UNIVERSITY of York

This is a repository copy of *In Vivo Proximity Cross-Linking and Immunoprecipitation of Cell Wall Epitopes Identify Proteins Associated with the Biosynthesis of Matrix Polysaccharides*.

White Rose Research Online URL for this paper: <u>https://eprints.whiterose.ac.uk/214764/</u>

Version: Published Version

Article:

Gomez, Leonardo Dario orcid.org/0000-0001-6382-9447 (2024) In Vivo Proximity Cross-Linking and Immunoprecipitation of Cell Wall Epitopes Identify Proteins Associated with the Biosynthesis of Matrix Polysaccharides. ACS Omega. ISSN 2470-1343

https://doi.org/10.1021/acsomega.4c00534

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/

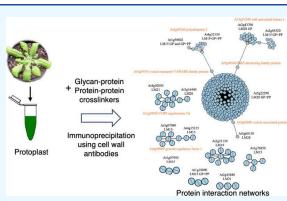


In Vivo Proximity Cross-Linking and Immunoprecipitation of Cell Wall Epitopes Identify Proteins Associated with the Biosynthesis of Matrix Polysaccharides

Pitchaporn Wannitikul, Issariya Dachphun, Jenjira Sakulkoo, Anongpat Suttangkakul, Passorn Wonnapinij, Rachael Simister, Leonardo D. Gomez, and Supachai Vuttipongchaikij*



ABSTRACT: Identification of proteins involved in cell wall matrix polysaccharide biosynthesis is crucial to understand plant cell wall biology. We utilized *in vivo* cross-linking and immunoprecipitation with cell wall antibodies that recognized xyloglucan, xylan, mannan, and homogalacturonan to capture proteins associated with matrix polysaccharides in *Arabidopsis* protoplasts. The use of cross-linkers allowed us to capture proteins actively associated with cell wall polymers, including those directly interacting with glycans via glycan-protein (GP) cross-linkers and those associated with proteins linked to glycans via a protein-protein (PP) cross-linker. Immunoprecipitations led to the identification of 65 *Arabidopsis* protein IDs localized in the Golgi, ER, plasma membrane, and others without subcellular localization data. Among these, we found several glycosyltransferases directly involved in polysaccharide synthesis,



along with proteins related to cell wall modification and vesicle trafficking. Protein interaction networks from DeepAraPPI and AtMAD databases showed interactions between various IDs, including those related to cell-wall-associated proteins and membrane/ vesicle trafficking proteins. Gene expression and coexpression analyses supported the presence and relevance of the proteins to the cell wall processes. Reverse genetic studies using T-DNA insertion mutants of selected proteins revealed changes in cell wall composition and saccharification, further supporting their potential roles in cell wall biosynthesis. Overall, our approach represents a novel approach for studying cell wall polysaccharide biosynthesis and associated proteins, providing advantages over traditional immunoprecipitation techniques. This study provides a list of putative proteins associated with different matrix polysaccharides for further investigation and highlights the complexity of cell wall biosynthesis and trafficking within plant cells.

INTRODUCTION

Downloaded via UNIV OF YORK on July 12, 2024 at 14:29:22 (UTC). See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

Plant cell wall biosynthesis is a complex and coordinated process, with numerous enzymes and regulatory proteins playing pivotal roles.¹ Unlike cellulose, which is synthesized from the cell membrane toward the cell wall, matrix polysaccharides undergo synthesis and modification in the Golgi apparatus before being transported to the cell walls through vesicles for final assembly.²⁻⁴ However, precise molecular mechanisms underlying cell wall biosynthesis and remodeling are not fully understood. Many proteins and enzymes involved in various stages of this biosynthetic pathway present challenges for a comprehensive identification of the biosynthesis components.⁵ While glycosyltransferases, as the primary biosynthesis enzymes for matrix polysaccharides, have been extensively studied and characterized, we have little knowledge about the complete set of proteins involved in processes such as side chain substitutions, polysaccharide delivery associated with membrane or vesicles, and regulatory proteins.⁶ Therefore, it is important to identify and characterize these associated proteins to understand plant cell wall biosynthesis, from glycan synthesis to delivery and wall assembly.

Plant cell wall matrix polysaccharides comprising hemicelluloses and pectins represent a diverse group of polysaccharides that play essential roles in maintaining cell wall integrity and function.^{7,8} Xyloglucan (XyG), a major hemicellulose found in primary cell walls of dicots and nongraminaceous monocots, contributes to cell wall strength and elasticity through interactions with cellulose microfibrils. Structurally, xyloglucans consist of a β -1,4-linked glucan backbone, which is similar to cellulose, with side chains composed of xylose residues attached to the backbone at regular intervals. These

Received:	January 18, 2024
Revised:	June 21, 2024
Accepted:	June 27, 2024

Α

xylose residues can be further substituted with other monosaccharides, such as galactose and fucose, forming a diverse array of xyloglucan structures.⁹ Xylans, which can have a wide range of structural arrangements including glucuronoxylan (GX), glucuronoarabinoxylan (GAX), and arabinoxylan (AX), are prominent hemicelluloses found in the secondary cell walls of many plant species. In some species, xylans can also be found in the primary cell walls of GX. GX is abundant in the secondary cell walls of dicots and the primary and secondary walls of grasses, with a structure comprising a backbone of β -1,4-linked xylose residues and occasional glucuronic acid substitutions, conferring rigidity to the cell wall. GAX and AX are characterized by the presence of arabinose and glucuronic acid side chains, which influence their functionality and interaction with other cell wall components.¹⁰ Mixed linkage glucan (MLG), a common hemicellulose (after xylan), is found in the primary cell walls of many grass species. MLG is characterized by a mixed β -1,3 and β -1,4-glucan backbone, contributing to the flexibility and strength of grass cell walls.¹⁰ Galactomannan (GM), prevalent in the primary cell walls of monocots, is characterized by a β -1,4-linked mannose backbone with galactose side chains.¹¹ On the other hand, pectins are structurally and functionally the most complex polysaccharides in plant cell walls, encompassing a family of galacturonic acid-rich polymers including homogalacturonan (HGA), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and xylogalacturonan (XGA).^{12,13} These polysaccharides have pivotal roles in cell wall integrity, plant growth, morphology, development, and defense. Furthermore, these matrix polysaccharides undergo modifications such as methyl and acetyl substitutions right upon biosynthesis for their functionality.^{12,14,15} For instance, recent work has shown that QUASIMODO2 (QUA2) is a pectin methyltransferase required for normal pectin biosynthesis, which is crucial for their roles in maintaining cell wall integrity.¹⁶

In recent years, efforts have been made to identify and characterize proteins associated with plant cell wall biosynthesis. Zhou et al.¹⁷ investigated protein interactions related to cell wall synthesis and found 100 protein candidates and selected 42 of them as the most reliable candidates for future study. Parsons et al.¹⁸ utilized a combination of density centrifugation and surface charge separation to isolate Golgi membranes from Arabidopsis, enabling proteomic analysis that led to the identification of 371 proteins localized in the Golgi and involved in matrix polysaccharide biosynthesis. Cai et al. employed microarray data and coexpression networks in Populus to identify gene candidates associated with plant cell wall synthesis. Moreover, Atmodjo et al.²⁰ focused on the immunoprecipitation of the GAUT1/GAUT7 complex, responsible for homogalacturonan synthesis, and identified 12 proteins involved in this biosynthetic complex, including glycosyltransferases, glycosidases, and other cell wall-related proteins. In parallel, other studies have aimed to identify proteins functioning within the cell wall itself, known as extracellular proteomes or cell wall proteomes.²¹ Interestingly, these investigations revealed the presence of noncanonical cell wall proteins, which were previously expected to reside in the endoplasmic reticulum (ER) or Golgi, indicating the complexity and diversity of the cell wall proteome. Collectively, these studies have provided an extensive list of proteins/genes involved in cell wall polysaccharide biosynthesis, including those directly participating in synthesis as well as those

functioning indirectly or in close proximity to the synthetic complex.

Notably, many previous studies have utilized nonspecific approaches to identify proteins involved in cell wall biosynthesis, such as total purified Golgi fractions and proteomic analysis.¹⁸ As a result, it is difficult to assign specific functions for these proteins to specific cell wall polysaccharides or other related processes. In light of this, our study aims to identify novel proteins involved in the biosynthesis of specific matrix polysaccharides, particularly during active synthesis within the cell. In this study, we devised a specific approach by using cell wall antibodies that recognized cell wall polysaccharides including XyG, GX, GM, and HGA. Arabidopsis protoplasts were used to ensure immunoprecipitation of cell wall epitopes occurring within the intracellular space. We treated the protoplasts with molecular cross-linkers to stabilize and capture proteins associated with matrix polysaccharides that are undergoing synthesis within the cells. Immunoprecipitation was then performed using glycan-specific monoclonal antibodies. Two types of cross-linkers were used, glycan-protein (GP) and protein-protein (PP) cross-linkers, to capture proteins both directly and indirectly associated with cell wall polymers. To verify the identified proteins, we conducted extensive analyses using a number of Arabidopsis gene databases, including those for subcellular localization, protein interactions, and gene expression and coexpression profiling. We identified candidate proteins that are directly or indirectly involved in the biosynthesis and delivery of four major plant cell wall matrix polysaccharides. Moreover, we selected three proteins for further investigation, analyzing T-DNA insertion mutants to assess their role in matrix polysaccharide synthesis. The proteins identified represent promising candidates for further investigation and potential modification aimed at enhancing cell wall matrix polysaccharides, which could lead to crop improvement and various biotechnological applications.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Arabidopsis Col-0 seeds were germinated on 1% (w v⁻¹) agar plates containing 1/2 strength Murashige and Skoog medium and 1% (w v⁻¹) sucrose for 7 days and then grown in compost under conditions: 16 h light (125 μ mol photons m⁻² s⁻¹) at 22 °C. The Arabidopsis Col-0 seeds (stock deposition number N1093) and T-DNA insertion lines (SALK_119422 for At2g43700, SALK_152917 for At5g12150, and SALK_203306C for At3g22460) were obtained from NASC (Nottingham Arabidopsis Stock Centre). All methods were performed in accordance with the relevant guidelines and regulations.

Protoplast Isolation. Protoplasts were isolated from leaves of 3-week-old plants using a modified version of the Tape– *Arabidopsis* Sandwich method.²² The upper epidermal surface of the leaves was placed on a glass slide, while the lower epidermal surface was attached to a strip of transparent tape. The lower epidermal cell layer was carefully peeled off and transferred to a Petri dish containing an enzyme solution (1% cellulase, 0.25% macerozyme, 0.4 M mannitol, 10 mM CaCl₂, 20 mM KCl, 0.1% BSA, and 20 mM MES at pH 5.7). The Petri dish was then shaken at 40 rpm at 25 °C for 2 h. The resulting protoplasts were separated by centrifugation at 150 rpm for 10 min, washed twice with prechilled modified W5 solution (154 mM NaCl, 125 mM CaCl₂, 5 mM KCl, 5 mM glucose, and 2 mM MES at pH 5.7), and incubated on ice for 30 min. Finally, the protoplasts were resuspended in modified MMg solution (0.4 M mannitol, 15 mM MgCl₂, and 4 mM MES at pH 5.7) to obtain a final concentration of 5×10^6 cells mL⁻¹. Protoplast concentrations were determined using a hemocytometer.

Immunoprecipitation and Protein Identification by Liquid Chromatography-Mass Spectrometry (LC-MS/ MS). Immunoprecipitation using glycan-specific antibodies was performed using Arabidopsis protoplasts treated with glycanprotein cross-linkers (GP) and glycan-protein and proteinprotein cross-linkers (GP+PP). Protoplasts were aliquoted into three groups (10⁶ cell each) before being added with GP crosslinkers, GP+PP cross-linkers, and a buffer with no cross-linker and incubated at 25 °C for 2 h. The GP cross-linkers were prepared by mixing MMg solution with 1 mM of KMUH, EMCH, BMPH, and MPBH. For GP+PP cross-linkers, the GP cross-linker solution was added with 1 mM BMOE. These cross-linkers were purchased from Thermo Fisher Scientific. Treated protoplasts were collected by centrifugation at 150 rpm for 10 min, and total proteins were extracted using HEPES protein extraction buffer (50 mM HEPES pH 6.8, 25 mM KCl, 0.25 mM MnCl₂, 0.25 mM MgCl₂, 2 mM EDTA, 40 mM CHAPs, 1X proteinase inhibitor, 1 mM PMSF, 50 mM DTT). After protein quantification using the Bradford assay, 1 mg of the total protein was used for immunoprecipitation using SureBeads protein G immobilized with cell wall specific antibodies including LM15, LM19, LM21, and LM28. Briefly, the magnetic beads were washed three times with PBST before incubation with an antibody (1:10 dilution with PBST) at room temperature for 1 h and then washed twice using PBS-T. The protein extract was added to the beads and incubated at room temperature for 1 h, followed by washing with PBS-T three times. The protein was eluted by incubating with 20 mM glycine pH 2.0 at room temperature for 5 min, and then 1 M phosphate buffer pH 7.4 was added to the protein. The eluted protein was briefly run in a 4% polyacrylamide stacking gel of SDS-PAGE, stained, and excised for LC-MS/MS analysis.

The gel band was subjected to in-gel trypsin digestion at 37 °C for 16 h. Peptides were extracted twice using a solution of 50% acetonitrile and 5% trifluoroacetic acid (TFA) and then dried in a vacuum centrifuge. Subsequently, peptides were reconstituted in 15 μ L of 0.1% formic acid (FA) before analysis. The LC-MS/MS system comprised a liquid chromatography component (Dionex Ultimate 3000, Thermo Scientific) coupled with an electrospray ionization (ESI)/ quadrupole ion trap mass spectrometer (model amazon SL, Bruker, Germany) at the Proteomics Services, Faculty of Medical Technology, Mahidol University (Salaya Campus), Nakonpathum, Thailand. LC separation was conducted on a reversed-phase column (Hypersil GoLD 50 \times 0.5 mm, 5 μ m C18) and safeguarded by a guard column (Hypersil GoLD 30 \times 0.5 mm, 5 μ m C18), with elution at a flow rate of 0.1 mL/ min under gradient conditions of 5-80% B over 50 min. Mobile phase A consisted of water/formic acid (99.9:0.1, v/v), while mobile phase B consisted of acetonitrile. Mass spectral data ranging from 300 to 1500 m/z were collected in positive ionization mode. Peptide mass fingerprinting was conducted using the Swiss-Prot and Arabidopsis thaliana databases through the MASCOT searching engine (http://www. matrixscience.com). Search parameters in a MASCOT MS/ MS iIon search included carbamidomethylation at cysteine residues as a fixed modification, oxidation at methionine residues as a variable modification, peptide tolerance of ± 1.2

Da, MS/MS fragment tolerance of ± 0.6 Da, and allowance for 1 missed trypsin cleavage site. Proteins with a Mascot score greater than the threshold, set with a *p*-value < 0.05, were considered significant.

Protein Identifications. The protein IDs were searched for *Arabidopsis* protein interactions using DeepAraPPI (based in deep learning-assisted prediction)²³ and AtMAD (based on experimental data such as FRET, yeast two-hybrid, coimmunoprecipitation, and affinity capture-MS).²⁴ For coexpression analysis, the *Arabidopsis* RNA-seq Database was used.²⁵ Protein interaction and coexpression networks were built using the igraph R package²⁶ (R Studio 2023.03.1 Build 446 2009–2023 Posit Software, PBC "Cherry Blossom" Release (6e31ffc3, 2023–05–09) for windows).

Yeast Two-Hybrid Assays. The coding sequences of selected genes were inserted into the pGBKT7 and pGADT7 yeast two-hybrid vector system (Takara Bio USA, Inc.). Yeast strains Y187 and AH109 were transformed with the constructs using an LiAC method. Transformants were selected on SD/-Trp agar and SD/-Leu agar for pGBKT7 and pGADT7 constructs, respectively, at 28 °C for 5–7 days. The two strains were mated and grown on SD/-Trp/-Leu (Media Double Dropouts, DDO), SD/-Trp/-Leu/-His (Media Triple Dropouts, TDO), and SD/-Trp/-Leu/-His/-Ade (Media Quadruple Dropouts, QDO) at 28 °C for 5 days. Mating between the constructs with pGBKT7 and an empty pGADT7 vector and vice versa was used as the background control.

Analysis of T-DNA Insertion Mutants. gDNA was isolated by the CTAB method using 2–3 young leaves. Homozygous insertion lines for mutants were identified using a primer in Table S1. PCR was performed using 50 ng of gDNA in a 20 μ L reaction volume containing 4 mM dNTPs, 30 mM MgCl₂, 0.5 μ M for each primer, and 1 unit of *Taq* polymerase (Vivantis, Malaysia) using 35 cycles of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 1.15 min, with a final extension for 5 min. Individual plants identified as negative for the gene-specific amplification and positive for the left border amplification were considered homozygous line candidates. Seed progenies derived from the candidates were retested with PCR before being designated as homozygous lines.

Cell Wall Preparations. AIRs were prepared using a modified method from Pettolino et al.²⁷ Leaves of three-weekold plants were groud in liquid nitrogen, washed three times in 80% ethanol, absolute ethanol, acetone, and methanol, respectively, and then dried in an incubator at 55 °C. AIRs were extracted with 50 mM CDTA pH 7.0 at room temperature by shaking for 18 h before being filtered and collected using nylon mesh. The residue was subsequently extracted with 4 M NaOH containing 1% (w w⁻¹) NaBH₄ at room temperature with shaking for 18 h. The soluble fraction was collected by filtering using nylon mesh before being neutralized using glacial acetic acid. Both soluble fractions were dialyzed against distilled water at room temperature and then freeze-dried. The remaining cellulose residue was dried at 55 °C.

Cell Wall Composition Analysis. The CDTA and NaOH fractions were hydrolyzed with 2 M trifluoroacetic acid at 100 °C for 4 h before separation by high-performance anion-exchange chromatography on a Dionex Carbopac PA-10 column with pulsed amperometric detection as previously described.²⁸ Separated monosaccharides were quantified by external calibration using an equimolar mixture of nine monosaccharide standards (arabinose, fucose, rhamnose,

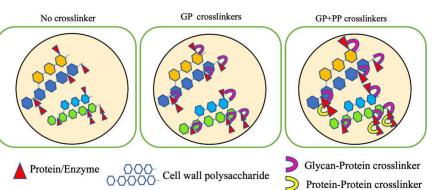


Figure 1. Schematic workflow of *in vivo* proximity cross-linking and immunoprecipitation of cell wall polysaccharides using *Arabidopsis* protoplasts. *Arabidopsis* protoplasts were prepared using young rosette leaves and subjected to nontreated or treated glycan-protein (GP) cross-linkers or glycan-protein and protein-protein (GP+PP) cross-linkers before immunoprecipitation using cell wall antibodies including LM15, LM28, LM21, and LM19. The illustration depicts cell wall polysaccharides that are being synthesized and interacted within the Golgi by various proteins or enzymes, which are then cross-linked to the polysaccharides by the treatment of GP cross-linkers. The treatment of GP+PP cross-linkers allows further proximity cross-linking of proteins to proteins cross-linked onto the polysaccharides.

xylose, glucose, galactose, mannose, glucuronic acid, and galacturonic acid). CDTA and NaOH fractions were calculated by combining the nine sugar contents. Cellulose content was quantified using Saeman hydrolysis²⁹ and Anthrone assay.³⁰

Saccharification Analysis. Saccharification was performed following Gomez et al.³¹ and Whitehead et al.³² in 96-wellplate formats in a robotic platform (Tecan Evo200; Tecan Group Ltd.). Four milligrams of AIR samples were loaded into 96-deep-well plates, pretreated with 350 μ L of 0.5 M NaOH solution at 90 °C for 30 min, washed for five times with 500 μ L of 25 mM sodium acetate buffer pH 4.5, and then incubated with the enzyme cocktail at 50 °C for 8 h. The enzyme cocktail includes Celluclast (cellulose from Trichoderma reesei) and Novozyme 188 (Novozymes A/S, Bagavaerd, Denmark) at a ratio of 4:1 at an enzyme loading of 22.5 Filter Paper Unit (FPU) per gram of material. Biomass hydrolysates were analyzed for reducing sugar released using a modified MBTH method.³³ The experiment was performed using three biological replicates, each with four technical replicates. OD reads were converted to amounts of reducing sugars released in nanomoles using glucose standards.

RESULTS

Identification of Proteins Involved in Matrix Polysaccharide Biosynthesis by Proximity Cross-Linkers and Immunoprecipitation. To identify the biosynthetic machinery responsible for the synthesis of cell wall matrix polysaccharides, we employed a strategic approach involving Arabidopsis protoplasts and intracellular molecular crosslinkers, followed by immunoprecipitation using cell wall antibodies. The antibodies included LM15 recognizing XyGs,³⁴ LM28 recognizing GX,³⁵ LM21 recognizing GM,³ and LM19 recognizing unesterified HGA.³⁷ Our approach is based on the concept that in vivo treatments of protoplast cells with cell-permeable GP cross-linkers would facilitate proximity cross-linking between cell wall matrix polysaccharides being assembled and cell wall biosynthetic enzymes or other related proteins actively functioning in the Golgi network through to secretory vesicles. Furthermore, PP cross-linkers can be added to further extend the network of targets to other associated proteins. Once cross-linked, different matrix polysaccharides, along with their cross-linked proteins, can be isolated through immunoprecipitation using cell wall specific antibodies,

Article

followed by protein identification using LC/MS/MS. For this instance, we selectively acquired cell-permeable crosslinkers based on the cross-linker selection tool (Thermo Fisher Scientific). There are five and six cell-permeable reagents for in vivo cross-linking of GP and PP interactions, respectively. Notably, BMOE (bismaleimidoethane) was chosen as a PP cross-linker, having been used for in vivo cross-linking experiments in various organisms including E. coli,^{38,39} Bacillus *subtilis*,⁴⁰ yeasts,⁴¹ and human endothelial cells and platelets.⁴² It is important to note that while GP cross-linkers have been used for in vitro cross-linking assays⁴³⁻⁴⁵ their in vivo crosslinking potential has remained unexplored. Here, we selected four cell-permeable GP cross-linkers, namely, KMUH (N-ĸmaleimidoundecanoic acid hydrazide), EMCH (N-E-maleimidocaproic acid hydrazide), BMPH (N- β -maleimidopropionic acid hydrazide), MPBH (4-(4-N-maleimidophenyl)butyric acid hydrazide), and BMOE (PP cross-linker), for in vivo cross-linking experiments. We used Arabidopsis protoplasts to avoid any interference from extracellular cell wall epitopes during the immunoprecipitation using cell wall antibodies. Therefore, only cell wall epitopes along with their cross-linked proteins that reside within the cell can be specifically targeted. The experimental workflow is illustrated in Figure 1. Protoplasts were isolated from Arabidopsis leaves using the Tape-Arabidopsis Sandwich method.²² Three treatments of Arabidopsis protoplasts were performed: (a) no cross-linker treatment (aimed to isolate proteins tightly associated with cell wall polymers); (b) GP cross-linkers (a mixture of KMUH, EMCH, BMPH, and MPBH at 1 mM each) targeting proteins directly interacting with glycans; and (c) combined GP+PP cross-linkers (the GP mixture and 1 mM BMOE) targeting those associated with proteins linked to glycans. The protoplasts were treated with the cross-linkers by incubation at 25 °C for 2 h before total protein extraction and immunoprecipitation.

