

The role of the receptor kinase STRUBBELIG in the cell wall stress response

Barbara Leśniewska

Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München zur Erlangung einer

Doktorin der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitz: Prof. Dr. Frank Johannes

Prüfer*innen der Dissertation:

1. Prof. Dr. Kay H. Schneitz
2. Prof. Dr. Brigitte Poppenberger-Sieberer

Die Dissertation wurde am 14.08.2023 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 22.11.2023 angenommen.

I. Table of Contents

I. Table of Contents

II. List of Figures

III. List of Tables

IV. Abbreviations

V. Summary

VI. Zusammenfassung

1. Introduction	1
1.1. The plant cell wall	1
1.1.1. Cellulose	1
1.1.2. Hemicelluloses	3
1.1.3. Pectin	3
1.1.4. Cell wall proteins	4
1.1.5. Lignin	4
1.1.6. Callose	8
1.2. The role of the cell wall in plant morphogenesis	9
1.3. The role of the cell wall in stress responses	9
1.4. Selected examples of cell wall integrity receptors	11
1.4.1. <i>Catharanthus roseus</i> RLK1-like kinases	11
1.4.2. Wall-Associated Kinases (WAKs)	12
1.4.3. Leucine-Rich Repeat Receptor Kinases (LRR-RK)	12
1.5. Downstream signaling processes involved in response to cell wall damage	13
1.6. The <i>STRUBBELIG</i> signaling pathway and its role in plant morphogenesis	14
1.6.1. <i>STRUBBELIG</i> is an atypical receptor kinase	14
1.6.2. <i>SUB</i> regulates tissue morphogenesis in <i>Arabidopsis</i>	15
1.6.3. Additional components of the <i>SUB</i> signaling pathway (<i>QKY</i> , <i>DOQ/AN</i> , <i>ZET</i>)	16
1.7. Role of receptor kinase <i>STRUBBELIG</i> in adapting to reduced cellulose biosynthesis in <i>Arabidopsis thaliana</i>	18
1.7.1. <i>SUB</i> is required for cellulose synthesis inhibition (CBI)-induced cell wall stress response	18

1.7.2. Morphogenesis in Arabidopsis during cellulose deficient condition is regulated by the activity of <i>SUB</i>	19
1.8. Objectives	19
2. Materials and Methods	21
2.1. Plant work and plant genetics	21
2.2. Arabidopsis genomic DNA extraction and genotyping PCR	22
2.3. Confocal laser scanning microscopy (CLSM)	23
2.4. Chemical treatments	23
2.5. Growth media, growth conditions and frequently used buffers	24
2.6. Bioinformatics	25
2.7. RNAseq and transcriptomic analysis	25
2.8. Callose accumulation assay	26
2.9. Lignin accumulation assay - phloroglucinol stain	26
2.10. Lignin accumulation assay – Basic Fuchsin	27
2.11. Statistics	27
3. Results	28
3.1. The Arabidopsis receptor kinase STRUBBELIG regulates cell wall remodeling in response to specific cell wall damage	28
3.1.1. Response to pectinase-induced cell wall damage requires <i>SUB</i> activity	28
3.1.2. PMR4 plays a critical role in the accumulation of CWD-induced callose	32
3.1.3. Cellulose-derived oligomers do not induce callose production	34
3.1.4. Treatment with cell wall-degrading enzymes promotes the internalization of <i>SUB</i>	36
3.1.5. Isoxaben-induced root lignification begins in the endodermis	43
3.1.6. <i>SUB</i> function required for lignin propagation	46
3.2. <i>QKY</i> is not required for the pectinase-induced CWD response	48
3.3. <i>ANGUSTUFOLIA</i> and <i>ZERZAUST</i> do not contribute to isoxaben-induced lignification	50
3.4. The <i>SCHENGEN</i> pathway is not essential for isoxaben-induced lignin accumulation	52
3.5. Screening potential candidates involved in the <i>SUB</i> signaling pathway	53
3.6. The <i>SUB</i> -dependent transcriptional response to isoxaben treatment	58

4. Discussion	69
4.1. The receptor kinase STRUBBELIG does not affect general cell wall damage responses	69
4.1.1. Callose synthase PMR4 contributes to callose production independent of stress	70
4.1.2. Inhibition of cellulose biosynthesis triggers different protective responses than degradation of existing cellulose	71
4.1.3. Enzymatic treatments lead to SUB internalization from the plasma membrane	72
4.1.4. Distinct mechanisms and cellular specificity of isoxaben-induced lignification	72
4.2. Differential role of <i>SUB</i> signaling pathway components in response to cell wall damage	74
4.3. Novel candidate factors of the <i>SUB</i> signaling pathway	76
4.4. Isoxaben-induced changes in lignin biosynthesis genes and the role of <i>SUB</i> in the transcriptome	78
5. Conclusions	80
6. Supplement	81
7. References	82
8. Acknowledgments	98

II. Lists of Figures

Figure 1. Schematic illustration of the plant cell wall.	2
Figure 2. Lignin biosynthesis pathway and a schematic illustration of root morphology.	7
Figure 3. Overview of the domain architecture of SUB and phenotypic comparison of Col-0 and <i>sub-9</i> .	15
Figure 4. Comparison of the overall above-ground morphology of <i>sub-1</i> , <i>doq-1</i> , <i>qky-8</i> , and <i>zet-2</i> mutants.	17
Figure 5. Effect of cell wall-degrading enzymes on root epidermal cell morphology.	29
Figure 6. Lack of lignin production upon the application of cell wall-degrading enzymes.	29
Figure 7. Callose accumulation upon active vs. heat-deactivated cell wall-degrading enzymes.	30
Figure 8. <i>SUB</i> affects pectinase-induced callose accumulation.	31
Figure 9. Involvement of <i>Cals7</i> in CWD-induced callose accumulation.	33
Figure 10. Cellulose-derived oligomers play a negligible role in callose accumulation.	35
Figure 11. Application of all three enzymes is followed by concentration-dependent reduction of reporter signal at the plasma membrane.	37
Figure 12. Influence of CWD enzymes on SUB abundance at the plasma membrane.	39
Figure 13. Prolonged incubation in mock solution results in lower SUB:EGFP signal at the plasma membrane.	40
Figure 14. Sub-cellular localization of SUB:EGFP upon cell wall damage.	41
Figure 15. Effect of CWD enzymes on root hair patterning.	42
Figure 16. Time course analysis of isoxaben-induced lignification in response to a reduction in cellulose content.	44
Figure 17. Comparison of wild-type and <i>sub</i> mutants in time course analysis of isoxaben-induced lignification.	47
Figure 18. Role of <i>QKY</i> in CWD-induced callose accumulation.	49
Figure 19. Influence of QKY on plasma membrane SUB:EGFP levels after pectinase treatment.	50
Figure 20. Role of <i>ZET</i> and <i>AN</i> in cell wall damage response.	51
Figure 21. Impact of <i>SGN3</i> on CBI-induced lignin accumulation.	52
Figure 22. Callose accumulation assay as a first screening factor.	55
Figure 23. Lignin accumulation assay as a second screening factor.	56
Figure 24. Impact of <i>CASPLP1B1</i> on CBI-induced lignin accumulation.	57

- Figure 25. Radar plot showing expression of *NST1*, *VND7*, and various *MYB* genes in response to isoxaben exposure. 59
- Figure 26. Radar plot showing expression of various *CCR*, *CAD*, *PAL*, and *4CL* genes in response to isoxaben exposure. 61
- Figure 27. Radar plot showing expression of *RBOHA*, *RBOHB*, *RBOHD*, and *RBOHF* genes in response to isoxaben exposure. 63
- Figure 28. Radar plot showing expression of various *LAC* genes in response to isoxaben exposure. 65
- Figure 29. Radar plot showing expression of various *PRX* genes in response to isoxaben exposure. 67

III. List of Tables

Table 1. List of candidate genes used in the study.	21
Table 2. PCR reaction mix and cyclers program.	22
Table 3. Cell wall degrading enzymes and oligomers used in this study.	24
Table S1. List of genotyping primers.	81

IV. Abbreviations

4CL8 - 4-HYDROXYCINNAMATE COA LIGASE 8

AN – ANGUSTIFOLIA

BRI1 - BRASSINOSTEROIDS INSENSITIVE1

CAD2 - CINNAMYL ALCOHOL DEHYDROGENASE 2

CalS – callose synthase

CASPL - CASPARIAN STRIP MEMBRANE DOMAIN-LIKE PROTEIN

CBI – cellulose synthesis-inhibition

CCR2 - CINNAMOYL COA REDUCTASE 2

CDPKs - calcium-dependent protein kinases

CESAs - cellulose synthase proteins

CLV1 - CLAVATA1

COBL7 - COBRA-LIKE PROTEIN 7

Col-0 - Columbia

CrRLK1L - Catharanthus roseus RECEPTOR-LIKE KINASE1-LIKE

CSC - cellulose synthase complex

CWD- cell wall damage

CWPs - cell wall proteins

CWS - cell wall sensing

DAMPs - damage-associated molecular patterns

DEGs - differentially expressed genes

DMSO - Dimethyl sulfoxide

DOQ - DETORQEO

ET - ethylene

FDR - false discovery rate

FER - FERONIA

GSL - GLUCAN SYNTHASE-LIKE

H - root hair cells

HERK2 – HERKULES2

HG – homogalacturonan

ISX - isoxaben

JA - jasmonic acid

LAC - LACCASE

Ler - Landsberg (erecta mutant)

LLGs - LORELEI-LIKE GLYCOSYLPHOSPHATIDYLINOSITOL (GPI)-ANCHORED PROTEINS

LRR-RK - Leucine-Rich Repeat Receptor Kinases

LRX - LEUCINE-RICH REPEAT EXTENSIN

MAPK - Mitogen-activated protein kinases

MIK2 - MDIS1-INTERACTING RECEPTOR LIKE KINASE 2

MS - Murashige and Skoog

MYB - MYELOBLASTOMA FAMILY

N - non-hair cells

OGs - oligogalacturonides

PAL2 - L-PHENYLALANINE AMMONIA-LYASE 2

PD – plasmodesmata

PER - PEROXIDASE

PGIPs - polygalacturonase inhibitors

PM - plasma membrane

PMEs - pectin methylesterases

PMEIs - pectin methylesterase inhibitors

PMR4 - POWDERY MILDEW RESISTANT 4

PRX - PEROXIDASE

QKY – QUIRKY

RALF - RAPID ALKALINIZATION FACTOR

RBOHF - RESPIRATORY BURST OXIDASE HOMOLOG F

RG-I - rhamnogalacturonan-I

RG-II - rhamnogalacturonan-II

RNAseq - RNA sequencing

ROS reactive oxygen species

RUL1 - REDUCED IN LATERAL GROWTH 1

SA - salicylic acid

SCM - SCRAMBLED

SERK - LRR-RKs SOMATIC EMBRYOGENESIS RECEPTOR KINASES

SGN3 – SCHENGEN 3

SIMP1 - SALT-INDUCED MALECTIN-LIKE DOMAIN-CONTAINING PROTEIN 1

SLM - STRUBBELIG-LIKE MUTANT

SND1/NST3 - SECONDARY WALL ASSOCIATED NAC DOMAIN PROTEIN1/NAC
SECONDARY WALL THICKENING PROMOTING FACTOR 3

SRF - STRUBBELIG-RECEPTOR FAMILY

SSE - pSUB::SUB:EGFP

SUB - STRUBBELIG

SUB-OE - pUBQ::SUB:mCherry

THE1 – THESEUS 1

VND6 - VASCULAR-RELATED NAC-DOMAIN 6

WAK - WALL-ASSOCIATED KINASE

ZET - ZERZAUST

V. Summary

The plant cell wall is a vital component in various aspects of plant existence. Composed primarily of complex carbohydrates, including cellulose, hemicellulose, and pectin, it forms a dynamic and multifunctional barrier surrounding plant cells (Lampugnani et al. 2018). The cell wall provides mechanical support, enabling plants to withstand environmental stresses and maintain their structural integrity (Cosgrove 2022). It also serves as a protective barrier, defending against physical injuries, pathogens, and herbivores (Malinovsky et al. 2014). In certain plant tissues, the secondary cell wall adds an extra layer of fortification through lignification (R. Zhong and Ye 2009). In addition to its structural and protective roles, the cell wall facilitates cell-to-cell communication and signaling. This communication network coordinates tissue and organ growth, as well as the transmission of stress and defense signals (Gigli et al. 2019). The cell wall undergoes dynamic changes during different stages of the plant life cycle. It must be modified to accommodate cell expansion, organ growth, and reproductive processes (B. Zhang et al. 2021). To manage all these functions, dedicated signaling mechanisms have evolved to convey various internal and external signals inside the cell. Receptor kinases constitute a major part of such a mechanism (Wolf 2022). In Arabidopsis, the atypical receptor kinase STRUBBELIG (*SUB*) integrates two main biological processes: development and defense. Initially, the importance of *SUB* was exclusively related to its regulatory function in floral organ morphogenesis (Vaddepalli et al. 2011; Kwak et al. 2014). Recent studies revealed that *SUB* is required for morphogenesis during cellulose deficiency and cell wall stress response induced by the cellulose biosynthesis inhibitor, isoxaben (Chaudhary et al. 2020; Chaudhary et al. 2021).

This work further investigated the specificity of *SUB* in the context of the cell damage caused by the activity of different enzymes that degrade the major components of the cell wall: cellulase, hemicellulase, and pectinase. Presented data depict *SUB* as a highly specific element of the cell wall integrity system. Similar to the response to isoxaben, the application of pectinase triggered *SUB*-dependent callose production. However, cellulase and xyloglucanase treatments resulted in a *SUB*-independent response. Furthermore, all tested enzymes contributed to decreased *SUB* abundance at the plasma membrane. The study further addressed the spatial and temporal distribution of lignin in roots subjected to reduced cellulose levels. The data show that ectopic lignification initially starts in the endodermis and later spreads to adjacent cell layers. Finally, new members of the *SUB* signaling pathway involved in isoxaben-induced lignification were identified and investigated.

VI. Zusammenfassung

Die pflanzliche Zellwand ist eine wichtige Komponente für verschiedene Aspekte der pflanzlichen Existenz. Sie besteht hauptsächlich aus komplexen Kohlenhydraten, darunter Zellulose, Hemizellulose und Pektin und bildet eine dynamische und multifunktionale Barriere um die einzelnen Pflanzenzellen (Lampugnani et al. 2018). Die Zellwand bietet mechanische Unterstützung und ermöglicht es den Pflanzen Umweltbelastungen zu widerstehen und ihre strukturelle Integrität zu erhalten (Cosgrove 2022). Sie dient auch als Schutzbarriere um physische Verletzungen, Krankheitserreger und Pflanzenfresser abzuwehren (Malinovsky et al. 2014). In bestimmten Pflanzengeweben fügt die sekundäre Zellwand durch Verholzung eine zusätzliche Schutzschicht hinzu (R. Zhong und Ye 2009). Zusätzlich zu ihren strukturellen und schützenden Funktionen erleichtert die Zellwand die Kommunikation zwischen den Zellen und die Signalübertragung. Dieses Kommunikationsnetzwerk koordiniert das Gewebe- und Organwachstum sowie die Übertragung von Stress- und Abwehrsignalen (Gigli et al. 2019). Die Zellwand unterliegt in den verschiedenen Phasen der Lebenszyklen einer Pflanze dynamischen Veränderungen. Sie muss modifiziert werden um die Zellexpansion, das Organwachstum und die Fortpflanzungsprozesse zu ermöglichen (B. Zhang et al. 2021). Um all diese Funktionen zu steuern haben sich spezielle Signalmechanismen entwickelt, die verschiedene interne und externe Signale innerhalb der Zelle übermitteln. Rezeptorkinasen sind ein wichtiger Teil eines solchen Mechanismus (Wolf 2022). In Arabidopsis ist die atypische Rezeptorkinase STRUBBELIG (SUB) an zwei wichtigen biologischen Prozessen beteiligt: Entwicklung und Abwehr. Ursprünglich wurde die Bedeutung von *SUB* ausschließlich mit seiner regulatorischen Funktion bei der Morphogenese der Blütenorgane in Verbindung gebracht (Vaddepalli et al. 2011; Kwak et al. 2014). Jüngste Studien zeigen, dass *SUB* für die Morphogenese unter Zellulosemangel und für den, durch den Zellulose-Biosynthese-Inhibitor Isoxaben ausgelösten Zellwandstresserforderlich ist (Chaudhary et al. 2020; Chaudhary et al. 2021).

In dieser Arbeit wurde die Spezifität von SUB im Zusammenhang mit der Zellschädigung durch verschiedenen Enzyme wie Zellulase, Hemizellulase und Pektinase, welche die wichtigsten Bestandteile der Zellwand abbauen, untersucht. Die vorliegenden Daten zeigen, dass SUB ein hochspezifisches Element des Systems der Zellwandintegrität darstellt. Ähnlich wie bei der Reaktion auf Isoxaben löste die Anwendung von Pektinase eine SUB-abhängige Kalloseproduktion aus. Die Behandlungen mit Zellulase und Xyloglucanase führten jedoch zu einer SUB-unabhängigen Reaktion. Außerdem führten alle getesteten Enzyme zu einer verringerten SUB-Präsenz an der Plasmamembran. Darüber hinaus wurde die räumliche und

zeitliche Deposition von Lignin, ausgerufen durch reduzierte Zellulose in Wurzeln, adressiert. Die Daten zeigen, dass die ektopische Lignifizierung zunächst in der Endodermis beginnt und sich später auf benachbarte Zellschichten ausbreitet. Hierdurch wurden neue Mitglieder des *SUB*-Signalwegs, die an der Isoxaben-induzierten Lignifizierung beteiligt sind, identifiziert und untersucht.

1. Introduction

Throughout their lifetime, plants, as immobile organisms, must constantly adjust to alterations in the environment they exist in. Plants regularly face adversities of biotic and abiotic nature, which evoke distinct defensive responses. To survive and thrive, plants evolved specialized mechanisms that can detect system alterations and initiate required reactions. An essential component of plant development, growth, and defense is the cell wall, a strong, complex outer layer composed mainly of carbohydrates such as cellulose, hemicellulose, and pectin. To maintain all necessary functions, the cell wall must be able to replace injured tissue and change its structure without losing its properties. The integrity of the cell wall is monitored by a diverse set of sensors and receptors, which convey a plethora of internal and external signals, ensuring proper developmental patterns and protective reactions (Lampugnani et al. 2018; Engelsdorf et al. 2018; Wolf 2022).

1.1. The plant cell wall

The plant cell wall is a complex yet highly regulated network crucial for plant growth, development, and protection (Wolf 2022). The primary cell wall is predominantly carbohydrate-based and consists of three main classes of polysaccharides: cellulose, hemicellulose, and pectin (Fig. 1). However, the exact composition of the wall is highly variable, depending on the plant species, tissue type, and developmental stage (Lampugnani et al. 2018; Cosgrove 2022). The primary cell wall also contains various enzymes, structural proteins, and signaling molecules that help to regulate the molecular mechanisms responsible for cell wall synthesis and remodeling (Franková et al. 2013; Gigli et al. 2019). Those cells that have completed their cellular development and need to brace the wall structure for functional reasons may be supported by the secondary cell wall, which also contains cellulose but is reinforced by the addition of lignin and xylans (Meents et al. 2018). The functional properties of the cell wall include cell expansion, shape determination, protection, cell-to-cell communication, and cell adhesion (B. Zhang et al. 2021).

1.1.1. Cellulose

Cellulose is the primary structural component of the plant cell wall, providing rigidity and mechanical strength to the plant body. It is a linear homopolysaccharide formed by repeated β -1,4-glycosidic bonds connecting glucose residues that create long and firm microfibrils (Polko

and Kieber 2019). These elastic fibers, together with hemicellulose and pectin, form the inner structure of the cell wall (Cosgrove 2016). Cellulose is produced in the plasma membrane by the cellulose synthase complex (CSC), which is composed of a hexameric cluster (rosette) of cellulose synthase proteins (CESAs) (Somerville et al. 2004). Recent studies suggest that each of the six subunits of the rosette includes 12 to 36 CESA proteins (Nixon et al. 2016). In *Arabidopsis thaliana*, ten isoforms of CESA proteins (CESA1-10) are responsible for cellulose synthesis in specific tissues and at particular stages of development. The different CESA proteins show some functional redundancy but have distinct roles in cell wall biosynthesis (Pedersen et al. 2023). For example, CESA1, CESA3, and CESA6 are involved in primary cell wall formation, whereas CESA4, CESA7, and CESA8 are involved in secondary cell wall formation (Persson et al. 2007; Gonneau et al. 2014; Hill et al. 2014). Mutations in *CESA* genes can lead to defects in cell wall synthesis, resulting in stunted growth, reduced mechanical strength, and increased susceptibility to biotic and abiotic stresses (Hu et al. 2018). Thus, cellulose is also involved in plant defense in addition to its structural role. In stressed plants, the mobility of CSCs on the plasma membrane is significantly reduced. To cope with adverse conditions, cellulose synthesis is rapidly adjusted by the endo- or exocytosis of CSCs. (Bashline et al. 2014; Lei et al. 2015).

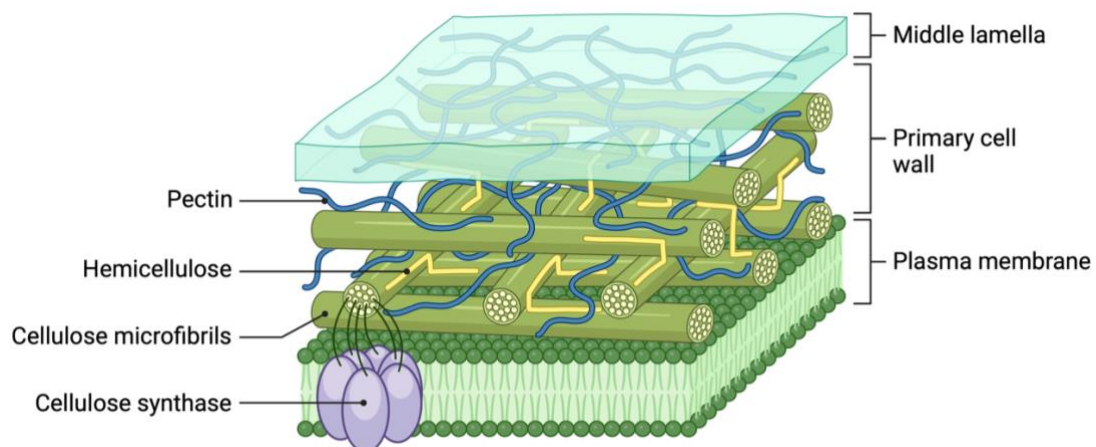


Figure 1. Schematic illustration of the plant cell wall.

The plant cell wall contains cellulose, pectin (homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II), hemicelluloses (xyloglucan, xylan, together with minor fractions of mannan and glucomannan). In some plant tissues, cells also produce a secondary cell wall composed of cellulose, hemicelluloses, and lignin. Adapted from “Plant Cell Wall Structure” by BioRender.com (2023). Retrieved from <https://app.biorender.com/biorender-templates>.

1.1.2. Hemicelluloses

Hemicelluloses belong to a heterogeneous group of polysaccharides that comprise a significant proportion of cell walls and have diverse functions in plant organisms. Hemicelluloses adopt numerous distinct forms by linking two or more monosaccharides, such as xylose, glucose, mannose, and galactose (Scheller and Ulvskov 2010). Their composition and specific arrangement differ according to plant species, tissue type, and developmental stage (W. Zhang et al. 2021). The main classes in primary cell walls are xyloglucans and arabinoxylans, while xylans and glucomannans are predominant in secondary cell walls. Hemicelluloses are synthesized by glycosyltransferases found in the Golgi membranes, and the supply of these components to the cell surface happens through secretory vesicles (Scheller and Ulvskov 2010). They are essential for the structural integrity and physical properties of the cell wall, as well as for cell expansion and growth (Park and Cosgrove 2015; W. Zhang et al. 2021). It has been shown that xyloglucans tether between cellulose microfibrils. However, the interaction seems to be not critical for maintaining the mechanical properties of the cell wall (Kuki et al. 2020; Cosgrove 2022). The biosynthesis and regulation of hemicelluloses represent complex processes involving several transporters and enzymes, such as glycosyltransferases and hydrolases (Scheller and Ulvskov, 2010).

1.1.3. Pectin

The third major category of cell wall polysaccharides is pectin. It is made up of a diverse range of building blocks such as homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and a compact but complicated rhamnogalacturonan-II (RG-II). Arabinans and galactans are generally covalently linked to the RG-I backbone and are also classified as pectic polysaccharides (Ropartz and Ralet, 2020). Cross-linked polymers form pectin-rich zones are found in the middle lamella which is located between the primary walls of neighboring cells and participate in their adhesion (Daher and Braybrook 2015). HG and other pectic polysaccharides are synthesized in the Golgi apparatus and then transported to the cell wall, where they can be incorporated into existing structures or used to build new ones (Yang and Anderson 2020). HG is supplied to the extracellular layer in methylesterified form, the degree of which is important for the mechanical state of the cell wall. Pectin methylesterases (PMEs) facilitate the removal of the methoxyl group, producing a pectic backbone with a free carboxylic acid group, methanol, and a proton. On the other hand, pectin methylesterase inhibitors (PMEIs) regulate the activity of PMEs by limiting their function, thereby controlling the extent of pectin

methylesterification (Yang and Anderson 2020). De-methylesterified sections may be more prone to pectin decomposition, which may promote the loosening of the cell wall (De Lorenzo et al. 2019). The degree of HG methyl esterification is also governed by pectin-degrading enzymes and their regulators, such as polygalacturonases, pectate lyases, and polygalacturonase inhibitors (PGIPs), respectively (Bonnin and Pelloux 2020). The balance between these two sets of enzymes is critical in modulating pectin properties necessary for maintaining optimal cell wall architecture. The egg-box structure is created when the non-methyl esterified portions of HG cross-links with Ca²⁺. This arrangement is essential for securing adhesion between cells and maintaining the robustness of the cell wall (Bonnin and Pelloux 2020). In addition, pectin also plays an important role in cell-cell communication and the regulation of cell wall remodeling (Shin et al. 2021).

1.1.4. Cell wall proteins

Plant cell walls also contain proteins. The main categories of these compounds commonly found in cell walls are hydrolases, proteases, oxidoreductases, signaling proteins, and structural proteins (Albenne et al. 2014). Cell wall proteins (CWPs) have multiple functions. They maintain the structure and function of plant cell walls and support physiological processes such as growth, development, and stress response (Srivastava et al. 2017).

1.1.5. Lignin

As mentioned above, there are two main types of cell walls in plants: primary and secondary. While there are some similarities between primary and secondary cell walls regarding their elemental composition and structure, their functions and developmental regulation are diverse and unique. The primary cell wall is thin and flexible, whereas the secondary wall is thicker and stiffer, consisting mainly of cellulose, lignin, xylans, and glucomannans (R. Zhong and Ye 2009). The secondary cell wall provides mechanical strength and support to the mature cell and is often deposited after the primary cell wall has stopped expanding (Cosgrove and Jarvis 2012). Secondary cell walls are typically found in specialized cells called sclerenchyma, composed of fibers, sclereids, and tracheary elements such as tracheids and vessels. They are also present in the xylem and anther cells (R. Zhong and Ye 2009).

One of the characteristic components of secondary cell walls is lignin. It is a heterogeneous aromatic polymer that provides additional support to plant cell walls (Meents et al. 2018). Literature reports that lignin biosynthesis is controlled by an extensive transcriptional network

(Rogers and Campbell 2004; Nakano et al. 2015; M. Xie et al. 2018). The transcription factors within the NAM, ATAF1,2, and CUC2 (NAC) family are recognized for their crucial function in controlling the formation of secondary cell walls. Elements such as *VASCULAR-RELATED NAC-DOMAIN 6 (VND6)*, *VND7*, *SECONDARY WALL ASSOCIATED NAC DOMAIN PROTEIN1/NAC SECONDARY WALL THICKENING PROMOTING FACTOR 3 (SND1/NST3)*, and *NST1* function as first-layer master switches (Kubo et al. 2005; Mitsuda et al. 2005; R. Zhong, Demura, and Ye 2006). They regulate the biosynthesis of lignin and other secondary cell wall components. Subsequently, the second-layer master switches, namely *MYB46* and *MYB83*, activate additional members of the MYB gene family. These members act as specific regulators of lignin biosynthesis genes encoding activators (such as *MYB20*, *MYB42*, *MYB43*, *MYB58*, *MYB63*, *MYB85*) or repressors (including *MYB4*, *MYB7*, *MYB32*) of this process (S. Zhong et al. 2008; R. Zhong et al. 2011; Taylor-Teeple et al. 2015a; Geng et al. 2020). *MYB46* is known to be a central element of the gene regulatory network that controls secondary cell wall formation (R. Zhong et al. 2007; Taylor-Teeple et al. 2015). Ectopic expression of *MYB46* has been associated with the induction of lignin biosynthetic gene expression (Zhong et al. 2007).

