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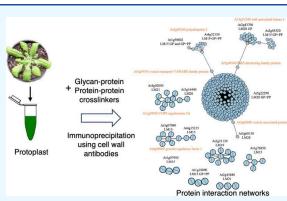


# *In Vivo* Proximity Cross-Linking and Immunoprecipitation of Cell Wall Epitopes Identify Proteins Associated with the Biosynthesis of Matrix Polysaccharides

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**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

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Plant cell wall biosynthesis is a complex and coordinated process, with numerous enzymes and regulatory proteins playing pivotal roles.<sup>1</sup> 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.<sup>2-4</sup> 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.<sup>5</sup> 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.<sup>6</sup> 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.<sup>7,8</sup> 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  $\beta$ -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

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xylose residues can be further substituted with other monosaccharides, such as galactose and fucose, forming a diverse array of xyloglucan structures.<sup>9</sup> 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  $\beta$ -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.<sup>10</sup> 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  $\beta$ -1,3 and  $\beta$ -1,4-glucan backbone, contributing to the flexibility and strength of grass cell walls.<sup>10</sup> Galactomannan (GM), prevalent in the primary cell walls of monocots, is characterized by a  $\beta$ -1,4-linked mannose backbone with galactose side chains.<sup>11</sup> 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).<sup>12,13</sup> 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.<sup>12,14,15</sup> 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.<sup>16</sup>

In recent years, efforts have been made to identify and characterize proteins associated with plant cell wall biosynthesis. Zhou et al.<sup>17</sup> 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.<sup>18</sup> 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.<sup>20</sup> 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.<sup>21</sup> 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.<sup>18</sup> 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<sup>-1</sup>) agar plates containing 1/2 strength Murashige and Skoog medium and 1% (w v<sup>-1</sup>) sucrose for 7 days and then grown in compost under conditions: 16 h light (125  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) 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.<sup>22</sup> 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 CaCl<sub>2</sub>, 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 CaCl<sub>2</sub>, 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 MgCl<sub>2</sub>, and 4 mM MES at pH 5.7) to obtain a final concentration of  $5 \times 10^6$  cells mL<sup>-1</sup>. 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 (10<sup>6</sup> 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 MnCl<sub>2</sub>, 0.25 mM MgCl<sub>2</sub>, 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  $\mu$ 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  $\mu$ m C18) and safeguarded by a guard column (Hypersil GoLD 30  $\times$  0.5 mm, 5  $\mu$ 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  $\pm 1.2$ 

Da, MS/MS fragment tolerance of  $\pm 0.6$  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.

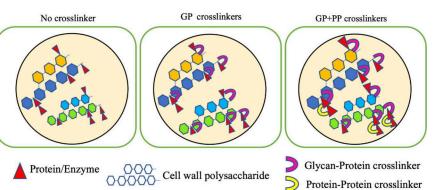
**Protein Identifications.** The protein IDs were searched for *Arabidopsis* protein interactions using DeepAraPPI (based in deep learning-assisted prediction)<sup>23</sup> and AtMAD (based on experimental data such as FRET, yeast two-hybrid, coimmunoprecipitation, and affinity capture-MS).<sup>24</sup> For coexpression analysis, the *Arabidopsis* RNA-seq Database was used.<sup>25</sup> Protein interaction and coexpression networks were built using the igraph R package<sup>26</sup> (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  $\mu$ L reaction volume containing 4 mM dNTPs, 30 mM MgCl<sub>2</sub>, 0.5  $\mu$ 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.<sup>27</sup> 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<sup>-1</sup>) NaBH<sub>4</sub> 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.<sup>28</sup> 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 hydrolysis<sup>29</sup> and Anthrone assay.<sup>30</sup>