Peptide analysis by LC-MS/MS from the four antibodies, each with the three treatments, resulted in a total of 218 peptide fragments ranging from 8 to 39 amino acids. BLASTP analysis of these peptides led to the identification of 110 protein identities (IDs) based on the *Arabidopsis* genome. The total peptide reads and protein IDs for each antibody and treatment are presented in Table S2. The reliability of our protein identification was supported by the confirmation of

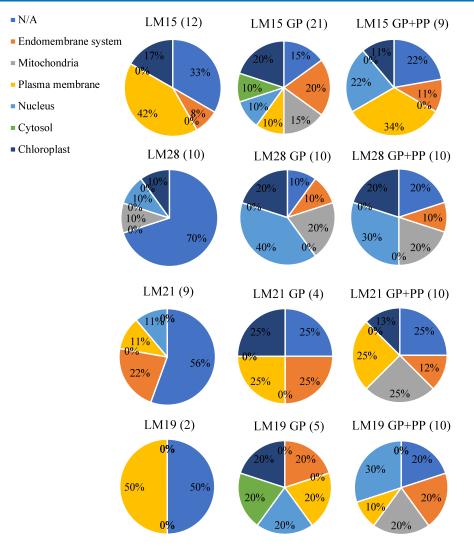


Figure 2. Proteomic analysis of immunoprecipitated products using cell wall antibodies. Identified proteins were classified based on annotated subcellular localizations. The number of proteins identified for each antibody and treatment are presented. Diagrams show percentages of proteins for each localization compartment.

several protein IDs through alignments with an average of two fragments (maximum of 11 fragments) located within each protein. In total, the numbers of proteins IDs identified for no cross-linkers, GP cross-linkers, and GP+PP cross-linkers for each antibody are as follows: 12, 21, and 9 IDs for LM15, 10, 10, and 10 IDs for LM28, 9, 4, and 10 IDs for LM21 and 2, 5, and 10 IDs for LM19. We classified these proteins based on their subcellular localizations using gene annotations and experimental reports (Figure 2 and Table S2). The identified protein originated from various cellular compartments, including endomembrane systems, mitochondria, plasma membrane, nucleus, cytoplasm, and chloroplast. However, a substantial number of these proteins lacked specific information regarding localization. Since our focus was on identifying proteins involved in cell wall matrix polysaccharides, we present protein IDs that were either reported or predicted to be localized in the Golgi, ER, endomembrane, plasma membrane, and those without localization data (Table 1). Accordingly, there are 26, 14, 17, and 8 protein IDs for LM15, LM28, LM21, and LM19, respectively. Notably, two proteins, At1g58602 from LM15 and At1g13210 from LM19, were identified in both GP and GP+PP treatments, suggesting their interaction with their respective cell wall polymers and affirming the reliability of our method.

We identified several glycosyltransferases directly involved in synthesizing cell wall polysaccharides in the Golgi, including galactomannan galactosyltransferase (MBGT1, At4g13990), putative GT14 Arabinogalactan synthesis (At3g03690), callose synthase (GSL4, At3g14570) from LM15 (GP), putative galacturonosyltransferase 2 (GAUT2, At2g46480) from LM28 (GP), and putative pectin acetyltransferase (TBL42, At1g78710) from LM21 (GP+PP). We observed other proteins involved in matrix polysaccharide biosynthesis and cell wall modification such as the UDP-xylose transporter (At2g30460) from LM15, beta-glucosidase 6 (At3g60130) from LM28, and beta-xylosidase (At5g09730) from LM21. Furthermore, we found the Rho GTPase activation protein (PHGAP1, At5g12150) from LM15 GP+PP that has been shown to be involved in cell wall patterning and a formation of pavement cell shape through an interaction with Rho-related GTPases. These findings demonstrate the effectiveness of our method, involving molecular cross-linkers, protoplasts, and immunoprecipitation using cell wall antibodies in isolating and identifying proteins actively involved in the process of matrix polysaccharide biosynthesis within the plant cell.

Table 1. Protein Identification Obtained from Immunoprecipitation of Leaf Protoplasts Using Cell Wall Antibodies^a