The fundamental elements of lignin are monolignols: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol (Boerjan, Ralph, and Baucher 2003). Polymerization of these components leads to the formation of three types of lignin: H lignin, G lignin, and S lignin (Dixon and Barros 2019). Monolignols are synthesized in the cytosol via the phenylpropanoid pathway, starting from L-phenylalanine. This pathway involves different types of reactions that are assisted by a variety of enzymes (Fig. 2). Certain monolignol derivatives assemble in the central vacuole, while the majority of the monolignol supply is directed to the apoplast. They are then transferred across the plasma membrane to the cell wall (Yao et al. 2021). The process of lignin synthesis also includes the oxidative radical-mediated coupling of monolignols within the cell wall. This reaction is catalyzed by peroxidases and laccases, resulting in the formation of a diverse and randomized polymer structure (Boerjan, Ralph, and Baucher 2003).

Particular laccases and peroxidases can act within various sections of the cell wall (Hoffmann et al. 2020). For instance, *LACCASE 4 (LAC4)*, *LAC17*, and *PEROXIDASE 72 (PRX/PER72)* are incorporated into the secondary cell wall of xylem vessels and fibers. On the other hand, *PER64* and *PER71*, together with the transitory *LAC4*, are found in the cell corners and middle lamellae of fibers during the initiation of lignification (Schuetz et al. 2014; Yi Chou et al. 2018; Hoffmann et al. 2020). In addition, monolignol activation by peroxidases requires

ROS production through NADPH-oxidase RESPIRATORY BURST OXIDASE HOMOLOG F (RBOHF) (Lee et al. 2013).

A distinctive instance of lignification takes place within a narrow section of the root endodermal wall called the Casparian strip, which forms a hydrophobic barrier between the stele and the cortex (Fig. 2) (Barbosa, Rojas-Murcia, and Geldner 2019). Crucial factors governing this process include an NADPH oxidase, a peroxidase, and CASPARIAN STRIP PROTEIN 1 (CASP1), which orchestrates the arrangement of membrane proteins at the Casparian strip (Roppolo et al. 2014; Fujita, De Bellis, et al. 2020).

Perturbation of lignin production can lead to the activation of a compensatory mechanism in the form of excessive activation of the SGN (SCHENGEN) pathway. This pathway serves as a monitoring system, overseeing the stability of the Casparian strips during regular plant growth (Pfister et al. 2014; Doblas et al. 2017). Furthermore, different types of lignin are deposited as a response to various negative biotic and abiotic factors. It has been observed that challenged plant tissues tend to reinforce the primary cell wall of cells that are not usually lignified. These sorts of lignin typically have lower levels of S-units and higher levels of G- and H-units, giving them different chemical and physical properties compared to regular lignins (Barros et al. 2015).

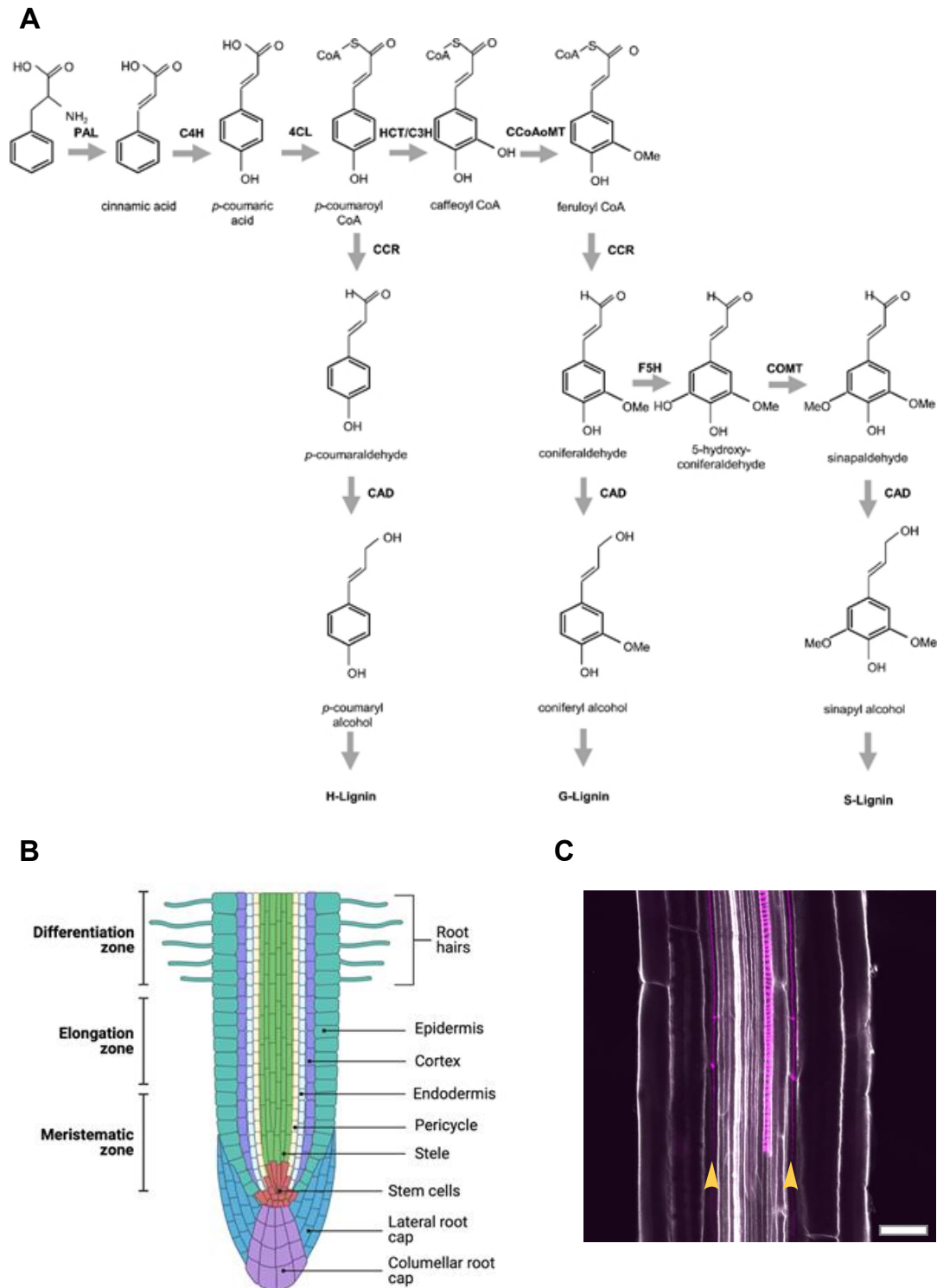


Figure 2. Lignin biosynthesis pathway and a schematic illustration of root morphology.

(A) Schematic representation of the enzymatic reactions involved in the biosynthesis of monolignol from L-phenylalanine. Enzymes are L-phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H); shikimate hydroxycinnamoyl transferase (HCT); coumaroyl shikimate 30-hydroxylase (C30H); coumarate 3-hydroxylase (C3H); caffeic acid/5-hydroxy-coniferaldehyde 3/5-O-methyltransferase (COMT); 4-hydroxycinnamate CoA ligase (4CL); caffeoyl CoA 3-O-methyltransferase (CCoAoMT); cinnamoyl CoA reductase (CCR); cinnamyl alcohol dehydrogenase (CAD); and ferulic acid/coniferaldehyde 5-hydroxylase (F5H). Modified from (Yoon, Choi, and An 2015).

(B) Schematic illustration of root cell layers. Adapted from “Root Meristem Anatomy” by BioRender.com (2023). Retrieved from <https://app.biorender.com/biorender-templates>.

(C) Confocal micrographs showing a cross-section of the Col-0 root. Lignin present in the xylem and in the Casparian strip is shown in magenta (stained with Basic Fuchsin). Cell walls are shown in gray (stained with Calcofluor White). Yellow arrowheads indicate Casparian strips in endodermal cells.

1.1.6. Callose

Callose is a linear β -1,3-glucan polysaccharide composed of glucose residues and bound by β -1,3-glycosidic bounds. It is synthesized by a plasma membrane-associated multisubunit enzyme complex, the major component of which is callose synthase (Verma and Hong 2001; J. Li et al. 2003). Twelve members of the CALLOSE SYNTHASE (CalS) family, also annotated as the GLUCAN SYNTHASE-LIKE (GSL) family, contribute to callose synthesis and exhibit time- and tissue-specific expression patterns (Verma and Hong 2001; Richmond and Somerville 2000). In plants, callose is involved in many important biological functions: it is deposited during cell plate formation (CalS1 and CalS10) and is crucial for proper pollen development (CalS5, CalS11, and CalS12) (Hong, Delauney, and Verma 2001; Hong et al. 2001; Enns et al. 2005; B. Xie, Wang, and Hong 2010; Ellinger and Voigt 2014). Callose is also found in sieve plates in the phloem (CalS7) and in the neck regions of plasmodesmata, helping to regulate permeability during stress (CalS3 and CalS10) (Vatén et al. 2011; Sevilem, Miyashima, and Helariutta 2013; Ellinger and Voigt 2014; Han et al. 2014). Furthermore, in response to pathogen attack, callose is produced precisely between the plasma membrane and the existing cell wall (CalS12) (Jacobs et al. 2003; Hessler et al. 2021). Fortification of the plant cell wall with callose serves as a robust chemical and physical defense strategy against invading pathogens. In addition, callose provides mechanical support against various environmental stresses such as injury, low temperature, and heavy metals (Piršelová and Matusšíková 2013). Thus, callose is an integral part of both developmental processes and stress responses in plants.

Each cell wall component has unique properties that together form a strong outer layer enclosing the plant cell. The mechanical features achieved by the composition of the plant cell wall ensure its participation in many physiological processes corresponding to plant development and adaptation. The functions of the plant cell wall are, therefore, rather broad.

1.2. The role of the cell wall in plant morphogenesis

Plant morphogenesis, a temporally controlled and spatially oriented biological process, is inherently linked to the plant cell wall. This complex and dynamic structure contributes fundamentally to the overall structure of the plant. Morphogenesis is viewed as a dynamic mechanism by which cells develop their external shape and internal organization, involving individual cells, tissues, and the entire plant (Marconi and Wabnik 2021). The plant cell wall provides critical mechanical support, acts as a signaling interface between the cell and its environment, and plays an important role in the development of plant tissues (Bacete and Hamann 2020). The composition and structural elements of the cell wall play a critical role in regulating cell shape and size by influencing the direction of cell expansion. In addition to cellulose microfibrils, which impart tensile strength and mechanical anisotropy to walls, polysaccharides such as pectin and hemicellulose also affect various phases of development and growth (De Lorenzo et al. 2019). Pectin, a major component of the middle lamella that cements adjacent cells together, regulates cell-to-cell adhesion and, when degraded, can lead to cell separation and tissue remodeling (Palin and Geitmann 2012). In addition to its mechanical properties, the cell wall is also involved in the transduction of various signals critical for plant morphogenesis (Jonsson et al. 2022). These signals are recognized by receptor kinases and other transmembrane proteins in the cell wall, initiating intracellular signaling cascades that regulate gene expression and other cellular processes (Wolf 2022). Furthermore, the mechanochemical makeup of the cell wall can be influenced by plant hormones such as auxin, ethylene, and jasmonate (Jonsson et al. 2022). The interplay between mechanical and hormonal signals is pivotal in directing plant development. Through mechanisms such as changes in gene expression and cell wall modifications, these hormones translate mechanical signals into growth responses and regulate morphogenetic processes (Jonsson et al. 2022). Thus, through the interplay of mechanical and chemical cues, the plant cell wall serves as a critical regulator of plant morphogenesis.

1.3. The role of the cell wall in stress responses

Due to their stationary growth mode, plants have adjusted to their restricted terrestrial habitats by displaying high adaptability in their responses to current conditions, including nutrient accessibility, light, and temperature levels, as well as toxic or harmful factors (Mareri, Parrotta, and Cai 2022). The cell wall biosynthesis undergoes significant modifications in response to environmental cues, serving as a common adaptive strategy (Malinovsky, Fangel, and Willats

2014; Engelsdorf et al. 2018). Plants approach constant biotic and abiotic stresses through two types of responses: immediate and long-term. Rapid reactions engage instant changes in the activities of proteins essential for cell wall synthesis within a short timeframe, while long-term responses involve the regulation of multiple genes associated with cell wall metabolism to sustain plant growth under unfavorable conditions (B. Zhang et al. 2021). In response to cell wall damage, the movement of CSCs at the plasma membrane is reduced, and CSCs are either internalized or released from the plasma membrane through endocytosis enabling fast adjustments in cellulose synthesis (Lei et al. 2015). To maintain the mobility of CSCs and sustain cellulose synthesis under prolonged saline conditions, cellulose synthase cofactors like CC1/2, which stabilize cortical microtubules (CMTs), play a crucial role (Endler et al. 2015). In addition to abiotic stresses, the cell wall serves as a physical barrier against pathogens. Plant innate immunity relies on the activation of defense responses upon recognition of pathogen-derived or damage signals. Damage-associated molecular patterns (DAMPs) play a crucial role in immune responses and tissue repair (Ferrari et al. 2013). The primary example of DAMPs are OGs released by the degradation of the pectin homogalacturonan by pectinases (Ferrari et al. 2013). *Arabidopsis* WALL-ASSOCIATED KINASE 1 (WAK1) functions as a receptor for OGs (Brutus et al. 2010) triggering early signaling events such as ROS and nitric oxide production, callose accumulation, and MAPK-mediated activation of defense marker gene expression (Galletti et al. 2008, 2011; Rasul, Dubreuil-Maurizi, et al. 2012). Further studies showed that cellobiose, a small oligomer of cellulose, can be sensed by *Arabidopsis* plants, resulting in the induction of a signaling cascade and greater expression of defense-related genes (de Azevedo Souza et al. 2017). Cellobiose treatment also stimulated an immediate but temporary increase in intracellular calcium levels, similar to other known DAMPs. Interestingly, when combined with other elicitors, cellobiose showed an additive or synergistic effect on calcium signatures, particularly with flg22 (de Azevedo Souza et al. 2017). Furthermore, cellobiose activated MAPKs at an early stage, suggesting that its perception may involve receptor-mediated processes at the cell surface, given the observed rapid response to this cellulose degradation product (de Azevedo Souza et al. 2017). In addition, recent studies have demonstrated that xyloglucan fragments derived from hemicellulose can elicit immune responses in both grapevine and *Arabidopsis*. These responses include the upregulation of defense genes, MAPK activation, as well as callose deposition (Claverie et al. 2018). Notably, the protective reactions induced by xyloglucan appear to be more transient compared to other well-known elicitors. While research has provided insight into the perception and signaling mechanisms of xyloglucan oligosaccharides, the exact receptor involved remains elusive

(Claverie et al. 2018). Understanding the responses of plant cell walls to environmental cues and their role in adaptive strategies is essential for improving plant resilience and productivity.

Further investigation is needed to elucidate the precise mechanisms of pattern recognition and signal transduction in response to different oligosaccharides derived from cellulose, pectin, and hemicellulose.

1.4. Selected examples of cell wall integrity receptors

1.4.1. *Catharanthus roseus* RLK1-like kinases

The *Catharanthus roseus* RECEPTOR-LIKE KINASE1-LIKE (CrRLK1L) protein family is involved in cell wall sensing (CWS) mechanisms. Although the binding mechanism is not fully understood, it has been proposed that CrRLK1L proteins interact with cell wall carbohydrates through their malectin-like extracellular domain (Schallus Thomas et al. 2009; Moussu et al. 2018). To maintain their activity, CrRLK1Ls also interact with LORELEI-LIKE GLYCOSYLPHOSPHATIDYLINOSITOL (GPI)-ANCHORED PROTEINS (LLGs) (Xiao et al. 2019). The intracellular region of CrRLK1Ls contains a kinase domain, but the exact role of kinase activity in the CrRLK1L function has yet to be elucidated. Intracellular domain-swapping studies suggest that downstream signaling may be shared between CrRLK1Ls, while the extracellular section determines their individual properties (Kessler et al. 2015). FERONIA (FER), the most studied member of the CrRLK1L family, acts as a receptor for RAPID ALKALINIZATION FACTOR 1 (RALF1) peptide and interacts with LEUCINE-RICH REPEAT EXTENSIN (LRX) proteins (Stegmann et al. 2017; Dünser et al. 2019). It has been reported that FER activity is required for multiple aspects of plant viability, including development, immunity, and cell wall sensing. For instance, it mediates vacuolar expansion as a reaction to signals sent by the cell wall (Dünser et al. 2019). FER is also involved in salt tolerance and response to mechanical stress. Increased salinity leads to diminished root growth and simultaneously to a loss of cell anisotropy and a reduction in the stiffness of the cell walls (Feng et al. 2018; Kong et al. 2023). THESEUS 1 (THE1) is another receptor involved in multiple processes related to CWI maintenance and adaptive reactions (Cheung and Wu 2011). It plays a role in controlling cell wall stiffness and is affected by changes in cell wall composition and structure (Bacete et al. 2022). A decrease in cellulose biosynthesis leads to various protective responses such as growth arrest, lignin production, and activation of defense genes. Previous research has shown that *THE1* activity is required for most of these responses (Hématy et al. 2007; Qu et al. 2017). It also acts as a receptor for the RALF peptide RALF34 and is involved in lateral root initiation (Gonneau et al. 2018). The interactions between

CrRLK1Ls, RALF peptides, LRX proteins, and cell wall components are complex and require further investigation. However, the importance of these receptor kinases in CWS and their diverse roles in plant expansion, development, and immunity are indisputable.

1.4.2. Wall-Associated Kinases (WAKs)

The wall-associated kinases (WAKs) are a group of receptor kinases that contain a cytoplasmic serine-threonine kinase, a transmembrane domain, a number of epidermal growth factor repeats, and a pectin-binding domain called GUB, which is situated in the extracellular part (Kohorn 2016). The *Arabidopsis* genome contains five WAK genes, which are closely clustered in a 30-kb locus on chromosome one and share a high degree of similarity in their kinase domains and extracellular regions (He et al. 1999). WAKs are considered to be plasma membrane receptors and are associated with the cell wall surface (Anderson et al. 2001; Wagner 2001). Previous research has shown that WAKs are necessary for cell expansion (Wagner 2001). In addition, pathogen attack, physical injury, and herbivory induce the production of oligogalacturonides (OGs) and simultaneously WAK expression (Harholt et al. 2010; Brutus et al. 2010; Kohorn and Kohorn 2012). In vitro, both WAK1 and WAK2 bind to different types of pectins, such as homogalacturonan, both rhamnogalacturonans, and OG fragments (Kohorn et al. 2009). Studies have shown that WAKs can activate different downstream pathways depending on the state of the cell wall, such as the cell expansion cascade with MPK3 when the walls are intact and expanding and the stress response with MPK6 when OGs are produced by wall disruption (Kohorn et al. 2009, 2012). In summary, at least some of the WAK genes are involved in regulating cell wall integrity, stress responses, and controlling growth and development. Therefore, a better understanding of the cell wall properties and the molecular mechanisms behind the processes occurring in the cell is of paramount importance.

1.4.3. Leucine-Rich Repeat Receptor Kinases (LRR-RK)

The leucine-rich repeat receptor kinase (LRR-RK) family is the largest class of plant receptor kinases, with over 200 members in *Arabidopsis thaliana* (Wu et al. 2016). These membrane-bound receptors consist of a leucine-rich extracellular domain, a transmembrane domain, and a cytoplasmic kinase domain with serine/threonine specificity. This arrangement suggests that LRR-RKs play a significant role in signal perception at the plasma membrane (Osakabe et al. 2013). It has been shown that LRR-RKs form heterodimers with co-receptors upon ligand

binding, activating the cytoplasmic kinase domain and initiating downstream signaling (Xi et al. 2019). Understanding the mechanism of biological processes mediated by LRR-RKs highly depends on their structural features and their complexes with corresponding peptide ligands and co-receptors. Functionally characterized LRR-RKs contribute to a wide variety of fundamental processes in the plant organism. For instance, certain LRR-RKs, such as CLAVATA 1 (CLV1), CLAVATA 2 (CLV2), and their ligand CLAVATA 3 (CLV3), are involved in maintaining the stability between cell proliferation and differentiation in the shoot meristem to ensure proper organ formation (Hu et al., 2018). BRASSINOSTEROIDS INSENSITIVE 1 (BRI1) is a key receptor involved in brassinosteroid signaling that promotes plant stature and shape (Nam and Li 2002). The LRR-RKs SOMATIC EMBRYOGENESIS RECEPTOR KINASES 1 and 2 (SERK1 and SERK2) are critical for anther development, an essential process in plant reproduction (Colcombet et al. 2005). Furthermore, FEI1 and FEI2 have been implicated in cell wall sensing (CWS) mechanisms. FEI1 and FEI2 are essential for cellulose synthesis, controlled cell elongation under high sugar conditions, and the correct structure of the seed coat mucilage (Basu et al. 2016). Another LRR-RK, MDIS1-INTERACTING RECEPTOR LIKE KINASE 2 (MIK2), is linked to THE1 and is required for the response to inhibition of cellulose biosynthesis and for combating abiotic and biotic stresses. The involvement of MIK2 in different processes suggests its role as a potential integrating element of developmental and protective responses (Van der Does et al. 2017).

Overall, LRR-RKs play diverse roles in cell wall sensing, development, and stress responses, highlighting the intricate integration of cell wall-related processes with different signaling pathways in plants.

1.5. Downstream signaling processes involved in response to cell wall damage (CWD)

In plants, the protective reaction to cell wall damage involves a series of downstream signaling events that employ diverse cellular and molecular responses. One of the key elements in initiating the necessary processes is the activation of cell surface receptor proteins, such as RLKs and RLPs, to recognize specific DAMPs released from the impaired cell wall (Escocard de Azevedo Manhães et al. 2021). The accumulation of reactive oxygen species (ROS) in the damaged cells serves as a warning mechanism that propagates the message to neighboring cells to amplify the response (Choudhury et al. 2017). In addition, ROS production activates MAPK cascades, which are a series of protein kinases that deliver information from the cell surface to the nucleus. The MAPK pathway plays a critical role in coordinating various intracellular responses, including gene expression, production of defense molecules, and reinforcement of

the cell wall (Son et al. 2011). Detection of the ongoing cell wall damage also leads to the influx of calcium ions (Ca²⁺) into the cytoplasm. Calcium is considered a second messenger that contributes to the activation of specific calcium-dependent protein kinases (CDPKs) and the regulation of gene expression (Ma et al. 2013). All of these signaling events ultimately result in the induction or repression of specific genes that control the defense and repair machinery. Consequently, many different transcription factors, such as MYB and WRKY, are often involved in regulating the expression of defense-related genes (X. Wang et al. 2021; H. Wang et al. 2023). In addition, the biosynthesis of hormones such as jasmonic acid (JA) and salicylic acid (SA) is also closely related to immune responses in plants (Kachroo and Kachroo 2012). To support the damaged area of the cell wall, plants deposit additional components in the form of callose and lignin, which were described in the chapter on the plant cell wall. Stress-induced callose synthesis and degradation can help control plasmodesmata permeability. During a pathogen attack, callose accumulates between the plasma membrane and the existing cell wall, forming a chemical and physical barrier (Y. Wang et al. 2021a). Lignin provides additional strength to the primary cell wall of challenged plant tissues (Le Gall et al. 2015).

In summary, plants are constantly exposed to harmful external cues and must resist them by launching a chain reaction of accurate internal processes.

1.6. The *STRUBBELIG* signaling pathway and its role in plant morphogenesis

1.6.1. *STRUBBELIG* is an atypical receptor kinase

STRUBBELIG (SUB), also known as *SCRAMBLED* (SCM), is an atypical leucine-rich repeat transmembrane receptor kinase (LRR-RK) found in *Arabidopsis*. SUB belongs to the LRR-V/*STRUBBELIG*-RECEPTOR FAMILY (SRF) of LRR RKs (Shiu and Bleecker 2001; Eyüboğlu et al. 2007). SUB is made up of three components: an extracellular domain, a transmembrane, and an intracellular domain (Fig. 3A). The extracellular domain includes a signal peptide, an amino-terminal SUB domain which is common among the LRR-V/SRF group, six LRRs, and a proline-rich region. The intracellular domain provides the juxtamembrane domain as well as a carboxyl-terminal kinase domain (Chevalier et al. 2005). This arrangement suggests that SUB is involved in signaling across the membrane and hence in intercellular communication. As previously stated, SUB appears to be a part of the atypical or 'dead' receptor kinases, as its *in vivo* activity does not require a functional kinase domain. However, atypical RKs are not excluded from being an important part of plant signaling pathways (Chevalier et al. 2005; Vaddepalli et al. 2011).

1.6.2. *SUB* regulates tissue morphogenesis in Arabidopsis

Phenotypic analysis of Arabidopsis mutants lacking *SUB* function revealed aberrant floral organ morphology, including unusual ovule integument initiation and outgrowth (Fig. 3B). Moreover, *sub* mutants exhibit stem and silique twisting, accompanied by abnormal leaf shape and reduced plant height (Fig. 4F-J) (Schneitz et al. 1997; Fulton et al. 2009). Further studies indicate that *SUB* signaling is involved in the positioning of cell division planes in the floral meristem layer (Vaddepalli et al. 2011; Kwak et al. 2014; Vaddepalli et al. 2017). *SUB* also supports undefined root epidermal cells to decode their position in correspondence to cortical cells and determine root hair cell patterning (Fig. 3C) (Kwak et al. 2005). The mutation in the *sub-2* allele is characterized by temperature sensitivity as well as defects in leaf morphology, suggesting that *SUB* activity affects leaf development (Lin et al. 2012).

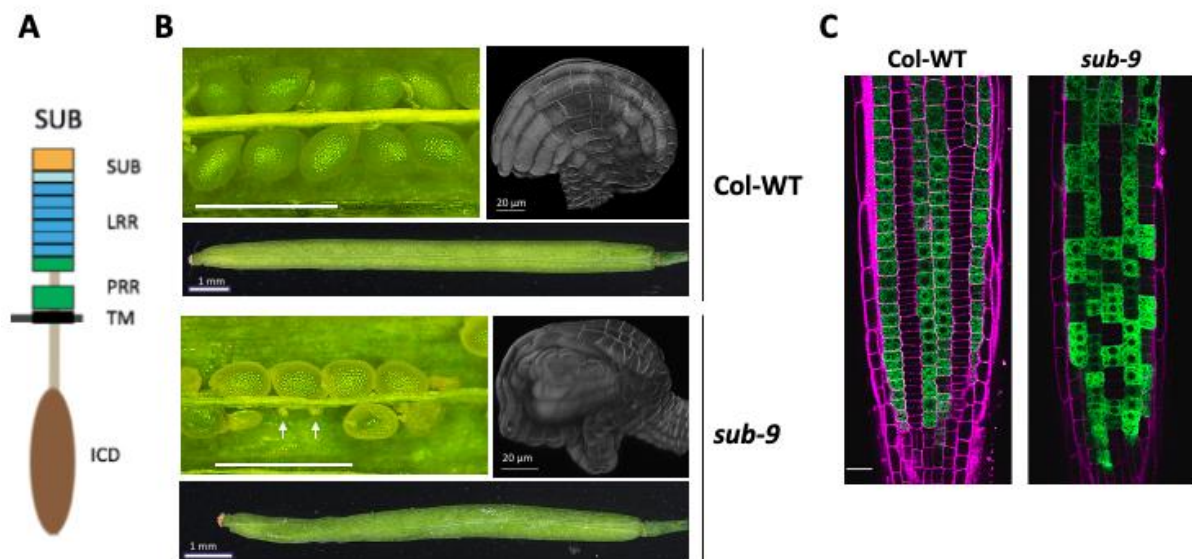


Figure 3. Overview of the domain architecture of SUB and phenotypic comparison of Col-0 and *sub-9*.

(A) Arrangement of SUB receptor kinase. Abbreviations: SUB, SUB-domain; LRR, leucine-rich repeat; PRR, proline-rich repeat; TM, transmembrane domain; ICD, intracellular domain including kinase domain. Length of SUB protein: 768 amino acids. (B) Comparison of seed development, ovule, and silique morphology between Col-WT and *sub-9*. Note aborted ovules, untypical integument outgrowth, and twisted siliques in *sub-9*. Genotype labeling is visible in the panel. (C) Root hair patterning differences between wild-type and *sub-9* visualized by pGL2::GUS:EGFP reporter line.

1.6.3. Additional components of the *SUB* signaling pathway (*QKY*, *DOQ/AN*, *ZET*)

To explore the signaling mechanisms of *SUB* a forward genetic approach was applied. This led to the discovery of recessive mutations in three genes, *DETORQEO* (*DOQ*), *QUIRKY* (*QKY*), and *ZERZAUST* (*ZET*), whose phenotypes resemble those of *sub* mutants (Fulton et al. 2009). Plants with defects in *DOQ*, *QKY*, and *ZET* displayed corresponding abnormalities in outer integument development, floral organ shape, stem twisting, floral meristem cellular defects, and root hair patterning (Fig. 4). These genes collectively constitute the STRUBBELIG-LIKE MUTANT (SLM) class (Fulton et al. 2009). Cytoplasm-localized *DOQ* has been identified as the *ANGUSTIFOLIA* (*AN*) protein (Bai et al. 2013). Molecular characterization of *DOQ/AN* has provided insights into its contributions to plant organogenesis and shed light on the complex mechanisms governing these developmental processes. In addition, *AN* is recognized as an important player in plant resistance to both hemibiotrophic and necrotrophic pathogens (M. Xie et al. 2020). *AN* exhibits antagonistic regulation of the salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET) signaling pathways. The co-regulation of *MYB46* and *WRKY33* by *AN* enables the coordination of the SA and JA/ET pathways, facilitating the plant's ability to mount effective defenses against different types of pathogens (M. Xie et al. 2020).

