the loss of cellulose or pectin, or that the CWI responses in *xxt1 xxt2* are more effective at compensating for the loss of xyloglucan and maintaining proper growth and development.

2.3 | Genes encoding wall structural proteins are differentially expressed in wall biosynthetic mutants, but glycosyltransferases that modify these proteins are not

Nineteen of the 51 wall-related genes that were differentially expressed in all the mutants encoded wall structural proteins (Figure 2b and Table S2). Cell wall proteins such as the proline-rich extensin-like family and glycine-rich proteins are structural proteins that respond to physical damage in the plant primary cell wall (Fowler et al., 1999). These proteins contain motifs for hydroxylation, glycosylation, and cell wall polymer cross-linking. We hypothesize that proteins containing these functional motifs, when upregulated, might serve as reinforcements to shore up defective or damaged walls.

The transcriptome analysis indicated that in the tested wall synthesis mutants, many arabinogalactan proteins (AGPs) were significantly downregulated, whereas most proline-rich extensin-like and glycine-rich protein genes were significantly upregulated (Table S2). Because the latter proteins are heavily *O*-glycosylated, we hypothesized that the upregulation of wall structural proteins might also be accompanied by the upregulation of genes involved in protein *O*-glycosylation. However, very few AGP and extension-related glycosyltransferases were differentially expressed (Table S2). Based on this finding, it is possible that these wall protein glycosyltransferases are less important for CWI responses. Due to the metabolic cost associated with glycosyltransferase synthesis and *O*-glycosylation, direct wall protein cross-linking might serve as a CWI response because extensins can form intramolecular isodityrosine cross-links or intermolecular di-isodityrosine or pulcherosine cross-links, potentially strengthening the wall (Brady et al., 1998). Similarly, they can form pectate–extensin complexes during cell wall assembly and repair (Cannon et al., 2008; Mishler-Elmore et al., 2021). However, it remains to be determined what the underlying physical and/or functional connections might be between wall-strengthening responses or structures in the absence of key polysaccharides and the machinery that senses and maintains CWI.

2.4 | CWI signaling genes are differentially regulated in wall biosynthetic mutants

Glycosylated wall proteins and wall polymer epitopes attached to RLKs, such as wall-associated kinases (WAKs), can elicit signals upon structural changes in the cell wall. Defects in wall polymer synthesis and/or wall assembly might interfere with epitope recognition by RLKs, thereby impacting CWI sensing and response (Engelsdorf & Hamann, 2014). Here, we observed significant changes in the expression of *WAK1* and *WAK5* in the wall synthesis mutants investigated, with *WAK1* being downregulated in *qua2* and *cesa3*^{je5} and upregulated in *xxt1 xxt2*, and *WAK5* being downregulated in all genotypes (Figure 2b and Table S2). Given the reduction in homogalacturonan content in *qua2* mutants (Du et al., 2020; Mouille et al., 2007), it is possible that wall modifications driven by reduced abundance of pectins or pectin fragments are associated with reduced expression of their interaction partners. If *WAK* gene expression is contingent upon the pool of WAKs binding pectic polymers, the reduced pectin abundance might trigger the downregulation of wall-related kinases such as *THESEUS1* (*THE1*), *FEI2*, *WAK1*, and *WAK5*, as observed in *qua2* mutants (Table S2). Conversely, overproduction of pectin fragments due to enhanced pectin degradation in *qua2* (Barnes et al., 2022) might overstimulate the CWI signaling associated with these wall-related kinases, resulting in their downregulation. *WAK1* was significantly upregulated in *xxt1 xxt2* mutants, consistent with earlier findings of upregulation of this gene in *xxt1 xxt2* and the idea that *WAK1* upregulation is a compensatory response to an increase in pectin abundance in the absence of xyloglucan (Xiao et al., 2016). We also observed a significant upregulation of *WAK*-like genes, especially in *qua2* mutants (Table S2). It should be noted that unchanged RLK expression does not exclude the possibility of RLK activation through phosphorylation (Faria-Blanc et al., 2018); therefore, further research is needed to delineate downstream signaling involving *THE1*, *FEI2*, *WAKs*, and *WAK*-like genes in response to cell wall defects and the functional specificity they confer with their interacting partners during CWI sensing and response.