LM15 GP - Y/Y ArtgSb602 LRR and NB-ARC domain containing dasase resistance protein 26 - Y/Y ArtgS1909 beta-GGM galactosyltransferase (beta-MBCT1) 26 Golgi N/N 4 ArtgS4000 satilate transporter 1; 2 36 PM N/Y 4 ArtgS400 sacetylserine (thiol) lyses (OAS-TL) isoform A2 34 - Y/Y 4 ArtgS4002 acetylserine (thiol) lyses (OAS-TL) isoform A2 34 - Y/Y 4 ArtgS4020 Resistance protein LGS1 3 - N/N 5 ArtgS4020 Resistance protein (GSL4) 13 - N/N 5 ArtgS4031 IRR and NB-ARC domain containing desase resistance protein 18 - Y/Y ArtgS4032 Givine-rick protein (GSL4) 13 - N/N 5 ArtgS4032 Givine-rick protein is domain containing desase resistance protein is 18 - Y/Y ArtgS4039 Desphate transporter 19 PM N/N 5	Protein Annotation Unit Unit Instrume Instrume Ligisse0 LRR and NB-ARC domain-containing disear resistance postein 26 Geligi N/N 46 Ligisse0 Sidints transporter II, 2. 36 PM N/N 47, 2 Ligisse0 Sidints transporter II, 2. 36 - N/N 49, 2 Ligisse0 Cacerzith annothing beat 1/6 N acetylghocanaminyltransferanc finally protein (GT14 19 ES N/N 51 Ligisse0 Cacerzith annothing beat 1/6 N acetylghocanaminyltransferanc finally protein (GT14 19 ES N/N 51 Ligisse0 Cacerzith annothing beat 1/6 N acetylghocanaminyltransferanc finally protein 13 - Y/Y 53 Ligisse0 Cacerzith annothing disear resitance protein 18 - Y/Y 53 Ligisse0 Calcun dependent protein issort annot and acear resitance protein 18 - Y/Y 54 Ligisse0 Calcun dependent protein issort annot annot acear annota				U		
Ald [5800] LRR and NB-ARC domain containing disease resistance protein 26 - Y/Y Atg [2800 Sulfate transporter 1, 2 36 Odgi N/N 44 Atg [2800 Sulfate transporter 1, 2 36 PM N/Y 44 Atg [2800 Sulfate transporter 1, 2 34 - N/Y 44 Atg [2800 Cacetylateine (thiol) lyses (0AST1) is oftom A1 9 PM N/N 44 Atg [2800 Caretylateine (thiol) lyses (0AST1) is oftom A2 34 - N/N 44 Atg [2800 Care 2, LF anothing beta 1, EA Acetylg [acosaminyltransferase family protein (GT14 19 ES N/N 5 Atg [2510 Clacan synthas-like protein (GSL4) 13 - N/N 5 Atg [2530 Glychne sch protein shase 1 CMI S (P+P) 19 PM N/Y 5 Atg [2530 Glychne sch protein shase 1 26 PM N/N 5 Atg [2530 Glychne sch protein shase 1 26 PM N/N 5 Atg [2530 Rio GTPase activation protein shase 1 29 - N/N	Ligs800LRR and NR-ARC domain-containing disease resistance protein26-··<	Protein	Annotation		Localization		Ref
Artg 1390beta GGM galactmyltransferase (beta MBGT1)26GrigtN/N4Artg 7800Sulfate transporter 1; 236PMN/Y4Actg 2480O-acetylterine (hind) lyase (OAS-TL) isoform A19-N/Y4Actg 2480O-acetylterine (hind) lyase (OAS-TL) isoform A234-Y/Y4Attg 1901Anthelpsis defension like protein hard12ESN/N5Attg 2480Resistance protein Let39PMN/N5Attg 254137Glacen synthase like protein (GSL4)13-N/N5Attg 25500Glycine rich protein (GSL4)13-N/N5Attg 25800Glycine rich protein 3 short isoform9ESY/Y5Attg 25800Glycine rich protein 3 short isoform9PMN/N5Attg 25800Glycine rich protein 3 short isoform18-Y/N5Attg 25130Rot Taxas critistion protein (HGAP1)33PMN/N5Attg 25130Rot Taxas critistion protein (HGAP1)33PMN/N5Attg 25130Rot CTaxas critistion protein (HGAP1)33PMN/N5Attg 25147Rot GTAxas critistion protein (HGAP1)33PMN/N5Attg 25130Rot CTaxas critistion protein (HGAP1)33PMN/N5Attg 25130Rot CTAxas critistion protein (HGAP1)33PMN/N5Attg 25130Rot CTAxas critistion protein sub	spin spin base CGAB glaboxplanaferase (hear-MBCT1) '' <th'''< th=""> <th'''< th=""> '''</th'''<></th'''<>		LM15 GP				
Alt groupSulfate transporter 1; 236PMN/Y4Attg14880O-acetylserine (thiol) hyse (OAS TL) isoform A19-N/Y4Attg1401Arabidapis defensin-like protein12ESN/N4Attg1401Arabidapis defensin-like protein12ESN/N4Attg1401Arabidapis defensin-like protein12ESN/N5Attg45030Core-2,1/Enanching beta-1,6/N-acetylglucosaminyltransferase family protein (GT14)19ESN/N5Attg45131Testraticnoperities repeat (TPR)-like superfamily protein13-N/N5Attg4530Chcan synthase-like protein (GS14)13-Y/Y5Attg4530Glycine-rich protein 3 short isoform9ESY/Y5Attg4530Chosphate transporter 119PMN/YSAttg1530Curreft-3 short isoform9ESY/YSAttg1530Curreft-3 short isoform9ESY/YSAttg1530UDP-Glycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase30-N/NSAttg1530UCF14se and NB-ARCAttg1530Curreft-44N/NSSAttg15428Race-laced protein RABA422PMY/NSAttg15452Attg1545Attg1545Attg1545Attg1545Attg1545Attg15452Attg1545Attg1545Attg1545Attg1545Attg1545Attg1545Attg1545 <t< td=""><td>ang 29000 Suffac range or right (AST-L) isoform A1 9 - N/Y 49,5 stigg2240 O acceyberine (thio) byse (OAST-L) isoform A2 34 - Y/Y 49,5 stigg2240 O acceyberine (thio) byse (OAST-L) isoform A2 34 - Y/Y 49,5 stigg2340 Carc-2/L-branching beta-L-6-X-actybilicosaninyltransferase family protein (GT14 19 ES N/N 51 stigg3400 Carc-2/L-branching beta-L-6-X-actybilicosaninyltransferase family protein (GT14 13 - N/N 52 stigg4507 Checa synthase like protein (CSL4) 13 - N/N 52 stigg4508 Checa synthase like protein (CPIA)-like synterfamily protein 18 - Y/Y 53 stigg4508 Checa mythase like protein (CPIA)-like synterfamily protein 19 PM N/N 53 stigg4508 Checa mythase like protein (CPIA)-like synterfamily protein 26 PM N/N 54 stigg4508 Checa machemic protein has horino form 9 ES Y/N 53 stigg4508 Checa machemic protein has horino 10 N/N 54 stigg4508 Checa machemic protein has horino 10 N/N 55 stigg4508 Checa machemic protein has horin</td><td>At1g58602</td><td>LRR and NB-ARC domain-containing disease resistance protein</td><td>26</td><td>-</td><td>Y/Y</td><td></td></t<>	ang 29000 Suffac range or right (AST-L) isoform A1 9 - N/Y 49,5 stigg2240 O acceyberine (thio) byse (OAST-L) isoform A2 34 - Y/Y 49,5 stigg2240 O acceyberine (thio) byse (OAST-L) isoform A2 34 - Y/Y 49,5 stigg2340 Carc-2/L-branching beta-L-6-X-actybilicosaninyltransferase family protein (GT14 19 ES N/N 51 stigg3400 Carc-2/L-branching beta-L-6-X-actybilicosaninyltransferase family protein (GT14 13 - N/N 52 stigg4507 Checa synthase like protein (CSL4) 13 - N/N 52 stigg4508 Checa synthase like protein (CPIA)-like synterfamily protein 18 - Y/Y 53 stigg4508 Checa mythase like protein (CPIA)-like synterfamily protein 19 PM N/N 53 stigg4508 Checa mythase like protein (CPIA)-like synterfamily protein 26 PM N/N 54 stigg4508 Checa machemic protein has horino form 9 ES Y/N 53 stigg4508 Checa machemic protein has horino 10 N/N 54 stigg4508 Checa machemic protein has horino 10 N/N 55 stigg4508 Checa machemic protein has horin	At1g58602	LRR and NB-ARC domain-containing disease resistance protein	26	-	Y/Y	
An-4/14880 O-acetylaerine (thiol) byas (OAS-TL) isoform A1 9 - N/Y 4 At2522460 O-acetylaerine (thiol) byas (OAS-TL) isoform A2 34 - Y/Y 4 At2642680 Resistance protein Let3 9 PM N/N At264850 Resistance protein Let3 9 PM N/N 5 At264850 Resistance protein Let3 9 PS N/N 5 At264850 Resistance protein (GTI4) 13 - N/N 5 At264850 Resistance protein (GTA) 13 - N/N 5 At264850 Resistance protein (GTA) 13 - N/N 5 At262503 Glychen efich protein 3 short isoform 9 ES Y/Y 5 At1618800 LAR and NB-ARC domain-containing diseaer resistance protein 18 - N/N 5 At161500 UDFGGroogNtransframes usepfinally protein, hydroxycinamate glucosyltransferase 30 - N/N 5 At161500 UDFGGroogNtransframes usepfinality protein, bydroxycinamate glucosyltransferase 30 - N/N 5 <td>upper lange of the constraint of the constr</td> <td>•</td> <td></td> <td>26</td> <td>e</td> <td></td> <td></td>	upper lange of the constraint of the constr	•		26	e		
Atf2240 O.acetylsenia (thiol) Page (OAS-TL) isoform A2 34 - Y/Y 4 Atf21900 Archdogris definishile protein 12 ES N/N N Atf24900 Resistance protein Ler3 9 PM N/N S Atf24507 Glean synthase-like protein (GS14) 13 - N/N S Atf24507 Glean synthase-like protein (GS14) 13 - N/N S Atf24508 Glycine-rich protein GS14) 18 - Y/Y S Atf25508 Glycine-rich protein 3 short isoform 9 ES N/N S Atf24508 Glycine-rich protein is short isoform 9 PS N/N S Atf24508 Glycine-rich protein is short isoform 9 PS N/N S Atf24508 Calcium dependent protein kinase 1 30 - N/N S Atf24508 UCTTSAA 24 - N/N S Atf24508 Mo GTPaes activation protein (PHGAP1) 3 PM Y/Y Y/Y Atf24508 Mo GTPaes activation protein shuhit 22b	Ligh2360Oacctyleiner (hilo) lyse (OAS TL) isofom A234-Y/40, 5Ligh2360Carc 2, Honnching beta 1,6'N acctylglacoxaninyltransferase family protein (GT14)19ESN/NLigh2360Carc 2, Honnching beta 1,6'N acctylglacoxaninyltransferase family protein (GT14)13-N/NS1Ligh2360Carc 2, Honnching beta 1,6'N acctylglacoxaninyltransferase family protein22ESN/NS2Ligh2361Tetratricopeptide repeat (TPR)-like superfamily protein22ESN/NS3Ligh2363Tetratricopeptide repeat (TPR)-like superfamily protein13-Y/YS3Ligh2363Glocine-rich protein is abort isoform9ESY/YS3Ligh3640Calcian-dependent protein kinase 126PMY/NS5Ligh3650Calcian-dependent protein kinase 1LM15-Y/NS6Ligh3680Calcian-dependent protein (PHGAP1)33PMN/NS5Ligh3680Tanscriptin factor MYB2524-N/NS5Ligh3680Excryst complex component SECSA28PMY/YULigh3680Excryst complex component SECSA28PMY/YULigh3680Excryst complex component SECSA28PMY/YULigh3680Excryst complex component SECSA28PMY/YULigh3681Boscnann-fold Malendami containing protein24EN/NS0Ligh3680Calcian endant dependen-containing p		-		PM		47, 48
Altgrößen Arabidapse defension-like protein 12 ES N/N Atsgrößen Core-2/1 branching beta-1,6-N-acetykglacosaminyltranaferase family protein (GT14) 19 ES N/N Atsgrößen Core-2/1 branching beta-1,6-N-acetykglacosaminyltranaferase family protein (GT14) 13 - N/N 5 Atsgrößen Suttasse för protein (GSL4) 13 - N/N 5 Atsgrößen Core-2/1 branching beta-1,6-N-acetykglacosaminyltranaferase family protein (GT14) 13 - N/N 5 Atsgrößen Core-2/1 branching beta-1,6-N-acetykglacosaminyltranaferase family protein (GT14) 13 - N/N 5 Attgrößen Rohe Statusse protein and the superfamily protein in klasse 1 26 PM N/Y 5 Attgrößen Oberphate transporter 1 13 2 PM N/N 5 Attgrößen Oberphate transporter in protein, hydroxycinnamate glacosyltranaferase 30 - N/N 5 Atsgrößen Oberphate transporter 2 33 PM N/N 5 Atsgrößen Rohe Arba polymerase II transcription subunit 22b 29 - N/N 5 Atsgrößen Carbon Orbits Rabpadote rapporter 2 31 BS N/N 5	tig 1940 Arbibagoi afemin-like protein (ST 14) 9 BM N/N = Sty4800 Bactors protein Le3 9 BM N/N = Sty4800 Bactors protein (ST 44) 13 5 PM N/N = ST 143 SG 24 SG 25 N/N = SG 24 SG	•		9	-		49, 50
At554620 Resistance protein Let3 9 PM N/N At5g05600 Core-2/Lbranching beta-1,6-N-acetylglucosaminylransferase family protein (CF14 19 ES N/N S At3g1570 Glucan synthase-like protein (CSL4) 13 - N/N S At3g1570 Ilkan and NF-ARC domain containing disease resistance protein 18 - V/Y At5g5050 Glycine-rich protein 3 short isoform 9 ES Y/Y S At1g58000 Core-rich protein 3 short isoform 9 ES Y/Y S At1g18800 Calcium-dependent protein insas 1 10 PM N/N S At1g18000 COF-Caycosytransferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N S At1g07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N N/N At5g62527 Rase-related protein RABA4 22 PM Y/N S At2g07940 Dessare resistance protein SEC5A 28 PM Y/Y At3g0707 At2g0553 MATH domain and ocolde-col domain-containing protein 21 <	StystessResistance protein Les ³ 9PMN/NVig09300Cor-2/-branching hetar-1.6-Nacrdylglacosamily/transferase family protein13-N/N\$5family)Tetratricopoptide repeat (TPR)-like superfamily protein22ESN/N\$52LM15 GP+PP-Y/Y\$53tigd5400IRR and NB-ARC domain containing disease resistance protein9ESY/Y\$53tigd5405Polychapite transporter19PMN/N\$54tigd5405Colcine-rich protein is short isoform9ESN/N\$55tigd5405Colcine-rich protein kinase 126PMN/N\$55tigd5405Colcine-rich protein (PHGAP1)33PMN/N\$57tigd550Racredien toritot in RNA polymerase II transcription subanti 22b-N/N\$58tigd550Racredien protein RABA422PMY/Y\$14tigd550Racredie protein RABA423PMY/Y\$12tigd550Racredie protein RABA423RemY/Y\$12tigd550Racredie protein RABA423RemN/N\$2tigd550Racredie protein RABA423RemN/N\$2tigd550Racredie of domain-containing protein24PMY/Y\$12tigd560Racredie of domain-containing protein26ERN/N\$2tigd560Racredie of domain-containing protein26ERN/N\$2 <t< td=""><td>•</td><td></td><td></td><td></td><td></td><td>49, 50</td></t<>	•					49, 50
A13g0369 Core-2.1/Esrnching beta-1,6-N-acetykglucosaminyltransferase family protein (GT14 19 ES N/N S A13g14570 Glucan synthase-like protein (GSL4) 13 - N/N S A12g5313 Tetratricopeptide repeat (TPR)-like superfamily protein 12 ES N/N S A12g5350 Qinenerch, protein is abort isoform 9 ES Y/Y S A15g5400 LRR and NB-ARC domain-containing disease resistance protein 18 - Y/Y S A15g54330 Phosphate transporter 1 19 PM N/N S Atfg15100 Dic-Qivcoynitransferase superfamily protein, hydroxycinnamate glucoyltransferase 0 - N/N S Atfg15200 Dic-Qivcoynitransferase superfamily protein, hydroxycinnamate glucoyltransferase 0 - N/N S Atfg15200 Moditor of RNA polymerase II transcription subunit 22b 29 - N/N S Atfg05207 Ras-related protein RABA4 22 PM Y/Y - Atfg05208 Machtor of RNA polymerase II transcription subunit 22b 23 Membrane Y/Y - <tr< td=""><td>LightSon Core 2/Libenching beta-1,6-N-acetylglucosaminylinanderase family protein (GT14 19 ES N/N vtgltA50 Glucan synthase-like protein (GSL4) 13 - N/N 51 UtgltA510 Tetratricopeptide repeat (TPR)-like superfamily protein 18 - N/N 51 UtgltA500 Glucian synthase-like protein 3 short isoform 18 - Y/Y 53 UtgltA500 Glucian-schink protein is short isoform 19 PM N/N 54 UtgltA500 Glucina-schink protein is short isoform 19 PM N/N 54 UtgltA500 UDP-Glycosyntrasferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 56 UtgltA500 UDP-Glycosyntrasferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 56 UtgltA500 UDP-Glycosyntrasferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 58 UtgltA500 UDP-Glycosyntrasferase grotein (PHIGAP1) 33 PM Y/N 54 UtgltA500 UDP-Solvase activation protein RA54 28 PM Y/N 54</td><td>•</td><td></td><td></td><td></td><td></td><td></td></tr<>	LightSon Core 2/Libenching beta-1,6-N-acetylglucosaminylinanderase family protein (GT14 19 ES N/N vtgltA50 Glucan synthase-like protein (GSL4) 13 - N/N 51 UtgltA510 Tetratricopeptide repeat (TPR)-like superfamily protein 18 - N/N 51 UtgltA500 Glucian synthase-like protein 3 short isoform 18 - Y/Y 53 UtgltA500 Glucian-schink protein is short isoform 19 PM N/N 54 UtgltA500 Glucina-schink protein is short isoform 19 PM N/N 54 UtgltA500 UDP-Glycosyntrasferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 56 UtgltA500 UDP-Glycosyntrasferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 56 UtgltA500 UDP-Glycosyntrasferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 58 UtgltA500 UDP-Glycosyntrasferase grotein (PHIGAP1) 33 PM Y/N 54 UtgltA500 UDP-Solvase activation protein RA54 28 PM Y/N 54	•					
rhmly) 13 - N/N S Ak2g153 Olicons synthas-like protein (GSL4) 13 - N/N S Al2g53130 Tetraticopeptide repeat (TPR)-like superfamily protein 12 ES N/N S Al1g58601 LIR and NB-ARC domain-containing disease resistance protein 18 - Y/Y S Al2g63530 Glycine rich protein 3 shot isoform 9 ES Y/Y S Al1g18890 Calcium-dependent protein kinase 1 26 PM Y/N S Al4g1550 UDP Citycosptransferase superfamily protein, hydroxycinamate glucosyltransferase 30 - N/N S Al5g1528 Ras related protein RABA4 22 PM Y/N S Al2g6980 Transcription factor MTB25 24 - N/N S Al2g69700 Disease resistance protein RABA4 22 PM Y/N S Al2g69701 Disease resistance protein RPM1 12 PM Y/Y S Al2g69701 Disease resistance protein RPM1 23 Membrane Y/Y S Al2g6958 <td>family family tdg51470 Class synthas-like protein (GSL4) 13 - N/N 51 tdg51430 Tetratricopeptide repeat (TPR)-like superfamily protein 22 ES N/N 52 tdg58602 LRR and NB-ARC domain-containing disease resistance protein 18 - Y/Y 53 tdg58508 Diophate transporter 1 9 PM N/N 54 tdg58508 Calcum-dependent protein kinase 1 26 PM N/N 55 tdg15108 Rob Grase activation protein (PIGAP1) 33 PM N/N 58 tdg20580 ranaccription factor MTB25 29 - Y/N 58 tdg50270 Ra-related protein RABA4a 22 PM Y/Y 59 tdg50380 ranaccription factor MTB25 26 PM Y/Y 54 tdg50308 Rober redated protein RABA4a 22 PM Y/Y 52 tdg50308 Rober redated protein RMI 28 S6 N/N 59 tdg5030</td> <td>•</td> <td>-</td> <td></td> <td></td> <td></td> <td></td>	family family tdg51470 Class synthas-like protein (GSL4) 13 - N/N 51 tdg51430 Tetratricopeptide repeat (TPR)-like superfamily protein 22 ES N/N 52 tdg58602 LRR and NB-ARC domain-containing disease resistance protein 18 - Y/Y 53 tdg58508 Diophate transporter 1 9 PM N/N 54 tdg58508 Calcum-dependent protein kinase 1 26 PM N/N 55 tdg15108 Rob Grase activation protein (PIGAP1) 33 PM N/N 58 tdg20580 ranaccription factor MTB25 29 - Y/N 58 tdg50270 Ra-related protein RABA4a 22 PM Y/Y 59 tdg50380 ranaccription factor MTB25 26 PM Y/Y 54 tdg50308 Rober redated protein RABA4a 22 PM Y/Y 52 tdg50308 Rober redated protein RMI 28 S6 N/N 59 tdg5030	•	-				
A12g3S130 Tetratricopeptide repeat (TPR)-like superfamily protein 22 ES N/N S L112SGP+PP IL1S GP+PP N/Y N/Y S A12g8S02 Clycine-rich protein 3 short isoform 9 ES Y/Y S A12g8S02 Clycine-rich protein Xinas 1 19 PM N/Y S A14g1880 Calcium-dependent protein Kinas 1 26 PM N/N S A14g1800 Calcium-dependent protein (PHGAP1) 33 PM N/N S A14g07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N S A12g07880 Transcription factor MTB25 24 - N/N S A12g0580 Diseave resistance protein RMA4a 22 PM Y/Y Y/Y A12g0580 Mediator of RNA polymerase II transcription subunit 22b 28 PM Y/Y Y/Y A12g0580 Mediator MAB4a 2 PM Y/Y Y/Y A12g0580 Masseriated protein RAB4a 2 PM Y/Y Y/Y A12g05400 Dibesaversitance protein	Large S130Terratricoperside repeat (TPR)-like saperfamily protein22ESN/NS2LIS GP+PIIV/YVIS2Lige S300Glycine-rich protein 3 short isoform9P.SY/YS3Step S130Phophabet transporter 119P.MN/NS4Us S1300Objects and protein kinks 126P.MN/NS5Us G1210Calcium-dependent protein kinks 126P.MN/NS5Us G1210Rob GP see activation protein (PHGAP1)31P.MN/NS5Us G23080Transcription factor MTB2529-N/NS6Us G23080Transcription factor MTB2524-N/NS6Us G23080Transcription factor MTB2524-N/NS6Us G23080Transcription factor MTB2524-N/NS9Us G23080Transcription factor MTB2524-N/NS9Us G23080Canaporter 231E.SN/NS9Us G3030Ross resistance protein RPM123MembraneY/YLig G306G1411Hand and colled-coll domain-containing protein28-N/NS0Us G3030Ross resistance protein RPM123GelgiN/N60Us G3030Ross resistance protein RPM123GelgiN/NS0Us G3030Ross resistance protein RPM123GelgiN/NS0Us G3030Ross resistance protein RPM1<		family)		ES		
LM15 GP+PF Arlg58602 GIR and NARC domain-containing disease resistance protein 18 - Y/Y Arlg205520 Glycine-rich protein 3 short isoform 9 ES Y/Y S. Arlg15860 Diposphate transporter 1 10 PM N/Y S. Arlg15850 UDP-Glycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N S. Arlg07550 Mcdiator of RNA polymerase II transcription subunit 22b 29 - Y/N S. Arlg07580 Mediator of RNA polymerase II transcription subunit 22b 24 - N/N S. Arlg05850 Transcription factor MYB25 24 - N/N S. Arlg5850 Excryst complex component SECSA 28 PM Y/Y Y/Y Arlg5050 BAC transporter 1 23 Rombrane Y/Y Y/Y Arlg5050 MATH domain and colled-coil domain-containing protein 28 - N/N Y/Y Arlg5050 BZIP transcription factor-like protein LM28 GP - N/N - Arlg50501 AAA-tyrpe ATPase family p	LM1s CP+PP LM1s CP+PP PI PI PI V12g0520 Glycine-rich protein 3 short isoform 9 ES YY 53 V12g0520 Glycine-rich protein is short isoform 9 PIS YY 53 V15g1630 Diosphate transporter 1 19 PM YY 55 V15g101 Diosphate transporter protein knase isperfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 55 V15g101 Rh6 GTPase activation protein (PHGAPI) Di LM1S - Y/N 52 V15g203 Reclator of RNA polymerase II transcription subunit 22b Di - N/N 58 V12g30500 Discase resistance protein RDM1 Discase resistance protein RDM1 21 PM Y/Y V13g30500 Discase resistance protein RDM1 Discase resistance protein RDM1 23 Membrane Y/Y V14g30500 Discase resistance protein RDM1 Discase resistance protein RDM1 23 Membrane Y/Y V1 V14g30501 Discase resistance protein RDM1 Discase	U U	· · · · · · · · · · · · · · · · · · ·				
AtlgS8602 LRR and NB-ARC domain-containing disease resistance protein 18 - Y/Y AtlgS502 AtlgGS502 Glycine-rich protein 3 short isoform 9 ES Y/Y S AtlgGS502 Calcium-dependent protein kinase 1 26 PM Y/N S Atlg15502 UDP Glycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N S Atlg07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N S Atlg07950 Mediator of RNA polymerase II transcription subunit 22b 29 - N/N S Atlg078950 Exacystat component SECSA 28 PM Y/Y Y/Y Atlg78680 Exacystat component SECSA 28 PM Y/Y Y/Y Atlg78040 UDP-xylose transporter 2 31 ES N/N 45 Atlg780510 Bease resistance protein RPM1 21 PM Y/Y Y/Y Atlg780530 Dessare resistance protein family morber 41 23 Membrane Y/Y Y/Y Atlg965598 BZIP transcription factor-like protein	shi g SeouL R and N N-ARC domain-containing disease resistance protein18-Y /YL2g0S20Gydne-rich protein 3 short isoform9ESY/YS3Styl320Phophale transporter 126PMY/YS4Ltg1S80Calcium-dependent protein kinase 126PMY/NS5Ltg1S10UP-Chycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase30-N/NS5Ltg1S10Reb GTPase activation protein (PHGAP1)3PMN/NS5Ltg07990Mediator of RNA polymerase II transcription subunit 22b29N/NS5Ltg07990Mediator of RNA polymerase II transcription subunit 22b29-N/NS5Ltg07900Mediator of RNA polymerase II transcription subunit 22b29-N/NS5Ltg07900Mediator of RNA polymerase II transcription subunit 22b29N/NS9Mig St0500Raser esistance protein RABA422PMY/YLtg2St050Raser esistance protein RABA428PMY/YLtg2St050Raser esistance protein RABA428N/NS9Mig St0500Raser esistance protein RPM120PMY/YLtg2St060DD-sylose transporter 2LM28 GP-N/NMig St0500Raser esimale proteinLM28 GP-N/NLtg2St07Cancarvalin A like lectin protein fung proteinLM28 GP-N/N <td>At2g35130</td> <td></td> <td>22</td> <td>ES</td> <td>N/N</td> <td>52</td>	At2g35130		22	ES	N/N	52
At2g05520Glycine-rich protein 3 short isoform9ESY/YSAt5q1330Phosphate transporter 119PMN/YSAt1g1880Calcium-dependent protein kinase 126PMY/NSAt4g15500UDP-Glyconyltransferase superfamily protein, hydroxycinnamate glucosyltransferase30-N/NSAt5g12150Kho GTPase activation protein (PHGAP1)33PMN/NSAt1g07950Mediator of RNA polymerase II transcription subunit 22b29-Y/NSAt2g3880Transcription factor MYB2524-N/NSAt2g30820Transcription factor MYB2524-N/NSAt2g30400UDP-xylose transporter 231ESN/NSAt3g07040Disease resistance protein RPM112PMY/YAAt3g0330Rosmann-fold NAD(P)-binding domain-containing protein28-N/NAt3g0589BZIP transcription factor-like protein15-N/NAt2g46480Galacturonosyltransferase 2 (GAUT2)22GolgiN/NAt2g45700Concanavalin A-like lectin protein family protein28-N/NAt2g45490Fobol-relukin-Resistance (TIR) domain family protein26ERN/YAt2g45490Galacturonosyltransferase 2 (GAUT2)22GolgiN/NAt2g45490Concanavalin A-like lectin protein kinase family protein31-N/NAt2g45400Fobox/RNI-like superfamily protein <td>LagdS520 Glycine-rich protein 3 short isoform 9 ES Y/Y \$3 UtSg4330 Phosphate transporter 1 19 PM N/Y \$44 UtSg4300 UDP-Glycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N \$55 UtSg4210 Rho GTPase activation protein (PHGAP1) 33 PM N/N \$57 UtSg4210 Rho GTPase activation protein (PHGAP1) 33 PM N/N \$57 UtSg4200 Transcription factor MM25 24 - N/N \$86 VL205080 Transcription factor MM25 24 - N/N \$86 VL205040 UDP-aylose transporter 2 31 ES N/N \$9 VL205040 UDP-aylose transporter 2 31 ES N/N \$9 VL3050307 Rossman-fold NAD(P)-binding domain-containing protein 24 - N/N \$15 VL3050308 ROSsman-fold NAD(P)-binding domain-containing protein 28 - N/N \$14 VL3050307 Rossman-fold NAD(P)-binding domain-containing protein 28 - N/N</td> <td></td> <td></td> <td></td> <td></td> <td> 6</td> <td></td>	LagdS520 Glycine-rich protein 3 short isoform 9 ES Y/Y \$3 UtSg4330 Phosphate transporter 1 19 PM N/Y \$44 UtSg4300 UDP-Glycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N \$55 UtSg4210 Rho GTPase activation protein (PHGAP1) 33 PM N/N \$57 UtSg4210 Rho GTPase activation protein (PHGAP1) 33 PM N/N \$57 UtSg4200 Transcription factor MM25 24 - N/N \$86 VL205080 Transcription factor MM25 24 - N/N \$86 VL205040 UDP-aylose transporter 2 31 ES N/N \$9 VL205040 UDP-aylose transporter 2 31 ES N/N \$9 VL3050307 Rossman-fold NAD(P)-binding domain-containing protein 24 - N/N \$15 VL3050308 ROSsman-fold NAD(P)-binding domain-containing protein 28 - N/N \$14 VL3050307 Rossman-fold NAD(P)-binding domain-containing protein 28 - N/N					6	