QKY is another gene belonging to the SLM class of genes in Arabidopsis. It has been molecularly characterized as a putative transmembrane protein carrying four C2 domains, suggesting its involvement in membrane trafficking in a calcium-dependent manner (Fulton et al. 2009). *QKY* is preferentially located at plasmodesmata (PD) and it has been shown that *QKY* promotes the progression of *SUB* signaling through direct interaction at the PD (Vaddepalli et al. 2014; Song et al. 2019). Furthermore, *QKY* is required to maintain *SUB* at the plasma membrane by preventing its internalization (Song et al. 2019; Chaudhary et al. 2020).

Finally, cell wall-localized *ZET* encodes a putative GPI-anchored β -1,3-glucanase, that is localized in the cell wall. Its precise role in callose metabolism is still unclear. Studies have revealed that *ZET* acts in a non-cell autonomous manner and is essential for cell wall assembly during tissue formation (Vaddepalli et al. 2017). The characterization of *ZET* has provided insights into its contribution to plant development and elucidated the molecular mechanisms underlying cell wall dynamics. Analysis of single and double-mutant combinations as well as a comprehensive comparison of transcript profiles during flower development demonstrated mutual but also recognizably different roles of *SLM* genes in plant morphogenesis (Fulton et al. 2009).

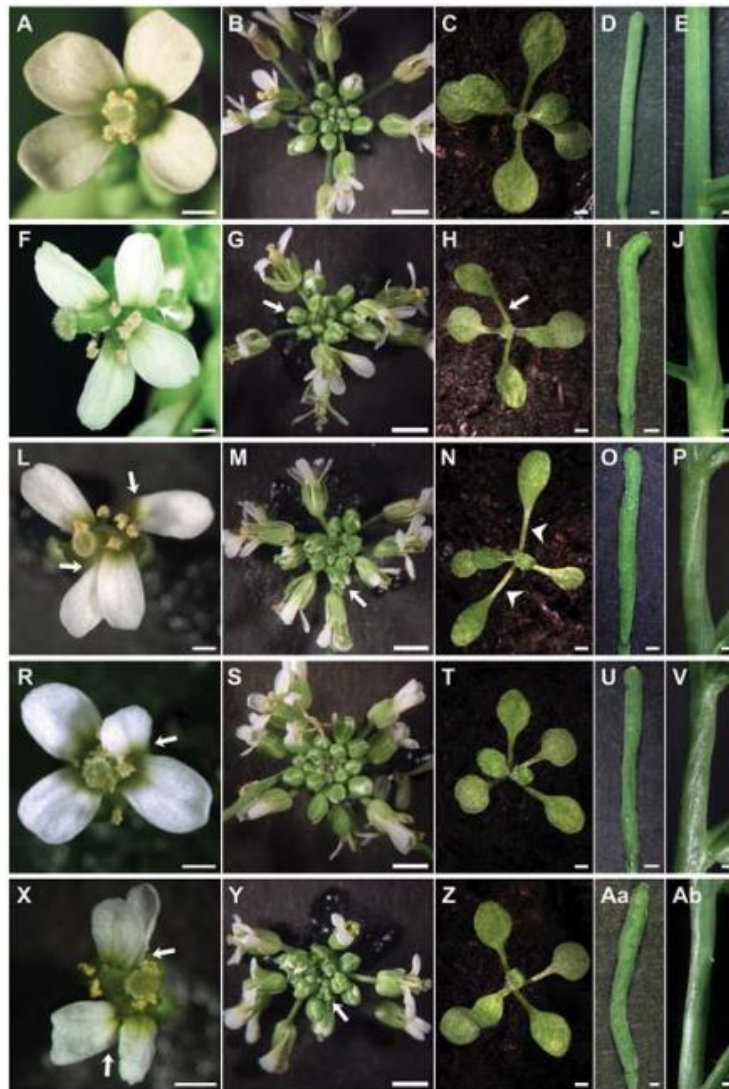


Figure 4. Comparison of the overall above-ground morphology of *sub-1*, *doq-1*, *qky-8*, and *zet-2* mutants.

(A–E) Wild-type Ler. (F–J) *sub-1*. (L–P) *doq-1*. (R–V) *qky-8*. (X–Ab) *zet-2*. (A, F, L, R, X) An open stage 13 flower from a 30-day-old plant. Note the misorientation of petals due to twisting in the basal end of the petal structure (arrows). (F, R, X) Petals can also show small notches. (B, G, M, S, Y) Top view of a 30-day inflorescence. (G, M, S, Y) Flower phyllotaxis is irregular. Arrows mark prematurely opened flower buds. (C, H, N, T, Z) Top view of a 12-day rosette. (H, N) Leaf petioles can be twisted (arrows). (N) *doq-1* leaves have longer petioles and narrow blades (arrowheads). (D, I, O, U, Aa) Morphology of mature siliques. Typical twisting was observed in (I) *sub-1*, (U) *qky-8* and (Aa) *zet-2* mutants. *doq-1* exhibits more subtle twisting in both (O) siliques and (P) stems. (E, J, P, V, Ab) A lateral view of a section of stems from a 30-day plant. (V) *qky-8* and (Ab) *zet-2* mutant show twisting in stems equivalent to (J) *sub-1* plants. Scale bars: (A, D, E, F, I, J, L, O, P, R, U, V, X, Aa, Ab) 0.5 mm, (B, C, G, H, M, N, S, T, Y, Z) 2 mm. Modified from Fulton et al. 2009.

1.7. Role of the receptor kinase STRUBBELIG in adaptation to reduced cellulose biosynthesis in *Arabidopsis thaliana*

In *Arabidopsis*, the atypical receptor kinase STRUBBELIG plays an important role in at least two different biological processes: the regulation of tissue morphogenesis and the response to cell wall damage. Obtained results indicate that *SUB* affects multiple aspects of the stress response induced by the cell wall damage (Chaudhary et al. 2020, 2021).

1.7.1. *SUB* is required for cellulose synthesis inhibition (CBI)-induced cell wall stress response

The herbicide isoxaben (ISX) is a well-characterized inhibitor of plant cellulose biosynthesis. It primarily targets the activity of cellulose synthase enzymes, which are responsible for generating cellulose, a crucial component of plant cell walls (Desprez et al. 2002). Isoxaben acts in a targeted manner by hindering the integration of glucose units into the acid-insoluble cell wall fraction (Heim et al. 1990). Additionally, at the cellular scale, isoxaben is able to initiate the rapid clearance of CESA6 from the plasma membrane into cytoplasmic vesicles, thereby causing a disturbance within the CESA complex (Paredes, Somerville, and Ehrhardt 2006). By interfering with cellulose production, isoxaben disrupts the integrity of the cell wall and leads to several adverse effects on normal plant growth and development. The main effect of isoxaben on plants is the disturbance in cell expansion and elongation during root growth (Engelsdorf et al. 2018). Since cellulose provides structural support to cell walls, its reduction hampers the ability of cells to stretch and enlarge, resulting in stunted growth and abnormal morphology.

Previous research suggests that the *SUB* function is necessary to maintain the size and form of root epidermal cells and recover root growth after temporary exposure to isoxaben. It has also been proposed that *SUB* signaling is a part of the initial increase of intracellular reactive oxygen species and induction of stress genes such as *CCR1*, *PDF1.2*, or *RBOHD* (Chaudhary et al. 2020). Furthermore, *SUB* reduces cell bulging and accelerates root growth recovery following exposure to isoxaben. Additionally, isoxaben-induced cell wall defects can trigger balancing reactions to maintain the structural integrity of the plant cell wall and minimize the damaging effects (Hamann et al. 2009). These responses include the deposition of additional components, such as lignin and callose, to reinforce weakened cell walls. Interestingly, seedlings lacking *SUB* activity do not properly promote these protective mechanisms when subjected to isoxaben treatment. These results suggest that *SUB* contributes to compensatory

lignification in roots and callose production in cotyledons upon cell wall stress (Chaudhary et al. 2020).

1.7.2. Morphogenesis in Arabidopsis during cellulose deficient condition is regulated by the activity of *SUB*

The presence of *SUB* is critical for the regulation of floral organ morphogenesis and root hair patterning, not only under normal conditions but also when subjected to stress (Kwak and Schiefelbein 2007; Vaddepalli et al. 2011; Chaudhary et al. 2021). Exposure to sub-lethal concentrations of isoxaben induces increased internalization of *SUB* and a dose-dependent decrease in *SUB* transcript levels. These effects on *SUB* abundance have profound consequences for floral organ morphogenesis and root hair patterning (Chaudhary et al. 2021). Downregulation of *SUB* by isoxaben treatment results in phenotypes similar to *sub* loss-of-function mutants. Apparent defects in floral organ development included abnormal floral meristem cell division planes and impaired ovule development (Chaudhary et al. 2021). In root hair patterning, isoxaben-induced reduction of *SUB* levels leads to perturbations in root hair distribution and differentiation, causing arrangement defects (Chaudhary et al. 2021). Importantly, the ectopic expression of *SUB* can attenuate the detrimental effects of isoxaben on these developmental processes. This suggests that maintaining adequate levels of *SUB* is biologically relevant for proper floral organ morphogenesis and root hair patterning (Chaudhary et al. 2021).

The role of *SUB* in these processes highlights its importance as a key regulator that integrates cell wall status and developmental signaling to ensure appropriate responses to CBI-induced cell wall stress.

1.8. Objectives

In Arabidopsis, the atypical receptor kinase *SUB* plays an important role in at least two different biological processes: the regulation of tissue morphogenesis and the response to cell wall damage (Chevalier et al. 2005; Vaddepalli et al. 2011; Chaudhary et al. 2020 and 2021). Although a few biological questions have been answered, many aspects remain to be explored. This research is centered around two key unknowns: first, the role of *SUB* in the cell wall damage response induced by treatments other than isoxaben, and second, the extent to which the role of *SUB* is universal across these processes. These questions have been addressed by using cell wall degrading enzymes, namely cellulase, pectinase, and xyloglucanase. The

degradation of essential cell wall components simulates the type of damage a plant cell might experience during a pathogen attack or environmental stress. The study was designed to gain insight into the mechanisms of maintenance and repair of cell wall integrity. Implementing a combination of genetic, biological, and pharmacological techniques, it has been possible to elucidate which specific cellular responses to cell wall damage require *SUB* function.

Understanding the mechanisms of isoxaben-induced lignification, a critical *SUB*-dependent cell wall damage response, was another important goal of this study. Delving deeper into the lignification contributed to a more comprehensive understanding of this process.

In addition, this research aimed to identify new potential candidates involved in the *SUB* pathway and to test their involvement in cell wall integrity responses.

2. Materials and Methods

2.1. Plant work and plant genetics

Arabidopsis thaliana (L.) Heynh. var. Columbia (Col-0) and var. Landsberg (erecta mutant) (Ler) were used as wild-type strains. Plants were grown as described earlier (Fulton et al., 2009). The *sub-1*, *qky-8*, *zet-1*, *an-1/doq-1* (all in Ler), and the *sub-9*, *sub-21*, and *qky-11* mutants (Col) have been characterized previously (Chevalier et al., 2005; Fulton et al., 2009; Vaddepalli et al., 2011; Vaddepalli et al., 2014, Chaudhary et al., 2020). The lines carrying pSUB::SUB:EGFP (*sub-9*, *sub-1*, *zet-1*, *an-1*, *qky-8*), pUBQ::SUB:mCherry (O3, L1, Col) and pGL2::GUS:EGFP O3 was generated previously (Vaddepalli et al., 2014). Seedlings were grown on half-strength Murashige and Skoog (1/2 MS) agar plates (Murashige and Skoog, 1962). Before sowing seeds on 1/2 MS, they were surface sterilized in 3.5% (V/V) sodium hypochlorite (NaOCl) plus 0.1% (V/V) Triton X-100 for 15 min on a rotator to prevent bacterial and fungal growth on plates. Seeds were washed four times with ddH₂O and stratified for 2d at 4°C prior to plating. Dry seeds were stratified for 2d at 4°C in ddH₂O and sown on soil (Patzer Einheitserde, extra- gesiebt, Typ T, Patzer GmbH & Co. KG, Sinntal-Jossa, Germany) situated above a layer of perlite, and placed in a long day cycle (16h light) using Philips SON-T Plus 400 Watt fluorescent bulbs. The light intensity was 120-150 μmol/m²sec. The plants were kept under a lid for 5-6 days to increase humidity (50-60%) and support equal germination.

Table 1. List of candidate genes used in the study.

No.	Gene name	AGI CODE	Line
1	<i>CASPL1B1</i>	AT5G44550	SAIL_114_C02
2	<i>COBL7</i>	AT4G16120	SALKseq_72381
3	<i>GSL5/PMR4</i>	AT4G03550	position 2060 G→A substitution
4	<i>GSL7/CalS7</i>	AT1G06490	SALK_048921, SAIL_114_A01
5	<i>HERK2</i>	AT1G30570	SALK_105055C
6	<i>LMK1</i>	AT1G07650	SALK_120356
7	<i>RUL1</i>	AT5G05160	SALK_121868C
8	<i>SIMP1</i>	AT2G37050	SAIL_308_C12
9	<i>SRF3</i>	AT4G03390	SALK_051048C
10	<i>SRF7</i>	AT3G14350	SALK_039120C

2.2. Arabidopsis genomic DNA extraction and genotyping PCR

DNA was extracted from a piece of leaf tissue approximately 1 cm in diameter. The leaf disc was frozen in liquid nitrogen and ground to a fine powder (Qiagen grinder). The homogenized tissue was suspended in 400 μ L gDNA extraction buffer and mixed by inverting Eppendorf tubes. The mixture was centrifuged at 13000 rpm for 15 minutes at 4°C. 280 μ L of the supernatant was transferred to a new tube. 300 μ l of isopropanol was added to the supernatant, mixed by inversion, incubated for 20 min at -20°C, and centrifuged at 13000 rpm for 15 min. The pellet was washed with 1 ml of 70% ethanol, dried completely and resuspended in 100 μ l of or ddH₂O. The whole preparation was stored at -20°C until use. PCR-based genotyping was performed using the genotyping primer combinations listed in Table S1. PCR reaction mix and cyclor program is presented in Table 2.

Table 2. PCR reaction mix and cyclor program.

Reaction mix for Taq polymerase based PCR amplification		
Components/reaction	Volume (μl)	
10x Standard Taq Reaction buffer	2.5	
2 mM dNTPs	1.5	
10 μ M Forward primer	1	
10 μ M Reverse primer	1	
MgCl ₂	0.25	
Taq polymerase (5 U/ μ l)	0.125	
Template DNA (100 ng made up to 1 μ l)	4	
Sterile double distilled water	14.625	
PCR Cyclor program for Taq polymerase		
Temperature	Time	Cycles
95 °C	5 min	1
95 °C	30 s	30-36
X °C	30 s	
72 °C	1 min/ kb	
72 °C	5 min	1

2.3. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy was performed using an Olympus FV1000 setup with an inverted IX81 stand and FluoView software (FV10-ASW version 01.04.00.09) (Olympus Europa GmbH, Hamburg, Germany) equipped with a water-corrected 40x and 60x objective (NA 0.9 and 1.20 respectively) at 3x digital zoom. For subcellular localization of SUB: EGFP after drug treatments or colocalization with an endosomal marker, confocal laser scanning microscopy was performed on epidermal cells of root meristems located approximately 100 μm above the quiescent center using an Olympus FV1000 microscope equipped with HSD and PMT detectors. The scanning speed was set to 400 Hz, the line average was set between 2 and 4, and the digital zoom was set to 3 (colocalization with FM4-64 and drug treatments) or 1 (root hair patterning, callose). EGFP fluorescence was excited at 488 nm with a multi-line argon laser (3-4 percent intensity) and detected at 502 to 536 nm. FM4-64, PI and Basic Fuchsin fluorescence was excited with a 561 nm laser (0.5-1 percent intensity) and detected at 610 to 672 nm. Calcofluor White and aniline blue were excited with a 405 nm laser and detected at 425 to 475 nm. For direct comparisons of fluorescence intensities, the laser, pinhole, and gain settings of the confocal microscope were kept identical when capturing images of seedlings from different treatments. The intensities of fluorescence signals at the PM were quantified using ImageJ/Fiji software. To measure the fluorescence levels at the PM, optimal optical sections of the root cells were used for measurements. The fluorescent circumference of a single cell (ROI) on the captured images was selected using the selection tool. The mean pixel intensities for the selected ROIs were recorded and calculated using the Corrected Total Cell Fluorescence (CTCF). To determine colocalization, the distance from the center of each EGFP spot to the center of the nearest FM4-64 signal was measured manually on individual optical sections using ImageJ/Fiji software (Schindelin et al., 2012). If the distance between two puncta was less than the resolution limit of the objective lens (0.24 μm), the signals were considered to colocalize (Ito et al., 2012). Arabidopsis seedlings were covered with a 22 \times 22 mm glass coverslip with a thickness of 0.17 mm (No. 1.5H, Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Images were adjusted for color and contrast using ImageJ/Fiji software.

2.4. Chemical treatments

Cell wall-degrading enzymes: pectinase, cellulase, and xyloglucanase were purchased from Megazyme (Table 3). Cello-oligomers were purchased from Sigma and Megazyme. Isoxaben (ISX) was obtained from Sigma-Aldrich and used from stock solutions in DMSO (1 mM ISX). FM4-64 was purchased from Invitrogen (1.7 mM stock solution in water). For FM4-64 staining

seedlings were incubated in 1 μ M FM4-64 in liquid half strength MS medium for 5 min prior to imaging.

Table 3. Cell wall degrading enzymes and oligomers used in this study.

Name	Details
cellobiose	D-(+)-Cellobiose
cellotriose	Product code: O-CTR-50MG
cellotetraose	Product code: O-CTE-50MG
cellopentaose	Product code: O-CPE-20MG
cellulase	Cellulase (<i>endo</i> -1,4- β -D-glucanase) (<i>Aspergillus niger</i>) Product code: E-CELAN
pectinase	<i>endo</i> -Polygalacturonanase M2 (<i>Aspergillus aculeatus</i>) Product code: E-PGALUSP
xyloglucanase	Xyloglucanase (GH5) (<i>Paenibacillus</i> sp.) Product code: E-XEGP

2.5. Growth media, growing conditions, and frequently used buffers

Ingredients are dissolved in deionized H₂O, and all growth media need to be autoclaved.

<p>For DNA gel electrophoresis</p> <p>5x TBE running buffer (pH should be 8.3)</p> <p>450 mM Tris Base</p> <p>400 mM boric acid</p> <p>10 mM EDTA pH 8</p>
<p>For DNA extraction</p> <p>100 mM Tris/HCl pH 8</p> <p>250 mM NaCl</p> <p>25 mM EDTA pH 8</p> <p>0.5% (v/v) SDS</p>
<p>For plant tissue culture $\frac{1}{2}$ Murashige-Skoog medium</p> <p>0.22% MS medium powder</p> <p>1% sucrose</p> <p>0.9% Agar (plant cell culture tested)</p>

For plant liquid culture ½ Murashige-Skoog medium

0.22% MS medium powder

1% sucrose

0.5g/L MES

ClearSee

xylitol (10%, w/v)

sodium deoxycholate (15%, w/v)

urea (25%, w/v)

Seedlings were grown on half strength MS with 1% sucrose at 22 °C and continuous light for 5-6 days.

2.6. Bioinformatics

Bioinformatic analysis was mainly performed using geneious software. Alignments were generated in geneious software using a ClustalW algorithm with BLOSUM62 matrix. Sequencing results were analyzed in geneious software using the map to reference tool with geneious mapper and highest sensitivity.

2.7. RNAseq and transcriptome analysis

Sterilized and stratified seeds were grown vertically on square plates with half strength MS medium and 1% sucrose supplemented with 0.9% agar for six days at 22°C under continuous light. Six-day-old seedlings were first transferred to multiwell plates containing liquid half strength MS medium supplemented with 1% sucrose for 24 hours. Then, without disturbing the seedlings, the medium was replaced with a fresh liquid medium containing either mock (DMSO) or treatment (600 nM isoxaben) for 30min, 5h, and 8h. Three replicate samples were collected for each genotype, treatment, and time point. Samples were immediately frozen in liquid nitrogen and stored at -80° C for RNA extraction.

The total RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN). Five hundred nanograms of total RNA from each sample was used for the cDNA and NGS library preparation using QuantSeq 3' mRNA-Seq Library Prep kit (FWD) (015, Lexogen) following the

manufacturer's user guide. Individual NGS libraries were pooled in equimolar ratios and sequenced on a NovaseqS4 in paired end-150 base mode.

The quality of raw reads was checked using FastQC (v0.11.8) and then aligned by HISAT2 (v2.1.0) on *A. thaliana* genome TAIR10. The aligned reads were counted using featureCounts (v2.0.1). DESeq2 (v2.14) was used to conduct differential expression analysis. The comparison expression levels within each genotype and the cut-offs of differentially expressed genes (DEGs) were as follows: $FDR < 0.05$ and $\log_2(\text{Fold Change}) > 1$. The clustering of DEGs was performed via R package stats (v3.6.2) and visualized using R package ggplot2 (v3.3.3). The enrichment of gene ontology (GO) terms was investigated using BinGO (v3.0.3) from Cytoscape (v3.8.0). Hypergeometric test corrected by Benjamini & Hochberg False Discovery Rate correction was used to determine the enrichment of GO terms, and $FDR < 0.5$ was considered a significant enrichment of GO terms.

The experiment and analysis were done with great help from Minsoo Choi, Balaji Enugutti and Rodion Boikine.

2.8. Callose accumulation assay

Sterilized and stratified seeds were grown vertically on square plates with half-strength MS medium and 1% sucrose supplemented with 0.9% agar for six days at 22°C under continuous light. Six-day-old seedlings were first transferred to multiwell plates containing half-strength liquid MS medium supplemented with 1% sucrose for 24 hours. Then, without disturbing the seedlings, the medium was replaced with a fresh liquid medium containing either mock (DMSO) or treatment (600 nM isoxaben, 0.1% pectinase, 0.5% xyloglucanase, 0.8% cellulase) for 24 h. Staining for callose (aniline blue) was performed as described in Schenk and Schikora, 2015. Imaging of cotyledons was carried out with an Olympus FV1000 under a 10x objective. Quantification of micrographs was performed in ImageJ/Fiji by measuring the area fraction of cotyledons with a strong aniline blue signal, indicating callose deposition.

2.9. Lignin accumulation assay– phloroglucinol stain

Sterilized and stratified seeds were grown vertically on square plates with half-strength MS medium and 1% sucrose supplemented with 0.9% agar for six days at 22°C under continuous light. Six-day-old seedlings were first transferred to multi-well plates containing half-strength liquid MS medium supplemented with 1% sucrose for minimum 6 hours. Then, without

disturbing the seedlings, the medium was replaced with a fresh liquid medium containing either mock (DMSO) or treatment (600 nM isoxaben, 0.1% pectinase, 0.5% xyloglucanase, 0.8% cellulase) for 16 hours. Root images were taken using the Leica SAPO stereomicroscope and Leica MC170HD camera. For quantification, a defined region of interest (ROI) located 300 μ m above the root tip (excluding the root cap) was used in all samples. Staining for lignin (phloroglucinol) was performed as described in Van der Does et al., 2017. Quantification of micrographs was performed in ImageJ/Fiji by measuring the phloroglucinol signal intensity, indicating lignification (Rueden et al., 2017).

2.10. Lignin accumulation assay – Basic Fuchsin

ClearSee-adapted cell wall staining was performed as described in recent publications (Kurihara et al. 2015; Ursache et al. 2018) . Briefly, after the treatment with ISX, five-day-old seedlings were fixed in 3 ml 1 \times PBS containing 4% paraformaldehyde for 1 h at room temperature in 12-well plates and washed twice with 3 ml 1 \times PBS. After fixation, the seedlings were cleared in 3 ml ClearSee solution with gentle shaking. After overnight clearing, the solution was replaced with a new ClearSee solution containing 0.2% Fuchsin and 0.1% Calcofluor White for lignin and cell wall staining, respectively. After overnight staining, the dye solution was removed and rinsed once with a fresh ClearSee solution. The samples were washed in fresh ClearSee solution for 30 minutes with gentle shaking and washed again in another fresh ClearSee solution for at least one night before observation.

2.11. Statistics

Statistical analysis was performed with PRISM8 software (GraphPad Software, San Diego, USA).

3. Results

3.1. The Arabidopsis receptor kinase STRUBBELIG regulates cell wall remodeling in response to specific cell wall damage

3.1.1. Response to pectinase-induced cell wall damage requires *SUB* activity

The response of young Arabidopsis seedlings to several cell wall degrading enzymes either applied as a mixture or individually, was previously reported to result in cell wall damage (Engelsdorf et al., 2018). The aim of the study was to investigate whether *SUB* is exclusively involved in the protective response to isoxaben-induced cellulose depletion or whether it also responds to other types of cell wall stress. The experiment involves cell wall degrading enzymes that, upon application, initiate the degradation of three major cell wall polysaccharide categories: cellulose, hemicellulose (xyloglucan), and pectin. The effects of enzyme activity on cell wall remodeling were studied by examining lignin and callose accumulation in roots and cotyledons of six-day-old seedlings. Lignin accumulation was assessed by phloroglucinol (Wiesner) staining, and the presence of callose was detected by aniline blue staining.

The activity of the enzymes was determined by incubating seedlings in solutions containing different concentrations of either cellulase, xyloglucanase, or pectinase. Apparent morphological changes in the form of damaged cells were first observed in the meristematic and transition zone of the root (Fig. 5). Alterations in tested tissue were detected using propidium iodide, a well-known indicator of damaged or dead cells (Deitch et al. 1982). Exposure to 0.8% cellulase, 0.5% xyloglucanase, or 0.1% pectinase for 24 hours caused noticeable changes in the epidermal cells of the analyzed seedlings (Fig. 5).

After confirming the effectiveness of the enzymes, seedlings were treated under these conditions to investigate the potential activation of the cell wall remodeling processes. Lignin and callose accumulation were compared between wild-type (*Col-0*), two null alleles *sub-21* and *sub-9*, and two independent *SUB* overexpression lines (*pUBQ10::SUB:mCherry*) *SUB-OE L1* and *O3* (Chaudhary et al. 2020). Obtained results showed that the application of cellulase, xyloglucanase, or pectinase for 16 hours did not lead to a visible buildup of phloroglucinol stain in the roots of wild-type seedlings, indicating that the impact of the treatments on lignin accumulation was negligible (Fig. 6).

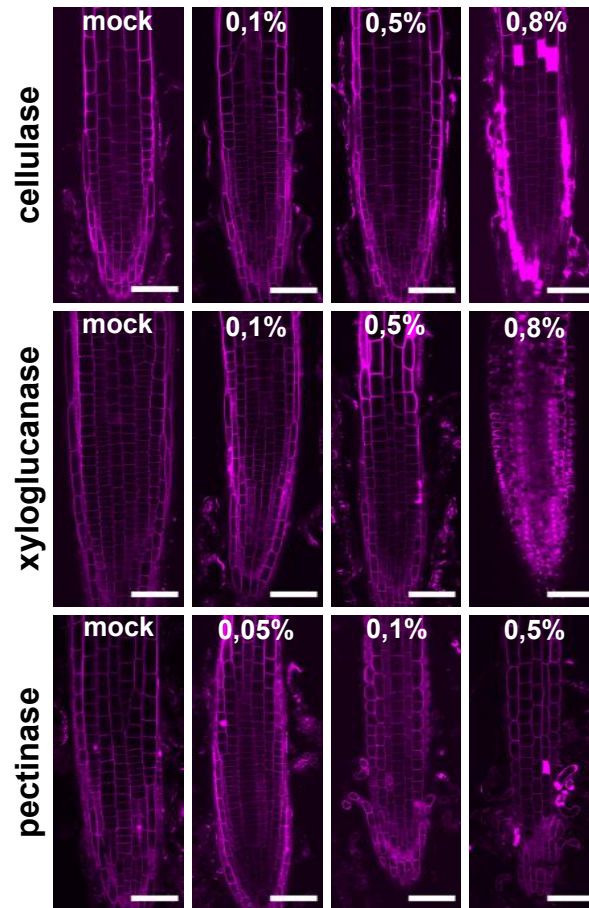


Figure 5. Effect of cell wall-degrading enzymes on root epidermal cell morphology.