Saccharification Analysis. Saccharification was performed following Gomez et al.<sup>31</sup> and Whitehead et al.<sup>32</sup> 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  $\mu$ L of 0.5 M NaOH solution at 90 °C for 30 min, washed for five times with 500  $\mu$ 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.<sup>33</sup> 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,<sup>34</sup> LM28 recognizing GX,<sup>35</sup> LM21 recognizing GM,<sup>3</sup> and LM19 recognizing unesterified HGA.<sup>37</sup> 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,

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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,<sup>38,39</sup> Bacillus *subtilis*,<sup>40</sup> yeasts,<sup>41</sup> and human endothelial cells and platelets.<sup>42</sup> It is important to note that while GP cross-linkers have been used for in vitro cross-linking assays<sup>43-45</sup> 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- $\beta$ -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.<sup>22</sup> 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

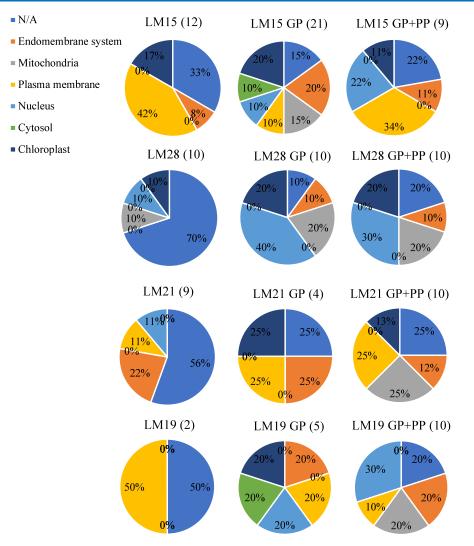


Figure 2. Proteomic analysis of immunoprecipitated products using cell wall antibodies. Identified proteins were classified based on annotated subcellular localizations. The number of proteins identified for each antibody and treatment are presented. Diagrams show percentages of proteins for each localization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

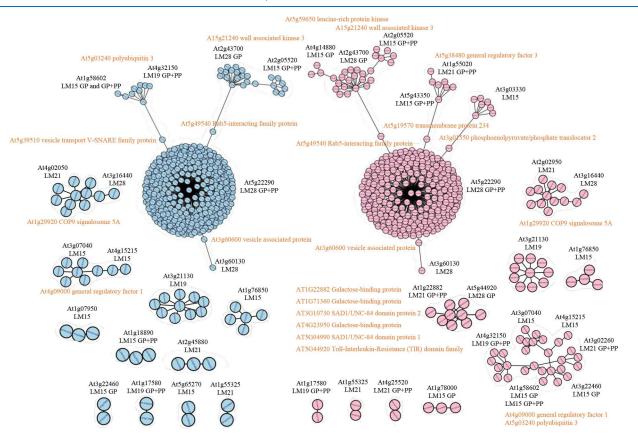
## Table 1. Protein Identification Obtained from Immunoprecipitation of Leaf Protoplasts Using Cell Wall Antibodies<sup>a</sup>