Afsg43350 Phosphate transporter 1 19 PM N/Y 5 A1g18890 Calcium-dependent protein kinase 1 26 PM Y/N 55 At4g15500 UDP-Glycosythansferase superfamily protein, hydroxycinnamate glucosyltransferase (UGT84A4) 30 - N/N 58 At5g12150 Rho GTPase activation protein (PHGAP1) 33 PM N/N 58 At3g3980 Transcription factor MYB25 29 - Y/N 54 At3g39500 Mediator of RNA polymerase II transcription subunit 22b 29 PM Y/N 54 At3g39500 Ras-related protein RABA4a 22 PM Y/N 54 At3g397040 Disease resistance protein RPM1 12 PM Y/Y 54 At3g30530 Rossmann-fold NAD(P)-binding domain-containing protein 28 - N/N 54 At3g033030 Rossmann-fold NAD(P)-binding domain-containing protein 28 - N/N 54 At4g06598 BZIP transcription factor-like protein 15 - N/N 54 At2g44400 Galacturonoosythransferase 2 (GADT2) 28	stsg4339 Phosphate transporter 1 19 PM N/Y 54 ttg18489 Calcium-dependent protein kinase 1 26 PM V/N 55 ttg1549 De Graves artistariane as uperfamily protein, hydroxycinnamate glucosyltransferase (UCT8444) 30 - N/N 56 ttg1219 Rho Graves activation protein (PHGAP1) 33 PM N/N 57 ttg209380 Transcription factor MTB25 24 - N/N 58 ttg209380 Transcription factor MTB25 24 - N/N 58 ttg209380 Transcription factor MTB25 24 - N/N 59 ttg209380 Transcription factor MTB25 24 N/N 59 utg07040 Disease resistance protein RPM1 12 PM V/Y - utg03230 Rossman-fold ADM(P)-binding domain-containing protein 23 Membrane V/Y - utg24540 Galacturonosytematicses 2 (GAUT2) 22 Golgit N/N 60 utg245400 Calcuronostenases are calcuranso domain containing protein 26 FS V/Y - </td <td></td> <td>· ·</td> <td></td> <td></td> <td></td> <td></td>		· ·				
Atlg18890Calcium-dependent protein kinase 126PMY/NS.Atlg1550UDP-Glycosyltransferase superfamily protein, hydroxycinnamate glucosyltransferase30-N/NS.Atlg1510Rho GTPase activation protein (PHGAP1)33PMN/NS.Atlg07950Mediator of RNA polymerase II transcription subunit 22b24-N/NS.Atlg07950Mediator of RNA polymerase II transcription subunit 22b24-N/NS.Atlg07950Excorpt complex component SECSA28PMY/NY/NAtlg26050Excorpt complex component SECSA31ESN/NS.Atlg20512ABC transporter 231ESN/NYAtlg20580MATH domain and colled-coil domain-containing protein28-N/NYAtlg20580MATH domain factor filk protein15-N/NAtlg41521Atlg20580ROBALTANSCHARD21-N/NAtlg42430Atlg406599BZIP transcription factor-like protein15-N/NAtlg42430Atlg4800Collacturonosyltransferase 2 (GAUT2)22GolgiN/N6Atlg48400Collacturonosyltransferase 2 (GAUT2)	thig is a section of protein kinase 126PMY/N55Ug 12-Cipcoxpiransferase superfamily protein, hydroxycinnamate glucoxyltransferase30-N/N56Ug 12-Cipcoxpiransferase superfamily protein, hydroxycinnamate glucoxyltransferase30-N/N57Ug 12-Cipcoxpiransferase superfamily protein, hydroxycinnamate glucoxyltransferase90-N/N57Ug 12-Cipcoxpiransferase superfamily protein subunit 22b29-N/N58Ug 23-200Transcription factor MTB2524-N/N58Ug 23-200Transcription factor MTB2524PMY/Y-Ug 23-200Cascest component SECSA28PMY/Y-Ug 23-200Disease resistance protein RPM112PMY/Y-Ug 23-200Disease resistance protein RPM123MembraneY/Y-Ug 23-200Disease resistance protein RPM123MembraneY/Y-Ug 23-200Disease resistance protein RPM123N/N-N/NUg 23-200Disease resistance protein RPM123MembraneY/Y-Ug 23-200Disease resistance protein RPM124-N/N-Ug 23-200Disease resistance protein RPM123MembraneY/Y-Ug 23-200Disease resistance protein RPM123MembraneY/Y-Ug 23-200Disease resistance protein RPM123MembraneY/Y-Ug 23-200Disease resi	•					
Ar4g15500 UDP (Gryovyltransferase superfamily protein, hydroxycinnamate glucosyltransferase 30 - N/N 5 Artsg12150 Rbo GTPase activation protein (PHGAP1) 33 PM N/N 5 Artsg12150 Rbo GTPase activation protein (PHGAP1) 29 - Y/N 5 Artsg59820 Transcription factor MYB25 29 - Y/N 5 Artsg655270 Ras-related protein RABA4a 22 PM Y/N 5 Artsg655270 Ras-related protein RABA4a 21 PM Y/Y 7 Artsg655270 Ras-related protein RABA4a 21 PM Y/Y 7 Artsg65406 UDP-xylose transporter 2 31 ES N/N 59 Artsg75400 Disease resistance protein RPM1 12 PM Y/Y 7 Artsg595500 MATH domain and coiled-coil domain-containing protein 14 ER, PM N/N 43 Artsg65570 Ras-replate family motein 15 - N/N 7 Artsg59580 MATH domain amily protein 35 ES 7 7 <	using 15500UDP-Gbroomptransferase superfamily protein, hydroxycinnamate glucosyltransferase30-N/N56Ktsg 12157Rho GTPase activation protein (PHGAP1)33PMN/N57LM1529N/N58Ktsg 120790Mediator of RNA polymerase II transcription subunit 22b2929.N/N58Ktsg 2508Rarelated protein RABA4a22PMY/N.58Ktsg 2508Raserlated protein RABA4a21PMY/N.59Ktsg 2508Raserlated protein RABA4a21PMY/Y <t< td=""><td>•</td><td></td><td></td><td></td><td></td><td></td></t<>	•					
AtSg12150 Rho GTPase activation protein (PHGAP1) 33 PM N/N S LM15 LM15 N/N S Atg07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N A239880 Atg565270 Ras-related protein RABA4a 22 PM Y/N S Atg76850 Exceyst complex component SECSA 28 PM Y/Y Atg70460 UDP-xylose transporter 2 31 ES N/N S Atg70470 Disease resistance protein RPM1 12 PM Y/Y A42g3980 Atg70520 AA-type ATPase family member 41 23 Membrane Y/Y A42g39380 Atg70530 Rossmann-folding domain-containing protein 28 - N/N A42g39380 MATH domain and coiled-coil domain-containing protein 23 - N/N A42g39370 Asg63740 Cancaravign factor-like protein 15 - N/N A2g46480 Glacturonosyltransferase 2 (GAUT2) LM28 GP+PP - N/N A42g34390 A12g46480 FS N/N A5g38396 Y/Y <td< td=""><td>Hord Area 33 PM N/N \$57 Ltg12150 Rbo GTPase activation protein (PHGAP1) LM15 - Y/N \$58 Ltg07950 Mediator of RNA polymerase II transcription subunit 22b 24 - N/N \$58 Ltg07505 Rear-related protein RABA4a 22 PM Y/N \$58 Ltg076850 Exocyst complex component SECSA 28 PM Y/Y \$59 Ltg07040 Disease resistance protein RPM1 12 PM Y/Y \$59 Ltg07040 Disease resistance protein RPM1 28 - N/N \$59 Ltg05050 MATH domain and coiled-coil domain-containing protein 28 - N/N \$45 Ltg05050 MATH domain and coiled-coil domain-containing protein 28 - N/N \$45 Ltg05050 MATH domain and coiled-coil domain-containing protein 28 - N/N \$45 Ltg05050 BZIP transcription factor-like protein LM28 GP - N/N \$45 Ltg05050 Foreprol</td><td>•</td><td></td><td></td><td>PM</td><td></td><td></td></td<>	Hord Area 33 PM N/N \$57 Ltg12150 Rbo GTPase activation protein (PHGAP1) LM15 - Y/N \$58 Ltg07950 Mediator of RNA polymerase II transcription subunit 22b 24 - N/N \$58 Ltg07505 Rear-related protein RABA4a 22 PM Y/N \$58 Ltg076850 Exocyst complex component SECSA 28 PM Y/Y \$59 Ltg07040 Disease resistance protein RPM1 12 PM Y/Y \$59 Ltg07040 Disease resistance protein RPM1 28 - N/N \$59 Ltg05050 MATH domain and coiled-coil domain-containing protein 28 - N/N \$45 Ltg05050 MATH domain and coiled-coil domain-containing protein 28 - N/N \$45 Ltg05050 MATH domain and coiled-coil domain-containing protein 28 - N/N \$45 Ltg05050 BZIP transcription factor-like protein LM28 GP - N/N \$45 Ltg05050 Foreprol	•			PM		
LM15 Art g07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N Ar2g39880 Transcription factor MYB25 24 - N/N Si Ar2g6527 Ras-related protein RABA4a 22 PM Y/N Artg76850 Exocyst complex component SEC5A 28 PM Y/Y Artg76850 Exocyst complex component SEC5A 28 PM Y/Y Artg76850 Disease resistance protein RPM1 12 PM Y/Y Artg25070 Artg15215 ABC transporter G family member 41 23 Membrane Y/Y Artg26720 Ar4g15215 ABC transporter family member 41 23 Membrane Y/Y Artg26720 Ar4g162520 AAA-type ATPase family protein 28 - N/N Artg65579 Artg65570 Rossmann-fold NAD (P)-binding domain-containing protein 28 - N/N Artg65659 Artg06598 BZIP transcription factor-like protein 15 - N/N Artg264640 Artg264598 BZIP transcription factor-like protein 15 - N/N Artg26	LM15 tilg07950 Mediator of RNA polymerase II transcription subunit 22b 29 - N/N 58 L3293880 Transcription factor MYB25 24 - N/N 58 L3293850 Transcription factor MYB25 24 - N/N 58 L3293850 Darscrelated protein RABA4a 22 PM Y/N 59 L3293040 DDrsylose transporter 5 SN/N 59 L3293051 ABC transporter 5 SN/N 59 L3293050 MATH domain and coiled-coil domain-containing protein 28 - N/N L4329330 Rosmann-fold NAD(P)-binding domain-containing protein 28 - N/N 4393330 Rosmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/Y 4394340 L4296789 RZIP transcription factor-like protein 15 - N/N L549420 Toil-Interleukin-Resistance (TIR) domain family protein 15 - N/N L5494500 Concanavalin A-like lectin protein kinase family protein 11 ER, nucleus N/Y 61 L522209 NAC domain containi	At4g15500	(UGT84A4)	30	-	N/N	56
A1g07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N A12g38980 Transcription factor MYB25 24 - N/N Si A15g65270 Ras-related protein RABA4a 22 PM Y/N Y/N A12g30460 UDP-xylose transporter 2 31 ES N/N Si A12g30460 UDP-xylose transporter 7 31 ES N/N Y/N A12g307040 Disease resistance protein RPM1 12 PM Y/Y Y/Y A13g07330 Rosmann-fold NAD(P)-binding domain-containing protein 28 - N/N X12g03330 At3g03330 Rossmann-fold NAD(P)-binding domain-containing protein 28 - N/N X12g03330 At4g03330 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/N X12g0344 At4g03588 BZIP transcription factor-like protein 15 - N/N X12g4449 At2g44590 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N X12g4549 At2g45300 Concanavalin A-like lectin motein kinase family protein 21 <td< td=""><td>tilg07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N tx2g3980 Tanscription factor MTB25 24 - N/N S8 tx2g3980 Except complex component SECSA 28 PM Y/Y tx2g3040 UDP-xylose transporter 2 31 ES N/N 59 tx3g30740 Disease resistance protein RPM1 23 Membrane Y/Y - tx3g30380 Nacssmann-fold NAD(P)-binding domain-containing protein 24 E.R. PM N/N - tx3g30380 Assessmann-fold NAD(P)-binding domain-containing protein 24 E.R. PM N/N - tx5g45720 AAA-type ATPase family protein LM28 GP - N/N - tx5g45720 Cacaravalin A-like lectin protein kinase family protein 35 E.S N/N 60 tx5g45720 Cacaravalin A-like lectin protein kinase family protein 31 - N/N - tx5g45720 Cacaravalin A-like lectin protein kinase family protein 23 - N/N - tx1g78400 Call-trefeuktin-Resistance (TIR) domain family protein 35</td><td>At5g12150</td><td>- · · · · ·</td><td>33</td><td>PM</td><td>N/N</td><td>57</td></td<>	tilg07950 Mediator of RNA polymerase II transcription subunit 22b 29 - Y/N tx2g3980 Tanscription factor MTB25 24 - N/N S8 tx2g3980 Except complex component SECSA 28 PM Y/Y tx2g3040 UDP-xylose transporter 2 31 ES N/N 59 tx3g30740 Disease resistance protein RPM1 23 Membrane Y/Y - tx3g30380 Nacssmann-fold NAD(P)-binding domain-containing protein 24 E.R. PM N/N - tx3g30380 Assessmann-fold NAD(P)-binding domain-containing protein 24 E.R. PM N/N - tx5g45720 AAA-type ATPase family protein LM28 GP - N/N - tx5g45720 Cacaravalin A-like lectin protein kinase family protein 35 E.S N/N 60 tx5g45720 Cacaravalin A-like lectin protein kinase family protein 31 - N/N - tx5g45720 Cacaravalin A-like lectin protein kinase family protein 23 - N/N - tx1g78400 Call-trefeuktin-Resistance (TIR) domain family protein 35	At5g12150	- · · · · ·	33	PM	N/N	57
At2g39800 Transcription factor MYB25 24 - N/N SI At2g39800 Rarelated protein RABA4a 22 PM Y/N At1g76850 Exocyst complex component SECSA 28 PM Y/Y At2g3040 Disease ransporter 2 31 ES N/N SI At3g07040 Disease resistance protein RPM1 12 PM Y/Y At3g07040 At3g0350 ABC transporter G family member 41 23 Membrane Y/Y At3g0330 At3g0330 Rossmann-fold NAD (P)-binding domain-containing protein 28 - N/N M At3g0330 Rossmann-fold NAD (P)-binding domain-containing protein 23 - N/N M At3g43900 Concanvalin Actor-like protein LM28 GP - N/N M At2g44800 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 At2g43700 Concanvalin A-like lectin protein finality protein 36 ES N/N 61 At2g43801 Feponid cyclases family protein 21 - N/N 61 At2g43802	12339880 Transcription factor MYB25 24 - N/N \$8 15365270 Ras-related protein RABA4a 22 PM Y/N 11276500 Excoyst complex component SECSA 28 PM Y/Y 11230460 UDP.xylose transporter 2 31 ES N/N \$9 11305070 Disease resistance protein RPM1 12 PM Y/Y 113050700 Disease resistance protein RPM1 23 Membrane Y/Y 113050700 Rost transporter 6 family member 41 23 Membrane Y/Y 113050700 Ack type ATPase family protein 12 P.M N/N 113050700 RAC transporter 6 family member 41 23 Membrane Y/Y 1145950700 Ack type ATPase family protein 13 N/N 11549507 Terpenoid cyclases family protein 13 S ES N/N 11553209 NAC domain containing protein 89 11 ER, nucleus Y/Y 115532209 NAC domain containing protein </td <td></td> <td>LM15</td> <td></td> <td></td> <td></td> <td></td>		LM15				
Atsg65270 Ras-related protein RABA4a 22 PM Y/N Attg76850 Excoyst complex component SECSA 28 PM Y/Y At2g030400 UDP-xylose transporter 2 31 ES N/N 54 At3g07040 Disease resistance protein RPM1 12 PM Y/Y 74 At4g15215 ABC transporter G family member 41 23 Membrane Y/Y 74 At3g02300 Rossmann-fold NAD(P)-binding domain-containing protein 28 - N/N 74 At4g05570 AAA.type ATPase family protein 23 - N/N 74 At4g06598 BZIP transcription factor-like protein 15 - N/N 74 At2g46400 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 64 At2g47800 Concanavalin A-like lectin protein family protein 35 ES Y/Y 64 At2g32290 NAC domain containing protein 89 11 ER, nucleus Y/Y 64 At3g3336 F-box/RNI-like superfamily protein 31 - N/N 64 At3g3336 <td< td=""><td>tisg65270Ras-related protein RABA4a22PMY/NVitg7680Excoyst complex component SECSA28PMY/NVitg7680Dory-shote transporter 231ESN/N\$9Vitg20400Disease resistance protein RPM112PMY/Y*Vitg20580RACT transporter G family member 4123MembraneY/Y*Vitg20580RATH domain and colled-coll domain-containing protein24ER, PMN/N*Vitg20580RASTH domain and colled-coll domain-containing protein23-N/N*Vitg20580RACT transporter 2LM28 GPN/N**Vitg406591EZIP transcription factor-like protein36ERN/N*Vitg46480Galacturonosyltransferase 2 (GAUT2)Concanavalin A-like lectin protein family protein36ESN/N*Vitg2209NAC domain containing protein21-N/N**Vitg2300Terpenoid cyclases family protein21-N/N*Vitg2209NAC domain containing protein 8911ER, nucleusY/Y*Vitg2209NAC domain containing protein23-N/N*Vitg24040Probable protein phosphatase 2C S825-N/N*Vitg20470Probable protein phosphatase 2C S824-N/N*Vitg20480Probable J-Nydroxyisobutyrate dehydrogenase-like 228-N/N*Vitg20471Probab</td><td>•</td><td>Mediator of RNA polymerase II transcription subunit 22b</td><td>29</td><td>-</td><td>Y/N</td><td></td></td<>	tisg65270Ras-related protein RABA4a22PMY/NVitg7680Excoyst complex component SECSA28PMY/NVitg7680Dory-shote transporter 231ESN/N\$9Vitg20400Disease resistance protein RPM112PMY/Y*Vitg20580RACT transporter G family member 4123MembraneY/Y*Vitg20580RATH domain and colled-coll domain-containing protein24ER, PMN/N*Vitg20580RASTH domain and colled-coll domain-containing protein23-N/N*Vitg20580RACT transporter 2LM28 GPN/N**Vitg406591EZIP transcription factor-like protein36ERN/N*Vitg46480Galacturonosyltransferase 2 (GAUT2)Concanavalin A-like lectin protein family protein36ESN/N*Vitg2209NAC domain containing protein21-N/N**Vitg2300Terpenoid cyclases family protein21-N/N*Vitg2209NAC domain containing protein 8911ER, nucleusY/Y*Vitg2209NAC domain containing protein23-N/N*Vitg24040Probable protein phosphatase 2C S825-N/N*Vitg20470Probable protein phosphatase 2C S824-N/N*Vitg20480Probable J-Nydroxyisobutyrate dehydrogenase-like 228-N/N*Vitg20471Probab	•	Mediator of RNA polymerase II transcription subunit 22b	29	-	Y/N	
AtigAtig28PMY/YAtigAtigUDP-xylose transporter 231ESN/N54AtigDisease resistance protein RPM112PMY/Y74AtigAbdCransporter G family member 4123MembraneY/Y74AtigAtigAtigSiAcA-type ATPase family member 4123MembraneY/Y74AtigAtigAsamann-fold NAD(P)-binding domain-containing protein14ER, PMN/Y74AtigAcA-type ATPase family protein23-N/N74AtigAtigAcA-type ATPase family protein15-N/N74AtigAdatope ATPase family protein15-N/N74AtigGalacturonosyltransferase 2 (GAUT2)22GolgiN/N64AtigAtigGalacturonosyltransferase 2 (GAUT2)22GolgiN/N64AtigAtigGalacturonosyltransferase 2 (GAUT2)22GolgiN/N64AtigAtigAtig11ER, nucleusY/Y64AtigAtigAtig31-N/N74AtigAtig11ER, nucleusY/Y64AtigAtigAtig32-N/N64AtigAtigAtig31-N/N74AtigAtigAtigAtig31-N/N74AtigAtigAtigAtig	thi g76850Excoyst complex component SECSA28PMY/YL2g3040UDP-xylose transporter 2S9M2g30400UDP-xylose transporter 2S9M307040Disease resistance protein RPM123MembraneY/YM329580MATH domain and coled-coli domain-containing protein23MembraneY/YM3293580Sosmann-fold NAD(P)-binding domain-containing protein24E.R., PMN/NM329330Rossmann-fold NAD(P)-binding domain-containing protein23-N/NM329330Rossmann-fold NAD(P)-binding domain-containing protein23-N/NM429530SZIP transcription factor-like protein156-N/NM329540Coll-Interleukin-Resistance (TIR) domain family protein36ERN/N60M3294300Concanavilin A-like lectin protein kinase family protein36SY60M3292290NAC domain containing protein goptein 8921-N/N61M329240Probable protein protein 8921-N/N61M329440Jacalin-related lectin 3224-N/N61M3294501Jacalin-related lectin 3224-N/N71M4294400Probable protein phosphatase 2C 5825-N/N14M3294501Jacalin-related lectin 3224-N/N71M4294502Hobilin-folding cofactor D30-N/N71M4294503MATH domain and coled-coli domain-containing		*	24	-	N/N	58
At2g30460 UDP-xylose transporter 2 31 ES N/N 5 At3g07040 Disease resistance protein RPM1 12 PM Y/Y At3g07040 Disease resistance protein RPM1 12 PM Y/Y At3g07040 Disease resistance protein RPM1 12 PM Y/Y At3g0300 Rostmann-fold NAD(P)-binding domain-containing protein 28 - N/N At3g03301 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/Y At4g06598 BZIP transcription factor-like protein 15 - N/N - At4g06598 BZIP transcription factor-like protein 15 - N/N - At4g06598 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 6 At2g46480 Galacturonosyltransferase 2 (GAUT2) 21 - N/N 6 At2g45300 Concanavalin A-like lectin protein kinase family protein 31 - N/N 6 At2g45480 Fobable protein phosphatase 2C 58 25 - N/N 4 At4g26400 Probable protein 32	x12g30460 UDP.xylose transporter 2 31 ES N/N \$9 x5g07040 Disease resistance protein RPM1 12 PM Y/Y vt4g15215 ABC transporter G family member 41 23 Membrane Y/Y x5g29580 MATH domain and colled-coil domain-containing protein 28 - N/N VI x15g3030 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/N VI x15g4570 AAA-type ATPase family protein 20 - N/N VI x15g4570 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 x12g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 61 x12g45800 Terpenoid cyclases family protein 35 ES Y/Y 61 x12g45800 Terpenoid cyclases family protein 20 - N/N 61 x12g45800 Terpenoid cyclases family protein 11 ER, nucleus Y/Y 61 x12g45800 Probable protein phosphatase 2C 58 25 - N/N 14 x12g60740 Jacalin-related	•		22	PM		
At3g07040Disease resistance protein RPM112PMY/YAt4g05215ABC transporter G family member 4123MembraneY/YAt4g29280MATH domain and colled-coil domain-containing protein28-N/NAt3g03330Rossmann-fold NAD(P)-binding domain-containing protein14ER, PMN/YAt5g4570AAA-type ATPase family protein23-N/NAt4g06598BZIP transcription factor-like protein15-N/NAt4g06598BZIP transcription factor-like protein36ERN/YAt4g04509Galacturonosyltransferase 2 (GAUT2)22GolgiN/N6At2g46480Galacturonosyltransferase 2 (GAUT2)22GolgiN/N6At2g45800Terpenoid cyclases family protein35ESY/Y6At5g22290NAC domain containing protein 8911ER, nucleusY/Y6At5g3330F-box/RNI-like superfamily protein31-N/N6At4g2400Probable protein phosphatase 2C 5825-N/N6At4g260740Tubulin-folding cofactor D30-N/N4At3g60740Tubulin-folding cofactor D30-N/N4At3g60740Tubulin-folding cofactor D30-N/N4At3g60740Tubulin-folding cofactor D30-N/N4At3g60740Tubulin-folding cofactor D30-N/N4At3g60740Tubulin-folding c	u3g07040Disease resistance protein RPM112PMY/Yu4g12515ABC transporter G family member 4123MembraneY/Yu3g29580MATH domain and coiled-coil domain-containing protein28 \cdot N/Nu3g29580Rossmann-fold NAD(P)-binding domain-containing protein14ER, PMN/Yu5g0330Rossmann-fold NAD(P)-binding domain-containing protein23 \cdot N/Nutsg45720AAA-type ATPase family protein23 \cdot N/Nutsg4588BZIP transcription factor-like protein15 $-$ N/Nut2g45480Galacturonosyltransferase 2 (GAUT2)22GolgiN/N60ut2g4570Concanavalin A-like lectin protein kinase family protein36ERN/N60ut2g4580Terpenoid cyclases family protein21 $-$ N/N61ut5g3839F-box/RNI-like superfamily protein31 $-$ N/N15utsg46440Jacalin-related lectin 321M2824 $-$ Y/Y61utsg7110Probable 3-hydroxylsobutyrate dehydrogenase-like 228 $-$ N/N14utsg7800Tubulin-folding cofactor D30 $-$ N/N14utsg78103Beta-glucosidase 1624 $-$ Y/Y14utsg7820MATH domain and coiled-coil domain-containing protein31 $-$ N/N14utsg78103Beta-glucosidase 1624 $-$ Y/Y14utsg7820MATH domain and coiled-coil domain-containi	At1g76850	Exocyst complex component SEC5A	28	PM	Y/Y	
At4g15215ABC transporter G family member 4123MembraneY/YAt3g29580MATH domain and colled-coil domain-containing protein28-N/NAt3g03330Rossmann-fold NAD(P)-binding domain-containing protein14ER, PMN/YAt5q54720AAA-type ATPase family protein23-N/NAt5q65720AAA-type ATPase family protein15-N/NAt4g06598BZIP transcription factor-like protein15-N/NAt5q644920Toll-Interleukin-Resistance (TIR) domain family protein36ERN/YAt2q64580Galacturonosyltransferase 2 (GAUT2)22GolgiN/N66At2q46480Galacturonosyltransferase 2 (GAUT2)22GolgiN/N66At2q64590Concanavalin A-like lectin protein kinase family protein31-N/NAt1g78500Terpenoid cyclases family protein21-N/NAt5g22290NAC domain containing protein 8911ER, nucleusY/Y6At5g38306F-box/RNI-like superfamily protein11-N/NAt4g28400Probable protein phosphatase 2C 5825-N/NAt1g71170Probable protein phosphatase 2C 5824-N/NAt3g60740Tubulin-folding cofactor D30-N/NAt3g60740Tubulin-folding cofactor D31-N/NAt3g60740Tubulin-folding cofactor D31 <td< td=""><td>tidg15215ABC transporter G family member 4123MembraneY/Ytd3g23580MATH domain and colled-coil domain-containing protein28-N/Ntd3g0330Rossmann-fold NAD(P)-binding domain-containing protein28-N/Ntd3g65780AAA-type ATPase family protein20-N/NLM28 GPLM28 GPUtg66588BZIP transcription factor-like protein15-N/NMy 4LM28 GPConcanavalin A-like lectin protein family protein20GolgiN/N60LM28 GP+PPtraget family protein21-N/N61LM28 GP+PPtraget family protein21-N/N61LM28 GP+PPtudg65490NAC domain containing protein 8911ER, nucleusY/Y61LM28 GP+PPtudg65400tudg65400Probable protein phosphatase 2C 5825-N/N42tudg65400-N/N1LM28 GP-N/N1tudg65400-N/Ntudg65400-N/N1tudg65401-N/N1tudg65401-N/N1tudg65401-N/N1tudg65401-N/N1</td><td>At2g30460</td><td></td><td>31</td><td>ES</td><td>N/N</td><td>59</td></td<>	tidg15215ABC transporter G family member 4123MembraneY/Ytd3g23580MATH domain and colled-coil domain-containing protein28-N/Ntd3g0330Rossmann-fold NAD(P)-binding domain-containing protein28-N/Ntd3g65780AAA-type ATPase family protein20-N/NLM28 GPLM28 GPUtg66588BZIP transcription factor-like protein15-N/NMy 4LM28 GPConcanavalin A-like lectin protein family protein20GolgiN/N60LM28 GP+PPtraget family protein21-N/N61LM28 GP+PPtraget family protein21-N/N61LM28 GP+PPtudg65490NAC domain containing protein 8911ER, nucleusY/Y61LM28 GP+PPtudg65400tudg65400Probable protein phosphatase 2C 5825-N/N42tudg65400-N/N1LM28 GP-N/N1tudg65400-N/Ntudg65400-N/N1tudg65401-N/N1tudg65401-N/N1tudg65401-N/N1tudg65401-N/N1	At2g30460		31	ES	N/N	59
At3g29580 MATH domain and coiled-coil domain-containing protein 28 - N/N At3g03330 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/Y At4g05330 Rossmann-fold NAD(P)-binding domain-containing protein 23 - N/N At4g06598 BZIP transcription factor-like protein 15 - N/N At4g06598 BZIP transcription factor-like protein 36 ER N/Y At2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 66 At2g453700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 6 At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38306 F-box/RNI-like superfamily protein 10 P. N/N 4 At4g264400 Probable protein phosphatase 2C 58 25 - N/N 4 At4g260740 Pubulin-folding cofactor D 30 - N/N 4 At4g260740 Pubulin-folding cofactor D 30 - N/N 4 At3g60740	H3g29580 MATH domain and colled-coil domain-containing protein 28 - N/N H3g0330 Rossmann-fold NAD (P)-binding domain-containing protein 14 ER, PM N/Y Usg45720 AAA-type ATPase family protein 23 - N/N LM28 GP - N/N - Usg45780 BZIP transcription factor-like protein 15 - N/N ttsg4420 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y ttsg45780 Cancanavalin A-like lectin protein family protein 36 ES Y/Y 60 ttsg45800 Terpenoid cyclases family protein 21 - N/N 61 ttsg38306 F-box/RNI-like superfamily protein 23 - N/N 61 ttsg38306 F-box/RNI-like superfamily protein 21 - N/N 61 ttsg38306 F-box/RNI-like superfamily protein 23 - N/N 42 ttsg38306 F-box/RNI-like superfamily protein 10 - N/N 42 ttsg38306 F-box/RNI-like superfamily protein 23 - <t< td=""><td>At3g07040</td><td>Disease resistance protein RPM1</td><td>12</td><td>PM</td><td>Y/Y</td><td></td></t<>	At3g07040	Disease resistance protein RPM1	12	PM	Y/Y	