Confocal micrographs show epidermal cell morphology and survival in 6-day-old Col-0 seedlings in the presence or absence of cellulase, xyloglucanase, and pectinase. The magenta color marks cell walls stained with propidium iodide. Note the increasing cell damage with rising enzyme concentrations. The experiment was performed twice with similar results. Treatments are indicated in the panels. $6 \leq n \leq 11$. Scale bars: 50 μm .

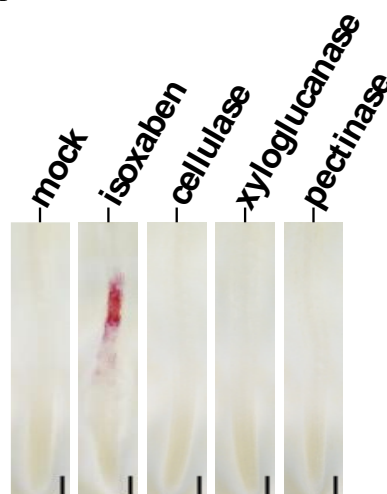


Figure 6. Lack of lignin production upon the application of cell wall-degrading enzymes.

Phloroglucinol signal strength indicates lignin accumulation in roots of 6-day-old seedlings exposed to 600 nM isoxaben, 0.8% cellulase, 0.5% xyloglucanase, and 0.1% pectinase for 16 hours. Genotype: Col-0. $7 \leq n \leq 10$. The experiment was performed twice with similar results. Scale bars: 100 μm .

The opposite effect was observed when six-day-old seedlings exposed to each of the enzymes for 24 hours were tested for the production of callose. All three treatments triggered an increase in the accumulation of callose in wild-type cotyledons, as indicated by aniline blue staining (Fig. 7A). To further confirm that callose formation was the result of the destructive action of the enzymes, the experiment was repeated using both active and heat-deactivated enzymes. Deactivation of the enzymes was achieved by incubating the solutions at 100°C for 20 minutes. The results showed that excessive callose deposition occurred only after the application of active enzymes and remained low when inactive enzymes were used (Fig. 7).

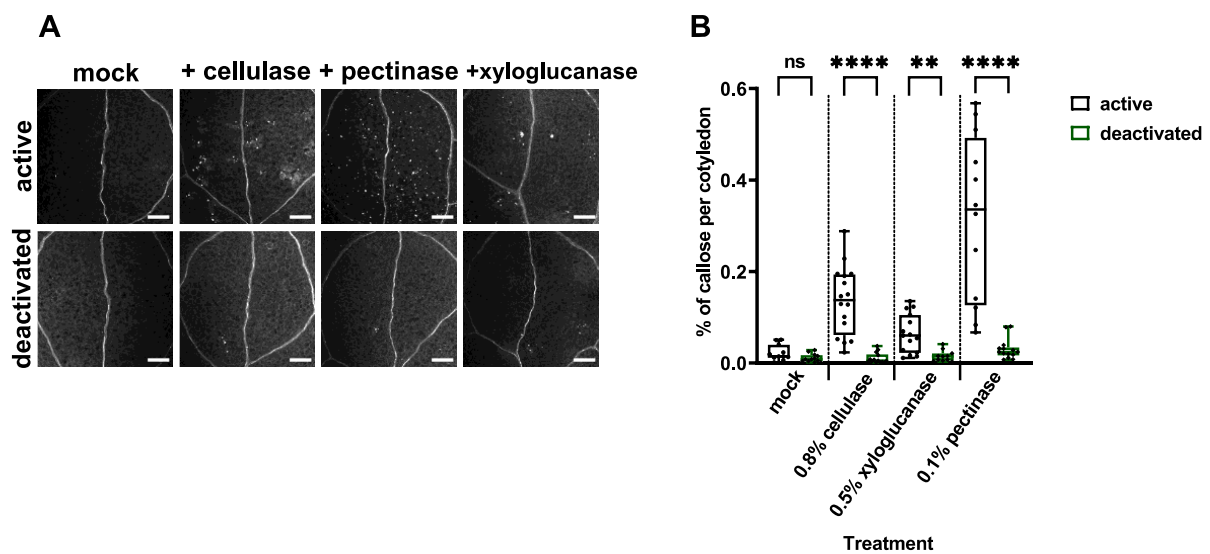


Figure 7. Callose accumulation upon active vs. heat-deactivated cell wall-degrading enzymes.

(A) Confocal micrographs show cotyledons of six-day-old Col-0 seedlings treated with active or heat-deactivated mock, 0.8% cellulase, 0.5% xyloglucanase, and 0.1% pectinase for 24 hours. Aniline blue fluorescence signal strength indicates callose accumulation. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $11 \leq n \leq 16$. Asterisks represent statistical significance. Adjusted P value **** $P < 0.0001$, ** $P = 0.0011$; unpaired t-test. The experiment was performed twice with similar results. Scale bars: 200 μm .

Further examination revealed that the application of pectinase did not result in a substantial increase in the aniline blue signal in *sub-21* or *sub-9* when compared to the mock treatment, and the levels reached by *SUB-OE1* and *O3* were similar to those observed in wild-type *Arabidopsis* seedlings (Fig. 8A, D). The application of cellulase or xyloglucanase appeared to have a minimal effect on callose production in plants with reduced or increased *SUB* activity (Fig. 8A-C).

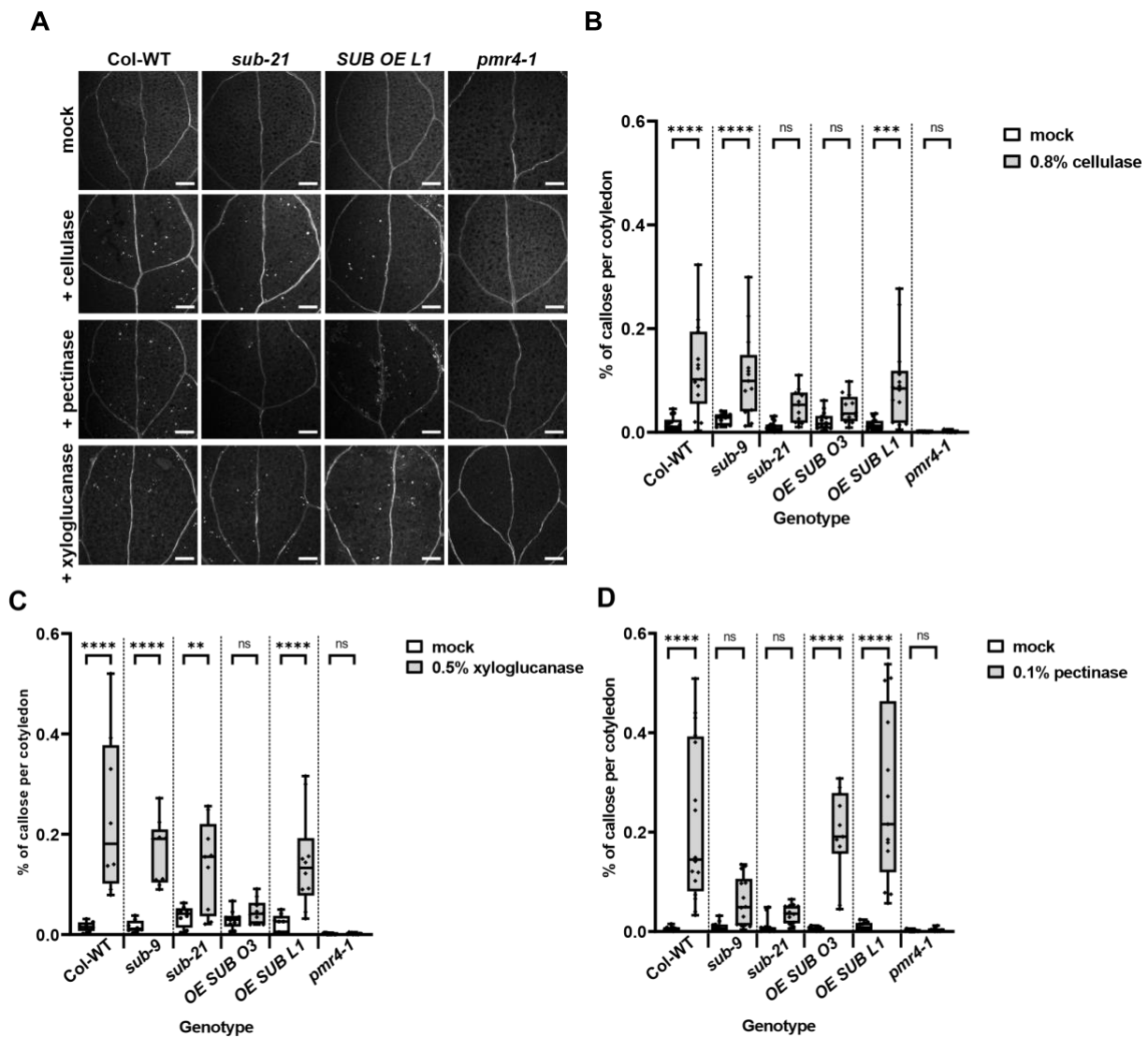


Figure 8. *SUB* affects pectinase-induced callose accumulation.

(A) Confocal micrographs show cotyledons of 6-day-old Col-0, *sub-21*, *SUB-OE L1*, and *pmr4-1* seedlings treated with mock, 0.8% cellulase, 0.5% xyloglucanase, and 0.1% pectinase for 24 hours. Aniline blue fluorescence signal strength indicates callose accumulation.

(B) Quantification of the results depicted in (A) for cellulase treatment; $11 \leq n \leq 16$.

(C) Quantification of the results depicted in (A) for xyloglucanase treatment; $8 \leq n \leq 14$.

(D) Quantification of the results depicted in (A) for pectinase treatment; $10 \leq n \leq 16$. (A-D) Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. Asterisks represent statistical significance. Adjusted P value **** $P < 0.0001$, *** $P = 0.0002$, ** $P = 0.0048$; one-way ANOVA followed by Šídák multiple comparison test. The experiment was performed three times with similar results. Scale bars: 200 μm .

3.1.2. *PMR4* plays a critical role in the accumulation of CWD-induced callose

Additionally, it was unknown which callose synthase promotes CWD-induced callose production in cotyledons. The callose synthase POWDERY MILDEW RESISTANT 4 (*PMR4*) is one of 12 members of the callose synthase family (*CalS12/GSL5*) (Ellinger and Voigt 2014). *PMR4* controls callose formation in several physiological processes, including cell wall modifications and defense responses to pathogen attacks (Jacobs et al. 2003, Y. Wang et al. 2021b). The involvement of *PMR4* in callose accumulation was assessed by performing enzymatic assays in the *pmr4-1* mutants. Exposure to the cell wall degrading enzymes failed to trigger callose deposition in *pmr4-1* cotyledons supporting the involvement of this gene in callose production (Fig. 8). Obtained results present *PMR4* as a curtail component of callose production upon applied stresses.

Knowing that CWD-induced callose accumulation is critically dependent on *PMR4*, a further experiment was performed to test a different callose synthase, *CalS7(GSL7)*. According to available studies, *CalS7* is mainly required for callose deposition in phloem sieve elements. Phenotypic analysis of Arabidopsis plants lacking *CalS7* function revealed only subtle alterations in stem and floral organ size (Barratt et al. 2011). To potentially uncover novel biological functions of this gene, *cals7-1* and *cals7-2* mutants were subjected to two cell wall disruption treatments, isoxaben and pectinase. Obtained results show that applied stress induced callose production, as indicated by increased aniline blue staining, in the wild-type cotyledons as well as in plants lacking *CalS7* activity. The amount of accumulated callose is similar between genotypes tested in this study, meaning that *CalS7* does not play an essential role in response to cell wall damage caused by isoxaben or pectinase (Fig. 9).

Taken together, the findings suggest that the use of cellulase, xyloglucanase, and pectinase under the conditions of this study does not induce lignin formation in roots but instead leads to *PMR4*-dependent callose accumulation in the cotyledons of young Arabidopsis seedlings. While cellulase and xyloglucanase treatments promote callose accumulation even in the absence of *SUB*, its presence is necessary for the pectinase-induced protective response in the form of callose.

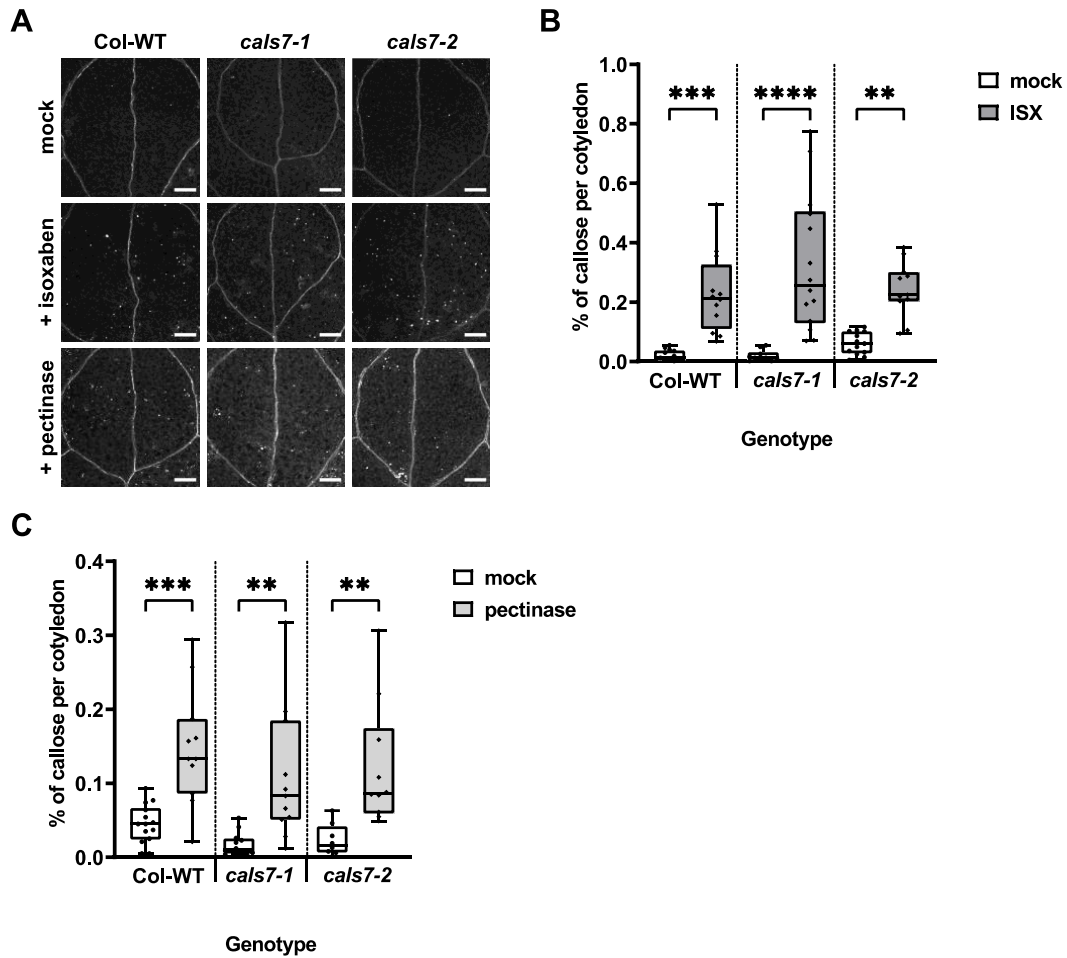


Figure 9. Involvement of *CalS7* in CWD-induced callose accumulation.

(A) Confocal micrographs show cotyledons of 6-day-old Col-0, *calS7-1* and *calS7-2* seedlings treated with mock, 600 nM isoxaben, or 0,1% pectinase for 24 hours. Aniline blue fluorescence signal strength indicates callose accumulation. (B) Quantification of the results depicted in A for ISX treatment. (C) Quantification of the results depicted in A for pectinase treatment. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $8 \leq n \leq 14$. Asterisks represent statistical significance. Adjusted P values *** $P=0.0003$, ** $P < 0.0034$; one-way ANOVA followed by Šidák multiple comparison test. The experiment was performed twice with similar results. Abbreviation: ISX, isoxaben. Scale bars: 200 μm .

3.1.3. Cellulose-derived oligomers do not induce callose production

Cellulose is a linear polymer consisting of glucose units linked by β -1,4 glycosidic bonds. When cellulase enzymes digest cellulose, it is hydrolyzed into shorter cello-oligosaccharides of varying lengths. These cello-oligosaccharides, such as cellobiose, cellotriose, and higher oligomers, are intermediate products created during cellulose degradation (Kluge et al. 2019). Further enzymatic or pathogen activity can break down these cello-oligomers into individual glucose monomers (Payne et al. 2015). Previous studies have reported that cellobiose and cellotriose induce specific immune responses in plants (de Azevedo Souza et al. 2017; Johnson et al. 2018). Callose production, a common reaction to stress, did not occur after cellobiose application (de Azevedo Souza et al. 2017).

The callose accumulation assay was performed using the four cello-oligomers to analyze whether higher oligomers are involved in this process. This experiment also aimed to determine whether callose production after cellulase treatment was induced only by enzymatic activity or also by cellulose degradation products. Six-day-old wild-type seedlings were incubated in a liquid medium containing 400 μ M of either cellobiose, cellotriose, cellotetraose, cellopentaose, or mock for 24 hours. The results confirmed the absence of callose deposition after cellobiose treatment. Moreover, none of the other three compounds were able to induce this protective response (Fig. 10). Data obtained from this study suggest that cello-oligomers of the degree of polymerization between 2 and 5 do not play a direct role in callose production.

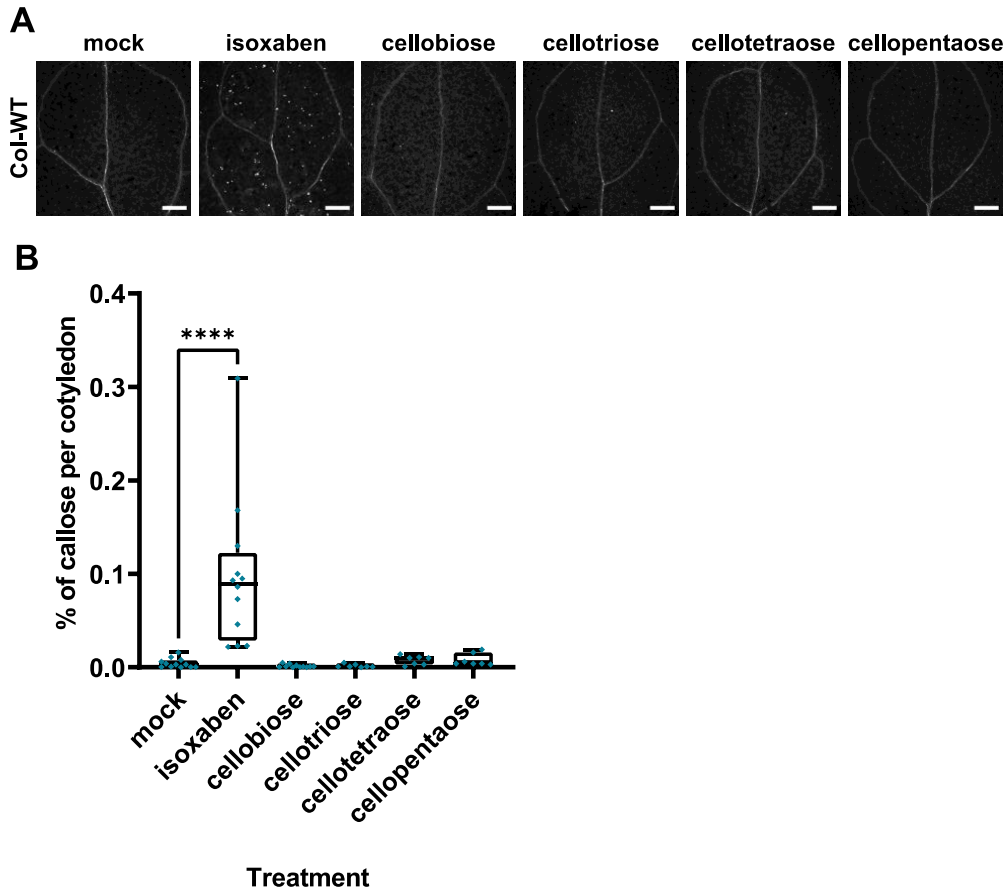


Figure 10. Cellulose-derived oligomers play a negligible role in callose accumulation.

(A) Confocal micrographs show cotyledons of 6-day-old seedlings treated with mock and various cello-oligomers for 24 hours. Genotypes: Col-0. Aniline blue fluorescence signal strength indicates callose accumulation. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $7 \leq n \leq 12$. Asterisks represent statistical significance. Adjusted P value **** $P < 0.0001$. One-way ANOVA followed by Dunnett's multiple comparison test. The experiment was performed twice with similar results. Scale bars: 200 μm .

3.1.4. Treatment with cell wall-degrading enzymes promotes the internalization of SUB

Previous studies have reported that *SUB* undergoes constitutive clathrin-mediated endocytosis (Gao et al. 2019; Song et al. 2019). It was shown that seedlings grown on agar plates containing 3 to 5 nM isoxaben or treated with 600 nM isoxaben for approximately 5 hours exhibited a significant and dose-dependent increase in the internalization of SUB. Consequently, prolonged exposure to isoxaben resulted in altered root hair patterning in the root meristem epidermis of young seedlings due to a decreased activity of *SUB* (Chaudhary et al. 2021).

Based on these findings, the next step was to determine whether exposure of seedlings to cellulase, xyloglucanase, and pectinase would also lead to reduced SUB levels at the plasma membrane and result in defects in root hair pattern formation. Roots of five-day-old seedlings were first tested to determine the abundance of SUB at the plasma membrane of epidermal cells of the meristem. For this purpose, the *sub-9* line carrying a complementing transgene encoding a SUB:EGFP translational fusion driven by its endogenous promoter (pSUB::SUB:EGFP; SSE) (Gao et al. 2019; Vaddepalli et al. 2011, 2014) was analyzed using a confocal microscope. The comparison of the GFP-based fluorescence signal produced by mock- and enzyme-treated seedlings served as an indicator of SUB present at the plasma membrane. Application of all three enzymes was followed by an apparent and concentration-dependent reduction of reporter signal at the plasma membrane within 30 minutes (Fig. 11).

The further experiment was conducted by subjecting seedlings to concentrations that caused morphological abnormality in roots (Fig. 5) and increased callose deposition in cotyledons (Fig. 8), namely 0.8% cellulase, 0.5% xyloglucanase or 0.1% pectinase. Surprisingly, increasing the exposure time of pectinase and xyloglucanase to 60 minutes did not further decrease the reporter signal at the plasma membrane compared to the mock treatment (Fig.12C-F). The correlation between incubation time and signal reduction was only observed for the cellulase treatment (Fig.12A-B). Furthermore, when compared to the start of the experiment, a significant loss of EGFP signal strength at the plasma membrane was observed during incubation with a mock treatment for 5 h, but not between 30 and 60 min (Fig. 13). It appears that prolonged incubation of seedlings in liquid medium has a negative effect on the SUB:EGFP expression at the plasma membrane.

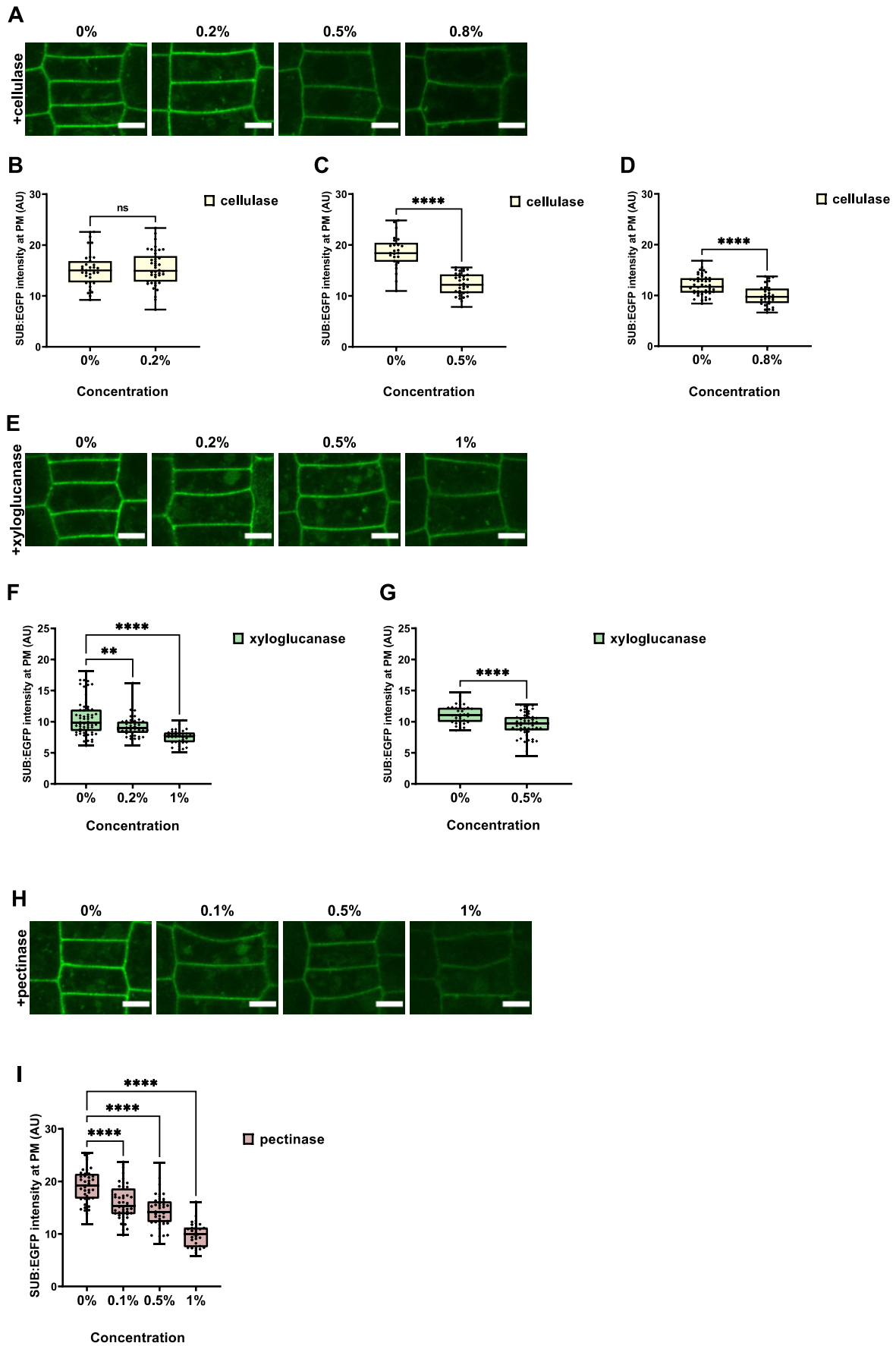


Figure 11. Application of all three enzymes is followed by concentration-dependent reduction of reporter signal at the plasma membrane.

The signal intensity of a functional pSUB::SUB:EGFP reporter in *sub-9* after 30 min exposure of seedlings to (A) mock or growing concentrations of cellulase:0%, 0.2%, 0.5%, 0.8%. (E) mock or growing concentrations of xyloglucanase: 0%, 0.2%, 0.5%, 1%. (H) mock or growing concentrations of pectinase: 0%, 0.1%, 0.5%, 1%. Note the reduction in signal in enzyme-treated root epidermal cells. (B, C, D) Quantification of the data shown in (A). $34 \leq n \leq 44$ (F, G) Quantification of the data shown in €. $36 \leq n \leq 60$ (I) Quantification of the data shown in (H). $32 \leq n \leq 47$. n=cell. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. Asterisks represent statistical significance. Adjusted P values **P=0.0034, *** P=0.0001, **** P < 0.0001; (B, C, D, G) unpaired t-test. (F, I) one-way ANOVA followed by Dunnett's multiple comparison test. The experiment was performed twice with similar results. Scale bars: 5 μ m.

The further analysis aimed to determine whether SUB endocytosis could be increased by treatment with the three enzymes in a manner analogous to isoxaben treatment. Following 5-minute incubation with the endocytic tracer dye FM4-64, seedlings were subjected to the respective enzymatic treatments for 30 min, and root epidermal cells were imaged. Colocalization was indicated by a previously established standard, where the distance between the centers of the SUB:EGFP and FM4-64 puncta was less than the resolution limit of the objective (0.24 μ m) (Gao et al. 2019; Ito et al. 2012). Following mock treatment for 30 minutes 64.8% (n=162) of all cytoplasmic SUB:EGFP foci were observed to be labeled with FM4-64. However, when treated with different enzymes for the same time, the percentage increased to 75.8% (n=273) for 0.8% cellulase, 72% (n=342) for 0.5% xyloglucanase, and 73% (n=198) for 0.1% pectinase (Fig. 14). This evidence suggests that the use of these enzymes promotes a mild increase in SUB internalization within the first 30 minutes.