| 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 =<br>Sty4800 Bactors protein Le3 9 BM N/N =<br>Sty4800 Bactors protein (ST 44) 13 5 PM N/N =<br>ST 143 SG 24 SG 25 N/N =<br>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 <sup>3</sup> 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<br>(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="">       -       -       &lt;</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<br>At1g03750  | Chromatin modification-related protein EAF1<br>LM21 GP+PP   | 34                               |                                    |                                 |        |
|   |  | At4g08560<br>At1g03750<br>At1g22882   | Chromatin modification-related protein EAF1<br>LM21 GP+PP<br>Galactose-binding protein  | 34<br>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<br>At1g03750<br>At1g22882<br>At1g55020  | Chromatin modification-related protein EAF1<br>LM21 GP+PP<br>Galactose-binding protein<br>Lipoxygenase 1  | 34<br>21<br>32                   | Nucleus, ER<br>-                   | N/Y<br>N/Y                      |        |
| At1g78710Trichome birefringence-like 42 (TBL42)29ESN/N  |  | At4g08560<br>At1g03750<br>At1g22882<br>At1g55020<br>At3g56480                           | Chromatin modification-related protein EAF1<br>LM21 GP+PP<br>Galactose-binding protein<br>Lipoxygenase 1<br>Myosin heavy chain-related  | 34<br>21<br>32<br>20             | Nucleus, ER<br>-<br>PM             | N/Y<br>N/Y<br>N/N               |        |
|   | 12-02260 Aurin transmistration BLC 21 DM $17/37$   | At4g08560<br>At1g03750<br>At1g22882<br>At1g55020<br>At3g56480<br>At1g78710              | Chromatin modification-related protein EAF1<br>LM21 GP+PP<br>Galactose-binding protein<br>Lipoxygenase 1<br>Myosin heavy chain-related<br>Trichome birefringence-like 42 (TBL42)                                | 34<br>21<br>32<br>20<br>29       | Nucleus, ER<br>-<br>PM<br>ES       | N/Y<br>N/Y<br>N/N<br>N/N        |        |
| At3g02260Auxin transport protein BIG21PMN/YAt4g25520Lim domain-binding protein37-N/Y  |  | At4g08560<br>At1g03750<br>At1g22882<br>At1g55020<br>At3g56480<br>At1g78710<br>At3g02260 | Chromatin modification-related protein EAF1<br>LM21 GP+PP<br>Galactose-binding protein<br>Lipoxygenase 1<br>Myosin heavy chain-related<br>Trichome birefringence-like 42 (TBL42)<br>Auxin transport protein BIG | 34<br>21<br>32<br>20<br>29<br>21 | Nucleus, ER<br>-<br>PM<br>ES<br>PM | N/Y<br>N/Y<br>N/N<br>N/N<br>N/Y |        |
|   | Ausin transport protein BIG 21 PM N/Y  | At4g08560<br>At1g03750<br>At1g22882<br>At1g55020<br>At3g56480<br>At1g78710              | Chromatin modification-related protein EAF1<br>LM21 GP+PP<br>Galactose-binding protein<br>Lipoxygenase 1<br>Myosin heavy chain-related<br>Trichome birefringence-like 42 (TBL42)                                | 34<br>21<br>32<br>20<br>29       | Nucleus, ER<br>-<br>PM<br>ES       | N/Y<br>N/Y<br>N/N<br>N/N        |        |

#### Table 1. continued

| Protein   | Annotation   | Peptide<br>length | Localization | DeepAraPPI/<br>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                  |        |

<sup>a</sup>The 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

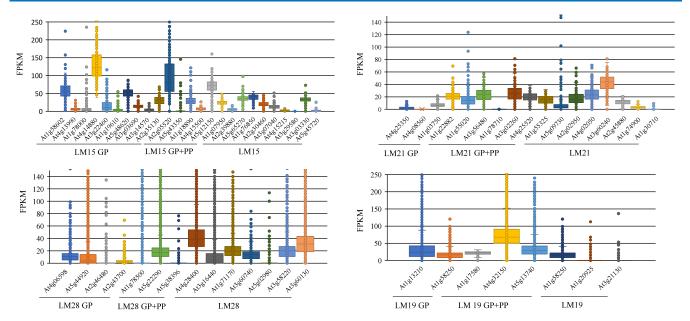


Figure 4. Gene expression levels of the protein IDs from four antibodies in *Arabidopsis* leaf tissues. Data was obtained from the *Arabidopsis* RNA-seq database.

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.