At3g03330 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/Y At5g45720 AAA-type ATPase family protein 23 - N/N LM28 GP At4g06598 BZIP transcription factor-like protein 15 - N/N At5g44920 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y At2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 At2g45700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 6 At5g33396 Febox/RNI-like superfamily protein 21 - N/N 6 At5g3396 Febox/RNI-like superfamily protein 21 - N/N 6 At4g28400 Probable protein phosphatase 2C 58 11 ER, nucleus Y/Y 6 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 7 At4g28400 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N 7 At4g28400 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 30 - <td>td3g0330 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/Y td5g45720 AAA-type ATPase family protein 23 - N/N td5g65720 AAA-type ATPase family protein 15 - N/N td9g05580 BZIP transcription factor-like protein 15 - N/N td2g4480 Galacturonosyltransferase 2 (GAUT2) 20 Golgi N/N 60 td2g4580 Concanavalin A-like lectin protein kinase family protein 21 - N/N 60 td2g4580 Concanavalin A-like lectin protein 89 11 ER, nucleus Y/Y 61 td5g3830 F-box/Rol-fike superfamily protein 21 - N/N 61 td5g3830 F-box/Rol-fike superfamily protein 25 - N/N 14 td5g3840 Jacalin-related lec</td> <td>•</td> <td>ABC transporter G family member 41</td> <td>23</td> <td>Membrane</td> <td>Y/Y</td> <td></td>	td3g0330 Rossmann-fold NAD(P)-binding domain-containing protein 14 ER, PM N/Y td5g45720 AAA-type ATPase family protein 23 - N/N td5g65720 AAA-type ATPase family protein 15 - N/N td9g05580 BZIP transcription factor-like protein 15 - N/N td2g4480 Galacturonosyltransferase 2 (GAUT2) 20 Golgi N/N 60 td2g4580 Concanavalin A-like lectin protein kinase family protein 21 - N/N 60 td2g4580 Concanavalin A-like lectin protein 89 11 ER, nucleus Y/Y 61 td5g3830 F-box/Rol-fike superfamily protein 21 - N/N 61 td5g3830 F-box/Rol-fike superfamily protein 25 - N/N 14 td5g3840 Jacalin-related lec	•	ABC transporter G family member 41	23	Membrane	Y/Y	
AtSg45720 AAA-type ATPase family protein 23 - N/N LM28 GP - N/N At4g06598 BZIP transcription factor-like protein 15 - N/N At5g44920 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y At2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 6 At2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y At1g78500 Terpenoid cyclases family protein 21 - N/N At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 7 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 7 At3g06740 Tubulin-folding cofactor D 30 - N/N 7 At5g02980 Putative F-box/kelch-repeat protein 31 - N/N 7 At3g06740 Tubulin-folding cofactor D 30 - N/N 7	xtsg45720 AAA-type ATPase family protein 23 - N/N LM28 GP I I - N/N xtsg405598 BZIP transcription factor-like protein 15 - N/N xtsg445420 Toll-Interletkin-Resistance (TIR) domain family protein 36 ER N/Y xtsg445420 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 xtsg45720 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 61 xtsg52290 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 xtsg38396 F-box/RNI-like superfamily protein 11 - N/N 61 xtsg45740 Yobable protein phosphatase 2C 58 25 - N/N 71 xtsg45740 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N 71 xtsg45209 MATH domain and coiled-coil domain-containing protein 29 - N/N 71 xtsg45204 Tubulin-folding cofactor D 30 - N/N 71 xtsg50208 MATH domain and coi	At3g29580	MATH domain and coiled-coil domain-containing protein	28	-		
LM28 GPAt4g06598BZIP transcription factor-like protein15-N/NAt5g44920Toll-Interleukin-Resistance (TIR) domain family protein36ERN/YAt2g46480Galacturonosyltransferase 2 (GAUT2)22GolgiN/N60At2g43700Concanavalin A-like lectin protein kinase family protein35ESY/Y60At2g43700Terpenoid cyclases family protein21-N/N60At5g22200NAC domain containing protein 8911ER, nucleusY/Y6At5g38396F-box/RNI-like superfamily protein31-N/N6At4g28400Probable protein phosphatase 2C 5825-N/N6At4g28400Probable protein phosphatase 2C 5825-N/N6At4g060740Tubulin-folding cofactor D30-N/N6At5g02980Putative F-box/kelch-repeat protein31-N/N6At3g0130Beta-glucosidase 1629-N/N6At4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N6	LM28 GP LM28 GP ut4g06598 BZIP transcription factor-like protein 15 - N/N ut5g44920 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y ut2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 ut2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 61 ut5g2520 NAC domain containing protein 89 11 ER nucleus Y/Y 61 ut5g238306 F-box/RNI-like superfamily protein 11 ER nucleus Y/Y 61 ut5g2400 Probable protein phosphatase 2C 58 25 - N/N 41 ut4g04400 Probable a-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N 14 ut3g60740 Tubalin-folding cofactor D 30 - N/N 14 ut3g60730 Beta-glucosidase 16 24 - N/N 14 ut3g5820 MATH domain and coiled-coil domain-containing protein 29 - N/N 14 ut3g5820 MATH domain and coiled-coil domain-containing protein <td>At3g03330</td> <td>Rossmann-fold NAD(P)-binding domain-containing protein</td> <td>14</td> <td>ER, PM</td> <td>N/Y</td> <td></td>	At3g03330	Rossmann-fold NAD(P)-binding domain-containing protein	14	ER, PM	N/Y	
At4g06598 BZIP transcription factor-like protein 15 - N/N At5g44920 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y At2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 66 At2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 67 At2g43700 Terpenoid cyclases family protein 21 - N/N 64 At5g22200 NAC domain containing protein 89 11 ER, nucleus Y/Y 66 At5g38396 F-box/RNI-like superfamily protein 25 - N/N 64 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 64 At3g16440 Jacalin-related lectin 32 24 - Y/Y 64 At3g60740 Tubulin-folding cofactor D 30 - N/N 64 At3g60740 Tubulin-folding cofactor D 31 - N/N 64 At3g60740 Tubulin-folding cofactor D 31 - N/N 64 At3g50208 Putati	https://doi.org/10.11111111111111111111111111111111111	At5g45720	AAA-type ATPase family protein	23	-	N/N	
Atsg44920 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y At2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 66 At2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 6 At1g78500 Terpenoid cyclases family protein 21 - N/N 6 At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 6 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 7 At4g28400 Jacalin-related lectin 32 24 - Y/Y 7 At4g28400 Jacalin-related lectin 32 28 - N/N 7 At3g16440 Jacalin-related lectin 32 30 - N/N 7 At3g60740 Tubulin-folding cofactor D 30 - N/N 7 At3g602980 Putative F-box/kelch-repeat protein 31 - N/N 7 At3g50220 MA	xtsg4492 Toll-Interleukin-Resistance (TIR) domain family protein 36 ER N/Y xt2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 xt2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 xt2g470 Concanavalin A-like lectin protein kinase family protein 36 ES N/N 60 xt2g4200 Terpenoid cyclases family protein 21 - N/N 61 xt5g2220 NAC domain containing protein 89 11 ER nucleus Y/Y 61 xt5g3336 F-box/RNI-like superfamily protein LM28 1 - N/N 61 xt5g2209 NAC domain containing protein 89 LM28 1 ER nucleus Y/Y 61 xt1g14640 Jacalin-related lectin 32 LM28 - N/N 1 1 xt1g1710 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 26 - N/N 1 xt1g01040 Tubulin-folding cofactor D 30 - N/N 1 xt1g50208 Putative F-box/kelch-repeat protein LM21 GP		LM28 GP				
At2g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 64 At2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 7 At1g78500 Terpenoid cyclases family protein 21 - N/N 7 6 At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 7 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 7 At1g71170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N 7 At3g60740 Tubulin-folding cofactor D 30 - N/N 7 At3g60730 Beta-glucosidase 16 29 - N/N 7 At3g60130 Beta-glucosidase 16 24 - Y/Y 7 At3g60130 Beta-glucosidase 16 29 - N/N 7 At3g60130 Beta-glucosidase 16 24 - Y/Y 7 <tr td=""> - - <</tr>	x12g46480 Galacturonosyltransferase 2 (GAUT2) 22 Golgi N/N 60 x12g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y 7 x11 ER, nucleus Y/Y 61 x15g25200 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 x15g25200 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 x15g25200 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 x15g25200 NAC domain containing protein 80 - N/N 61 x15g25200 NAC domain containing protein 10 - N/N 61 x15g25200 Probable protein phosphatase 2C 58 25 - N/N 12	At4g06598		15	-	N/N	
At2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y LM28 GP+PP 21 - N/N At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 6 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 7 At3g16440 Jacalin-related lectin 32 24 - Y/Y 6 At3g60740 Tubulin-folding cofactor D 30 - N/N 7 At3g6030 Petative F-box/kelch-repeat protein 31 - N/N 7 At3g6030 Beta-glucosidase 16 24 - N/N 7 7 At3g6030 Putative F-box/kelch-repeat protein 31 - N/N 7 At3g6030 Beta-glucosidase 16 24 - Y/Y 7 At3g5030 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 7	x2g43700 Concanavalin A-like lectin protein kinase family protein 35 ES Y/Y LM28 GP+PP LM28 GP+PP 11 ER, nucleus Y/Y 61 xt5g2209 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 xt5g2209 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 xt5g2209 Probable protein phosphatase 2C 58 1 - N/N 1 xt4g28400 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N 1 xt4g28401 Tubulin-folding cofactor D 30 - N/N 1 1 xt3g60290 Putative F-box/kelch-repeat protein 31 - N/N 1 1 xt3g60208 Putative F-box/kelch-repeat protein 31 - N/N 1 <td>•</td> <td></td> <td>36</td> <td></td> <td>N/Y</td> <td></td>	•		36		N/Y	
LM28 GP+PP At1g78500 Terpenoid cyclases family protein 21 - N/N At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 6 LM28 LM28 11 ER, nucleus Y/Y 6 At4g28400 Probable protein phosphatase 2C 58 25 - N/N At4g28400 Jacalin-related lectin 32 24 - Y/Y At1g71170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N At3g60740 Tubulin-folding cofactor D 30 - N/N At3g502280 Putative F-box/kelch-repeat protein 31 - N/N At3g60130 Beta-glucosidase 16 24 - Y/Y At3g60130 Beta-glucosidase 16 29 - N/N At3g60130 Y/Y - LM21 GP LM21 GP LM21 GP - N/N 6	LM28 GP+PP 21 - N/N kt1g78500 Terpenoid cyclases family protein 21 - N/N kt5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 61 kt5g38396 F-box/RNI-like superfamily protein 31 - N/N kt4g28400 Probable protein phosphatase 2C 58 25 - N/N kt4g28401 Jacalin-related lectin 32 24 - N/N kt4g26404 Jacalin-related lectin 32 24 - N/N kt3g60740 Tubulin-folding cofactor D 30 - N/N kt3g60740 Tubulin-folding cofactor D 30 - N/N kt3g60740 Putsuive F-box/kelch-repeat protein 31 - N/N kt3g60740 Beta-glucosidase 16 24 - Y/Y kt4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 62 kt4g08560 Pumilio-family RNA binding repeat (PUF) 36 ES N/N 62 kt4g08560 Pumilio-family RNA binding repeat (PUF) 36 ES N/N<	•	•	22			60
At1g78500 Terpenoid cyclases family protein 21 - N/N At5g22290 NAC domain containing protein 89 11 ER, nucleus Y/Y 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 6 At5g38396 F-box/RNI-like superfamily protein 31 - N/N 6 At4g28400 Probable protein phosphatase 2C 58 25 - N/N 6 At3g16440 Jacalin-related lectin 32 24 - Y/Y 6 At1g71170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N 7 At3g60740 Tubulin-folding cofactor D 30 - N/N 7 At3g58220 MATH domain and coiled-coil domain-containing protein 29 - N/N 7 At3g60130 Beta-glucosidase 16 2M21 GP - - N/N 6 At4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 6	thtg7850Terpenoid cyclases family protein21-N/Ntx5g2220NAC domain containing protein 8911ER, nucleusY/Y61tx5g3330F-box/RNI-like superfamily protein31-N/N1LM28tx4g2840Probable protein phosphatase 2C 5825-N/N1tx4g2840Probable protein phosphatase 2C 5825-N/N1tx4g2840Probable a-hydroxyisobutyrate dehydrogenase-like 224-N/N1tx6g0740Tubulin-folding cofactor D30-N/N1tx6g0740Tubulin-folding cofactor D31-N/N1tx6g0740MATH domain and coiled-coil domain-containing protein31-N/N1tx6g0740St S (ERD1/XPR1/SYG1) family protein31-N/N62tx1g2530EXS (ERD1/XPR1/SYG1) family protein31-N/N62tx1g2 Galactose-binding protein31-N/N62tx1g2 Galactose-binding protein31-N/N62tx1g2 Galactose-binding protein31-N/N62tx1g2 GB-N/N62tx1g2 Galactose-binding protein31-N/N62tx1g2 GB-N/N-tx1g2 Galactose-binding protein31-N/N	At2g43700	Concanavalin A-like lectin protein kinase family protein	35	ES	Y/Y	
At5g2220NAC domain containing protein 8911ER, nucleusY/Y6At5g38396F-box/RNI-like superfamily protein31-N/NLM28At4g28400Probable protein phosphatase 2C 5825-N/NAt3g16440Jacalin-related lectin 3224-Y/YAt1g71170Probable 3-hydroxyisobutyrate dehydrogenase-like 228-N/NAt3g60740Tubulin-folding cofactor D30-N/NAt3g58220MATH domain and coiled-coil domain-containing protein31-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N6	http://tipNAC domain containing protein 8911ER, nucleusY/Y61http://tip31-N/Nhttp://tip11ER, nucleusY/Y61http://tip11ER, nucleusY/Y61http://tip11Fobable 3-hydroxyisobutynate11Fobale 3-hydroxyisobutynatehttp://tip111212N/N11http://tipProbable 3-hydroxyisobutynate dehydrogenase-like 228-N/Nhttp://tip13-N/N111111http://tipProbable 3-hydroxyisobutynate dehydrogenase-like 228-N/N11http://tiphttp://tiphttp://tip11111111N/N1111http://tip11121112N/N12http://tip12121211 <td< td=""><td></td><td>LM28 GP+PP</td><td></td><td></td><td></td><td></td></td<>		LM28 GP+PP				
At5g38396F-box/RNI-like superfamily protein31-N/NLM28LM28N/NAt4g28400Probable protein phosphatase 2C 5825-N/NAt3g16440Jacalin-related lectin 3224-Y/YAt1g71170Probable 3-hydroxyisobutyrate dehydrogenase-like 228-N/NAt3g60740Tubulin-folding cofactor D30-N/NAt3g02980Putative F-box/kelch-repeat protein31-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N6	Kt5g38396 F-box/RNI-like superfamily protein 31 - N/N LM28 LM28 N/N N/N kt4g28400 Probable protein phosphatase 2C 58 25 - N/N kt4g28401 Jacalin-related lectin 32 24 - Y/Y kt1g71170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N kt3g06740 Tubulin-folding cofactor D 30 - N/N kt3g5820 Putative F-box/kelch-repeat protein 31 - N/N kt3g60130 Beta-glucosidase 16 29 - N/N kt4g25350 EXS (ERD1/XPR1/SYG1) family protein 21 - N/N 62 kt4g08560 Pumilio-family RNA binding repeat (PUF) 36 ES N/N kt1g22882 Galactose-binding protein EM21 GP+PP - - - kt1g22882 Galactose-binding protein EM21 GP+PP - - - - kt1g255020 Lipoxygenase 1 LM21 GP+PP 32 - N/Y - kt1g55020 Lipoxygenase 1 32	At1g78500	Terpenoid cyclases family protein	21	-	N/N	
LM28 At4g28400 Probable protein phosphatase 2C 58 25 - N/N At3g16440 Jacalin-related lectin 32 24 - Y/Y At1g71170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N At3g60740 Tubulin-folding cofactor D 30 - N/N At5g02980 Putative F-box/kelch-repeat protein 31 - N/N At3g58220 MATH domain and coiled-coil domain-containing protein 29 - N/N At3g60130 Beta-glucosidase 16 24 - Y/Y LM21 GP	LM28 kt4g28400 Probable protein phosphatase 2C 58 25 - N/N kt3g16440 Jacalin-related lectin 32 24 - Y/Y kt1g71170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N kt3g60740 Tubulin-folding cofactor D 30 - N/N kt3g02980 Putative F-box/kelch-repeat protein 31 - N/N kt3g58220 MATH domain and coiled-coil domain-containing protein 29 - N/N kt3g60130 Beta-glucosidase 16 24 - Y/Y LM21 GP LM21 GP LM21 GP LM21 GP LM21 GP LM21 GP+PP	At5g22290	01		ER, nucleus	Y/Y	61
At4g28400Probable protein phosphatase 2C 5825-N/NAt3g16440Jacalin-related lectin 3224-Y/YAt1g71170Probable 3-hydroxyisobutyrate dehydrogenase-like 228-N/NAt3g60740Tubulin-folding cofactor D30-N/NAt5g02980Putative F-box/kelch-repeat protein31-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/NAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N6	httq28400 Probable protein phosphatase 2C 58 25 - N/N httq21640 Jacalin-related lectin 32 24 - Y/Y httq21170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N httq26040 Tubulin-folding cofactor D 30 - N/N httq203080 Putative F-box/kelch-repeat protein 31 - N/N httq263030 Beta-glucosidase 16 24 - N/N - httq25350 EXS (ERD1/XPR1/SYG1) family protein 21 - N/N 62 httq035050 Pumilio-family RNA binding repeat (PUF) 36 ES N/N 62 httq25350 EXS (ERD1/XPR1/SYG1) family protein EAF1 36 PM N/N 62 httq035050 Chromatin modification-related protein EAF1 36 ES N/N 7 httg258250 Galactose-binding protein EM21 GP+PP - - - - httg25826 Galactose-binding protein EAF1 36 ES N/N - httg25826 Galactose-binding protein EAF1	At5g38396		31	-	N/N	
At3g16440Jacalin-related lectri 3224-Y/YAt1g71170Probable 3-hydroxyisobutyrate dehydrogenase-like 228-N/NAt3g60740Tubulin-folding cofactor D30-N/NAt5g02980Putative F-box/kelch-repeat protein31-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/NAtSSSSSS	htt3g16440Jacalin-related letti 3224-Y/Ytt3g16440Jacalin-related letti 3228-N/Ntt1g71170Probable 3-hydroxyisobutyrate dehydrogenase-like 228-N/Ntt3g60740Tubulin-folding cofactor D30-N/Ntt3g02980Putative F-box/kelch-repeat protein31-N/Ntt3g58220MATH domain and coiled-coil domain-containing protein29-N/Ntt3g58210MATH domain and coiled-coil domain-containing protein29-N/Ntt3g58220EXS (ERD1/XPR1/SYG1) family protein21-N/N62tt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N62tt4g08560Pumilio-family RNA binding repeat (PUF)36ESN/N14tt1g22882Galactose-binding proteinAPMN/N14tt1g22882Galactose-binding protein21Nucleus, ERN/Y14tt1g55020Lipoxygenase 132-N/Y14tt3g56480Myosin heavy chain-related20PMN/N14						
At1g71170Probable 3-hydroxyisobutyrate dehydrogenase-like 228-N/NAt3g60740Tubulin-folding cofactor D30-N/NAt5g02980Putative F-box/kelch-repeat protein31-N/NAt3g58220MATH domain and coiled-coil domain-containing protein29-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N6	http://170 Probable 3-hydroxyisobutyrate dehydrogenase-like 2 28 - N/N htt3g60740 Tubulin-folding cofactor D 30 - N/N ht3g60740 Tubulin-folding cofactor D 30 - N/N ht3g60740 Putative F-box/kelch-repeat protein 31 - N/N ht3g58220 MATH domain and coiled-coil domain-containing protein 29 - N/N ht3g58230 Beta-glucosidase 16 24 - Y ht4g25350 EXS (ERD1/XPR1/SYG1) family protein 29 - N/N 62 ht4g08560 Pumilio-family RNA binding repeat (PUF) 36 ES N/N 41 ht1g03750 Chromatin modification-related protein EAF1 34 PM N/N 14 ht1g22882 Galactose-binding protein EAF1 32 - N/Y 14 ht1g55020 Lipoxygenase 1 32 - N/Y 14 14 ht1g556480 Myosin heavy chain-related 20 PM N/N 14				-		
At3g60740Tubulin-folding cofactor D30-N/NAt3g60740Putative F-box/kelch-repeat protein31-N/NAt3g02980Putative F-box/kelch-repeat protein31-N/NAt3g58220MATH domain and coiled-coil domain-containing protein29-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N	xtzg60740 Tubulin-folding cofactor D 30 - N/N xtzg02980 Putative F-box/kelch-repeat protein 31 - N/N xtzg02980 MATH domain and coiled-coil domain-containing protein 29 - N/N xtzg0310 Beta-glucosidase 16 24 - Y/Y LM21 GP xt4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 62 xt4g25350 EXS (ERD1/XPR1/SYG1) family protein 36 ES N/N 62 xt4g08560 Pumilio-family RNA binding repeat (PUF) 36 ES N/N 62 xt1g2282 Galactose-binding protein EAF1 34 PM N/N xt1g22882 Galactose-binding protein XI Y xt1g22882 Galactose-binding protein N/Y XI xt1g55020 Lipoxygenase 1 N/Y xt3g56480 Myosin heavy chain-related 20 PM N/N		•	24	-		
At5g02980Putative F-box/kelch-repeat protein31-N/NAt3g58220MATH domain and coiled-coil domain-containing protein29-N/NAt3g60130Beta-glucosidase 1624-Y/YLM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N	AttSg02980Putative F-box/kelch-repeat protein31-N/Nkt3g05820MATH domain and coiled-coil domain-containing protein29-N/Nkt3g60130Beta-glucosidase 1624-Y/YLM21 GPtt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N62tt4g208560Pumilio-family RNA binding repeat (PUF)36ESN/N62tt1g20852Galactose-binding proteinEAF134PMN/N62tt1g22882Galactose-binding proteinEAF132-N/Ytt1g55020Lipoxygenase 132-N/Ytt3g56480Myosin heavy chain-related20PMN/N	-			-		
At3g58220 MATH domain and coiled-coil domain-containing protein 29 - N/N At3g60130 Beta-glucosidase 16 24 - Y/Y LM21 GP - N/N 64 At4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 64	At3g58220MATH domain and coiled-coil domain-containing protein29-N/NLt3g60130Beta-glucosidase 1624-Y/YLM21 GPat4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N62at4g08560Pumilio-family RNA binding repeat (PUF)36ESN/N62at1g03750Chromatin modification-related protein EAF134PMN/N62at1g22882Galactose-binding proteinLM21 GP+PP1Nucleus, ERN/Yat1g55020Lipoxygenase 132-N/Y1at3g56480Myosin heavy chain-related20PMN/N1	-		30	-		
At3g60130 Beta-glucosidase 16 24 - Y/Y LM21 GP At4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 62	At3g60130Beta-glucosidase 1624-Y/YLM21 GPIM21 GP31-N/N62t44g08560Pumilio-family RNA binding repeat (PUF)36ESN/N62t41g03750Chromatin modification-related protein EAF134PMN/N62LM21 GP+PPtt1g22882Galactose-binding proteinEAF121Nucleus, ERN/Ytt1g22882Galactose-binding proteinLM21 GP+PP32-N/Ytt1g55020Lipoxygenase 132-N/Ytt3g56480Myosin heavy chain-related20PMN/N			31	-		
LM21 GP At4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 62	LM21 GPAt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N62At4g08560Pumilio-family RNA binding repeat (PUF)36ESN/N1At1g03750Chromatin modification-related protein EAF134PMN/N1LM21 GP+PPLM21 GP+PPLM21 GP+PP21Nucleus, ERN/YAt1g22882Galactose-binding proteinAt1g55020Lipoxygenase 132-N/YAt3g56480Myosin heavy chain-related20PMN/N	•	MATH domain and coiled-coil domain-containing protein	29	-		
At4g25350 EXS (ERD1/XPR1/SYG1) family protein 31 - N/N 62	kt4g25350EXS (ERD1/XPR1/SYG1) family protein31-N/N62kt4g08560Pumilio-family RNA binding repeat (PUF)36ESN/N1kt1g03750Chromatin modification-related protein EAF134PMN/N1LM21 GP+PPkt1g22882Galactose-binding protein21Nucleus, ERN/Y1kt1g55020Lipoxygenase 132-N/Ykt3g56480Myosin heavy chain-related20PMN/N	At3g60130		24	-	Y/Y	
	At4g08560Pumilio-family RNA binding repeat (PUF)36ESN/Nkt1g03750Chromatin modification-related protein EAF134PMN/NLM21 GP+PPLM21 GP+PP21Nucleus, ERN/YAt1g52882Galactose-binding protein32-N/YLt325020Lipoxygenase 132-N/YLt3g56480Myosin heavy chain-related20PMN/N						
Attacks Dumilio family DNA hinding report (DUE)	At1g03750 Chromatin modification-related protein EAF1 34 PM N/N LM21 GP+PP LM21 GP+PP 21 Nucleus, ER N/Y At1g55020 Lipoxygenase 1 32 - N/Y At3g56480 Myosin heavy chain-related 20 PM N/N	At4g25350	EXS (ERD1/XPR1/SYG1) family protein				62
	LM21 GP+PP kt1g22882 Galactose-binding protein kt1g55020 Lipoxygenase 1 kt3g56480 Myosin heavy chain-related			26	ES	N/N	
	httg22882Galactose-binding protein21Nucleus, ERN/Yhttg55020Lipoxygenase 132-N/Yhttg556480Myosin heavy chain-related20PMN/N	At4g08560					
	At1g55020Lipoxygenase 132N/Yat3g56480Myosin heavy chain-related20PMN/N		Chromatin modification-related protein EAF1		РМ	N/N	
	t3g56480 Myosin heavy chain-related 20 PM N/N	At4g08560 At1g03750	Chromatin modification-related protein EAF1 LM21 GP+PP	34			
		At4g08560 At1g03750 At1g22882	Chromatin modification-related protein EAF1 LM21 GP+PP Galactose-binding protein	34 21		N/Y	
	t_{1} $\sqrt{20}$ Twish small birst birst finance is a like t_{2} (TPI t_{2}) 20 EC t_{1}	At4g08560 At1g03750 At1g22882 At1g55020	Chromatin modification-related protein EAF1 LM21 GP+PP Galactose-binding protein Lipoxygenase 1	34 21 32	Nucleus, ER -	N/Y N/Y	
At1g78710Trichome birefringence-like 42 (TBL42)29ESN/N		At4g08560 At1g03750 At1g22882 At1g55020 At3g56480	Chromatin modification-related protein EAF1 LM21 GP+PP Galactose-binding protein Lipoxygenase 1 Myosin heavy chain-related	34 21 32 20	Nucleus, ER - PM	N/Y N/Y N/N	
	12-02260 Aurin transmistration BLC 21 DM $17/37$	At4g08560 At1g03750 At1g22882 At1g55020 At3g56480 At1g78710	Chromatin modification-related protein EAF1 LM21 GP+PP Galactose-binding protein Lipoxygenase 1 Myosin heavy chain-related Trichome birefringence-like 42 (TBL42)	34 21 32 20 29	Nucleus, ER - PM ES	N/Y N/Y N/N N/N	
At3g02260Auxin transport protein BIG21PMN/YAt4g25520Lim domain-binding protein37-N/Y		At4g08560 At1g03750 At1g22882 At1g55020 At3g56480 At1g78710 At3g02260	Chromatin modification-related protein EAF1 LM21 GP+PP Galactose-binding protein Lipoxygenase 1 Myosin heavy chain-related Trichome birefringence-like 42 (TBL42) Auxin transport protein BIG	34 21 32 20 29 21	Nucleus, ER - PM ES PM	N/Y N/Y N/N N/N N/Y	
	Ausin transport protein BIG 21 PM N/Y	At4g08560 At1g03750 At1g22882 At1g55020 At3g56480 At1g78710	Chromatin modification-related protein EAF1 LM21 GP+PP Galactose-binding protein Lipoxygenase 1 Myosin heavy chain-related Trichome birefringence-like 42 (TBL42)	34 21 32 20 29	Nucleus, ER - PM ES	N/Y N/Y N/N N/N	