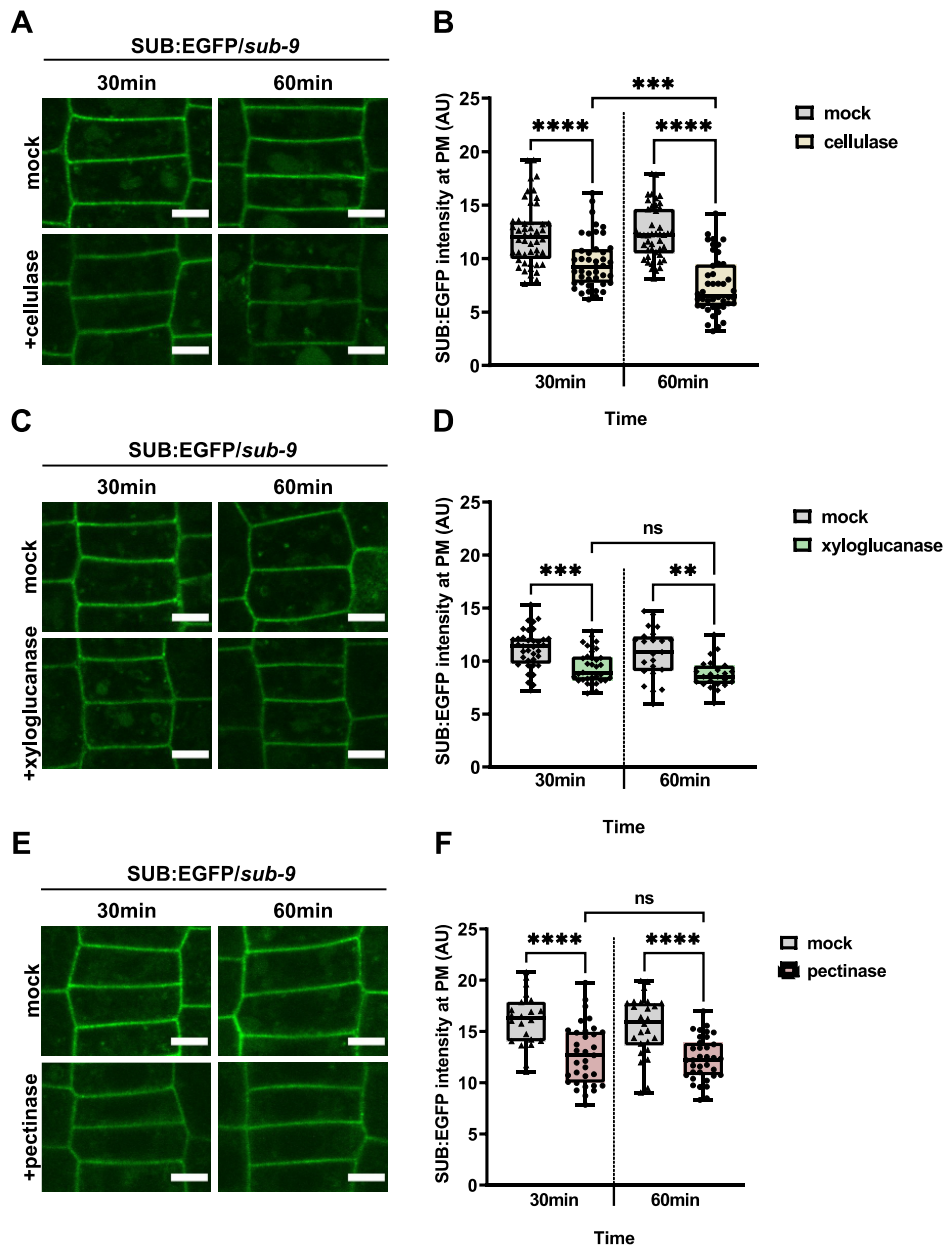


Figure 12. Influence of CWD enzymes on SUB abundance at the plasma membrane.

The signal intensity of a functional pSUB::SUB:EGFP reporter in *sub-9* after 30 min and 60 min exposure of seedlings to (A) mock or cellulase. (C) mock or xyloglucanase. (E) mock or pectinase. Note the reduction in signal in enzyme-treated root epidermal cells. (B) Quantification of the data shown in (A). $43 \leq n \leq 48$ (D) Quantification of the data shown in (C). $23 \leq n \leq 36$ (F) Quantification of the data shown in (E). $22 \leq n \leq 35$. $n = \text{cell}$. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. Asterisks represent statistical significance. Adjusted P values *** $P = 0.0001$, **** $P < 0.0001$; unpaired t-test. The experiment was performed three times with similar results. Scale bars: 5 μm .

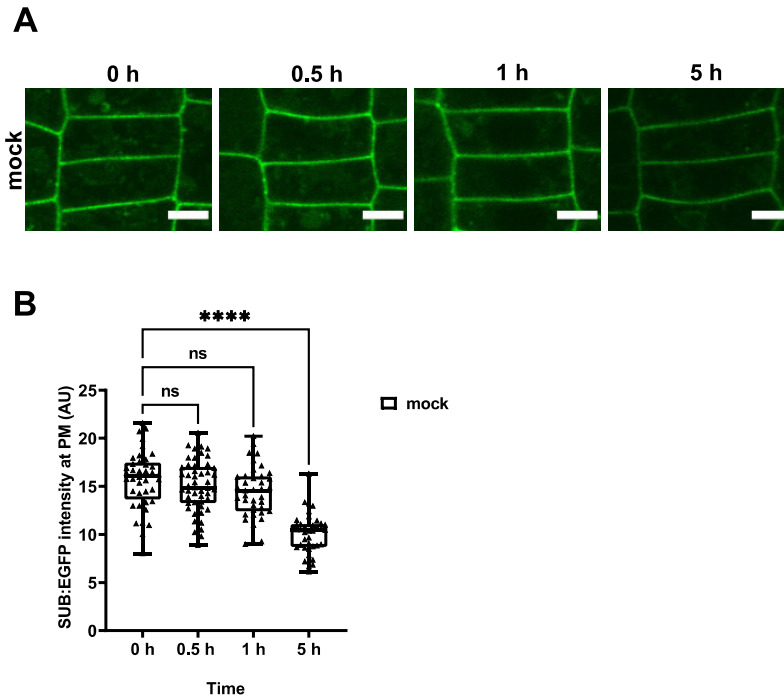


Figure 13. Prolonged incubation in mock solution results in lower SUB:EGFP signal at the plasma membrane.

(A) Signal intensity of a functional pSUB::SUB:EGFP reporter in *sub-9* after 0 min, 0.5, 1 and 5h exposure of seedlings to mock. (B) Quantification of the data shown in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $41 \leq n \leq 61$, $n = \text{cell}$. Asterisks represent statistical significance. Adjusted P value **** $P < 0.0001$; unpaired t-test. The experiment was performed three times with similar results. Scale bar: 5 μm .

It has been reported that the application of isoxaben leads to a *sub*-like phenotype in wild-type plants (Chaudhary et al. 2021). Changes in root hair patterning, resembling those seen in *sub-9* mutants, were visible in five-day-old seedlings exposed to 3 nM isoxaben for 48 hours. This altered patterning is reflected at the molecular level using Col-0 plants carrying a pGL2::GUS:EGFP reporter. The *GLABRA2* (*GL2*) promoter drives expression in non-hair (N) cells, but not hair (H) cells, of the root epidermis, making it a useful tool for analyzing root hair patterning (Kwak et al. 2005; Masucci et al. 1996). Further study was conducted to determine whether exposure of wild-type seedlings to the major cell wall degrading enzymes for 48 hours would result in changes in root hair patterning. To achieve this objective, the expression pattern of a pGL2::GUS:GFP reporter was examined to assess whether root hair patterning was affected at the molecular level. The number of hair and non-hair cells in the H and N positions of the root epidermis was compared between untreated *sub-9* and treated wild-type seedlings. The

results showed that no critical misexpression in root hair patterning was observed after the application of any concentration of the three enzymes (Fig. 15).

The data gathered in this study suggest that the action of three cell wall degrading enzymes causes a decrease in SUB levels at the plasma membrane by enhancing endocytosis, but this reduction does not result in abnormal root hair pattern formation. This contrasts with observations involving isoxaben treatment, where exposure to this drug leads to reduced *SUB* activity, increased SUB endocytosis, and altered root hair patterning.

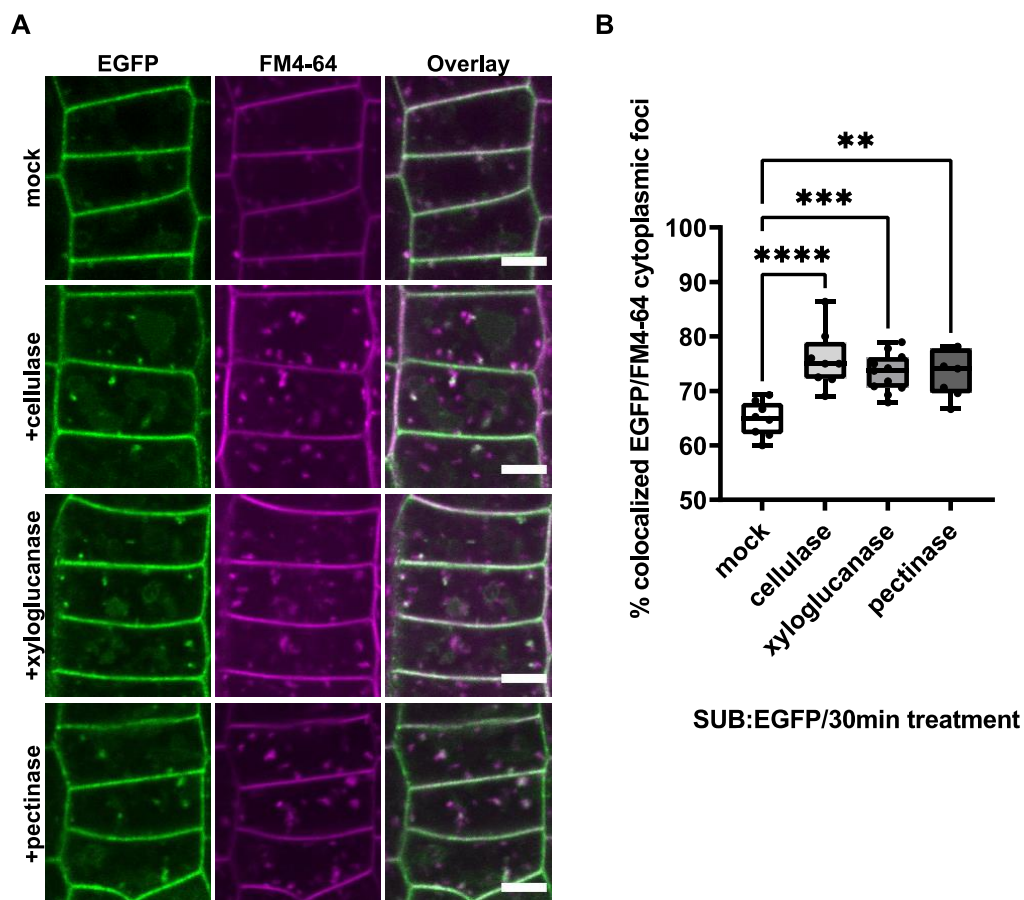


Figure 14. Sub-cellular localization of SUB:EGFP upon cell wall damage.

Fluorescence micrographs show optical sections of epidermal cells of root meristems of 5-day-old seedlings. (A) Partial colocalization of SUB:EGFP and FM4-64 foci upon pre-staining seedlings with FM4-64 for 5 min and later incubating them in either mock, 0.8% cellulase, 0.5% xyloglucanase or 0.1% pectinase for 30min. (B) Box-and-whiskers plot depicting the results of a quantitative colocalization analysis of SUB:EGFP-positive foci shown in (A). Data points indicate the percentage of colocalization in 3 to 6 cells per analyzed root. $7 \leq n \leq 11$. 2–17 SUB:EGFP foci were analyzed per cell. Asterisks represent statistical significance. Adjusted P values ****P < 0.0001, ***P=0.0003, **P=0.0015; one-way ANOVA followed by Dunnett’s multiple comparison test. The experiment was performed twice with similar results. Scale bars: 5 μ m.

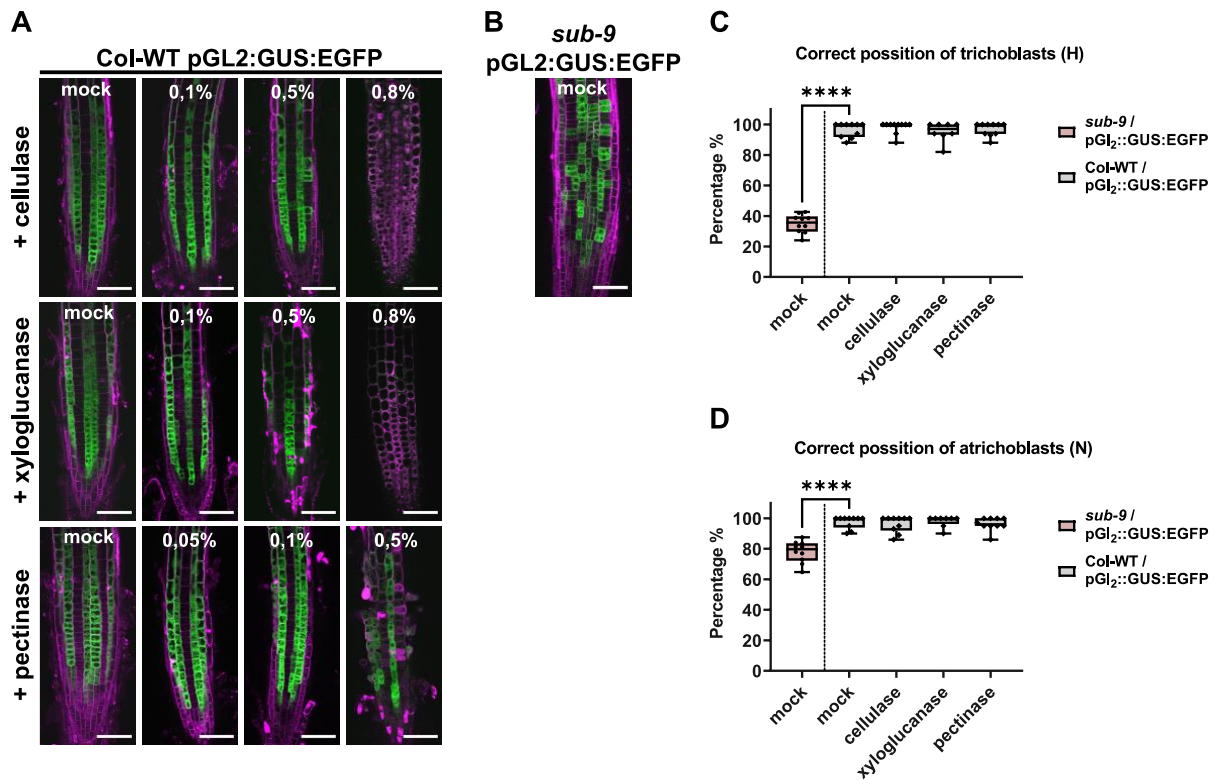


Figure 15. Effect of CWD enzymes on root hair patterning.

(A) Confocal micrographs depicting the epidermal cell layer of roots of 7-day-old Col-0 seedlings showing pGL2::GUS:EGFP reporter signal. The cell wall was counterstained with propidium iodide. (B) Confocal micrographs depicting the epidermal cell layer of *sub-9* seedlings showing pGL2::GUS:EGFP reporter signal. The cell wall was counterstained with propidium iodide. (C) Quantification of the data shown in (A); the correct position of H cells after 48h treatment with 0,1% of CWD enzymes. (D) Quantification of the data shown in (A); the correct position of N cells after 48h treatment with 0,1% of CWD enzymes. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $8 \leq n \leq 10$. Asterisks represent statistical significance. Adjusted P value ****P < 0.0001; one-way ANOVA followed by Šidák multiple comparison test. The experiment was performed twice with similar results. Genotypes and treatments are indicated in the panels. Scale bars: 50 μ m.

3.1.5. Isoxaben-induced root lignification begins in the endodermis

Under unstressed conditions, different patterns of lignified cell walls are associated with diverse functions in various plant tissues, such as xylem vessels as water pipes and Casparian strips as apoplastic barriers (Emonet and Hay 2022). These well-studied functions rely on the stiffness and hydrophobic effect that lignin polymers impart onto the cell wall (Ménard et al. 2021; Fujita et al. 2020; Reyt et al. 2021). In contrast, the detailed description of lignin produced during stress requires further investigation. In particular, the cellular distribution of lignin in the seedling root upon isoxaben was not known. Therefore, the following analysis was designed to examine the time course and specific site of lignification induced by reduced cellulose levels.

A commonly used method to visualize lignin is phloroglucinol staining, which binds to the polymer accumulated in the internal tissues of young seedling roots (Pradhan Mitra and Loqué 2014). Isoxaben-induced ectopic lignin production seems to be restricted to a specific region approximately 450 μm from the root tip, which appears particularly sensitive to the cell wall stress (Chaudhary et al. 2020; Hamann et al. 2009). This root area also shows reduced stiffness, as shown by Brillouin microscopy (Bacete et al. 2022). It roughly coincides with the transition zone and the early elongation zone, where rapid cell expansion is commonly observed. Initially, six-day-old wild-type seedlings were exposed to 600 nM isoxaben for an increasing number of hours to determine the earliest point of noticeable lignification and the general time course of the process. Previous studies have shown that the increase in phloroglucinol staining can be detected after 4-6 hours of isoxaben application (Hamann et al. 2009). In the presented experiment, the earliest signs of phloroglucinol staining were detected at approximately 6 hours of isoxaben exposure in small spots located in the transition and early elongation zone of the root (Fig. 16 A). A constant increase in signal intensity was observed during the 8 to 24 hours exposure period (Fig. 16 A, B). Additionally, the root section with a prominent signal was never present in the root tip or regions higher up in the main root, corroborating previous findings (Hamann et al. 2009).

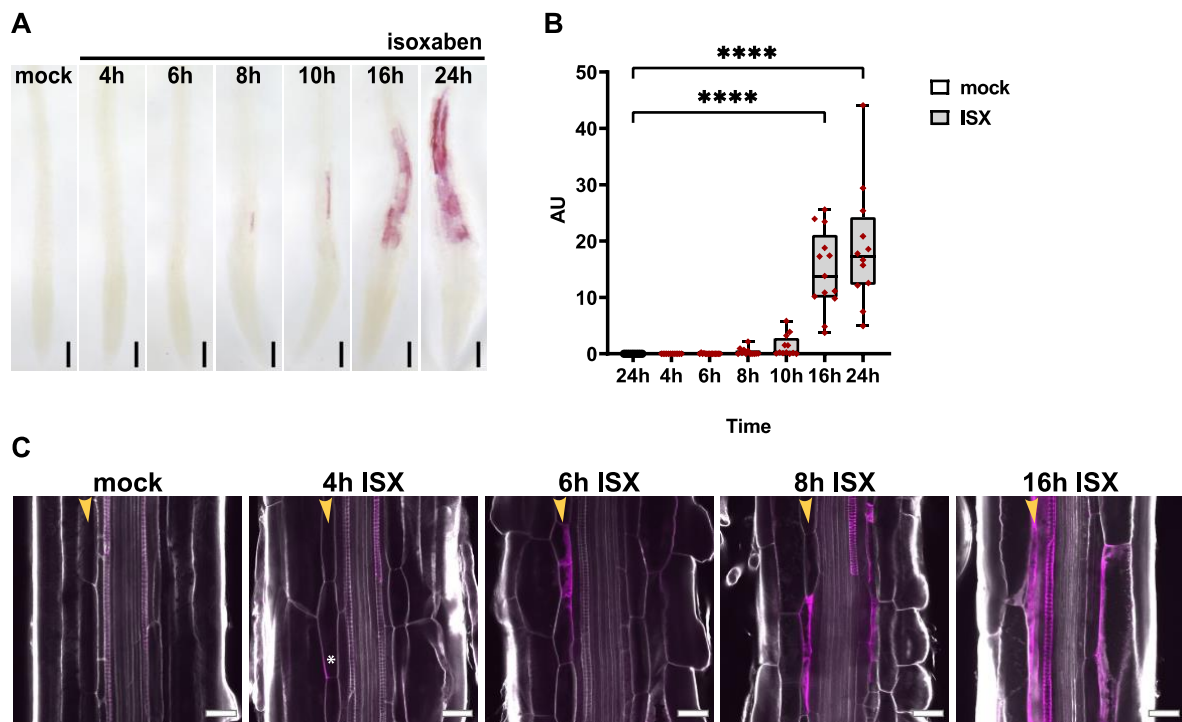


Figure 16. Time course analysis of isoxaben-induced lignification in response to a reduction in cellulose content.

(A) Phloroglucinol signal strength indicating lignin accumulation in roots of 6-day-old Col-0 seedlings exposed to 600 nM isoxaben for 16 hours. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $11 \leq n \leq 15$. Asterisks represent statistical significance. Adjusted P value ****P < 0.0001; one-way ANOVA followed by Dunnett's multiple comparison test. The experiment was performed three times with similar results. (C) Confocal micrographs showing a cross-section of Col-0 roots treated with mock or 600 nM isoxaben for 4-16 h. Lignin and cell walls are shown in magenta (stained with Basic Fuchsin) and gray (stained with Calcofluor White), respectively. Yellow arrowheads indicate endodermal cells. The experiment was performed twice with similar results. Scale bars: (A): 100 μm . (C): 20 μm .

Despite the widespread use of phloroglucinol to visualize lignin accumulation, this method has limitations. To obtain cellular resolution and a more detailed picture of the initial isoxaben-induced lignification, the assay was repeated using the ClearSee-adapted method (Kurihara et al. 2015; Fujita et al. 2020). This approach consisted of clearing fixed seedlings in ClearSee solution and staining the cell wall with Calcofluor White and lignin with Basic Fuchsin, allowing the deep imaging of roots with cellular resolution (Dharmawardhana et al. 1992; Ursache et al. 2018). This technique allowed the detection of Fuchsin signal in scattered individual endodermal cells in seedlings treated with isoxaben for 4 hours (Fig. 16C). Isoxaben treatment for a similar duration has been reported to activate lignin biosynthesis genes (Hamann

et al. 2009), while ectopic lignification is also observed in *prc1-1* mutants grown in the dark (Hématy et al. 2007). With extended exposure to the herbicide, the Fuchsin signal became more pronounced in the endodermis cells and occasionally in cortex cells. Initially, the staining appeared to be concentrated at the cell perimeter, but after 6 to 8 hours of treatment, staining was also present throughout the endodermal cell and more frequently at the cortical cell periphery (Fig. 16C). Treatment lasting 16 hours resulted in a greater spread of Fuchsin staining which was detected not only in the endodermis but also in adjacent cortex cells and sporadically in the pericycle (Fig. 16C). Apart from the xylem, Fuchsin signal was rarely observed in the stele and epidermis during the studied time.

The findings imply that within the region of the seedling root affected and sensitive to isoxaben treatment, lignification begins approximately 4 hours after the start of the exposure. Initiation of the process happens in individual endodermal cells and ultimately spreads throughout the endodermis and both adjacent cell layers.

3.1.6. *SUB* function required for lignin propagation

The protein *SUB* has been identified as necessary for isoxaben-induced lignin accumulation in the root system of seedlings (Chaudhary et al. 2020). Interestingly, in the loss-of-function alleles *sub-9* and *sub-21*, a significant reduction but not elimination of lignification is observed (Chaudhary et al. 2020). To better understand the role of *SUB* in isoxaben-induced lignification, the cellular signal distribution of Fuchsin-stained Col-0, *sub-9*, and *sub-21* seedlings exposed continuously to 600 nM isoxaben for intervals of 4, 6, and 8 hours were examined. After 4 hours of exposure, signs of ectopic lignin accumulation were noticed in all tested genotypes (Fig. 17). Fuchsin staining was detected irregularly at the periphery of individual endodermal cell walls. At this time point, *sub-9* appears to have more lignification at the endodermal and cortical cell wall periphery compared to wild-type seedlings (Fig. 17). However, the results obtained for the *sub-21* mutant are similar to those of the wild type. With increasing exposure time to 6 hours, more endodermal cells show lignin accumulation at the edge of the cell wall and inside the cell. In addition, after 6 hours of isoxaben treatment, more cortical cells showed detectable lignification (Fig.17). Interestingly, the number of lignified endodermal cells remained similar between genotypes. After 8 hours of exposure, the differences between the genotypes are more pronounced. In contrast to wild-type seedlings, where entire endodermal cells showed Fuchsin staining, endodermal cells in both *sub-9* and especially *sub-21* often showed only peripheral staining (Fig. 17). The number of complete endodermal cells with intense Fuchsin staining is significantly higher in wild-type seedlings compared to *sub-9* and *sub-21*. However, the number of cortical and endodermal cells with fuchsin staining only at the cell wall periphery remains similar.

In conclusion, these results are consistent with the idea that *SUB* is not critical for the initiation of isoxaben-induced lignification but plays a role in maintaining and spreading this process within the cell. It is important to note that the experiment was performed only once with the help of bachelor student Jasmin Xander, and additional replicates will be necessary to confirm this observation.

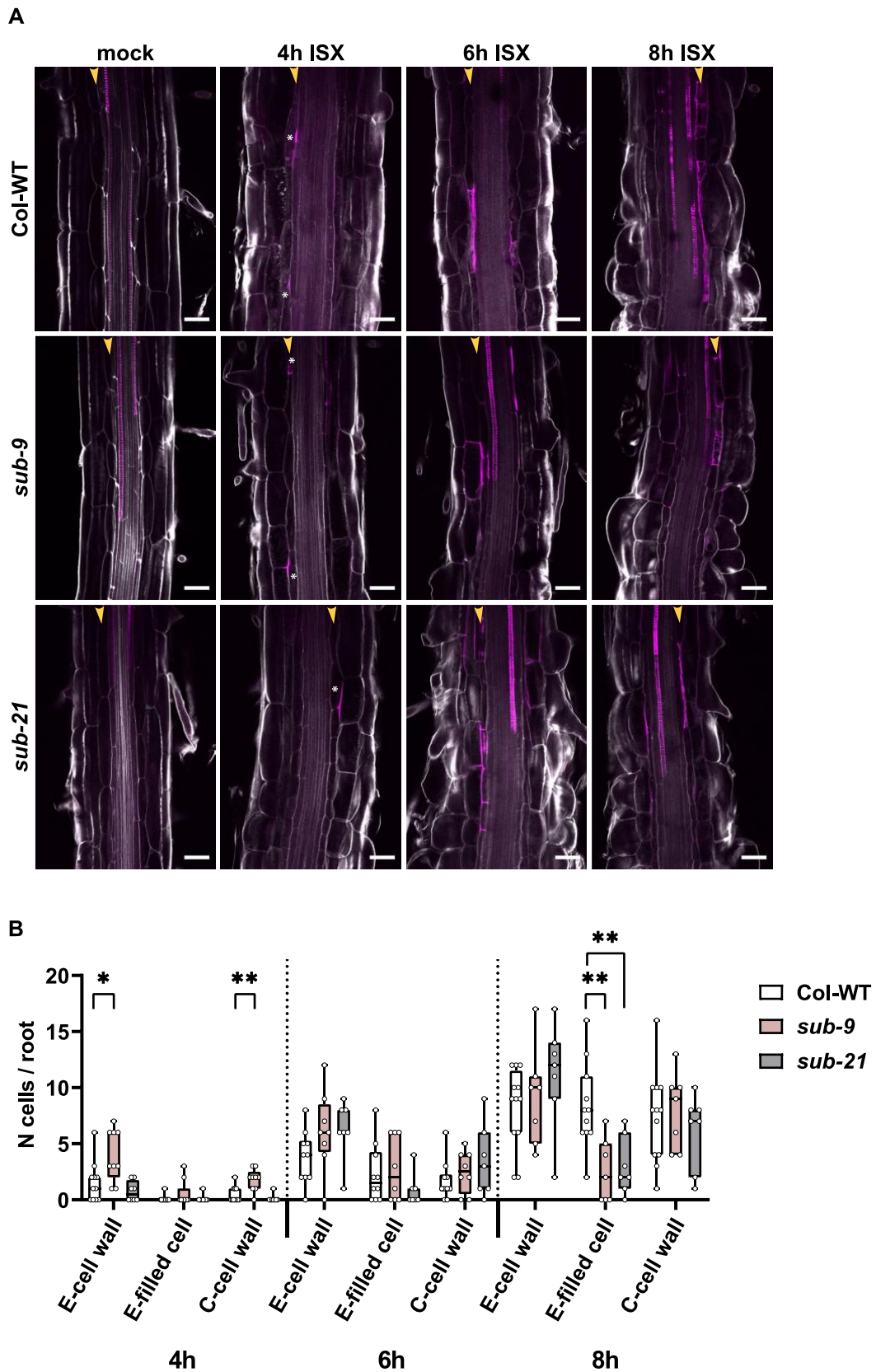


Figure 17. Comparison of wild-type and *sub* mutants in time course analysis of isoxaben-induced lignification.