2.5 | Gene ontologies of differentially expressed genes align with defects in specific wall components

To further investigate the wider effects of primary cell wall modifications in relation to plant growth, we examined other signaling and adaptive response pathways by analyzing cell wall-related gene ontology (GO) terms that were enriched in our dataset. GO terms related to wall organization and modification were highly enriched in both *qua2* and *cesa3*^{je5} mutants, but pectin-related GO terms were more specific to the *qua2* mutant (Figure 3 and Table S3). For the *xxt1 xxt2* mutant, xyloglucan metabolic process was the most enriched wall-related GO term, but further scrutiny revealed that this gene group contained only xyloglucan endo-transglycosylases/hydrolases, implying that these genes might be co-regulated in response to an absence of xyloglucan.

2.6 | Weighted gene co-expression network analysis identifies functional modules that are unique to different wall synthesis mutants

To elucidate the molecular responses of cell wall synthesis mutants, correlations between gene expression patterns and traits, defined as the different mutants, were investigated using weighted gene co-

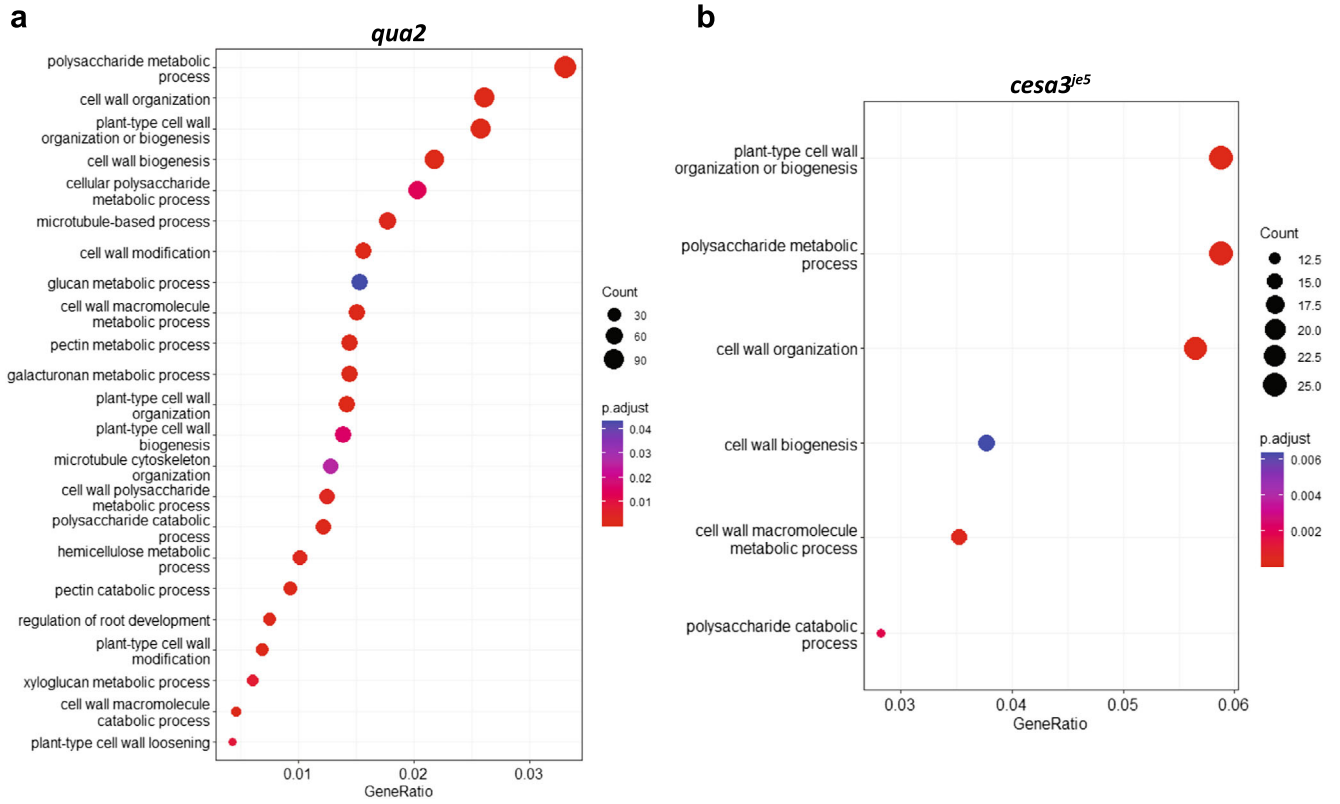

FIGURE 3 Functional categories of differentially expressed genes in the gene ontology. Significant gene ontology categories for *qua2* (a) and *cesa3*^{je5} (b) that were enriched for the indicated biological processes.