Table 1. continued

Protein	Annotation	Peptide length	Localization	DeepAraPPI/ ATMAD	Ref
	LM21				
At1g55325	Mediator of RNA polymerase II transcription subunit 13	34	-	Y/Y	
At5g09730	Beta-D-xylosidase 3	33	ES	N/N	
At2g02950	Phytochrome kinase substrate 1	25	-	N/Y	
At4g02050	Sugar transport protein 7	32	PM	Y/N	
At3g60240	Eukaryotic translation initiation factor 4G	21	-	N/N	
At2g45880	Beta-amylase 7	34	-	Y/N	
At1g74900	Pentatricopeptide repeat-containing protein	27	-	N/N	
At1g30710	Berberine bridge enzyme-like 14	29	ES	N/N	
	LM19 GP				
At1g13210	Probable phospholipid-transporting ATPase 11	13	PM	N/N	
At1g58250	Golgi-body localization protein domain; RNA pol II promoter Fmp27 protein domain	24	Golgi	N/N	63
	LM19 GP+PP				
At1g17580	Myosin 1	26	-	Y/Y	64
At4g32150	Vesicle-associated membrane protein 711	36	VM	Y/Y	65, 66
At5g13740	Zinc induced facilitator 1	37	VM	N/N	67
At1g13210	Probable phospholipid-transporting ATPase 11	13	PM	N/N	
	LM19				
At1g20925	Protein PIN-LIKES 1	28	Membrane	N/N	
At3g21130	Putative F-box protein	11	-	Y/Y	

^aThe longest peptide chains obtained from LC-MS/MS for each protein. Protein localization data were obtained from the literature and ThaleMine database. Protein interaction data were obtained from the DeepAraPPI and ATMAD database. GP: glycan-protein crosslinker, PP: protein-protein crosslinker. Plasma membrane: PM, endomembrane system: ES, vacuole membrane: VM.

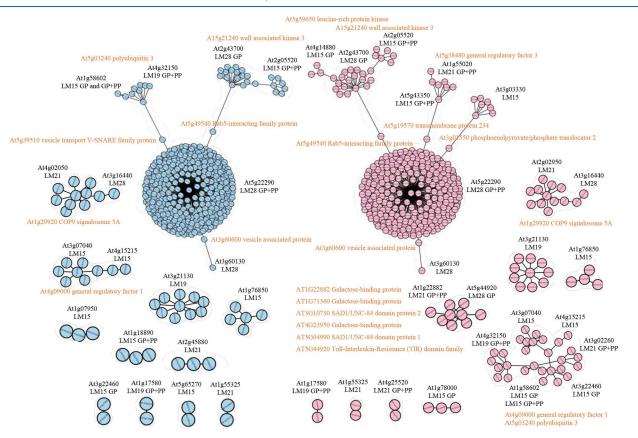


Figure 3. Protein interaction networks based on DeepAraPPI (in blue) and AtMAD (in pink). Each cluster represents a protein ID (*Arabidopsis* accession and its pull down condition) as a center connected to a number of interacting proteins, as found in each database. The networks were built based on common *Arabidopsis* gene accessions found for each protein ID. Interacting proteins known to be associated with the cell wall and those that connect between clusters are indicated.

In addition, the identified protein IDs also include proteins that are typically targeted to the cell walls or involved in cellular machinery for vesicle trafficking. Notably, disease resistance protein RPM1 (At3g07040) from LM15, resistant

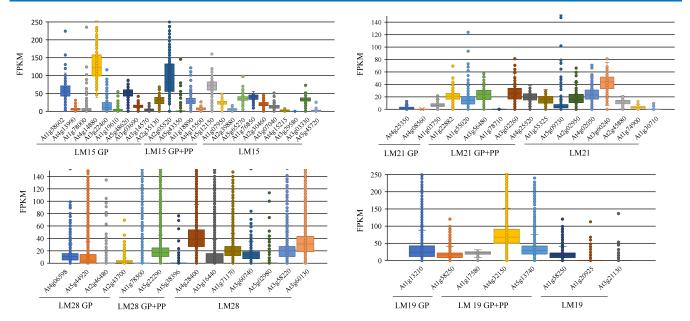


Figure 4. Gene expression levels of the protein IDs from four antibodies in *Arabidopsis* leaf tissues. Data was obtained from the *Arabidopsis* RNA-seq database.

proteins (At5g48620) from LM15 (GP), Arabidopsis defensinlike protein (At1g19610) from LM15 (GP), and glycine-rich protein 3 (At2g05520) from LM15 (GP+PP) were found in the cell wall and are known to play roles in conferring resistance to various pathogens and pests. Furthermore, exocyst complex proteins (At1g76850) from LM15, Golgi localization protein domain (At1g58250) from LM19 (GP), and vesicle-associated membrane protein 711 (At4g32150) from LM19 (GP+PP) were identified, indicating their association with matrix polysaccharides and their involvement in Golgi and vesicle protein components. Interestingly, there are some proteins that have never been reported to be associated with the plant cell wall, such as the kinesin motor family protein, sulfate transporter 1, and tetratricopeptide repeat. This result suggests that these proteins may have a potential association with the synthesis of matrix polysaccharides, representing novel candidates for further investigation. Taken together, our results have identified proteins that are directly or indirectly involved in cell wall matrix polysaccharides, from their synthesis to delivery. The use of cross-linkers has shown their ability to capture these associations, providing insights for further detailed functional studies.

Identification of Proteins Based on Protein Interactions. To identify the relationship of these proteins to the plant cell wall, we performed searches for Arabidopsis protein interactions using DeepAraPPI (based on deep learningassisted prediction)²³ and AtMAD (based on experimental data such as FRET, yeast-2-hybrid, coimmunoprecipitation, and affinity capture-MS)²⁴ databases. Protein/gene IDs that matched the protein interaction databases are indicated in Table 1. Out of the 63 IDs, 19 and 24 were found in DeepAraPPI and AtMAD databases, respectively. Figure 3 shows a summary of the protein interaction networks from the two databases (the full lists of interacting proteins for each ID are presented in Files S1 and S2). The protein interaction networks obtained from both databases showed considerable similarity. Notably, a large network was observed for At5g22290 (NAC domain containing protein 89; LM28 GP +PP) in connection with five or seven IDs (depending on the

databases) obtained from the four antibodies and with a large number of proteins (314 and 238 proteins for DeepAraPPI and AtMAD, respectively). This suggests that At5g22290 may have extensive interactions with other proteins related to cell wall biosynthesis. Among these interactions, we found connections to cellulose synthase-like proteins (CSLs), arabinogalactan proteins (AGPs), glycosylhydrolase (GHs), pectin methylesterase inhibitors (PMEIs), pectin lyase-like proteins, nucleotide sugar transporters, and other cell-wall-related proteins. Additionally, At5g22290 indirectly interacts with other proteins, including At2g43700 (Concanavalin A-like lectin protein kinase family protein) from LM28 GP, At4g32150 (vesicle-associated membrane protein 711) from LM19 GP+PP, and At3g60130 (beta-glucosidase 16) from LM28, through membrane and vesicle trafficking proteins, including the Rab5-interacting family protein, V-SNARE family protein, and vesicle-associated protein. The cross interactions among the proteins identified with the four antibodies suggest proximity networks of the proteins associated with the four polysaccharides from both hemicelluloses and pectins. This observation validates our method for in vivo proximity crosslinking immunoprecipitation.

Article

Interestingly, we found a branch in the network of At2g43700 that connects to At2g05520 (glycine-rich protein 3 short isoform) via wall associated kinase 3 and to At4g14880 (O-acetylserine (thiol) lyase) via a leucine-rich repeat protein kinase family protein. At2g05520 itself was also found to interact with wall-associated kinases. This observation may indicate a potential signaling network linking membrane/ vesicle systems to cell wall polysaccharides.

While smaller networks with a few or single IDs were also observed, they did not show further associations with cell-wallrelated proteins. Alternatively, we found networks for At4g02050-At3g16440 and At3g07040-At4g15215 from DeepAraPPI and AT2g02950-At3g16440 and the network of the five IDs (At4g32150, At3g07040, At4g15215, At3g02260, At3g22460, and At1g58602) from AtMAD involving the protein degradation pathway, such as COP9 signalosome SA, polyubiquitin 3, and, perhaps, general

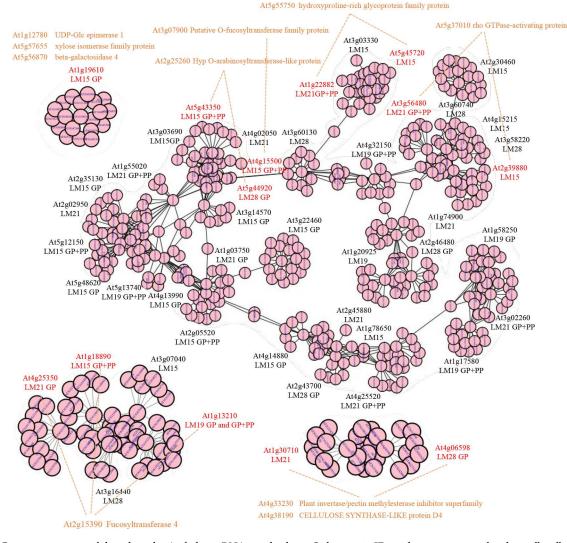


Figure 5. Coexpression network based on the *Arabidopsis* RNA-seq database. Only protein IDs with coexpressors related to cell walls (in red) and those IDs that are connected through the networks are presented. Protein IDs with their pulldown conditions are presented for each cluster. Coexpressors related to cell wall processes are presented in orange text with connected lines to their corresponding IDs.

regulatory factor 1. Furthermore, the At1g22882–At5g44920 network from AtMAD forms a network among galactose binding protein/SAD1/UNC-84 domain protein/Toll-Inter-leukin-Resistance (TIR) domain family protein, and this may play a role in the TLR signaling pathway. Although further investigation is required to confirm these interactions and their functional relevance, our results indicate that the molecular cross-linkers, cell wall antibodies, and the immunoprecipitation method employed in this study have identified putative proteins associated with matrix polysaccharide biosynthesis occurring within plant cells.

To further validate the identified proteins and their interactions, we sought to perform yeast-two-hybrid assays. However, directly testing glycan-protein interactions was challenging, and we were unable to verify this aspect. Nevertheless, for the protein IDs identified through GP+PP cross-linkers, which were based on protein-protein interactions, we conducted yeast-two-hybrid assays to assess interaction within each antibody group (see Table S3 for the list). After careful assay with negative controls, we did not observe any direct interactions among the proteins. Instead, we

found evidence of homomultimeric proteins (indicated in Table S3).

Identification of Proteins Based on Gene Expression and Coexpression Analysis. To validate the presence and relevance of the identified protein IDs in leaf tissues, we examined the expression levels of genes encoding these proteins using data obtained from the *Arabidopsis* RNA-seq database.²⁵ Figure 4 shows that the genes encoding the protein IDs are generally expressed in the leaf, with some of the IDs showing very low expression levels. Among them, At4g14880 (LM15 GP) and At2g05520 (LM15 GP+PP) exhibited the highest expression levels. The RNA-Seq data confirm that these genes are expressed in leaf tissues and are functional in the protoplasts.

Next, we examined coexpression analysis of the genes encoding the protein IDs from the *Arabidopsis* RNA-seq Database. Due to the large number of coexpressors for each ID, we selected the top 20 candidates and examined their functional annotations (see the lists of coexpression for each gene ID in File S3). Figure 5 presents the coexpression networks of the top candidates, focusing on those that are associated with cell wall-related genes. Out of the 63 gene IDs,

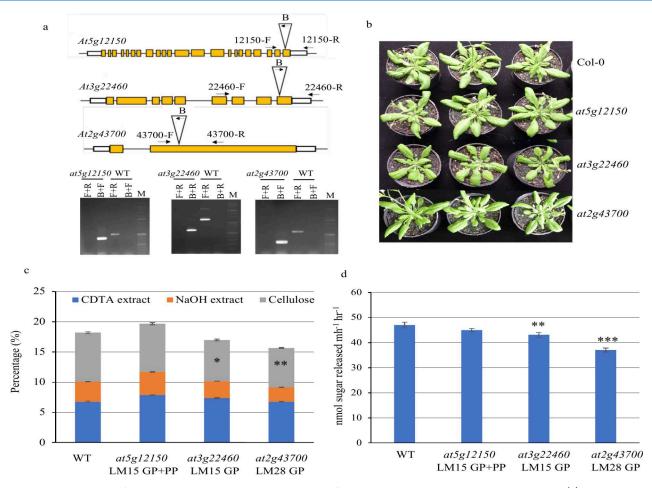


Figure 6. Characterization of T-DNA insertion mutants on proteins identified using cross-linking and immunoprecipitation. (a) T-DNA insertion positions are indicated within the gene structures. Arrows indicate the direction of PCR primers for genotyping including forward (F) and reverse (R) primers and the border (B) primer. The lower panel shows identification of homozygous lines using flanking and border primer combinations. M indicates DNA ladder (see full-length gels in Figure S1). (b) Four-week old plants of three mutants at an early bolting stage grown alongside WT plants. (c) Mass yield of CDTA extract, NaOH extract, and cellulose residue of AIRs obtained from leaves. Data were obtained from three biological replicates presented with SE (n = 3). (d) Saccharification analysis of the three mutants presented as reducing sugars released by enzymatic hydrolysis of pretreated AIR samples (three biological replicates and each with four technical replicates) (n = 3). Asterisks indicate significant differences from WT using student's t test ($*P \le 0.05$, $**P \le 0.01$, $***P \le 0.001$).

13 were found to have coexpression with cell wall-associated genes. Interestingly, we observed a large coexpression network comprising seven genes that are coexpressed with cell wallassociated genes and 31 genes without coexpression with cell wall genes. This network is associated with cell wall genes through a hydroxyproline-rich glycoprotein (HRGP), HRGP O-arabinosyltransferase, O-fucosyltransferase, and rho GTPase-activating protein genes. Additionally, we found that genes At1g19610 had coexpressors that interacted with cell wall-associated genes such as UDP-Glu epimerase, xylose isomerase, and beta-galactosidase 4. At1g30710 and At4g06598 are coexpressed with a putative PMEI (At4g33230) and CSLD4 (At4g38190). Furthermore, we identified a coexpression network involving five genes (At4g25350, At1g18890, At3g07040, At1g13210, and At3g16440) that connected to At2g15390 (arabinogalactan protein fucosyltransferase 4, AtFUT4), suggesting the potential function of these genes with the biosynthesis of cell wall components. These coexpression networks provide evidence that these proteins may indeed be associated with plant cell walls, despite not having been previously reported in this context. The expression analysis and coexpression networks

provide further support for the involvement of the proteins identified in leaf cell wall biosynthesis.