(A) Confocal micrographs showing a cross-section of Col-0, *sub-9*, and *sub-21* roots treated with mock or 600 nM isoxaben for 4h, 6h, and 8h. Lignin and cell walls are shown in magenta (stained with Basic Fuchsin) and gray (stained with Calcofluor White), respectively. Yellow

arrowheads indicate endodermis. White asterisks mark the start of lignification in endodermal cells. (B) Quantification of the data shown in (A); the number of cells affected by transient exposure to isoxaben for 4 h, 6 h, and 8 h. Endodermis-cell wall (E-cell wall), endodermal cells with Fuchsin signal on the cell wall periphery. Endodermis-filled cell (E-filled cell), endodermal cells with Fuchsin signal within the cell. Cortex-cell wall (C-cell wall), cortex cell with Fuchsin stain on the cell wall periphery. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $7 \leq n \leq 12$; $n = \text{root}$. Asterisks represent statistical significance. Adjusted P values $**P \leq 0.005$, $*P = 0.0153$. t-test. The experiment was performed once. Scale bars: 30 μm .

3.2. *QKY* is not required for the pectinase-induced CWD response

Previous studies have reported that *SUB* and *QKY* contribute in distinct ways to the isoxaben-induced CBI response. In contrast to *SUB*, the *QKY* function is only required for CBI-induced lignin production but not for callose production (Chaudhary et al. 2020). To examine the role of *QKY* in the isoxaben-induced downregulation of *SUB*, the plasma membrane signal of the pSUB::SUB:EGFP *qky-8* reporter was analyzed in the root epidermal cells of young seedlings. The results showed an additive reduction of the plasma membrane signal intensity of the reporter when isoxaben-treated seedlings were compared to untreated plants (Chaudhary et al. 2021). This suggests that isoxaben and *QKY* influence the amount of *SUB* at the plasma membrane through parallel pathways.

The aim of the following experiments was to investigate the potential specificity of *QKY* in contributing to the CWD response in seedlings. The importance of *QKY* in callose deposition caused by pectinase was evaluated based on the area with a visibly strong aniline blue signal in cotyledons. Similar to the wild type, a significant difference in the area covered by callose was observed between mock and pectinase-treated *qky-11* seedlings. Despite the treatment, approximately the same amount of callose accumulated in the cotyledons of *sub-21* (Fig. 18). These results suggest that *QKY* does not affect callose accumulation after pectinase treatment.

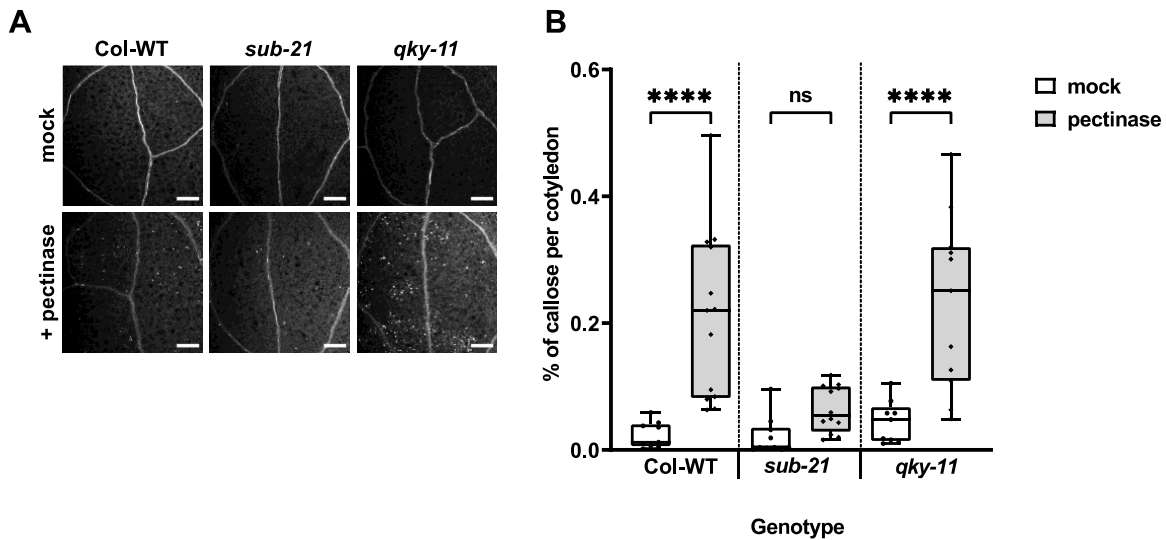


Figure 18. Role of *QKY* in CWD-induced callose accumulation.

(A) Confocal micrographs show cotyledons of 6-day-old Col, *sub-21*, and *qky-11* seedlings treated with mock and 0,1% pectinase for 24 hours. Aniline blue fluorescence signal strength indicates callose accumulation. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $9 \leq n \leq 13$. Asterisks represent statistical significance; Adjusted P value **** $P < 0.0001$; one-way ANOVA followed by Šídák multiple comparison test. The experiment was performed three times with similar results. Scale bars: 200 μm .

The next step was to measure the amount of SUB at the plasma membrane after treatment with pectinase. Five-day-old *sub-1* and *qky-8* seedlings expressing pSUB::SUB:EGFP were incubated for 30 min in a solution containing either 0.1% pectinase or pure half-strength MS medium. Obtained results confirm the visible reduction of SUB at the plasma membrane of epidermal root cells in *qky-8* seedlings under unstressed conditions (Fig. 19). Interestingly, the application of the enzyme did not lead to further depletion of SUB in *qky-8* plants (Fig. 19). The opposite effect can be observed in SSE *sub-1* treated with pectinase, where the signal intensity is significantly lower compared to mock-treated seedlings. The finding indicates that QKY may directly or indirectly influence the process of transferring SUB from the cell surface into the cell after pectinase treatment.

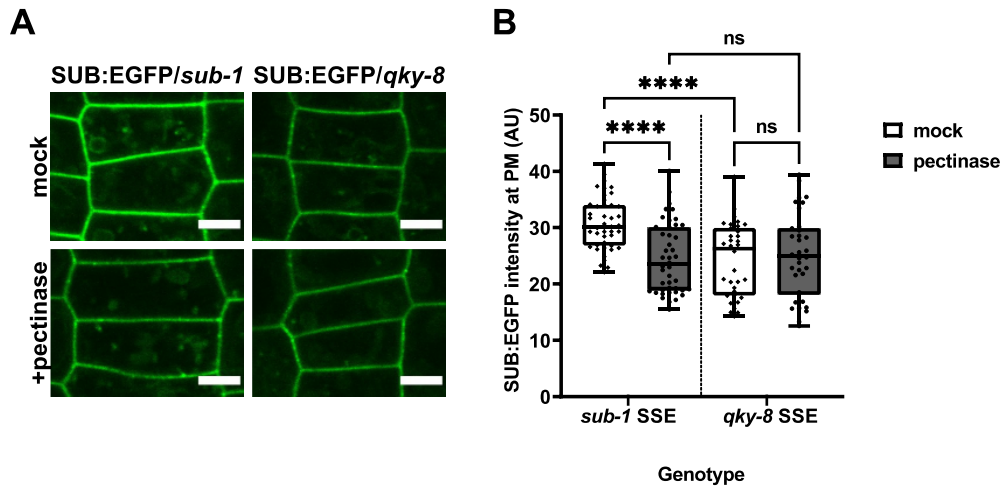


Figure 19. Influence of QKY on plasma membrane SUB:EGFP levels after pectinase treatment.

(A) Signal intensity of a functional pSUB::SUB:EGFP (SSE) reporter in *sub-1* and *qky-8* after 30 min exposure of seedlings to mock or pectinase. (B) Quantification of the data shown in (A). Note the reduction in signal in mock-treated SSE *qky-8* and enzyme-treated root epidermal cells of SSE *sub-1* and SSE *qky-8*. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $34 \leq n \leq 44$. $n = \text{cell}$. Asterisks represent statistical significance. Adjusted P value **** $P < 0.0001$; unpaired t-test. The experiment was performed three times with similar results. Scale bars: 5 μm .

3.3. *ANGUSTIFOLIA* and *ZERZAUST* do not contribute to isoxaben-induced lignification

ZET and *AN* are two additional components of the *SUB* signaling pathway. Following experiments aimed to uncover novel functions of these genes in the context of cell wall damage responses. Interestingly, *AN* has been described as a transcriptional repressor of *MYB46*, which encodes a transcriptional regulator of secondary cell wall formation, including lignin accumulation (Xie et al. 2020; Nakano et al. 2015).

The impact of *AN* and *ZET* on isoxaben-induced lignin formation was investigated by subjecting six-day-old seedlings of Ler, *sub-1*, *an-1*, and *zet-1* to 600 nM isoxaben for 16 hours. The analysis was done by comparing isoxaben-treated wild-type plants with *an-1* or *zet-1* plants carrying strong loss-of-function alleles. No obvious change in the phloroglucinol signal indicating lignification was observed after quantifying the results obtained from this assay (Fig. 20C, D). These results imply that *AN* and *ZET* do not control this process in a significant way.

The next experiment aimed to determine whether *AN* or *ZET* affects the abundance of *SUB* present at the plasma membrane of epidermal cells in the roots treated with pectinase. Five-day-old *sub-1*, *an-1*, and *zet-1* seedlings expressing pSUB::SUB:EGFP were incubated in 0.1%

pectinase or mock solution for 30 min. The results showed no detectable impact of AN on the internalization of SUB. However, the reporter signal detected in SSE *zet-1* plants after applying 0.1% pectinase did not exhibit a statistically significant difference compared to the control treatment (Fig. 20A, B). These observations suggest that ZET may have a direct or indirect role in facilitating the process of SUB internalization following pectinase treatment.

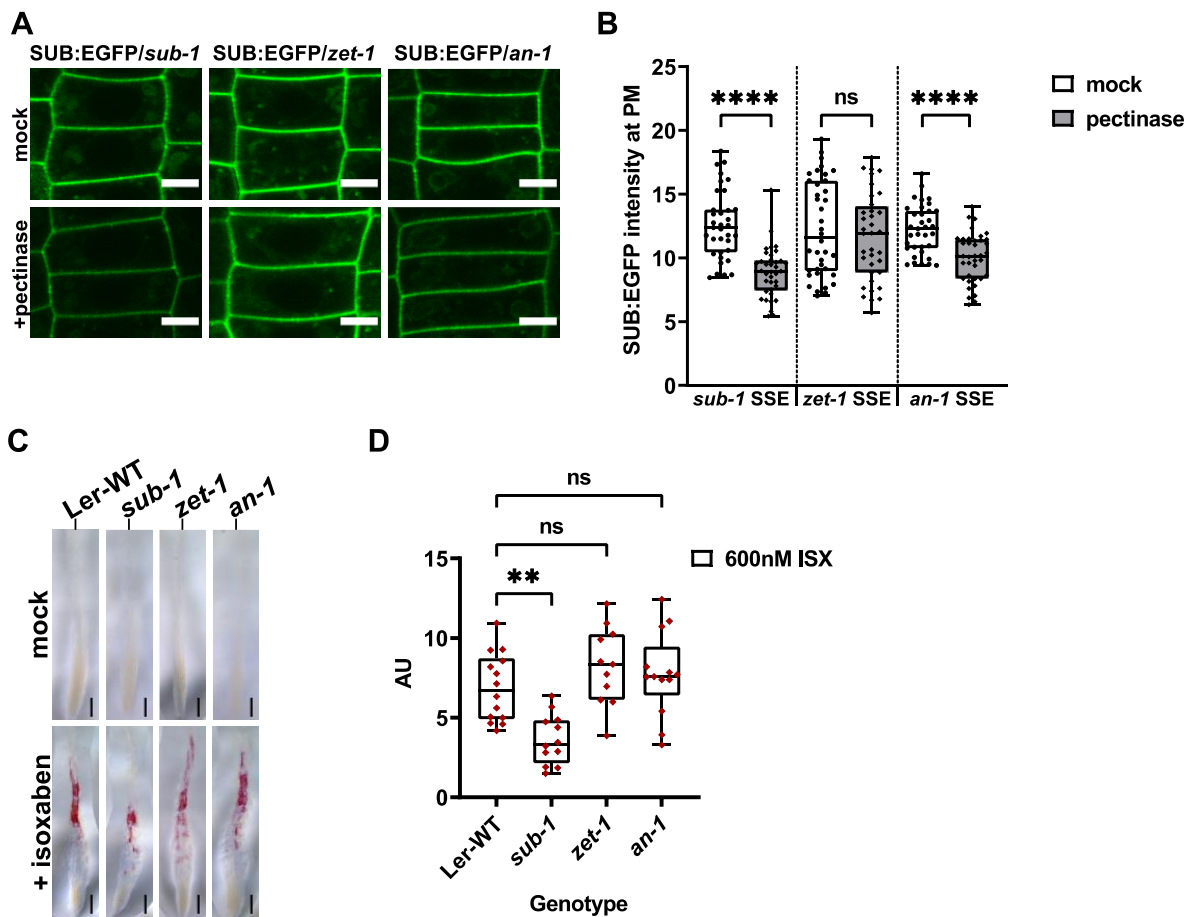


Figure 20. Role of ZET and AN in cell wall damage response.

(A) Signal intensity of a functional pSUB::SUB:EGFP (SSE) reporter in *sub-1*, *zet-1*, *an-1* after 30 min exposure of seedlings to mock or pectinase. (B) Quantification of the data shown in A. $35 \leq n \leq 39$. $n = \text{cell}$. Note the reduction in signal in enzyme-treated root epidermal cells of SSE *sub-1* and SSE *an-1*. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. Asterisks represent statistical significance. Adjusted P value **** $P < 0.0001$; unpaired t test. The experiment was performed three times with similar results. (C) Phloroglucinol signal strength indicates lignin accumulation in roots of 6-day-old seedlings exposed to 600 nM isoxaben for 16 hours. Genotypes: Ler, *sub-1*, *zet-1*, *an-1*. (D) Quantification of the results depicted in C. Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $11 \leq n \leq 14$. Asterisks represent statistical significance. Adjusted P value ** $P = 0.0016$; one-way ANOVA followed by Dunnett's multiple comparison test. The experiment was performed three times with similar results. Scale bars: (A): 5 μm . (C): 100 μm .

3.4. The *SCHENGEN* pathway is not essential for isoxaben-induced lignin accumulation

The findings presented above demonstrate the crucial involvement of the endodermis in the lignification process induced by isoxaben treatment in the roots of young seedlings. Under proper developmental conditions, the endodermis forms the Casparian strip, a major apoplastic diffusion barrier containing lignin (Barbosa et al., 2019). In addition, when the Casparian strip is impaired, a compensatory production of lignin begins in the middle lamella of the cell corners, and the continuously active Schengen signaling pathway mediates it. This process involves several molecular components, such as the receptor kinase *SGN3/GSO1* and its ligands *CIF1* and *CIF2*. It leads to localized ROS generation and lignification in the root through phosphorylation of *RBOHD/F* (Fujita et al., 2020; Lee et al., 2013; Pfister et al., 2014; Alassimone et al., 2010; Doblus et al., 2017).

To investigate whether the *SGN3* also participates in lignification caused by isoxaben stress, a phloroglucinol-based lignin accumulation assay was performed. For the analysis, six-day-old seedlings of wild-type and mutant plants carrying a null allele of *SGN3* (*sgn3-3*) and *SUB* (*sub-21*) were exposed to 600 nM isoxaben for 16 hours. Surprisingly, no significant difference in phloroglucinol staining intensity was detected between Col-0 and *sgn3-3* (Fig. 21). This suggests that *SGN3* does not play an essential role in lignin production upon isoxaben treatment. Notably, *in vitro* studies have shown that the ectodomains of *SUB* and *SGN3* do not interact, which is consistent with the results obtained in this experiment (Okuda et al., 2020).

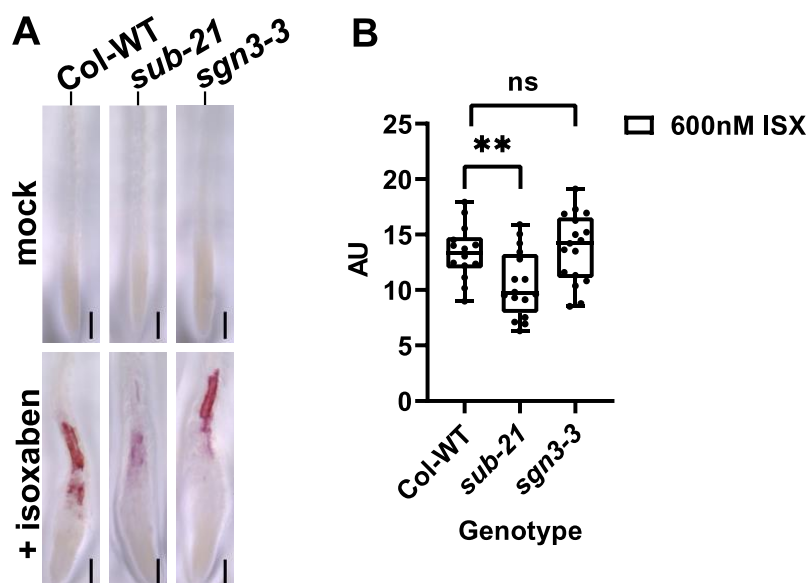


Figure 21. Impact of *SGN3* on CBI-induced lignin accumulation.

(A) Phloroglucinol signal strength indicating lignin accumulation in roots of 6-day-old seedlings exposed to 600 nM isoxaben for 16 hours. Genotypes: Col-0, *sub-21*, *sgn3-3*. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $14 \leq n \leq 17$. Asterisks represent statistical significance. Adjusted P value $**P = 0.0071$; one-way ANOVA followed by Dunnett's multiple comparison test. The experiment was performed three times with similar results. Scale bars: 200 μm .

3.5. Screening potential candidates involved in the *SUB* signaling pathway

In nature, there are instances where a single protein is responsible for one function, but protein complexes determine many vital functions in the plant life cycle. To find candidates involved in putative *SUB* complexes, a co-immunoprecipitation experiment was performed. A former Ph.D. student, Dr. Ajeet Chaudhary, carried out the study. The analysis used *sub-9* seedlings expressing pSUB::SUB:EGFP subjected either to mock or to 0.1% pectinase for 15min. The obtained protein identifiers were grouped into several classes according to their characteristics. The largest and most relevant group consisted of receptor kinases, receptor-like proteins, and GPI-anchored proteins. Many proteins found in this set were considered for further analysis. Other categories included cell wall localized proteins, kinases, phosphatases, plasma membrane proteins, proteins related to BR signaling, transporters, and more. The mutant alleles of the respective candidate proteins used in this study are listed in Table 1.

One of the putative members of the *SUB* signaling pathway is COBRA-LIKE PROTEIN-7 (COBL7). It has been reported that COBL7, together with its close homolog COBL9, plays an important role in regulating the growth and development of root hairs, which are important for water and mineral uptake and plant-microbe interactions (Z. Li et al. 2022). In addition, mutations in *COBL7* and *COBL9* resulted in impaired root hair growth and reduced cellulose accumulation in the growing tips of the hairs (Z. Li et al. 2022). Two of the nine members of the STRUBBELIG-RECEPTOR FAMILY (SRF) were also found in the co-IP screen. SRF3 and SRF7 are leucine-rich repeat receptor kinases whose functions are still not fully understood. Expression profiling suggests that SRF3 might be involved in lignification and pectin biosynthesis, while SRF7 may participate in primary cell wall biosynthesis and processes requiring cellulose synthase activity (Eyüboğlu et al. 2007). These roles align well with the search for potential *SUB* candidates.

Other notable proteins on the list included RUL1, LMK1, SIMP1, and HERK2. The REDUCED IN LATERAL GROWTH1 (RUL1), a receptor kinase has been identified as a positive regulator of cambium activity, which is responsible for the secondary plant growth and the production of woody stems (Agusti et al. 2011). RUL1 is expressed in the cambium of the shoot and the procambium cells of the leaf primordia. Identifying other LRR-RKs expressed in the cambium suggests a complex network of membrane-bound receptors involved in cambial regulation (Agusti et al. 2011). LMK1 is a receptor kinase with a malectin-like domain in its extracellular region. It has been demonstrated to induce cell death in plant leaves (X. Li et al. 2020). SALT-INDUCED MALECTIN-LIKE DOMAIN-CONTAINING PROTEIN1 (SIMP1) is a receptor kinase involved in plant salt tolerance (He et al. 2021). However, its exact function has yet to be discovered, HERK2, along with 5 CrRLK1Ls family members, might work in coordinating cell expansion, cell-cell communication, and reshaping cell walls during both vegetative and reproductive growth (Lindner et al. 2012).

The criteria to be considered was the responsiveness of the selected candidates to CBI-induced stress. First, the callose accumulation assay after isoxaben treatment was performed to test whether any candidate genes were involved in this response. Six-day-old seedlings of different genotypes were subjected to the standardized isoxaben treatment. The results show that all of the tested mutants exhibit a significant increase in callose deposition when exposed to isoxaben compared to mock (Fig. 22). The response of *cobl7*, *herk2*, *simp1*, *lmk1*, and *rull1* to applied stress was similar to that of the wild type and opposite to that of the *sub-21* cotyledons (Fig. 22).

Obtained results imply that none of the tested candidates contribute significantly to the callose production induced by the reduction of cellulose synthesis.

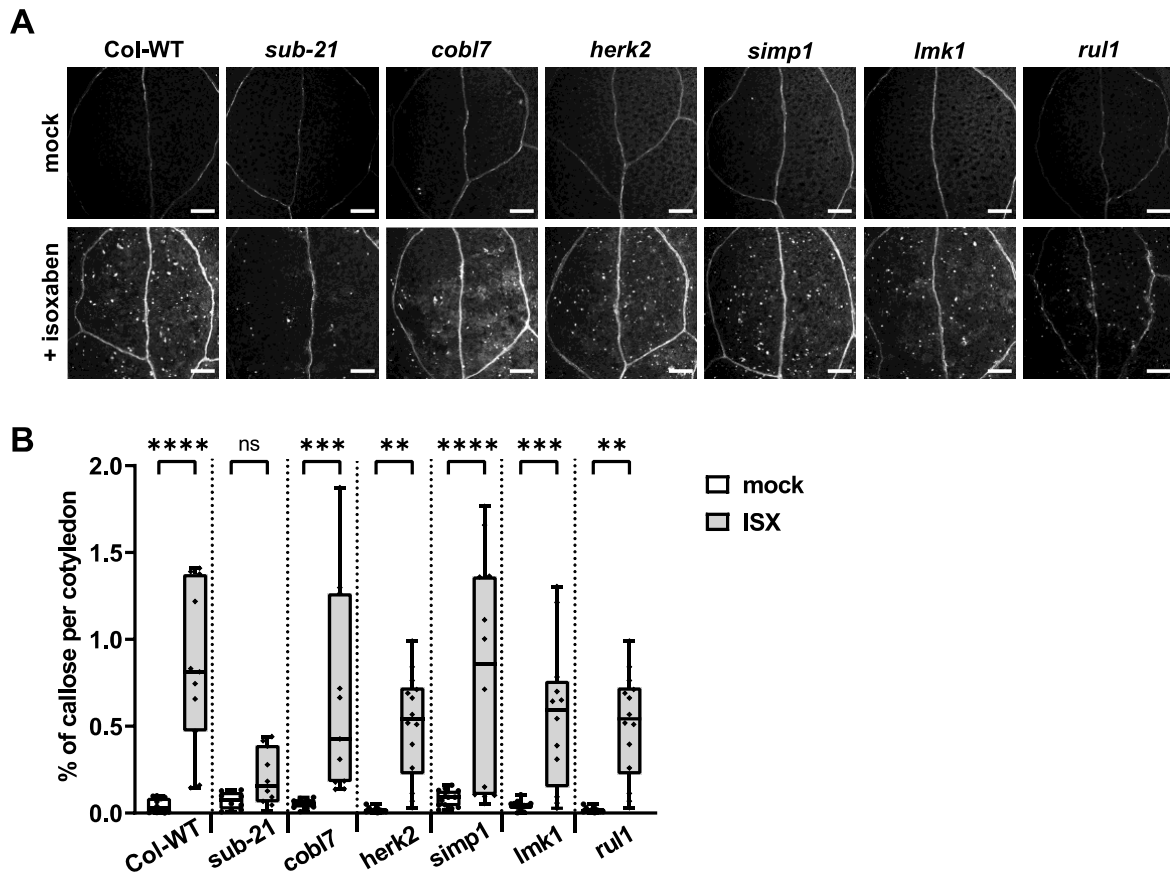


Figure 22. Callose accumulation assay as a first screening factor.

(A) Confocal micrographs show cotyledons of 6-day-old seedlings treated with mock and ISX for 24 hours. Genotypes: Col-0, *sub-21*, *cobl7*, *herk2*, *simp1*, *lmk1*, *rul1*. Aniline blue fluorescence signal strength indicates callose accumulation. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $10 \leq n \leq 14$. Asterisks represent statistical significance; Adjusted P value **** $P < 0.0001$; one-way ANOVA followed by Šídák multiple comparison test. The experiment was performed three times with similar results. Abbreviation: ISX, isoxaben. Scale bars: 200 μm .

The screening of the candidates was continued with an assay for the accumulation of lignin. Six-day-old seedlings of Col-0, *sub-9*, *cobl7*, *rul1*, *srf3*, *srf7*, *herk2*, *simp1* and *lmk1* were analyzed for ectopic lignin production after 16h of 600nM isoxaben treatment. Lignification, visualized by the phloroglucinol staining, was observed in the transition and elongation zones of the stressed roots (Fig. 23). A quantitative comparison of the signal intensity between wild-type and mutant roots revealed a reduced phloroglucinol signal in seedlings of *simp1* and plants lacking the *SUB* function. Interestingly, seedlings of *cobl7* produced significantly more lignin compared to wild-type plants (Fig. 23). Furthermore, no distinct changes in the phloroglucinol signal were detected in mutants of other genotypes with respect to the wild-type plants (Fig. 23).

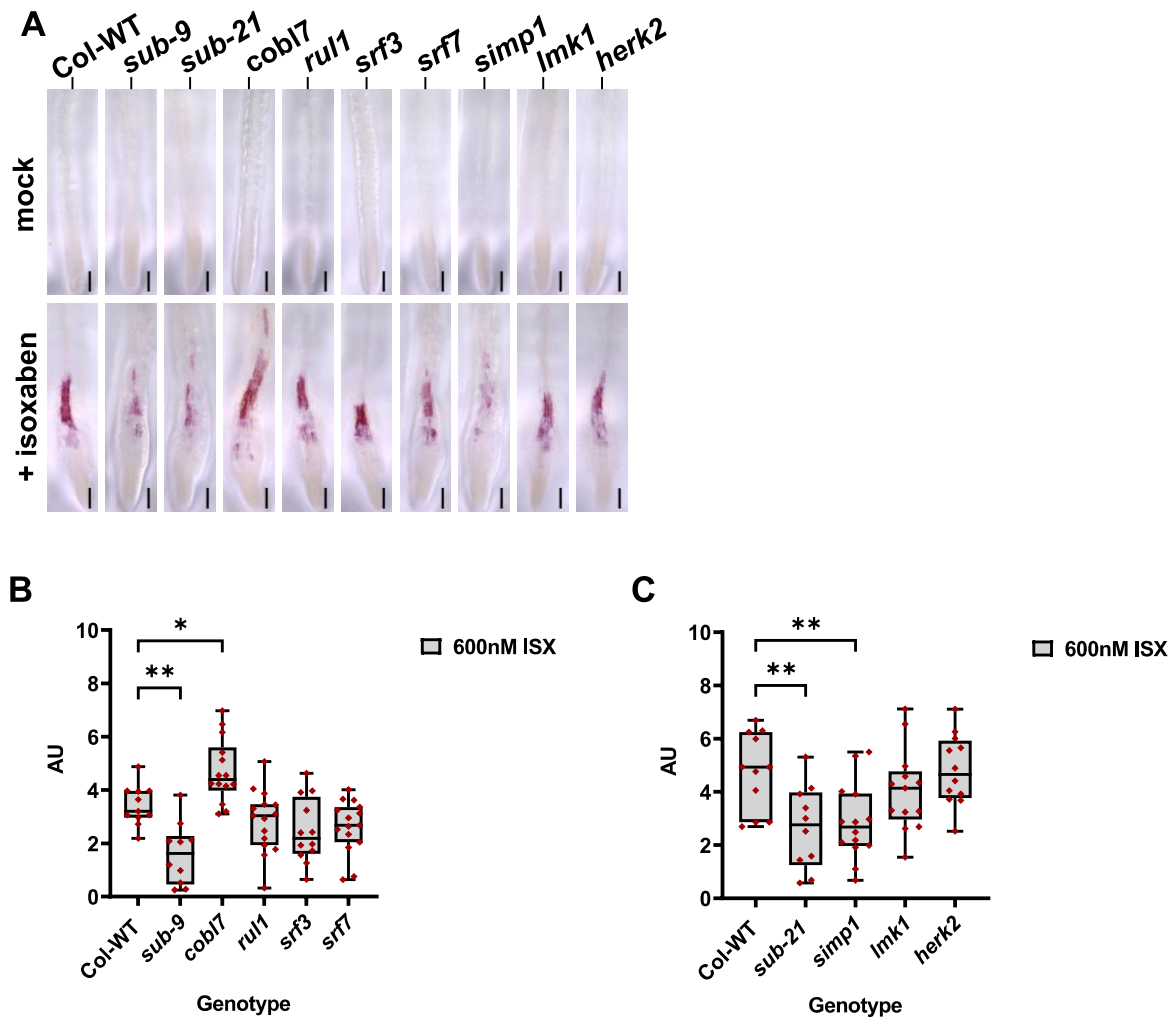


Figure 23. Lignin accumulation assay as a second screening factor.