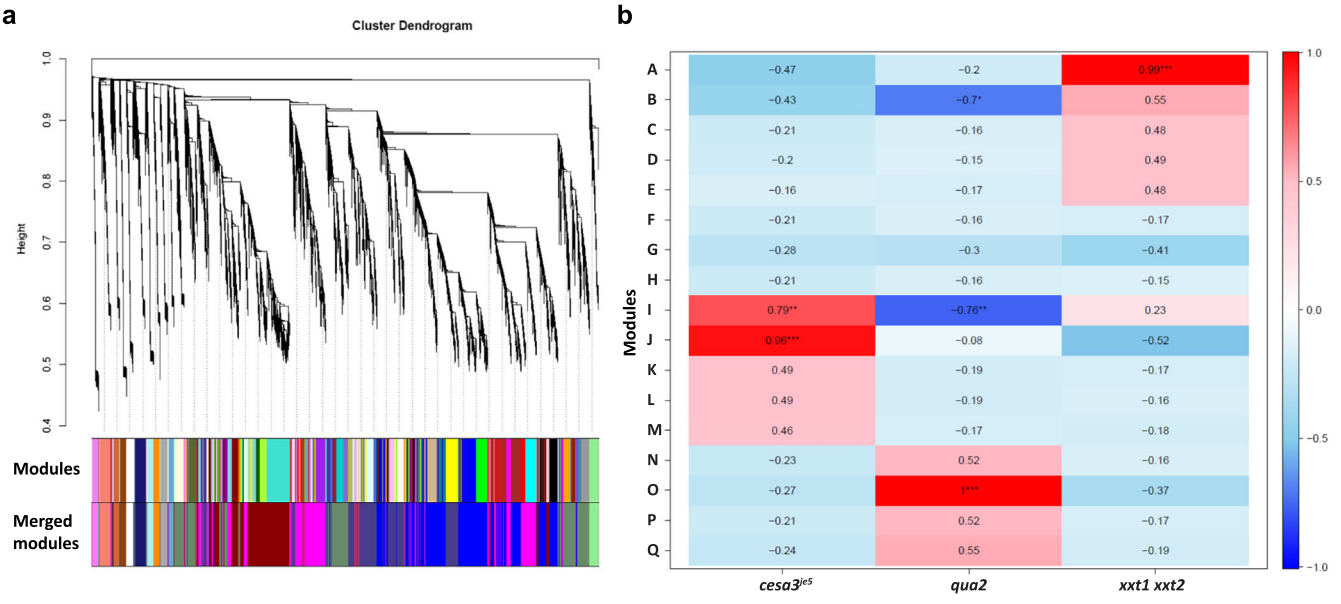

FIGURE 4 Weighted gene co-expression network analysis comparing gene expression profiles to cell wall mutants. (a) Hierarchical cluster tree showing co-expression modules identified by the Dynamic Tree Cut method. Each leaf, or short vertical line, corresponds to a gene. The branches of the dendrogram group together densely interconnected modules and are labeled with different colors. Genes with high co-expression levels (correlation ≥ .6) were merged into one module; 18 modules were obtained in total, with the gray module representing a set of unassigned genes. (b) Correlations of cell wall mutants with weighted gene co-expression network analysis modules. Each row corresponds to a module, and columns correspond to the three cell wall mutants. The color of each cell indicates the correlation coefficient between the module and traits: Red represents positive correlation, and blue represents negative correlation. Asterisks indicate *p*-values with *** ($p < .0001$), ** ($p < .001$), and * ($p < .01$).

expression network analysis (WGCNA). Cluster analysis of the wall mutants showed that the *xxt1 xxt2* mutant was in proximity to the WT, whereas the *cesa3je5* and *qua2* mutants were both clustered together further from the WT (Figure S1A). We also identified 18 distinct co-expression modules (Figure 4a), with each module containing clustered genes (Dataset S1).