Characterization of Cell Wall Associated Proteins Using Arabidopsis T-DNA Insertion Mutants. To validate our method, we employed reverse genetics to target three of the proteins identified. At5g12150 (LM15 GP+PP) was chosen as Rho GTPase activation proteins (PHGAP1 and PHGAP2), and Rho GTPase have been shown to be involved in cell wall patterning and directing the formation of cell wall pits in metaxylem vessel cells.⁶⁸⁻⁷⁰ At3g22460 (LM15 GP), an O-acetylserine (thiol) lyase, which plays a role in the final step in the cysteine biosynthetic pathway, has never been shown to be related to the plant cell wall. At2g43700 (LM28 GP), a Concanavalin A-like lectin protein kinase family protein, was selected based on its protein interaction data, suggesting associations with other cell wall components, including Wall Associated Kinases, via the At5g22290 protein. T-DNA insertion mutants for these genes were obtained, and homozygous lines were identified through PCR genotyping (Figure 6a). No visible alterations were observed in these mutants, except for slightly slower growth in the at3g22460 mutant compared to WT. However, when we performed cell

wall compositional analysis on these mutants, we found significant reductions (P < 0.05) in cellulose content in the at3g22460 and at2g43700 mutants. Subsequently, saccharification analysis using Alcohol Insoluble Residue (AIR) from leaves revealed that both mutants had significantly less saccharification potential compared to the WT (P < 0.01). This reduction in saccharification potential is likely a consequence of the reduced cellulose content in the cell walls of these mutants. Furthermore, the monosaccharide composition of the CDTA extracts of both mutants showed reductions in Xyl and GluA, along with increases in GalA, relative to the WT (Table 2). Likewise, substantial changes in all monosaccharides, except Glu and Man, were found in the NaOH extract of both mutants. However, no alterations in cell wall and monosaccharide compositions were observed in the at5g12150 mutant. Since At3g22460 and At2g43700 proteins were identified through LM15 and LM28 with GP crosslinkers, respectively, it is likely that these proteins were present in close proximity to XyG and GX, respectively, during the biosynthesis of these polysaccharides. Indeed, the reductions of Xyl and GluA in CDTA and NaOH extracts reflect the potential relationships of these proteins to XyG and GX, while changes in various sugar compositions in the NaOH extract suggest the impact of these proteins on other polysaccharides. These results indicate that T-DNA insertions in At3g22460 and At2g43700 lead to changes in the cell wall, resulting in reduced cellulose content and altered matrix polysaccharide compositions. The changes in the cell wall of these mutants support their potential roles in cell wall biosynthesis.

DISCUSSION

We present a new approach for identifying proteins involved in cell wall matrix polysaccharide biosynthesis by utilizing in vivo proximity cross-linking and immunoprecipitation in Arabidopsis protoplasts. We used protoplast to ensure the exclusion of immunoprecipitation of epitopes in the cell wall. This approach allowed us to capture proteins actively associated with cell wall polymers: proteins directly interacting with glycan and those associated with protein linked to glycans. We focused on cell wall-related proteins by narrowing down the IDs to those predicted or reported to be localized in the Golgi, ER, plasma membrane, and those without specific localization data. We found several glycosyltransferases directly involved in cell wall polysaccharide synthesis, along with other proteins involved in cell wall modification and those typically targeted to cell walls or involved in vesicle trafficking. Protein interaction networks showed interactions between various proteins, including those related to cell wall-associated proteins and membrane/vesicle trafficking proteins. Gene expression and coexpression analysis supported the presence and relevance of the proteins identified in leaf tissues. Reverse genetic studies using T-DNA insertion mutants of selected proteins revealed changes in cell wall composition, monosaccharide composition and saccharification potential supporting their potential roles related to cell wall biosynthesis. Further investigations are necessary to unravel the precise functions of these proteins in the complex process of cell wall biosynthesis.

Chemical cross-linkers are compounds designed to covalently bind to specific functional groups on molecules, facilitating their physical interaction and stabilization. In our study, we employed four different chemical cross-linkers, KMUH, EMCH, BMPH, and MPBH, to specifically cross-link

Monosacchari	Monosaccharide per mg of Biomass ^a	imass ^a				4			
	Fuc	Ara	Rha	Gal	Glu	Xyl	Man	GalA	GluA
				CDJ	CDTA extract				
WT	1.40 ± 0.04	6.94 ± 0.08	6.41 ± 0.11	9.05 ± 0.06	14.96 ± 1.2	4.10 ± 0.06	1.24 ± 0.06	22.91 ± 1.54	0.71 ± 0.02
at5g12150	1.43 ± 0.15	5.77 ± 0.07	6.68 ± 0.25	8.75 ± 0.34	18.64 ± 2.7	3.53 ± 0.04	1.53 ± 0.28	31.54 ± 3.87	1.03 ± 0.05
at3g22460	1.19 ± 0.21	5.21 ± 0.35	5.22 ± 0.36	7.53 ± 0.7	13.26 ± 2.78	$2.33 \pm 0.28^{*}$	1.05 ± 0.24	37.35 ± 3.46	$0.61 \pm 0.03^{*}$
at2g43700	1.43 ± 0.09	5.32 ± 0.19	5.48 ± 0.11	6.97 ± 0.03	7.94 ± 1.65	$2.58 \pm 0.11^{*}$	0.85 ± 0.08	$36.58 \pm 1.85^{*}$	$0.54 \pm 0.04^{*}$
				NaC	NaOH extract				
WT	1.61 ± 0.1	3.66 ± 0.21	1.60 ± 0.18	6.22 ± 0.31	13.00 ± 4.3	5.92 ± 0.13	1.85 ± 0.37	3.50 ± 0.16	0.83 ± 0.07
at5g12150	$1.87 \pm 0.06^{*}$	3.69 ± 0.3	1.60 ± 0.08	7.04 ± 1	10.88 ± 1.78	6.38 ± 0.89	2.31 ± 0.41	3.70 ± 0.27	0.72 ± 0.13
at3g22460	$1.23 \pm 0.02^{*}$	$2.27 \pm 0.04^{**}$	$0.96 \pm 0.14^{**}$	$5.49 \pm 0.12^{*}$	9.61 ± 0.73	$4.26 \pm 0.31^{**}$	1.48 ± 0.04	$2.22 \pm 0.04^{**}$	$0.42 \pm 0.02^{**}$
at2g43700	$1.24 \pm 0.06^{*}$	$2.20 \pm 0.35^{**}$	$0.92 \pm 0.05^{*}$	4.97 ± 0.81	7.32 ± 1.56	$3.54 \pm 0.62^{*}$	1.34 ± 0.25	$1.96 \pm 0.13^{***}$	$0.41 \pm 0.01^{**}$
^a Data were obt	ained from three bio	ological replicates pre	sented with SE $(n =$	3). Asterisks indica	tte significant differe	nces from WT using	student's t-test (*	^a Data were obtained from three biological replicates presented with SE $(n = 3)$. Asterisks indicate significant differences from WT using student's t-test (* $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$).	$^{***P} \leq 0.001$).

Table 2. Cell Wall Monosaccharide Composition Analysis of CDTA and NaOH Extracts of Leave Samples from the Selected Mutants, Expressed in µg of Each

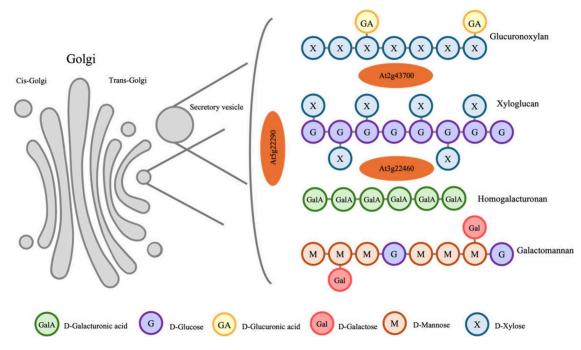


Figure 7. Schematic representation of the localization of matrix cell wall polysaccharide biosynthesis within the Golgi apparatus. Matrix polysaccharides are represented within the Golgi network. Specifically, matrix polysaccharides are synthesized entirely within the trans-Golgi and subsequently transported to the extracellular space via secretory vesicles. Our study shows the presence of matrix polysaccharides for both hemicelluloses and pectins within the Golgi and secretory vesicles, indicating their close proximity and polysaccharide–protein interacting networks. We identified At5g22290 as a candidate hub protein interacting with other cell wall-related proteins involved in XyG, GX, and GM biosynthesis. Furthermore, At2g43700 was identified as a protein interacting with GX and linked to other proteins involved with XyG, while At3g22460 interacts with XyG and is linked to other proteins involved with GM and HGA.

glycans or carbohydrates to proteins. These are hydrazide cross-linkers with a maleimide group that reacts with free thiol groups (-SH) on cysteine residues in proteins, enabling the preservation of glycan-protein interactions in their native state as they form stable covalent bonds. This approach has been previously used for studying glycan-protein interactions and their roles in various cellular processes.⁷¹ Additionally, our method employed BMOE as a chemical cross-linker to specifically cross-link proteins to proteins through two maleimide groups that can react with free thiol groups on cysteine residues in different proteins, forming covalent bonds between them. While no cross-linker treatments represent proteins that directly and tightly bind to matrix polysaccharides without the help from cross-linkers, the use of chemical crosslinkers in our experiment serves as a valuable tool to preserve and stabilize glycan-protein interactions and protein-protein interactions.

We observed that the protein IDs identified through LC-MS/MS analysis originated from various subcellular localizations including those of nontargeted organelles such as the nucleus, vacuole, chloroplast, and mitochondria. The high sensitivity of LC-MS/MS for protein identification is advantageous, allowing us to detect very small amounts of proteins. However, this sensitivity also poses a potential challenge as it increases the likelihood of detecting cross-contamination with nontargeted proteins during the immunoprecipitation process. This issue is not unique to our study as other protein identification methods face similar challenges.^{18,72,73} In our case, it is possible that certain organelles may interact with the magnetic beads or antibodies used in the immunoprecipitation process, leading to the identification of nontargeted proteins. This cross-contamination could arise from shared binding properties or nonspecific interactions, complicating the interpretation of the results. Nonetheless, in our case, we can eliminate certain protein IDs, which are known to be localized in the nontargeted organelles.

We identified several glycosyltransferases, which are enzymes directly involved in cell wall polysaccharide synthesis, among other proteins associated with the cell wall, such as the UDP-xylose transporter, beta-glucosidase 6, beta-xylosidase, and PHGAP1. We conducted yeast-two-hybrid assays to validate interactions among the identified protein IDs, however, no direct interactions were observed. Alternative methods to verify these interactions are required. One potential approach could be to use the luciferase protein complementation assay, which has been employed to study protein interaction networks for XyG biosynthesis enzymes in the Golgi.⁷⁴ To compare our results with existing studies related to cell wall biosynthesis, we examined other reported proteins for protein interactions, immunoprecipitations, and proteomic analyses, such as Zhou et al.,¹⁷ Parsons et al.¹⁸ and Atmodjo et al.²⁰ However, we did not find a match between the proteins identified in our work and the data from these studies. Moreover, we investigated whether our protein IDs possessed the Lewis A glycans for proteins involved in cell wall biosynthesis,⁷⁵ but none of our identified proteins showed a match with them. Thus, it is probable that our approach has identified specific proteins associated with different polysaccharides, in which they may be present in low abundance and have not been previously detected in other studies.

Based on data from protein interaction databases, At5g22290 stands out as a hub protein interacting with numerous proteins related to cell wall biosynthesis. We observed connections between At5g22290 and cell wallassociated proteins, including cellulose synthase-like proteins, arabinogalactan proteins, glycosyl hydrolases, pectin methylesterase inhibitors, and nucleotide sugar transporters. Furthermore, At5g22290 indirectly interacted with other proteins linking membrane and vesicle trafficking proteins with cell wall polysaccharides, including XyG, GX, and GM (Figure 7). No interactions were found for glycosyltransferases. Furthermore, we observed that many proteins, as per the protein interaction databases, interact with protein degradation factors. However, this does not necessarily mean that degradation was occurring during our analysis. Instead, these results suggest that these proteins may interact with protein degradation components under specific conditions for apoplastic delivery.

We note that our approach differed from previous reports on immunoprecipitation, particularly with regard to Golgi and GAUT complex pull down studies.²⁰ Those studies focused on pulling protein complexes at early stages of the biosynthesis, while our approach captures fully formed wall epitopes representing later stages of biosynthesis. Interestingly, our protein interaction analysis revealed interconnections between proteins associated with hemicelluloses and pectins. Our findings suggest that hemicellulose and pectin polymers may be present in the Golgi in close proximity to one another (Figure 7). However, it is important to acknowledge that our method makes it difficult to determine whether the captured epitopes originate from the Golgi or from Golgi derived vesicles.

Zhang and Staehelin⁷⁶ as well as Young et al.⁷⁷ have demonstrated that XyGs and pectins are synthesized within the Golgi stacks. Moore et al.⁷⁸ provided insight into the organization of different assembly pathways including glycoproteins and complex polysaccharides within the Golgi stacks. Polysaccharides and glycoproteins traverse through cisternae and are then packed into secretory vesicles to be transported to the trans-Golgi network. A study by Meents et al." investigated xylan biosynthesis in the Golgi and found that the backbone synthesis enzyme, IRX9, predominantly localizes to the ring of the inner margins of medial cisternae, while the xylan products accumulate at the margins of trans-cisternae and the trans-Golgi network. Similarly, a subcompartment localization study of XyG biosynthesis enzymes indicated their presence in the cis- and medial- cisternae.⁸⁰ This discrepancy in the location of synthesis enzymes and final products may explain why our immunoprecipitation approach did not yield a higher number of glycosyltransferases, as the localization of the synthetic enzymes is different from that of the final products recognized by the glycan antibodies. Since cell wall polysaccharides are enriched in the trans-Golgi network,⁸¹ it is plausible for proteins associated with a specific polysaccharide to be detected by other glycan-specific antibodies. Moreover, the presence of protein interaction networks representing associations with different matrix polysaccharides could suggest a shared location of these polysaccharides during the downstream biosynthesis in the Golgi, and potentially within secretory vesicles as well (Figure 7). This shared localization may play a role in the intricate process of polysaccharide biosynthesis and trafficking within the plant cell.

In recent years, several essential players involved in matrix polysaccharide biosynthesis have been discovered beyond the well-known glycosyltransferases responsible for their synthesis. Notably, a number of proteins localized in the Golgi network have been identified, each playing a crucial role in cell wall

biosynthesis. For instance, MSR accessory proteins have been found to be important for mannan biosynthesis.^{82,83} Additionally, CGR3 has been shown to influence the methyl esterification of HGA, and its identification was facilitated through coexpression analysis with cell wall synthesis genes.⁸⁴ The manganese transporter PML3 regulates plant growth through Golgi glycosylation and cell wall biosynthesis.85 Similarly, BICAT3 is involved in matrix polysaccharide biosynthesis, with Mn being necessary for the normal cell wall biosynthesis process, likely without direct interactions with other biosynthesis enzymes. ER-localized cell wallmodifying enzymes, such as RWA2, are required for unspecifically acetylated cell wall polysaccharides.⁸⁶ In this study, we present a list of putative cell wall-related proteins involved in cell wall biosynthesis. In particular, we show through mutant studies that At3g22460 and At2g43700 knockouts show changes in polysaccharide composition, suggesting their role related to biosynthesis of XyG and GX, respectively.

Immunoprecipitation typically requires selecting a specific bait protein to capture nearby interacting proteins. This can be challenging when searching for unknown proteins involved in plant cell wall biosynthesis. Glycosyltransferases and glycan synthases are key targets for cell wall polysaccharide biosynthesis, but studying them using immunoprecipitation is difficult as they are membrane bound or membrane spanning proteins.⁸⁷ However, our approach, pulling the whole polysaccharide molecule with cross-linkers, broadens the targets to the polysaccharide itself rather than specific protein baits, eliminating the need to select specific protein baits and also avoiding the need for expression constructs or transformation. By using cross-linkers, we can explore additional proteins that may be localized in close proximity to the targeted polysaccharide. This method allows us to isolate proteins related to matrix polysaccharides throughout their biosynthesis, starting from a stage recognized by the antibody to their delivery in vesicles. Furthermore, our approach using glycan antibodies can be applied to directly targeting various polysaccharides based on the available antibodies. Over 200 cell wall antibodies recognizing 78 cell wall epitopes have been produced.⁸⁸ In summary, our method offers a means of studying cell wall polysaccharide biosynthesis and associated proteins, providing advantages over traditional immunoprecipitation techniques. Our study provides a list of putative proteins associated with the four different matrix polysaccharides for further investigation.

ASSOCIATED CONTENT

Data Availability Statement

All data generated and used in this study are available as Supporting Information for this article.

G Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00534.

DeepArappi interaction (XLSX)

ATMAD protein interaction (XLSX)

Coexpression RNA-seq database (XLSX)

Figure S1. Full-length gels for T-DNA insertion lines. Table S1. Primer sequences for identifications of T-DNA insertion lines. Table S2. Protein identifications of glycan-protein (GP), glycan-protein and protein– protein (GP+PP) cross-linked and no cross-linked immunoprecipitation via cell wall specific antibodies. Peptide sequences and matching positions to *Arabidopsis* proteins are presented. Table S3. Lists of proteins used for yeast-two-hybrid assays with. Protein IDs within each antibody groups were assayed in all combination. Protein IDs with self-interaction based on yeast-two-hybrid assays are indicated using asterisks (PDF)

AUTHOR INFORMATION

Corresponding Author

Supachai Vuttipongchaikij – Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; Center of Advanced Studies for Tropical Natural Resources, Kasetsart University, Bangkok 10900, Thailand; Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU), Bangkok 10900, Thailand; orcid.org/0000-0002-1194-3552; Email: supachai.v@ ku.th

Authors

- Pitchaporn Wannitikul Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
- Issariya Dachphun Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
- Jenjira Sakulkoo Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
- Anongpat Suttangkakul Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; Center of Advanced Studies for Tropical Natural Resources, Kasetsart University, Bangkok 10900, Thailand; Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU), Bangkok 10900, Thailand
- Passorn Wonnapinij Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; Center of Advanced Studies for Tropical Natural Resources, Kasetsart University, Bangkok 10900, Thailand; Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU), Bangkok 10900, Thailand
- Rachael Simister CNAP, Department of Biology, University of York, Heslington, York YO10 5DD, United Kingdom
- Leonardo D. Gomez CNAP, Department of Biology, University of York, Heslington, York YO10 5DD, United Kingdom

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c00534

Author Contributions

S.V. and P.Wan. conceived the project and designed experiments. P.Wan., I.D., J.S., A.S., P.Wo., R.S., and L.G. performed the experiments and analyzed the data. S.V., P.Wan., and P.Wo. prepared the Figures and Tables and wrote the manuscript. L.G. revised the manuscript. All authors have approved the final version.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Research Council of Thailand: NRCT5-RSA63002-02; The Office of the Ministry of Higher Education, Science, Research and Innovation; and the Thailand Science Research and Innovation through the Kasetsart University Reinventing University Program 2021, Kasetsart University Research and Development Institute (KURDI). We are also grateful for the support of the Biotechnology and Biological Sciences Research Council (grant reference BB/Y51424X/1 - BBSRC International Institutional Sponsorship Awards Tranche 1 York). P.Wan. was supported by Science Achievement Scholarship of Thailand (SAST). P.Wo. was supported by NRCT-N42A650286.

REFERENCES

(1) Zhang, B.; Gao, Y.; Zhang, L.; Zhou, Y. The plant cell wall: Biosynthesis, construction, and functions. *J. Integr. Plant Biol.* **2021**, 63 (1), 251–272.

(2) Drakakaki, G. Polysaccharide deposition during cytokinesis: challenges and future perspectives. *Plant Science* 2015, 236, 177–184.
(3) Cosgrove, D. J. Catalysts of plant cell wall loosening. *F1000Research* 2016, 5, 119.

(4) Van de Meene, A. M. L.; Doblin, M. S.; Bacic, A. The plant secretory pathway seen through the lens of the cell wall. *Protoplasma* **2017**, *254*, 75–94.

(5) Hoffmann, N.; King, S.; Samuels, A. L.; McFarlane, H. E. Subcellular coordination of plant cell wall synthesis. *Dev. Cell.* 2021, 56 (7), 933–948.

(6) Amos, R. A.; Mohnen, D. Critical review of plant cell wall matrix polysaccharide glycosyltransferase activities verified by heterologous protein expression. *Front. Plant Sci.* **2019**, *10*, 915.

(7) Zhang, Y.; Yu, J.; Wang, X.; Durachko, D. M.; Zhang, S.; Cosgrove, D. J. Molecular insights into the complex mechanics of plant epidermal cell walls. *Science* **2021**, *372* (6543), 706–711.

(8) Kirui, A.; Du, J.; Zhao, W.; Barnes, W.; Kang, X.; Anderson, C. T.; Xiao, C.; Wang, T. A pectin methyltransferase modulates polysaccharide dynamics and interactions in Arabidopsis primary cell walls: Evidence from solid-state NMR. *Carbohydr. Polym.* 2021, 270, No. 118370.

(9) Fry, S. C.; York, W. S.; Albersheim, P.; Darvill, A.; Hayashi, T.; Joseleau, J. P.; Kato, Y.; Lorences, E. P.; Maclachlan, G. A.; McNeil, M.; Mort, A. J.; et al. An unambiguous nomenclature for xyloglucanderived oligosaccharides. *Physiologia Plantarum* **1993**, *89* (1), 1–3.

(10) Scheller, H. V.; Ulvskov, P. Hemicelluloses. Annual review of plant biology 2010, 61, 263-289.

(11) Ishida, K.; Ohba, Y.; Yoshimi, Y.; Wilson, L. F.; Echevarría-Poza, A.; Yu, L.; Iwai, H.; Dupree, P. Differing structures of galactoglucomannan in eudicots and non-eudicot angiosperms. *PLoS One* **2023**, *18* (12), No. e0289581.

(12) Mohnen, D. Pectin structure and biosynthesis. *Current opinion in plant biology* **2008**, *11* (3), 266–277.

(13) Harholt, J.; Suttangkakul, A.; Vibe Scheller, H. Biosynthesis of pectin. *Plant physiology* **2010**, *153* (2), 384–395.

(14) Schultink, A.; Naylor, D.; Dama, M.; Pauly, M. The role of the plant-specific ALTERED XYLOGLUCAN9 protein in Arabidopsis cell wall polysaccharide O-acetylation. *Plant Physiology* **2015**, *167* (4), 1271–1283.

(15) Temple, H.; Phyo, P.; Yang, W.; Lyczakowski, J. J.; Echevarría-Poza, A.; Yakunin, I.; Parra-Rojas, J. P.; Terrett, O. M.; Saez-Aguayo, S.; Dupree, R.; Orellana, A.; et al. Golgi-localized putative S-adenosyl methionine transporters required for plant cell wall polysaccharide methylation. *Nature Plants* **2022**, *8* (6), 656–669.