(A) Phloroglucinol signal strength indicating lignin accumulation in roots of 6-day-old seedlings exposed to 600 nM isoxaben for 16 hours. Genotypes: *Col*, *sub-9*, *sub-21*, *cob17*, *rull1*, *srf3*, *srf7*, *simp1*, *lmk1*, *herk2*. (B-C) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $9 \leq n \leq 15$. Asterisks represent statistical significance. Adjusted P values $*P = 0.0204$; $0.0015 \leq **P \leq 0.0077$; one-way ANOVA followed by Dunnett's multiple comparison tests. The experiment was performed three times with similar results. Abbreviation: ISX, isoxaben. Scale bars: 200 μm .

Another interesting candidate discovered during the co-IP experiment is a protein related to a class of proteins associated with forming the lignin-based Casparian strip. Furthermore, the previous chapter showed that isoxaben-induced lignin production begins to accumulate in the endodermis, which is the same cell layer where the Casparian strip is present. The protein found in the co-IP screen belongs to the large class of CASPARIAN STRIP

MEMBRANE DOMAIN-like proteins (CASPL). Little is known about individual members of this gene family, but they might be necessary for membrane scaffolding and regulation of various cell wall-modifying processes (Roppolo et al. 2014). The involvement of *CASP-LIKE PROTEIN 1B1* (*CASPLP1B1*) in the CBI-induced lignification was tested in another set of experiments. The same procedure of isoxaben as treatment and phloroglucinol as a detection method was used in this study. Overnight incubation of young seedlings in media containing 600nM isoxaben caused the expected inhibition of cellulose biosynthesis and, consequently, the fortification of root tissues in the form of lignin. Analysis of the susceptible part of the root revealed significantly reduced signal intensity of the stain accumulated by seedlings of the *sub-21*, but also of the *casplp1b1* mutant (Fig. 24).

Presented results reveal that *CASPLP1B1* and *SIMP1* may potentially be part of a genetic pathway involved in lignification caused by isoxaben-induced cell wall disruption.

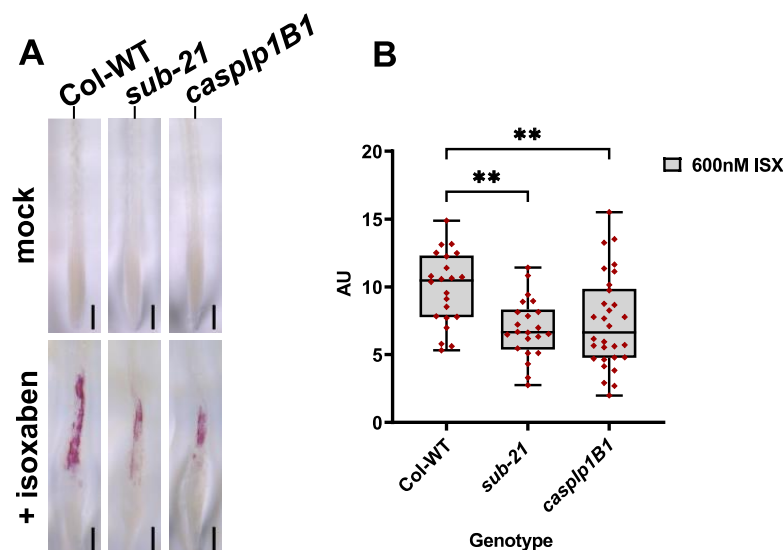


Figure 24. Impact of *CASPLP1B1* on CBI-induced lignin accumulation.

(A) Phloroglucinol signal strength indicating lignin accumulation in roots of six-day-old seedlings exposed to 600 nM isoxaben for 16 hours. Genotypes: Col, *sub-21*, *casplp1B1*. (B) Quantification of the results depicted in (A). Box and whisker plots are shown. Whisker ends mark the minimum and maximum of all the data. $22 \leq n \leq 30$. Asterisks represent statistical significance. Adjusted P values $**P < 0.0059$; one-way ANOVA followed by Dunnett's multiple comparison tests. The experiment was performed three times with similar results. Abbreviation: ISX, isoxaben. Scale bars: 200 μm .

3.6. The *SUB*-dependent transcriptional response to isoxaben treatment

The previous investigation of the transcriptional response of seedlings exposed to 600 nM isoxaben for 36 hours provided valuable insights (Hamann et al. 2009; Bacete et al. 2022). The present study aimed to identify the isoxaben-responsive genes in young seedlings sensitive to *SUB* activity at the mRNA level. Therefore, the focus was on the *SUB*-dependent transcriptome of six-day-old seedlings exposed to either mock or 600 nM isoxaben for 30 min, 5 hours, or 8 hours. This investigation used RNA sequencing (RNAseq) analysis, and the results were compared between wild-type, *sub-21*, and *SUB-OE LI* plants. The experiment included three independent biological replicates for each genotype and time point.

Considering that *SUB* is essential for controlling isoxaben-induced lignification, the study was directed toward the effect of isoxaben on the expression levels of genes participating in lignin biosynthesis. The collected data set contains more than 150 expressed genes related to lignin production. The search for differentially expressed genes (DEGs) after isoxaben treatment compared to mock led to filtering of the data based on a false discovery rate (FDR) adjusted p-value of ≤ 0.05 and a log₂ fold change (log₂FC) of ≥ 1 and ≤ -1 . An initial comparison was made with the wild-type expression profile after 8 hours of isoxaben exposure, using the results from previous studies (Hamann et al. 2009; Bacete et al. 2022). Increased gene expression after 8 hours of treatment included *PDF1.2*, *TOUCH4*, *OPCL1*, *RBOHD*, and *PAD4*, while a decrease in *TIP2;3* was confirmed. However, no induction of *ERD1*, *RD26*, or *RLP12* was detected, possibly due to the different growth conditions or detection methods used (RNA-seq versus Affymetrix microarray analysis in Hamann et al. 2009).

The data set obtained from this experiment contains several *MYB*-related genes known to act as regulators, namely genes encoding activators or repressors of the lignin biosynthetic process. *MYB46* expression in wild-type plants showed no significant changes at the 30 min and 8 hour-time points, while an induction was observed at the 5 hours mark. In comparison, *sub-21* showed no effect at 30 min, but a significant reduction was observed at 5 and 8 hours (Fig. 25). Interestingly, overexpression of *SUB* led to significant increases at 5 and 8 hours while showing no effect at 30 min. *MYB83* was induced in the wild-type at 30 min but decreased over time, with a slight reduction observed at 8 hours. The *sub-21* mutant showed less induction at 30 min and no effect at the subsequent 5- and 8-hour time points. In *SUB* overexpressing line, *MYB83* was repressed at 30 min, induced at 5 hours, and showed no effect at 8 hours (Fig. 25). As for downstream activators, *MYB63* showed an interesting pattern with general downregulation at 30 min followed by upregulation at 5 hours and even more so at 8 hours (Fig. 25). *MYB85* followed a similar trend with downregulation at 30 min, upregulation at 5 hours,

but no effect or slight downregulation at 8 hours in wild-type plants. Regarding the downstream repressors, *MYB4*, *MYB7*, and *MYB32* showed no significant effects (Fig. 25). These data show that *SUB* has different effects on the expression of *MYB46* and *MYB83*.

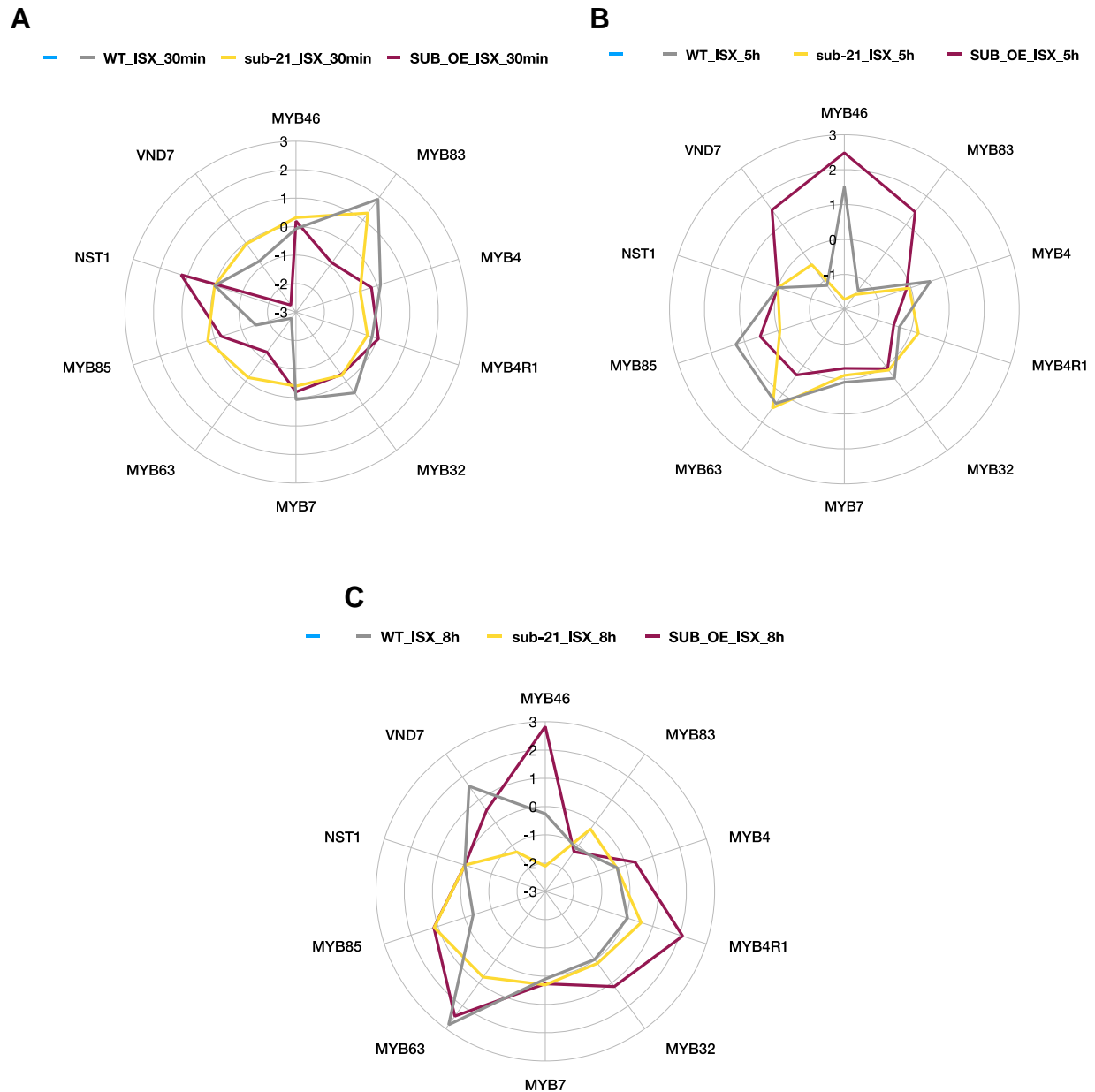


Figure 25. Radar plot showing expression of *NST1*, *VND7*, and various *MYB* genes in response to isoxaben exposure.

Radar plots present results of RNAseq analysis compared between wild-type (gray), *sub-21* (yellow), and *SUB-OE L1* (magenta) seedlings. Data based on a false discovery rate (FDR) adjusted p-value of ≤ 0.05 and a \log_2FC of ≥ 3 and ≤ -3 . (A) Radar plot of *NST1*, *VND7*, and various *MYB* gene expression after 30min of 600 nM ISX treatment. (B) Radar plot of *NST1*, *VND7*, and various *MYB* gene expressions after 5h of 600 nM ISX treatment. (C) Radar plot of *NST1*, *VND7*, and various *MYB* gene expressions after 8h of 600 nM ISX treatment. Abbreviation: ISX, isoxaben.

Differential induction of gene expression was discovered while tracing the temporal patterns of expression levels of genes involved in monolignol biosynthesis during isoxaben treatment. Induction of the genes encoding *4-HYDROXYCINNAMATE COA LIGASE 8 (4CL8)*, *CINNAMYL ALCOHOL DEHYDROGENASE 2 (CAD2)*, and *CAD6* was observed in wild-type after 30 min of treatment (Fig. 26). The 4CL enzymes are integral to the phenylpropanoid pathway and perform an intermediate step in monolignol biosynthesis, while the CAD enzymes catalyze the final step (Yao et al. 2021).

A sustained induction of *4CL8* was observed after 5 hours of treatment (Fig. 26B). Conversely, an upregulated expression of *L-PHENYLALANINE AMMONIA-LYASE 2 (PAL2)*, *4CL5*, *CINNAMOYL COA REDUCTASE 2 (CCR2)* and *CAD6* was observed at the 8-hour time point (Fig. 26C). Consequently, there is a pronounced increase in the expression of several genes encoding critical enzymes in the phenylpropanoid pathway after 8 hours of isoxaben treatment. These enzymes, starting with *PAL2*, function in a sequential manner during the process of monolignol biosynthesis (Rohde et al. 2004). The next step was to examine how the changes in *SUB* activity affected the expression of genes involved in monolignol biosynthesis. Notably, a significant decrease in *CAD2* expression was observed at the 30-minute time point in the *sub-21* sample, a reduction that was not seen in the *SUB-OE L1*. In addition, no expression was detected for *CAD6* in *sub-21*, but it was downregulated in *SUB-OE L1* (Fig. 26A). At the 5-hour time point, there was no induction of *4CL8* in *sub-21*, while *SUB-OE L1* showed a slightly higher level than the wild type. *CAD2* expression was slightly induced in *sub-21* but repressed in wild-type and *SUB-OE L1*. Another observation was that *SUB* overexpression led to upregulating *PAL1*, *4CL5*, *4CL8*, and *CCR2* (Fig. 26B). At the 8-hour time point, *CAD6* induction was absent in *sub-21*, and *CAD2*, *CAD3*, and *4CL8* repression was not noticed in *sub-21*. Meanwhile, minimal effects were observed in *sub-21*, but *SUB* overexpression led to increased *PAL1*, *4CL1*, *4CL5*, and *CCR2* levels (Fig. 26C).

In conclusion, the data suggest that the major effects of modifying *SUB* activity on the isoxaben-responsive expression of monolignol biosynthetic genes revolve around *4CL8*, *CAD2*, and *CAD6* in a time-dependent manner. The requirement of *SUB* activity for *CAD2* and *CAD6* induction at the 30 min time point is obvious, but the influence of other factors is also apparent. The induction of *4CL8* after 5 hours of exposure to isoxaben seems to be directly controlled by *SUB*. However, *CAD6* induction at the 8-hour time point requires *SUB* and additional factors.

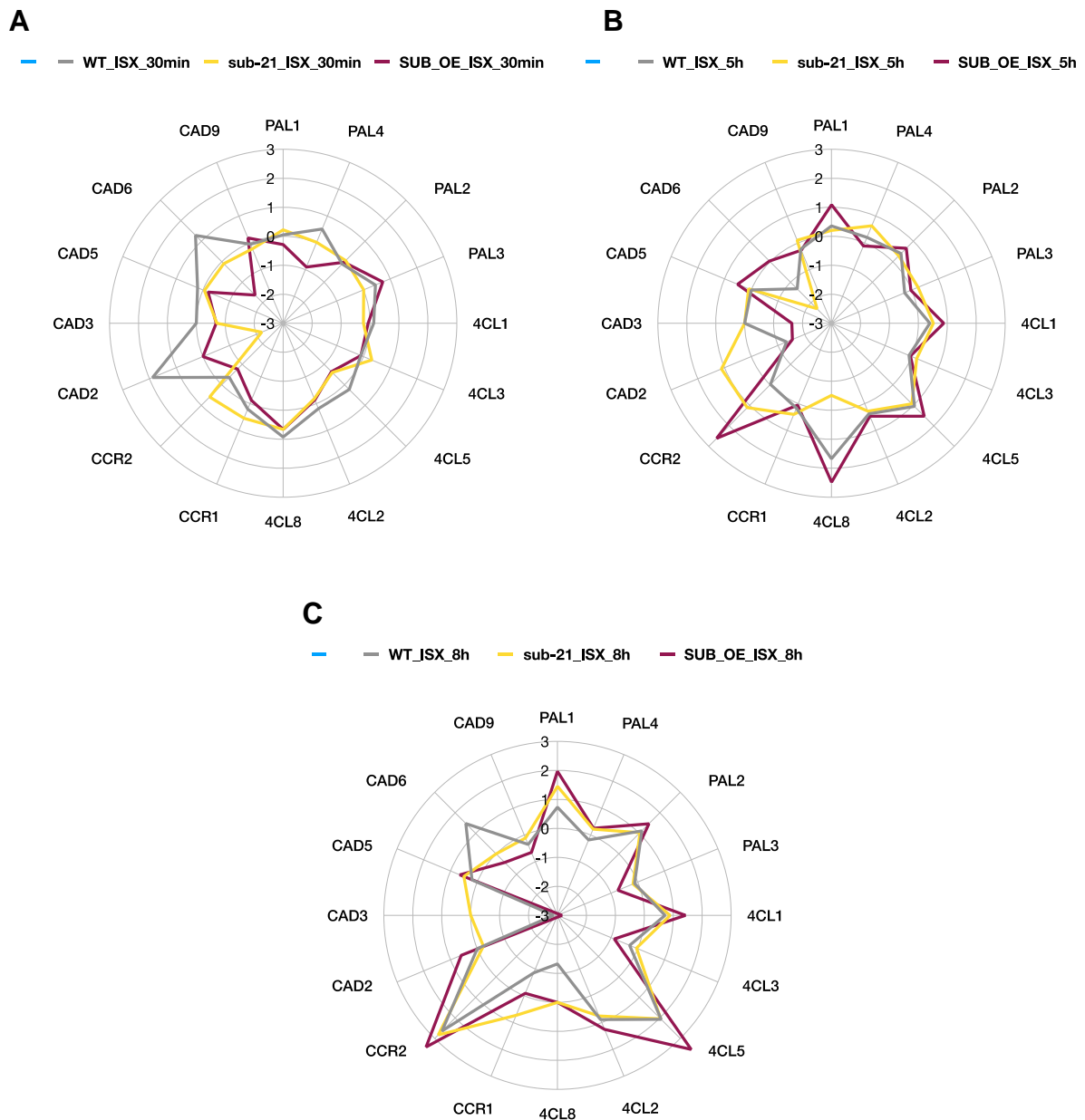


Figure 26. Radar plot showing expression of various *CCR*, *CAD*, *PAL*, and *4CL* genes in response to isoxaben exposure.

Radar plots present results of RNAseq analysis compared between wild-type (gray), *sub-21* (yellow), and *SUB-OE LI* (magenta) seedlings. Data based on a false discovery rate (FDR) adjusted p-value of ≤ 0.05 and a \log_2FC of ≥ 3 and ≤ -3 . (A) Radar plot of various *CCR*, *CAD*, *PAL*, and *4CL* gene expression after 30min of 600 nM ISX treatment. (B) Radar plot of various *CCR*, *CAD*, *PAL*, and *4CL* gene expressions after 5h of 600 nM ISX treatment. (C) Radar plot of various *CCR*, *CAD*, *PAL*, and *4CL* gene expressions after 8h of 600 nM ISX treatment. Abbreviation: ISX, isoxaben.

ROS play a central role in cell wall development, particularly through apoplastic peroxidases that use ROS as co-substrates to facilitate monolignol oxidation, leading to cell wall lignification (Torres and Dangl 2005; Barbosa, Rojas-Murcia, and Geldner 2019). Recent research has identified the NADPH oxidase RBOHF as essential for localized lignin formation in the root endodermis (Lee et al., 2013). Four of the ten Arabidopsis *RBOH* genes - *RBOHA*, *RBOHB*, *RBOHD*, and *RBOHF* - were detected in the presented data set (Fig. 27). In the wild-type, *RBOHA* showed a dynamic expression pattern with downregulation at 30 min and 8-hour time points, whereas a slight induction was observed for *RBOHB* over all time points. Confirming previous findings (Hamann et al. 2009), *RBOHD* was induced at the 5- and 8-hour time points (Fig. 27). *RBOHF*, however, showed little change in expression over the analyzed time course.

SUB appears to have differential effects on *RBOHA/B/D* expression (Fig. 27). *SUB* is necessary for the downregulation of *RBOHA* at the 8-hour incubation time and for the low induction of *RBOHB* after 30 minutes and 5 hours of treatment. Interestingly, *SUB* appears to promote the upregulation of *RBOHD* at the 5-hour time point while preventing its upregulation at the 8-hour time point. Overexpression of *SUB* leads to further induction of *RBOHD* after 5 hours of treatment (Fig. 27). While the genetics suggest that *SUB* directly regulates *RBOHD* expression, the effects observed are minimal.

In summary, the data indicate that *SUB* positively regulates *RBOHB* and *RBOHD* at the 5-hour time point and negatively regulates *RBOHA* at the 8-hour time point.

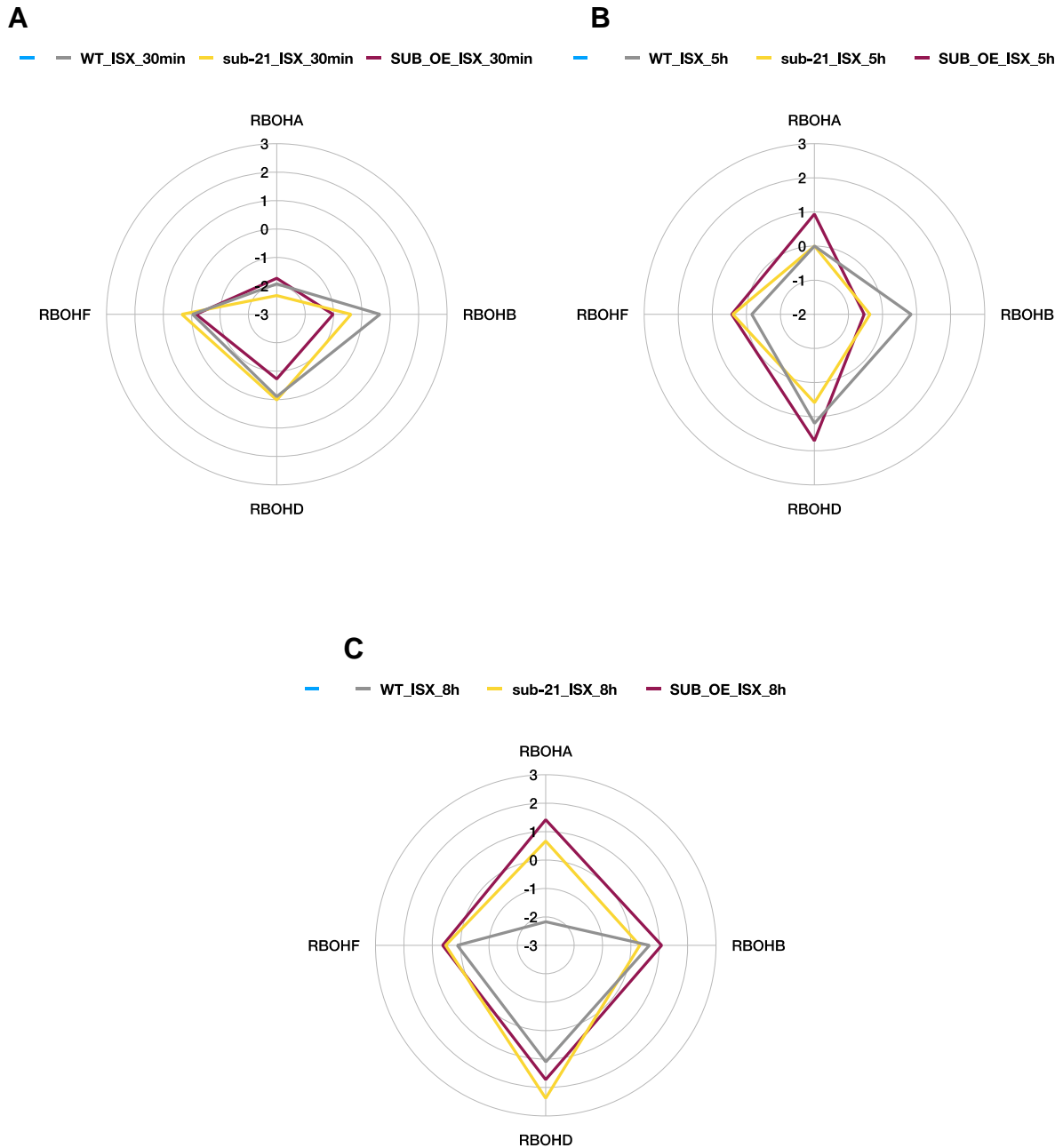


Figure 27. Radar plot showing expression of *RBOHA*, *RBOHB*, *RBOHD*, and *RBOHF* genes in response to isoxaben exposure.

Radar plots present results of RNAseq analysis compared between wild-type (gray), *sub-21* (yellow), and *SUB-OE LI* (magenta) seedlings. Data based on a false discovery rate (FDR) adjusted p-value of ≤ 0.05 and a \log_2FC of ≥ 3 and ≤ -3 . (A) Radar plot of *RBOHA*, *RBOHB*, *RBOHD*, and *RBOHF* gene expression after 30min of 600 nM ISX treatment. (B) Radar plot of *RBOHA*, *RBOHB*, *RBOHD*, and *RBOHF* gene expression after 5h of 600 nM ISX treatment. (C) Radar plot *RBOHA*, *RBOHB*, *RBOHD*, and *RBOHF* gene expression after 8h of 600 nM ISX treatment. Abbreviation: ISX, isoxaben.

The expression levels of several members of the Arabidopsis *LACCASE* gene family (Turlapati et al. 2011) were also examined in response to isoxaben treatment (Fig. 28). No consistent increase in expression levels was found for any of the *LAC* genes across the time points evaluated. Induction of *LAC5* was observed in wild-type after 30 min and of *LAC2*, *LAC10*, and *LAC12* after 5 hours of treatment. *LAC2* has been shown to reduce lignin formation in roots under water deficit or limited inorganic phosphate (Pi) conditions (Khandal et al. 2020). Recent studies have shown that *LAC5*, *LAC10*, and *LAC12* mediate vascular lignification (Blaschek et al. 2023). However, after 8 hours of isoxaben treatment, no significant increase in the expression of any of the *LAC* genes was detected, although *LAC16* was significantly downregulated. Interestingly, the isoxaben responsiveness of *LAC5*, as well as *LAC2/10/12*, requires *SUB* (Fig. 28). In *sub-21*, *LAC5* reduction was observed at the 30-minute time point. Furthermore, *LAC2/10* were downregulated, and *LAC12* did not show induction at the 5 hours of incubation time.

Finally, the downregulation of *LAC16* did not occur in *sub-21* at the 8-hour time point. Overexpression of *SUB* resulted in the upregulation of *LAC1* and *LAC14* at 30 minutes, *LAC1* at 5 hours, and *LAC11/12/14* at 8 hours (Fig. 28).

These results indicate that altering *SUB* activity influences the isoxaben-responsive expression of several *LAC* genes involved in lignification.