Module–trait associations (Figure 4b) indicated that for *qua2*, module **O** exhibited the highest positive correlation ($r = 1$; $p < .001$), with 2277 out of 3595 genes associated with this module ($p < .01$) (Dataset S1). Modules **B** and **I** displayed a negative correlation to *qua2* ($r = -.7$ for **B** and $r = -.76$ for **I**; $p < .001$), with 1027 out of 5723 genes and 238 out of 548 genes associated with modules **B** and **I**, respectively (Dataset S1). Functional enrichment analysis of these three modules against *qua2* revealed that most of the cell wall-related GO terms belonged to modules **B** and **O** (false discovery rate [FDR] < .1) and consist primarily of general cell wall organization/biogenesis-related terms and specific pectin/galacturonan metabolic processes (Dataset S1). Module **I**, however, does not have any GO terms associated with cell wall or pectin metabolism and was mostly actin related (FDR > .1) (Dataset S1). Of the 2277 genes in module **O**, 1066 were differentially expressed in *qua2* as compared with WT, as well as 613 out of 1027 genes and 67 out of 238 genes in modules **B** and **I**, respectively, suggesting that these genes might mediate the CWI response in *qua2* (Dataset S1). Among the top 10 genes in module **O** associated with *qua2*, we found *PECTIN ACETYL ESTERASE12* (AT3G05910) and *ALTERED XYLOGLUCAN8* (AT4G34260) to be cell wall-related hub genes that were downregulated in *qua2* mutants relative to WT (Dataset S1, bold red). Similarly, among the top 10 genes in module **B** that were associated with *qua2*, we identified *POLYGALACTURONASE INVOLVED IN EXPANSION3* (*PGX3*; AT1G48100), *GALACTURONOSYLTRANSFERASE4* (*GAUT4*; AT5G47780), and *BETA-XYLOSIDASE1* (AT5G49360) hub genes to be cell wall related, with *PGX3* and *BETA-XYLOSIDASE1* downregulated in *qua2* mutants and *GAUT4* not differentially expressed. The downregulation of these genes supports their co-expression status in each assigned module and positions them as potential regulators of CWI responses in *qua2* mutants. One gene in module **I**, *COMPANION OF CELLULOSE SYNTHASE1* (*CC1*), although it was among the top 10 genes associated with *qua2* and was co-expressed with other differentially expressed genes, was not differentially expressed (Dataset S1). Together, these data show that co-expression does not necessitate differential expression, at least in the case of *qua2*.

Two modules, **J** and **I**, were positively associated with *cesa3*^{je5} ($r = .96$; $p < .001$ for **J**; $r = .79$; $p < .001$ for **I**), with 2391 out of 4188 genes and 176 genes out of 548 genes belonging to modules **J** and **I**, respectively (Dataset S1). Unlike for *qua2*, very few wall-associated GO terms were detected for module **J** (Dataset S1). Instead, the most enriched GO terms were response to jasmonic acid and signaling (Dataset S1) (FDR < .1). Previous work involving the *korrigan1* (*kor1*) mutant indicated that altered cell walls induce jasmonic acid biosynthesis, and mutation in *ESMERALDA1* (*ESMD1*) suppresses jasmonic acid signaling in *kor1* (Mielke et al., 2021). The *esmd1* mutation also rescues cell adhesion defects in *qua2* (Verger et al., 2016), so the appearance of jasmonic acid and signal transduction categories in *cesa3*^{je5} implies that jasmonic acid signaling might function in responses to cell wall defects, as described in previous studies (Denness et al., 2011; Engelsdorf et al., 2019). For module **A**, associated with *xxt1 xxt2* ($r = .99$; $p < .001$), none of the 2787 out of 3802 genes associated with this module ($p < .01$) were linked to wall-related GO terms (Dataset S1). When all three mutants were jointly considered, we found that *qua2* contained the most wall-related genes, especially in module **O**.