(16) Du, J.; Kirui, A.; Huang, S.; Wang, L.; Barnes, W. J.; Kiemle, S. N.; Zheng, Y.; Rui, Y.; Ruan, M.; Qi, S.; Kim, S. H.; et al. Mutations in the pectin methyltransferase QUASIMODO2 influence cellulose biosynthesis and wall integrity in Arabidopsis. *Plant Cell* **2020**, *32* (11), 3576–3597.

(17) Zhou, C.; Yin, Y.; Dam, P.; Xu, Y. Identification of Novel Proteins Involved in Plant Cell-Wall Synthesis Based on Protein-Protein Interaction Data. *J. Proteome Res.* **2010**, *9* (10), 5025–5037. (18) Parsons, H. T.; Christiansen, K.; Knierim, B.; Carroll, A.; Ito, J.; Batth, T. S.; Smith-Moritz, A. M.; Morrison, S.; McInerney, P.; Hadi, M. Z.; Auer, M.; et al. Isolation and proteomic characterization of the Arabidopsis Golgi defines functional and novel components involved in plant cell wall biosynthesis. *Plant Physiol* **2012**, *159* (1), 12–26.

(19) Cai, B.; Li, C. H.; Huang, J. Systematic identification of cell-wall related genes in Populus based on analysis of functional modules in co-expression network. *PloS One* **2014**, *9* (4), No. e95176.

(20) Atmodjo, M. A.; Sakuragi, Y.; Zhu, X.; Burrell, A. J.; Mohanty, S. S.; Atwood III, J. A.; Orlando, R.; Scheller, H. V.; Mohnen, D. Galacturonosyltransferase (GAUT) 1 and GAUT7 are the core of a plant cell wall pectin biosynthetic homogalacturonan: galacturonosyltransferase complex. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (50), 20225–20230.

(21) Albenne, C.; Canut, H.; Jamet, E. Plant cell wall proteomics: the leadership of Arabidopsis thaliana. *Front. Plant Sci.* 2013, 4, 111.

(22) Wu, F. H.; Shen, S. C.; Lee, L. Y.; Lee, S. H.; Chan, M. T.; Lin, C. S. Tape-Arabidopsis Sandwich-a simpler Arabidopsis protoplast isolation method. *Plant Methods* **2009**, *5*, 1–10.

(23) Zheng, J.; Yang, X.; Huang, Y.; Yang, S.; Wuchty, S.; Zhang, Z. Deep learning-assisted prediction of protein–protein interactions in Arabidopsis thaliana. *Plant J.* **2023**, *114* (4), 984–994.

(24) Lan, Y.; Sun, R.; Ouyang, J.; Ding, W.; Kim, M. J.; Wu, J.; Li, Y.; Shi, T. AtMAD: Arabidopsis thaliana multi-omics association database. *Nucleic Acids Res.* **2021**, *49* (D1), D1445–D1451.

(25) Zhang, H.; Zhang, F.; Yu, Y.; Feng, L. I.; Jia, J.; Liu, B. O.; Li, B.; Guo, H.; Zhai, J. A comprehensive online database for exploring~20,000 public Arabidopsis RNA-seq libraries. *Mol. Plant* **2020**, *13* (9), 1231–1233.

(26) Csardi, G.; Nepusz, T. The igraph software package for complex network research. *Inter Journal, complex systems* **2006**, *1695* (5), 1–9.

(27) Pettolino, F. A.; Walsh, C.; Fincher, G. B.; Bacic, A. Determining the polysaccharide composition of plant cell walls. *Nature protocols* **2012**, *7* (9), 1590–1607.

(28) Jones, L.; Milne, J. L.; Ashford, D.; McQueen-Mason, S. J. Cell wall arabinan is essential for guard cell function. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (20), 11783–11788.

(29) Morse, E. E. Anthrone in estimating low concentration of sucrose. *Anal. Chem.* **1947**, *19*, 1012–1013.

(30) Saeman, J. F. Kinetics of wood saccharification-hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. *Industrial & Engineering Chemistry* **1945**, 37 (1), 43–52. (31) Gomez, L. D.; Whitehead, C.; Barakate, A.; Halpin, C.; McQueen-Mason, S. J. Automated saccharification assay for determination of digestibility in plant materials. *Biotechnol. Biofuels* **2010**, 3, 1–12.

(32) Whitehead, C.; Gomez, L. D.; McQueen-Mason, S. J. The analysis of saccharification in biomass using an automated high-throughput method. In *Methods in enzymology*; Academic Press, 2012; Vol. *510*, pp 37–50.

(33) Anthon, G. E.; Barrett, D. M. Determination of reducing sugars with 3-methyl-2-benzothiazolinonehydrazone. *Analytical biochemistry* **2002**, 305 (2), 287–289.

(34) Marcus, S. E.; Verhertbruggen, Y.; Hervé, C.; Ordaz-Ortiz, J. J.; Farkas, V.; Pedersen, H. L.; Willats, W. G.; Knox, J. P. Pectic homogalacturonan masks abundant sets of xyloglucan epitopes in plant cell walls. *BMC Plant Biol.* **2008**, *8* (1), 1–12.

(35) Cornuault, V.; Buffetto, F.; Rydahl, M. G.; Marcus, S. E.; Torode, T. A.; Xue, J.; Crépeau, M. J.; Faria-Blanc, N.; Willats, W. G.; Dupree, P.; Ralet, M. C.; et al. Monoclonal antibodies indicate lowabundance links between heteroxylan and other glycans of plant cell walls. *Planta* **2015**, *242* (6), 1321–1334.

(36) Marcus, S. E.; Blake, A. W.; Benians, T. A.; Lee, K. J.; Poyser, C.; Donaldson, L.; Leroux, O.; Rogowski, A.; Petersen, H. L.; Boraston, A.; Gilbert, H. J.; et al. Restricted access of proteins to mannan polysaccharides in intact plant cell walls. *Plant J.* **2010**, *64* (2), 191–203.

(37) Verhertbruggen, Y.; Marcus, S. E.; Haeger, A.; Ordaz-Ortiz, J. J.; Knox, J. P. An extended set of monoclonal antibodies to pectic homogalacturonan. *Carbohydr. Res.* **2009**, 344 (14), 1858–1862.

(38) Lee, J.; Tomasek, D.; Santos, T. M.; May, M. D.; Meuskens, I.; Kahne, D. Formation of a β -barrel membrane protein is catalyzed by the interior surface of the assembly machine protein BamA. *Elife* **2019**, *8*, No. e49787.

(39) Flack, C. E.; Parkinson, J. S. Structural signatures of Escherichia coli chemoreceptor signaling states revealed by cellular crosslinking. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (28), No. e2204161119.

(40) Antar, H.; Soh, Y. M.; Zamuner, S.; Bock, F. P.; Anchimiuk, A.; Rios, P. D. L.; Gruber, S. Relief of ParB autoinhibition by parS DNA catalysis and recycling of ParB by CTP hydrolysis promote bacterial centromere assembly. *Sci. Adv.* **2021**, *7* (41), No. eabj2854.

(41) Nozawa, K.; Takizawa, Y.; Pierrakeas, L.; Sogawa-Fujiwara, C.; Saikusa, K.; Akashi, S.; Luk, E.; Kurumizaka, H. Cryo–electron microscopy structure of the H3-H4 octasome: A nucleosome-like particle without histones H2A and H2B. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (45), No. e2206542119.

(42) Goyal, P.; Pandey, D.; Brünnert, D.; Hammer, E.; Zygmunt, M.; Siess, W. Cofilin oligomer formation occurs in vivo and is regulated by cofilin phosphorylation. *PLoS One* **2013**, *8* (8), No. e71769.

(43) Ishmael, S. S.; Ishmael, F. T.; Jones, A. D.; Bond, J. S. Protease domain glycans affect oligomerization, disulfide bond formation, and stability of the meprin A metalloprotease homo-oligomer. *J. Biol. Chem.* **2006**, *281* (49), 37404–37415.

(44) Gunning, A. P.; Bongaerts, R. J.; Morris, V. J. Recognition of galactan components of pectin by galectin-3. *FASEB J.* **2009**, *23* (2), 415–424.

(45) Hozumi, K.; Nomizu, M. Cell adhesion activity of peptides conjugated to polysaccharides. *Curr. Protoc. Cell Biol.* **2018**, 80 (1), No. e53.

(46) Yu, L.; Yoshimi, Y.; Cresswell, R.; Wightman, R.; Lyczakowski, J. J.; Wilson, L. F.; Ishida, K.; Stott, K.; Yu, X.; Charalambous, S.; Wurman-Rodrich, J.; et al. Eudicot primary cell wall glucomannan is related in synthesis, structure, and function to xyloglucan. *Plant Cell* **2022**, *34* (11), 4600–4622.

(47) Yamaguchi, C.; Takimoto, Y.; Ohkama-Ohtsu, N.; Hokura, A.; Shinano, T.; Nakamura, T.; Suyama, A.; Maruyama-Nakashita, A. Effects of cadmium treatment on the uptake and translocation of sulfate in Arabidopsis thaliana. *Plant Cell Physiol* **2016**, *57* (11), 2353–2366.

(48) Liu, X.; Wu, F. H.; Li, J. X.; Chen, J.; Wang, G. H.; Wang, W. H.; Hu, W. J.; Gao, L. J.; Wang, Z. L.; Chen, J. H.; Simon, M.; et al. Glutathione homeostasis and Cd tolerance in the Arabidopsis sultr1; 1-sultr1; 2 double mutant with limiting sulfate supply. *Plant Cell Rep* **2016**, 35, 397–413.

(49) Shirzadian-Khorramabad, R.; Jing, H. C.; Everts, G. E.; Schippers, J. H.; Hille, J.; Dijkwel, P. P. A mutation in the cytosolic O-acetylserine (thiol) lyase induces a genome-dependent early leaf death phenotype in Arabidopsis. *BMC Plant Biol.* **2010**, *10*, 1–12.

(50) Birke, H.; De Kok, L. J.; Wirtz, M.; Hell, R. The role of compartment-specific cysteine synthesis for sulfur homeostasis during H2S exposure in Arabidopsis. *Plant Cell Physiol* **2015**, *56* (2), 358–367.

(51) Maeda, H.; Song, W.; Sage, T.; DellaPenna, D. Role of callose synthases in transfer cell wall development in tocopherol deficient Arabidopsis mutants. *Front. Plant Sci.* **2014**, *5*, 46.

(52) Sugita, M. An overview of pentatricopeptide repeat (PPR) proteins in the moss Physcomitrium patens and their role in organellar gene expression. *Plants* **2022**, *11* (17), 2279.

(53) Mangeon, A.; Pardal, R.; Menezes-Salgueiro, A. D.; Duarte, G. L.; de Seixas, R.; Cruz, F. P.; Cardeal, V.; Magioli, C.; Ricachenevsky, F. K.; Margis, R.; Sachetto-Martins, G. AtGRP3 is implicated in root size and aluminum response pathways in Arabidopsis. *PLoS One* **2016**, *11* (3), No. e0150583.

(54) Chien, P. S.; Chao, Y. T.; Chou, C. H.; Hsu, Y. Y.; Chiang, S. F.; Tung, C. W.; Chiou, T. J. Phosphate transporter PHT1; 1 is a key determinant of phosphorus acquisition in Arabidopsis natural accessions. *Plant Physiol* **2022**, *190* (1), 682–697.

(55) Zou, J. J.; Wei, F. J.; Wang, C.; Wu, J. J.; Ratnasekera, D.; Liu, W. X.; Wu, W. H. Arabidopsis calcium-dependent protein kinase CPK10 functions in abscisic acid- and Ca2+-mediated stomatal regulation in response to drought stress. *Plant Physiol* **2010**, *154* (3), 1232–1243.

(56) Meißner, D.; Albert, A.; Böttcher, C.; Strack, D.; Milkowski, C. The role of UDP-glucose: hydroxycinnamate glucosyltransferases in phenylpropanoid metabolism and the response to UV-B radiation in Arabidopsis thaliana. *Planta* **2008**, *228*, 663–674.

(57) Lauster, T.; Stoeckle, D.; Gabor, K.; Haller, T.; Krieger, N.; Lotz, P.; Mayakrishnan, R.; Späth, E.; Zimmermann, S.; Livanos, P.; Mueller, S. Arabidopsis pavement cell shape formation involves spatially confined ROPGAP regulators. *Curr. Biol.* **2022**, *32* (3), 532– 544.

(58) Beathard, C.; Mooney, S.; Al-Saharin, R.; Goyer, A.; Hellmann, H. Characterization of Arabidopsis thaliana R2R3 S23 MYB transcription factors as novel targets of the ubiquitin proteasomepathway and regulators of salt stress and abscisic acid response. *Front. Plant Sci.* **2021**, *12*, No. 629208.

(59) Ebert, B.; Rautengarten, C.; Guo, X.; Xiong, G.; Stonebloom, S.; Smith-Moritz, A. M.; Herter, T.; Chan, L. J. G.; Adams, P. D.; Petzold, C. J.; Pauly, M.; et al. Identification and characterization of a Golgi-localized UDP-xylose transporter family from Arabidopsis. *Plant Cell* **2015**, *27* (4), 1218–1227.

(60) Caffall, K. H.; Pattathil, S.; Phillips, S. E.; Hahn, M. G.; Mohnen, D. Arabidopsis thaliana T-DNA mutants implicate GAUT genes in the biosynthesis of pectin and xylan in cell walls and seed testa. *Mol. Plant* **2009**, *2* (5), 1000–1014.

(61) Klein, P.; Seidel, T.; Stöcker, B.; Dietz, K. J. The membranetethered transcription factor ANAC089 serves as a redox-dependent suppressor of stromal ascorbate peroxidase gene expression. *Front. Plant Sci.* **2012**, *3*, 247.

(62) Kang, X.; Li, W.; Zhou, Y.; Ni, M. A WRKY transcription factor recruits the SYG1-like protein SHB1 to activate gene expression and seed cavity enlargement. *PLoS Genet* **2013**, *9* (3), No. e1003347.

(63) Pietra, S.; Gustavsson, A.; Kiefer, C.; Kalmbach, L.; Hörstedt, P.; Ikeda, Y.; Stepanova, A. N.; Alonso, J. M.; Grebe, M. Arabidopsis SABRE and CLASP interact to stabilize cell division plane orientation and planar polarity. *Nat. Commun.* **2013**, *4* (1), 2779.

(64) Ojangu, E. L.; Tanner, K.; Pata, P.; Järve, K.; Holweg, C. L.; Truve, E.; Paves, H. Myosins XI-K, XI-1, and XI-2 are required for the development of pavement cells, trichomes, and stigmatic papillae in Arabidopsis. *BMC Plant Biol.* **2012**, *12* (1), 81.

(65) Leshem, Y.; Golani, Y.; Kaye, Y.; Levine, A. Reduced expression of the v-SNAREs AtVAMP71/AtVAMP7C gene family in Arabidopsis reduces drought tolerance by suppression of abscisic acid-dependent stomatal closure. *J. Exp. Bot.* **2010**, *61* (10), 2615–2622.

(66) Leshem, Y.; Melamed-Book, N.; Cagnac, O.; Ronen, G.; Nishri, Y.; Solomon, M.; Cohen, G.; Levine, A. Suppression of Arabidopsis vesicle-SNARE expression inhibits fusion of H2O2-containing vesicles with tonoplast and increases salt tolerance. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (47), 18008–18013.

(67) Haydon, M. J.; Kawachi, M.; Wirtz, M.; Hillmer, S.; Hell, R.; Krämer, U. Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in Arabidopsis. *Plant Cell* **2012**, *24* (2), 724–737.

(68) Lauster, T.; Stoeckle, D.; Gabor, K.; Haller, T.; Krieger, N.; Lotz, P.; Mayakrishnan, R.; Späth, E.; Zimmermann, S.; Livanos, P.; Mueller, S. Arabidopsis pavement cell shape formation involves spatially confined ROPGAP regulators. *Curr. Biol.* **2022**, *32* (3), 532– 544.

(69) Oda, Y.; Fukuda, H. Rho of plant GTPase signaling regulates the behavior of Arabidopsis kinesin-13A to establish secondary cell wall patterns. *Plant Cell* **2013**, *25* (11), 4439–4450.

(70) Sugiyama, Y.; Nagashima, Y.; Wakazaki, M.; Sato, M.; Toyooka, K.; Fukuda, H.; Oda, Y. A Rho-actin signaling pathway shapes cell wall boundaries in Arabidopsis xylem vessels. *Nat. Commun.* **2019**, *10* (1), 468.

(71) Alavarse, A. C.; Frachini, E. C. G.; da Silva, R. L. C. G.; Lima, V. H.; Shavandi, A.; Petri, D. F. S. Crosslinkers for polysaccharides and proteins: Synthesis conditions, mechanisms, and crosslinking efficiency, a review. *Int. J. Biol. Macromol.* **2022**, *202*, 558–596.

(72) Fasimoye, R.; Dong, W.; Nirujogi, R. S.; Rawat, E. S.; Iguchi, M.; Nyame, K.; Phung, T. K.; Bagnoli, E.; Prescott, A. R.; Alessi, D. R.; Abu-Remaileh, M. Golgi-IP, a tool for multimodal analysis of Golgi molecular content. *Proc. Natl. Acad. Sci. U. S. A.* **2023**, *120* (20), No. e2219953120.

(73) Xu, S. L.; Shrestha, R.; Karunadasa, S. S.; Xie, P. Q. Proximity labeling in plants. *Annu. Rev. Plant Biol.* **2023**, *74*, 285–312.

(74) Lund, C. H.; Bromley, J. R.; Stenbæk, A.; Rasmussen, R. E.; Scheller, H. V.; Sakuragi, Y. A reversible Renilla luciferase protein complementation assay for rapid identification of protein–protein interactions reveals the existence of an interaction network involved in xyloglucan biosynthesis in the plant Golgi apparatus. *J. Exp. Bot.* **2015**, *66* (1), 85–97.

(75) Beihammer, G.; Maresch, D.; Altmann, F.; Van Damme, E. J.; Strasser, R. Lewis A glycans are present on proteins involved in cell wall biosynthesis and appear evolutionarily conserved among natural Arabidopsis thaliana accessions. *Front. Plant Sci.* **2021**, *12*, No. 630891.

(76) Zhang, G. F.; Staehelin, L. A. Functional compartmentation of the Golgi apparatus of plant cells: immunocytochemical analysis of high-pressure frozen-and freeze-substituted sycamore maple suspension culture cells. *Plant Physiol* **1992**, *99* (3), 1070–1083.

(77) Young, R. E.; McFarlane, H. E.; Hahn, M. G.; Western, T. L.; Haughn, G. W.; Samuels, A. L. Analysis of the Golgi apparatus in Arabidopsis seed coat cells during polarized secretion of pectin-rich mucilage. *Plant Cell* **2008**, 20 (6), 1623–1638.

(78) Moore, P. J.; Swords, K. M.; Lynch, M. A.; Staehelin, L. A. Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the Golgi apparatus of plants. *J. Cell Biol.* **1991**, *112* (4), 589–602.

(79) Meents, M. J.; Motani, S.; Mansfield, S. D.; Samuels, A. L. Organization of xylan production in the Golgi during secondary cell wall biosynthesis. *Plant Physiol* **2019**, *181* (2), 527–546.

(80) Chevalier, L.; Bernard, S.; Ramdani, Y.; Lamour, R.; Bardor, M.; Lerouge, P.; Follet-Gueye, M. L.; Driouich, A. Subcompartment localization of the side chain xyloglucan-synthesizing enzymes within Golgi stacks of tobacco suspension-cultred cells. *Plant J.* **2010**, *64* (6), 977–989.

(81) Wilkop, T.; Pattathil, S.; Ren, G.; Davis, D. J.; Bao, W.; Duan, D.; Peralta, A. G.; Domozych, D. S.; Hahn, M. G.; Drakakaki, G. A hybrid approach enabling large-scale glycomic analysis of post-Golgi vesicles reveals a transport route for polysaccharides. *Plant Cell* **2019**, *31* (3), 627–644.

(82) Wang, Y.; Mortimer, J. C.; Davis, J.; Dupree, P.; Keegstra, K. Identification of an additional protein involved in mannan biosynthesis. *Plant J.* **2013**, *73* (1), 105–117.

(83) Voiniciuc, C.; Dama, M.; Gawenda, N.; Stritt, F.; Pauly, M. Mechanistic insights from plant heteromannan synthesis in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (2), 522–527.

(84) Held, M. A.; Be, E.; Zemelis, S.; Withers, S.; Wilkerson, C.; Brandizzi, F. CGR3: a Golgi-localized protein influencing homogalacturonan methylesterification. *Mol. Plant* **2011**, *4* (5), 832–844. (85) Yang, C. H.; Wang, C.; Singh, S.; Fan, N.; Liu, S.; Zhao, L.; Cao, H.; Xie, W.; Yang, C.; Huang, C. F. Golgi-localised manganese transporter PML3 regulates Arabidopsis growth through modulating Golgi glycosylation and cell wall biosynthesis. *New Phytol* **2021**, 231 (6), 2200–2214.

(86) Manabe, Y.; Nafisi, M.; Verhertbruggen, Y.; Orfila, C.; Gille, S.; Rautengarten, C.; Cherk, C.; Marcus, S. E.; Somerville, S.; Pauly, M.; Knox, J. P.; et al. Loss-of-function mutation of REDUCED WALL ACETYLATION2 in Arabidopsis leads to reduced cell wall acetylation and increased resistance to Botrytis cinerea. *Plant Physiol* **2011**, *155* (3), 1068–1078. (87) Amos, R. A.; Mohnen, D. Critical review of plant cell wall matrix polysaccharide glycosyltransferase activities verified by heterologous protein expression. *Front. Plant Sci.* **2019**, *10*, 915.

(88) Ruprecht, C.; Bartetzko, M. P.; Senf, D.; Dallabernadina, P.; Boos, I.; Andersen, M. C.; Kotake, T.; Knox, J. P.; Hahn, M. G.; Clausen, M. H.; Pfrengle, F. A synthetic glycan microarray enables epitope mapping of plant cell wall glycan-directed antibodies. *Plant Physiol* **2017**, *175* (3), 1094–1104.