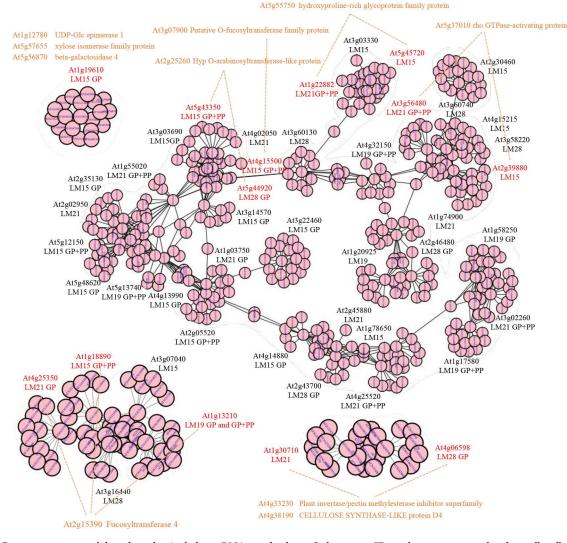
Identification of Proteins Based on Protein Interactions. To identify the relationship of these proteins to the plant cell wall, we performed searches for Arabidopsis protein interactions using DeepAraPPI (based on deep learningassisted prediction)<sup>23</sup> and AtMAD (based on experimental data such as FRET, yeast-2-hybrid, coimmunoprecipitation, and affinity capture-MS)<sup>24</sup> 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.

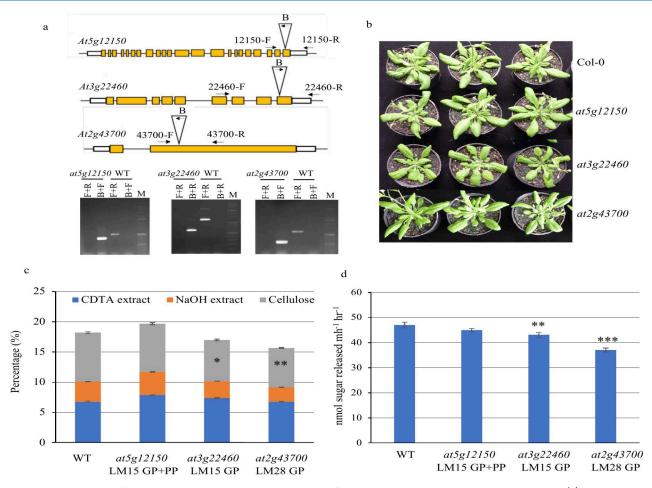
regulatory factor 1. Furthermore, the At1g22882–At5g44920 network from AtMAD forms a network among galactose binding protein/SAD1/UNC-84 domain protein/Toll-Inter-leukin-Resistance (TIR) domain family protein, and this may play a role in the TLR signaling pathway. Although further investigation is required 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.<sup>25</sup> 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.<sup>68-70</sup> 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.