3 | CONCLUSIONS

We have identified a core set of genes with putative functions in CWI signaling based on their shared and consistent transcriptional responses to defects in pectin, cellulose, and xyloglucan. Although we cannot exclude post-transcriptional and post-translational modifications that influence wall assembly and formation, we conclude that investigating the transcriptome offers exciting clues as to how plants respond to and compensate for cell wall defects to allow for plant survival and reproduction. In addition, GO enrichment and WGCNA analyses of *qua2* and *cesa3*^{je5} showed differences (*qua2* included cell wall biosynthesis and pectin metabolism, whereas the *cesa3*^{je5} mutant included jasmonic acid biosynthesis in addition to cell wall biogenesis) that hint at unique CWI signaling responses to wall defects. This may partly be explained by the fact that pectin has cross-linking sites and can act as a ligand for RLKs (Feng et al., 2018; Huerta et al., 2023; Kohorn et al., 2009; Lin et al., 2022). This suggests the presence of distinct CWI pathways that might inspire the future engineering of stress-resilient cell walls and plants. Earlier work showed fewer transcriptomic changes in response to secondary wall defects (Faria-Blanc et al., 2018). The core set of genes functioning in CWI in plants represents an attractive target for genetic manipulation of plant cell walls for improved biomass yields or characteristics and/or the production of wall polymers with new structural and functional properties.

4 | METHODS

4.1 | Plant materials, seed sterilization, and growth conditions

All Arabidopsis plants used in this study were from the Columbia (Col-0) background. The seeds of homozygous T-DNA insertion mutant lines *qua2*, *cesa3*^{je5}, *xxt1 xxt2* mutants, and WT (Col-0) were sterilized and transferred to ½ strength Murashige and Skoog medium with .8% agar and 0% sucrose and kept in the dark for 48 h at 4°C. The seeds were then grown for 8 days at 22°C in the absence of light. Three biological replicates, consisting of batches of sterilized seeds, were prepared for each genotype. Approximately 250 hypocotyls from each of the three biological replicate pools were immediately frozen in liquid nitrogen prior to total RNA isolation.

4.2 | RNA extraction, preparation of digital expression libraries, and Illumina sequencing

Total RNA was extracted with the QIAGEN RNeasy® Plant Mini Kit (QIAGEN, Cat. No. 74903), while RNA integrity was assessed using an Agilent Bioanalyzer. The Illumina Stranded mRNA TruSeq kit was used for library preparation, while sequencing was carried out at the Penn State University Genomics Core Facility using the NextSeq 2000 P2 100-nt single-read sequencing run.

4.3 | Differential gene expression analysis

Raw data were filtered to eliminate reads containing adapter, poly-N, and low-quality reads, and cleaned-up reads were mapped to the *A. thaliana* TAIR10 reference genome using HISAT2, following instructions in the manual (Kim et al., 2019). HTSeq v0.6.1 was used to calculate the number of reads mapped to each gene (read count data) (Putri et al., 2022). Differential expression analysis was performed using the DESeq2 R package Version 1.12.0 (Love et al., 2014), and $\log_2$ fold change ≥ ±1 was used as the threshold to assess the significance of differences in gene expression. GO over-representation analysis was carried out using clusterProfiler 4.0 in Bioconductor (Wu et al., 2021).

4.4 | Weighted gene co-expression network analysis

Gene-expressed values for WT and mutant transcriptomes were selected for WGCNA using the R package WGCNA (Zhang & Horvath, 2005). A suitable soft threshold of five was selected as the minimum power according to the preconditions of approximate scale-free topology and subsequently used for module construction. Following dynamic branch cutting with a merging threshold of .4, 18 modules were obtained. The intramodular connectivity of genes in the corresponding modules of interest was measured using module eigengene-based connectivity (kME). We removed the gray module for downstream analysis as genes within the gray module corresponded to the set of genes that were not clustered in any module. The correlation between module eigengenes and cell wall mutant traits was analyzed and displayed as heat maps to identify modules of interest that were significantly associated with cell wall mutant traits. In addition, the correlation between gene significance (the absolute value of the correlation between gene and trait) and module membership (defined as the correlation of the module eigengenes and gene expression profile) was further examined to identify hub genes, while significant genes associated with modules of interest were further compared with differentially expressed genes between WT and each wall mutant. For functional enrichment analysis of modules of interest, genes in each significant module were extracted for GO analysis to identify significant GO categories at a threshold cutoff of $p < .05$.

AUTHOR CONTRIBUTIONS

OA and CTA designed the research; OA performed research and analyzed data; and OA, EZ, and CTA wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors did not report any conflict of interest.

PEER REVIEW

The peer review history for this article is available in the Supporting Information for this article.

DATA AVAILABILITY STATEMENT

All data used in the manuscript are presented in the main figures and Supplementary Data. Raw transcriptome data are available from the authors upon request.

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SUPPORTING INFORMATION

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