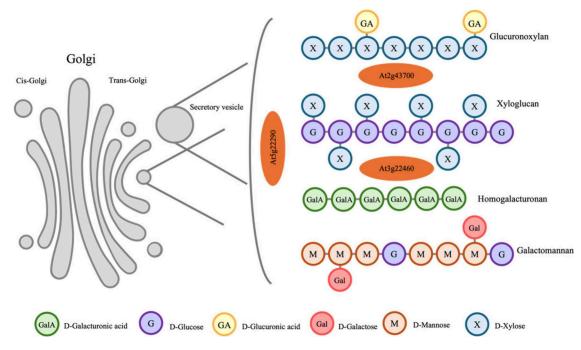
### DISCUSSION

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 <sup>a</sup> | imass <sup>a</sup>      |                       |                      |                         | 4                    |                     |   |                         |
|----------------------------|---|-------------------------|-----------------------|----------------------|-------------------------|----------------------|---------------------|---|-------------------------|
|                            | Fuc   | Ara                     | Rha                   | Gal                  | Glu                     | Xyl                  | Man                 | GalA  | GluA                    |
|                            |   |                         |                       | CDJ                  | CDTA extract            |                      |                     |   |                         |
| WT                         | $1.40 \pm 0.04$                               | $6.94 \pm 0.08$         | $6.41 \pm 0.11$       | $9.05 \pm 0.06$      | $14.96 \pm 1.2$         | $4.10 \pm 0.06$      | $1.24 \pm 0.06$     | $22.91 \pm 1.54$  | $0.71 \pm 0.02$         |
| at5g12150                  | $1.43 \pm 0.15$                               | $5.77 \pm 0.07$         | $6.68 \pm 0.25$       | $8.75 \pm 0.34$      | $18.64 \pm 2.7$         | $3.53 \pm 0.04$      | $1.53 \pm 0.28$     | $31.54 \pm 3.87$  | $1.03 \pm 0.05$         |
| at3g22460                  | $1.19 \pm 0.21$                               | $5.21 \pm 0.35$         | $5.22 \pm 0.36$       | $7.53 \pm 0.7$       | $13.26 \pm 2.78$        | $2.33 \pm 0.28^{*}$  | $1.05 \pm 0.24$     | $37.35 \pm 3.46$  | $0.61 \pm 0.03^{*}$     |
| at2g43700                  | $1.43 \pm 0.09$                               | $5.32 \pm 0.19$         | $5.48 \pm 0.11$       | $6.97 \pm 0.03$      | $7.94 \pm 1.65$         | $2.58 \pm 0.11^{*}$  | $0.85 \pm 0.08$     | $36.58 \pm 1.85^{*}$  | $0.54 \pm 0.04^{*}$     |
|                            |   |                         |                       | NaC                  | NaOH extract            |                      |                     |   |                         |
| WT                         | $1.61 \pm 0.1$                                | $3.66 \pm 0.21$         | $1.60 \pm 0.18$       | $6.22 \pm 0.31$      | $13.00 \pm 4.3$         | $5.92 \pm 0.13$      | $1.85 \pm 0.37$     | $3.50 \pm 0.16$   | $0.83 \pm 0.07$         |
| at5g12150                  | $1.87 \pm 0.06^{*}$                           | $3.69 \pm 0.3$          | $1.60 \pm 0.08$       | $7.04 \pm 1$         | $10.88 \pm 1.78$        | $6.38 \pm 0.89$      | $2.31 \pm 0.41$     | $3.70 \pm 0.27$   | $0.72 \pm 0.13$         |
| at3g22460                  | $1.23 \pm 0.02^{*}$                           | $2.27 \pm 0.04^{**}$    | $0.96 \pm 0.14^{**}$  | $5.49 \pm 0.12^{*}$  | $9.61 \pm 0.73$         | $4.26 \pm 0.31^{**}$ | $1.48 \pm 0.04$     | $2.22 \pm 0.04^{**}$  | $0.42 \pm 0.02^{**}$    |
| at2g43700                  | $1.24 \pm 0.06^{*}$                           | $2.20 \pm 0.35^{**}$    | $0.92 \pm 0.05^{*}$   | $4.97 \pm 0.81$      | $7.32 \pm 1.56$         | $3.54 \pm 0.62^{*}$  | $1.34 \pm 0.25$     | $1.96 \pm 0.13^{***}$   | $0.41 \pm 0.01^{**}$    |
| <sup>a</sup> 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 (* | <sup>a</sup> 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.<sup>71</sup> 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.<sup>18,72,73</sup> 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.<sup>74</sup> 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.,<sup>17</sup> Parsons et al.<sup>18</sup> and Atmodjo et al.<sup>20</sup> 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,<sup>75</sup> 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.<sup>20</sup> 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 Staehelin<sup>76</sup> as well as Young et al.<sup>77</sup> have demonstrated that XyGs and pectins are synthesized within the Golgi stacks. Moore et al.<sup>78</sup> 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.<sup>80</sup> 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,<sup>81</sup> 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.<sup>82,83</sup> 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.<sup>84</sup> 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.<sup>86</sup> 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.<sup>87</sup> 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.<sup>88</sup> 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)

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#### **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.

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