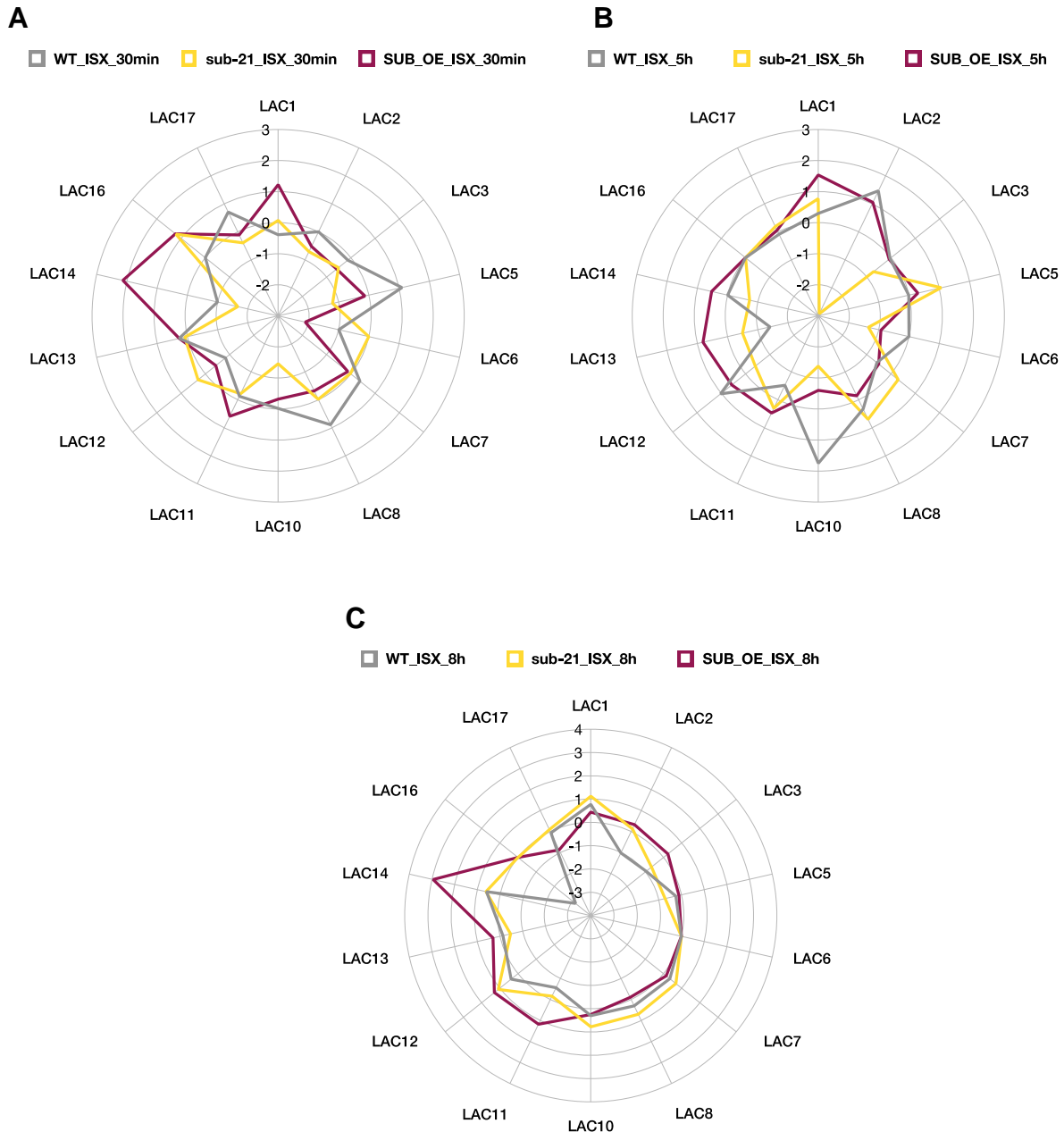


Figure 28. Radar plot showing expression of various *LAC* genes in response to isoxaben exposure.

Radar plots present results of RNAseq analysis compared between wild-type (gray), *sub-21* (yellow), and *SUB-OE LI* (magenta) seedlings. Data based on a false discovery rate (FDR) adjusted p-value of ≤ 0.05 and a \log_2FC of ≥ 4 and ≤ -3 . (A) Radar plot of *LAC* genes expression after 30min of 600 nM ISX treatment. (B) Radar plot of *LAC* genes expression after 5h of 600 nM ISX treatment. (C) Radar plot *LAC* genes expression after 8h of 600 nM ISX treatment. Abbreviation: ISX, isoxaben.

Additional investigations were conducted on members of the *PER/PRX* gene family (Duroux and Welinder 2003), many of which have been implicated in lignification (Dixon and Barros 2019; Blaschek and Pesquet 2021) (Fig. 29). The majority of *PRX* genes in the dataset were found to encode class III peroxidases. In particular, *PRX4* (*PER4*) was induced at all time points recorded. A log₂FC close to 3 for *PRX4* was observed after 30 min of isoxaben treatment. This was maintained at the 5- and 8-hour time points with a log₂FC of approximately 1.5 relative to mock (Fig. 29). *PRX4* is a prime candidate for a genetically direct transcriptional downstream gene of *SUB*, as its upregulation was consistently reduced in *sub-21* but enhanced in *SUB-OE L1* at 5 and 8 hours compared to the wild type. *PRX4* has been implicated in lignin formation (Fernández-Pérez et al. 2015), as well as in germination, photoperiod stress, and oligogalacturonide-induced immunity (Rasul, Lamotte, et al. 2012; Arnaud et al. 2017; Abuelsoud et al. 2020; Jemmat et al. 2021).

PRX28 also showed increased expression at all three time points evaluated, although it was reduced in *sub-21* after treatment for 30 minutes or 5 hours. In addition, expression of *PRX01* (*PR9*), which is involved in cold stress and root hair growth (Marzol et al. 2022), increased from a log₂FC of 1.5 at 5 hours to approximately 3 at an 8-hour time point (Fig. 29). This increase in expression was not seen in *sub-21*.

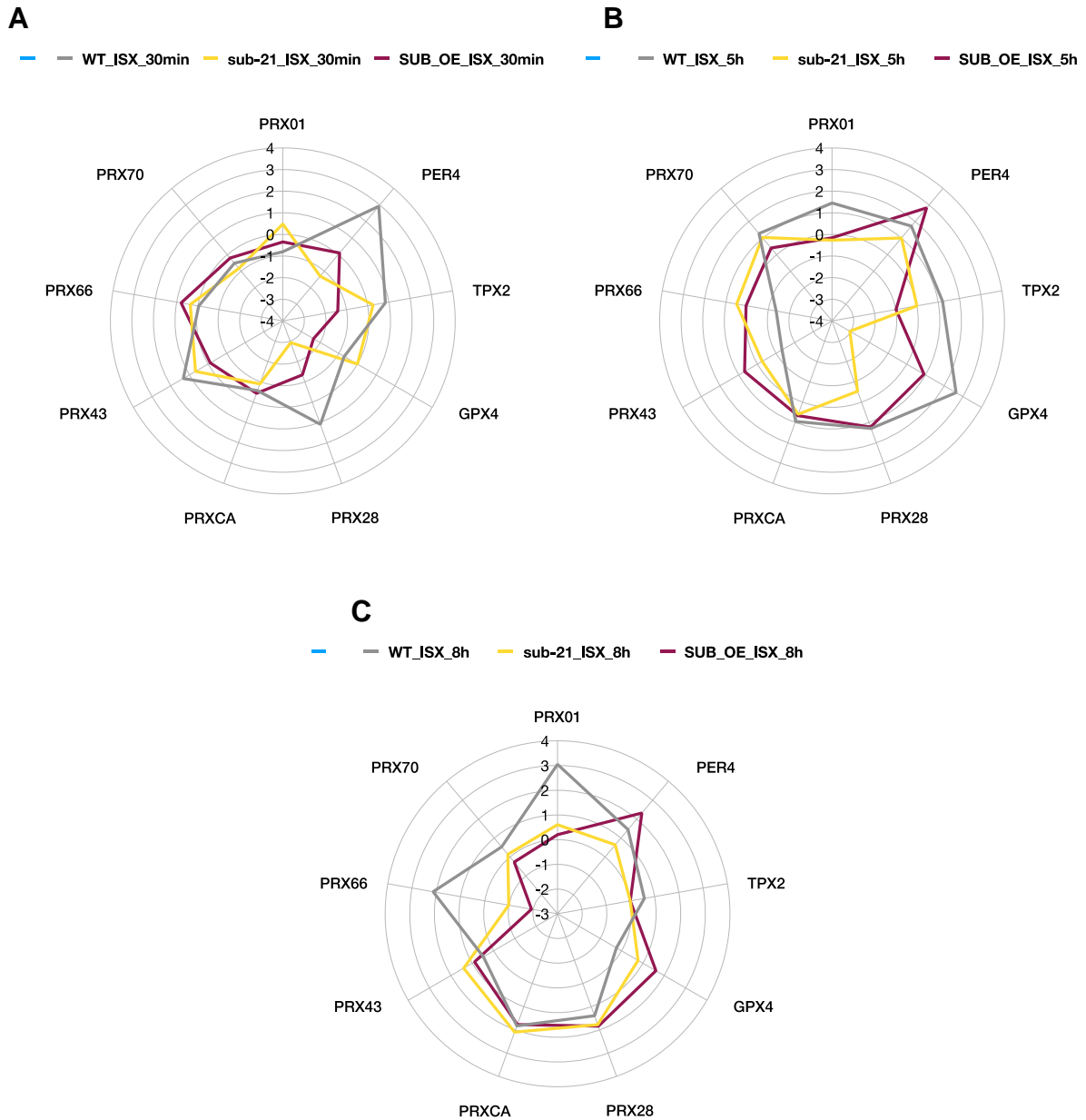


Figure 29. Radar plot showing expression of various *PRX* genes in response to isoxaben exposure.

Radar plots present results of RNAseq analysis compared between wild-type (gray), *sub-21* (yellow), and *SUB-OE LI* (magenta) seedlings. Data based on a false discovery rate (FDR) adjusted p-value of ≤ 0.05 and a \log_2FC of ≥ 4 and ≤ -4 . (A) Radar plot of *PRX* genes expression after 30min of 600 nM ISX treatment. (B) Radar plot of *PRX* genes expression after 5h of 600 nM ISX treatment. (C) Radar plot *PRX* genes expression after 8h of 600 nM ISX treatment. Abbreviation: ISX, isoxaben.

Isoxaben induction above the cut-off of $\log_2FC \geq 1$ was also observed for other *PRX* genes, but this was limited to single time points. After 30 minutes of treatment, an increase in *PRX43* expression was detected, which was absent in *sub-21* (Fig. 29). After 5 hours, *PRX70* showed higher expression, unaffected by altered *SUB* activity. Finally, after 8 hours, an increase in *PRX33* (*PRXCA*), involved in the control of the oxidative burst upon pathogen or microbe-associated molecular pattern (MAMP) exposure (Daudi and O'brien 2012; Kámán-Tóth et al. 2019), and *PRX66*, possibly involved in lignification (Tokunaga et al. 2009), was observed. In both cases, induction was not dependent on *SUB* (Fig. 29). All genes mentioned above encode putative apoplastic peroxidases and could therefore influence the isoxaben-induced lignification process.

However, *SUB*-dependent induction of *TPX2*, a predicted cytosolic thioredoxin-dependent peroxidase, and *GPX4*, a plasma membrane-anchored glutathione peroxidase-like enzyme (Attacha et al. 2017), was also observed at the 5-hour time point (Fig. 29). The potential involvement of these two enzymes in lignification remains unclear. They may contribute to the formation of cytoplasmic lignin oligomers or influence other *SUB*-dependent and isoxaben-induced cellular processes.

In conclusion, a consistent induction of monolignol biosynthesis genes is observed at the 8-hour mark. *LAC* gene expression is less affected, while the response of a group of class III *PRX* genes to isoxaben treatment is variable.

4. Discussion

Cell walls are critical in various cellular processes, including growth, development, and defense mechanisms. To efficiently orchestrate these functions, plants have evolved a sophisticated system consisting of diverse plasma membrane-based sensors and pattern recognition receptors (Bacete et al. 2018). These receptors are responsible for accurately transmitting a wide range of internal and external cues to downstream pathways, leading to an appropriate response (Wolf 2017).

4.1. The receptor kinase STRUBBELIG does not affect general cell wall damage responses

Besides its role in developmental processes, *SUB* also influences numerous aspects of the cell wall damage response induced by isoxaben, an inhibitor of cellulose biosynthesis (Chaudhary et al., 2020). Previous research suggests that *SUB* signaling contributes to the initial increase in intracellular ROS, activation of stress genes, and ectopic lignin and callose production in seedlings subjected to isoxaben. In addition, exposure to isoxaben leads to changes in *SUB* complexes at the plasma membrane, increased internalization of *SUB*, and a decrease in *SUB* transcript levels (Chaudhary et al., 2021).

In this study, the assessment of lignin and callose accumulation in young seedlings served as an indirect measure to examine whether *SUB* influences the cell wall remodeling response to various forms of cell wall damage. Young *Arabidopsis* seedlings were challenged by the application of enzymes that degrade the major components of the cell wall: cellulose, hemicellulose, and pectin. Pectin is an essential component of the cell wall, holding the other structural elements together. Pectinase enzymes degrade this polysaccharide, which can lead to significant structural damage. Callose acts as a protective, reinforcing agent in the cell wall, contributing to its resistance and adaptation to stress. The study shows the pectinase-induced protective response, as indicated by increased callose production in the cotyledons of treated wild-type seedlings. The lack of a significant callose formation in *sub-21* and *sub-9* mutants indicates that *SUB* is required to mediate this specific stress response. The results further suggest that *SUB* is needed but not sufficient for pectinase-induced callose accumulation, as overexpression of *SUB* did not lead to an increased callose deposition beyond that observed in the wild-type. Opposite with its direct genetic role in isoxaben-induced callose and lignin accumulation.

Treatment with cellulase or xyloglucanase resulted in a significant increase in callose production in the absence or presence of *SUB*, suggesting that the protective response to cellulose and hemicellulose degradation can occur independently of this gene.

The outcome of this study highlights the important role of the *SUB* gene in the remodeling response to isoxaben- and pectinase-induced cell wall damage in Arabidopsis. The results suggest that *SUB* activity is not universally required for the induction of the cell wall damage response but has specific functions related to different types of stress.

The observed differential response to cellulase and pectinase, resulting in different downstream responses, is consistent with previous research. It was demonstrated that young seedlings, when treated with these two enzymes, exhibited differences in phytohormone accumulation (Engelsdorf et al. 2018). Specifically, it was found that pectinase treatment stimulated the accumulation of both jasmonic acid (JA) and salicylic acid (SA), whereas cellulase treatment only resulted in an increase in SA accumulation. A similar pattern was observed with isoxaben treatment, which also increased the levels of both JA and SA (Hamann et al. 2009; Engelsdorf et al. 2018). However, genetic data indicated that *SUB* was not involved in isoxaben-induced JA production (Chaudhary et al. 2020).

Taken together, the collected data indicate that isoxaben, cellulase, and pectinase applications induce related but uniquely different downstream responses. These differences are displayed in various forms, such as phytohormone regulation, lignification, and *SUB*-mediated cell wall modifications.

4.1.1. Callose synthase PMR4 contributes to callose production independent of stress

Callose production is often observed in response to biotic factors (Luna et al. 2011). It is also a characteristic feature of Driselase treatment, which uses a complex mixture of enzymes to degrade the cell wall (Engelsdorf et al. 2018). In this study, all applied enzymes led to callose formation. However, the specific callose synthase responsible for mediating callose biosynthesis during these treatments was still being determined. PMR4 (GSL5, CaLS12) is a callose synthase that has been associated with responses to various biotic and abiotic components. Examples found in the literature describe the involvement of PMR4 in reacting to fungal pathogens (Kopischke et al. 2013; Ellinger and Voigt 2014) and its role in callose priming during mycorrhiza-induced resistance (Sanmartín et al. 2021). PMR4 has also been implicated in cell wall deposition during temperature-induced autoimmunity (Hessler et al.

2021). Based on the genetic data obtained, *PMR4* was characterized as the callose synthase gene essential for the response to damage caused by cell wall degrading enzymes. There was minimal callose accumulation in plants lacking *PMR4* activity, suggesting that *PMR4* is the primary callose synthase responsible for these processes.

In a broader context, callose is an essential component of the plant cell wall. This element is critical for plant development, stress responses, and defense mechanisms against pathogens. In *Arabidopsis*, the callose synthase family responsible for callose synthesis consists of 12 members (Ellinger and Voigt 2014). Among them, *CalS7* (*GSL7*) is mainly involved in callose deposition in phloem sieve elements (Barratt et al. 2011). However, this study investigated the role of *CalS7* after cell wall disruption, considering the important role of other callose synthases, such as *PMR4*, in callose accumulation during cell wall damage. The results indicate that *CalS7* does not contribute significantly to the callose response induced by isoxaben or pectinase treatments.

The findings highlight the specificity of different callose synthase members during plant stress responses and underscore the need to explore further the role of other members of the callose synthase family in response to cell wall damage.

4.1.2. Inhibition of cellulose biosynthesis triggers different protective responses than degradation of existing cellulose

The response of the seedlings to treatment with isoxaben is clearly different from that with cellulase. This is not surprising because isoxaben and cellulase have very distinct modes of action on plant cells, targeting different aspects of cell structure and metabolism. Isoxaben is an herbicide that specifically targets the process of cell wall biosynthesis in plants. It inhibits the synthesis of cellulose, an essential component of the plant cell wall. This disrupts the proper formation of the cell wall, causing abnormal cell shapes, stunted growth, and, eventually, plant death (Heim et al. 1990; Scheible et al. 2001). Isoxaben doesn't affect existing cell walls but prevents the generation of new ones. It is, therefore, most effective in rapidly growing plants where new cell walls are constantly being formed. Cellulase is an enzyme that breaks down cellulose in the existing cell wall. There are several types of cellulases that work synergistically to break down cellulose into various polysaccharide fragments and simple sugars such as glucose (Bayer et al. 1998; Kubicek et al. 2014). When applied to plant cells, cellulase can disrupt the cell wall structure by breaking down its cellulose content, causing the cell to burst due to osmotic imbalance and ultimately leading to cell death (Lampugnani et al. 2018).

While isoxaben induced *SUB*-dependent lignification and callose accumulation, cellulase treatment led to callose formation in a *SUB*-independent manner. Previous studies have suggested that certain cello-oligosaccharides, such as cellobiose and cellotriose, can induce immune responses in plants. Interestingly, treatment of seedlings with the disaccharide did not result in lignification or callose production (Souza et al. 2017), suggesting that other types of cell wall-derived carbohydrates released upon cellulase application lead to these responses. Conducted analysis revealed that also longer oligosaccharides, namely cellotetraose, and cellopentaose, did not trigger callose and lignin accumulation.

4.1.3. Enzymatic treatments lead to *SUB* internalization from the plasma membrane

The four treatments enhanced the internalization of *SUB*:EGFP, which led to a similar decrease in its signal intensity at the plasma membrane. However, only exposure to isoxaben resulted in *SUB*-dependent changes in root hair patterning (Chaudhary et al. 2021) while still ensuring the involvement of *SUB* in the CBI response. Previously, it was thought that the response to isoxaben required higher levels of *SUB* at the plasma membrane for root hair patterning and lower levels for its role in the CBI response (Chaudhary et al. 2021). The current findings do not prove the accuracy of this model. Although all treatments cause similar reductions in *SUB*:EGFP abundance, they induce different outcomes, suggesting the existence of different types of *SUB*-containing receptor complexes. The short ectodomain of *SUB* has six LRRs (Vaddepalli et al. 2011), suggesting that *SUB* may act as a co-receptor, similar to *SERK* co-receptors family that control diverse processes by interacting with numerous signaling receptor kinases (Liu, Li, and Shan 2020). Further research is needed to validate this model by analyzing the composition of the proposed *SUB* complexes. Another explanation is that severe and extensive cell wall damage could lead to overall cellular stress and deterioration, which could then indirectly affect the abundance of proteins at the plasma membrane.

4.1.4. Distinct mechanisms and cellular specificity of isoxaben-induced lignification

The reason for the restriction of isoxaben-induced lignin to the narrow internal region in the root tip remains elusive. The most plausible explanation is the overlap of the lignification region with the transition and early elongation zone of the root, an area of rapid cell expansion. Cell

walls in this region were found to be softer compared to other root zones, consistent with the proposed explanation (Bacete et al. 2022). In addition, these cells show increased sensitivity to cellulose reduction, as demonstrated by localized cell swelling upon isoxaben application (Hamann et al. 2009; Chaudhary et al. 2020; Bacete et al. 2022). Consequently, diminished cellulose content could lead to a compensatory boost in lignin incorporation to counteract the drop in rigidity. Recent findings highlighted a correlation between an increase in coniferylaldehyde (G_{CHO}) residues in lignin and an increase in tracheary element stiffness (Ménard et al. 2022). In particular, the G_{CHO}-specific phloroglucinol stain (Blaschek et al. 2020) may indicate a strengthening of the cell wall in affected tissues, following stronger phloroglucinol signals in isoxaben-treated seedlings. If true, the reduction in phloroglucinol signals in *sub* mutants could lead to the conclusion that *SUB*, along with other RKs such as *THE1* and *MIK2* (Denness et al. 2011; Van der Does et al. 2017; Engelsdorf et al. 2018), affects the proportion of G_{CHO} in total lignin content and, consequently, cell wall stiffness.

The stiffness of cell walls in the xylem and sclerenchyma, induced by lignin, provides the structural support that allows plants to resist gravity and achieve upright growth (Vanholme et al. 2010; Barros et al. 2015). However, elevated stiffness induced by lignin could interfere with cell growth. The increase in cell wall rigidity due to *SUB*-dependent lignification does not clarify the beneficial role of *SUB* in inhibiting root growth in light-grown *prc1-1* seedlings (Chaudhary et al. 2020). While both *SUB* and *THE1* facilitate lignification after isoxaben exposure, only *SUB* is needed for root growth inhibition in the *prc1-1* background, whereas *THE1* is not required (Chaudhary et al. 2020). Furthermore, no significant ectopic lignin accumulation is observed in light-grown *prc1-1* seedlings (Hématy et al. 2007). Under these circumstances, it appears that *SUB* alone inhibits root growth, a process that is apparently independent of substantial lignin formation. This implies that the interplay between lignin production and growth inhibition is highly related to the situation.

Lignin production is triggered by developmental signals in specific cell types, as illustrated by the formation of the Casparian strip within the endodermal cells (Lee et al. 2013). Nonetheless, various biotic and abiotic stressors can also stimulate lignin production (Barros et al. 2015). When triggered by stress, lignification often occurs in cells that do not typically undergo this process. A unique case is the root endodermis, where ectopic lignin accumulates in case of abnormal formation of the Casparian strip, a process reliant on the *SGN* receptor module (Fujita, Bellis, et al. 2020; Reyt et al. 2021). Earlier studies revealed that lignin formation induced by isoxaben occurs in the endodermis of the hypocotyl in *Arabidopsis* seedlings grown in darkness (Hématy et al. 2007). In this study, the location of isoxaben-

induced lignin buildup in the root was investigated. Sequential experiments using Fuchsin demonstrated that this process also initiates in the endodermis of the seedling root, beginning approximately 4 hours after the start of treatment. Notably, the Fuchsin signal ultimately extends to the cortex and, to a lesser degree, the pericycle, suggesting that lignification expands to the two cell layers adjacent to the endodermis. Preliminary results shed light on the possible involvement of *SUB* in lignin propagation. Initially, Fuchsin staining, indicating ectopic lignin formation induced by isoxaben, appeared to be concentrated at the endodermal cell periphery in wild-type and *sub* mutants. However, after prolonged exposure of seedlings to isoxaben, staining was also detected throughout the endodermal cells and more frequently at the cortical cell periphery. Comparison between wild-type and *sub* mutants revealed significantly more endodermal cells with broad Fuchsin staining in wild-type roots. The widespread cellular signal observed in this study is likely due to Fuchsin binding to small lignin oligomers produced in the cell via intracellular monolignol coupling. This is supported by a previous study showing the presence of small, glycosylated lignin oligomers in Arabidopsis leaf vacuoles (Dima et al. 2015).

The initial specificity towards the endodermis and how it's regulated is currently uncertain. The *SGN3*-dependent ectopic lignification at the corners of endodermal cells, which happens in response to irregular Casparian strip formation, has similarities to the early stages of isoxaben-induced lignification. However, the results obtained in this study indicate that *SGN3* does not contribute to this process, implying that different mechanisms mediate the lignification of endodermal cells during isoxaben stress and Casparian strip malformation. Furthermore, the formation of the Casparian strip does not appear to be affected in *sub* mutants, as indicated by Fuchsin staining. This observation aligns with the finding that the expression level of *MYB36*, a transcription factor responsible for regulating lignin formation in the Casparian strip (Lieberman et al. 2015; Kamiya et al. 2015), is not altered by *SUB*. Therefore, it appears that isoxaben-induced lignification in the endodermis of the seedling root is unrelated to the established mechanism regulating the two distinct forms of Casparian strip-related lignin accumulation. Unraveling the molecular mechanism of this process is an exciting challenge for future research.

4.2. Differential role of *SUB* signaling pathway components in response to cell wall damage

Previous studies have shown that both *QKY* and *SUB* are involved in cell wall damage responses (Chaudhary et al. 2020). However, they play different roles in this context. Upon cell wall

damage, *SUB* facilitates lignin deposition as well as callose accumulation. This dual response mechanism indicates that *SUB* is a versatile player in reacting to cell wall damage, capable of activating both lignification and callose deposition pathways (Chaudhary et al. 2020). In contrast, *QKY* appears to have more limited functionality. Although it is part of the same protein complex as *SUB*, the function of *QKY* is largely restricted to CBI-induced lignification. Experimental evidence shows that regardless of the treatment applied, *QKY* does not contribute to callose accumulation. Neither isoxaben (Chaudhary et al. 2020) nor pectinase induced callose production. This suggests that although *QKY* is involved in the cell wall damage response, its function is more specialized than that of *SUB*. The differential involvement of *QKY* and *SUB* in lignification and callose deposition illustrates the complexity of combating cell wall damage. It shows that although both genes are part of the same signaling complex, they might be responsible for different aspects of the response to cell wall disruption. This divergence in their roles could be due to different downstream targets or because of interactions with distinct sets of proteins. In addition, the study revealed a possible function of *QKY* in facilitating the internalization of *SUB* after pectinase application. The results indicated that the presence of *SUB* at the plasma membrane exhibits no significant change between pectinase-treated *qky* mutant plants and mock-treated plants.

Further research is necessary to fully elucidate the specific mechanisms by which *QKY* and *SUB* contribute to the cell wall damage response and to understand why their roles differ despite their participation in the same protein complex. This would provide valuable insights into the intricate cellular machinery that plants utilize to withstand and recover from cell wall damage.

ZET and *AN* have previously been identified as contributors to *SUB*-dependent organogenesis and have been implicated in a variety of developmental processes. *ZET* is known to encode a cell wall-localized β -1,3-glucanase that may be involved in modifying cell wall structure or composition (Fulton et al. 2009). On the other hand, *AN* is located in the cytoplasm and is known to be a transcriptional repressor of *MYB46*, a regulator of secondary cell wall formation, including lignin accumulation (Bai et al. 2013; M. Xie et al. 2020). In the context of cell wall damage responses, specifically isoxaben-induced lignification, the roles of *AN* and *ZET* appear to be less prominent. Experiments with loss-of-function alleles of *AN* and *ZET* showed no significant change in isoxaben-induced lignification compared to wild-type plants. This indicates that these proteins may not have a direct role in controlling lignification under these conditions. However, their involvement in other facets of the cell wall stress response

cannot be excluded and requires further investigation. Similarly to QKY, the study showed a potential role for ZET in the internalization of SUB following pectinase-induced cell wall damage. The data indicated that the abundance of SUB at the plasma membrane did not show a significant difference in *zet* mutant plants treated with pectinase compared to controls. Although this does not definitively prove a role for ZET in SUB internalization, it suggests a possible involvement and provides a direction for future studies. On the other hand, the role of AN in SUB internalization was found to be negligible based on the observed data.

4.3. Novel candidate factors of the *SUB* signaling pathway

The search for members of the SUB protein complex is crucial as these components potentially hold the key to understanding the functionality and interactions of *SUB*-dependent signaling in plant tissue morphogenesis. Identification of these putative *SUB* candidates will help elucidate the complex response mechanism of the plant to stimuli and perturbations, such as cell wall disruption. Proteins often do not function in isolation but in complexes or networks, and therefore identifying potential partners of SUB may provide insight into their biological function. The large number of candidates identified is partly due to the nature of protein complexes and signaling pathways, which often involve multiple components with different functions. In addition, the multi-layer nature of cellular responses to stimuli, such as cell wall disruption, requires the involvement of multiple proteins.

The present study identified several promising candidates involved in different aspects of plant growth and stress response. However, when their participation in the isoxaben-induced callose production was tested, none of the genes showed a significant contribution, thus ruling out their involvement in this aspect of the CBI-induced stress response. Nevertheless, the potential role of some candidates in lignification provides intriguing leads for further investigation. The reduced lignin production in *simp1* and *casplp1b1* or the increased production in *cobl7* mutants in response to isoxaben treatment indicate their impact on the CBI-induced lignification process.

SGN3 and *CASPLP1B1* appear to be involved differently in lignin formation. *SGN3* is involved in the signaling pathway that ensures proper formation and sealing of the Casparian strip in the endodermis. The *SGN3* pathway also mediates compensatory lignin formation in response to a defective Casparian strip (Pfister et al. 2014; Doblaz et al. 2017; Fujita, De Bellis, et al. 2020). However, it is not required for ectopic lignification following isoxaben treatment, suggesting that isoxaben-induced lignification is independent of the *SGN3*-mediated pathway. In contrast, *CASPLP1B1* may be involved in the pathway leading to lignification in response to

cell wall stress, such as that caused by inhibition of cellulose biosynthesis. The role of *CASPLP1B1* may be related to its putative function in membrane scaffolding and regulation of cell wall modification processes. In response to cellular stress, *CASPLP1B1* may help restore structural stability by modulating the lignification process, either by directly participating in lignin biosynthesis, organizing a scaffold for lignin precursors and enzymes, or assisting in the recruitment and activation of other lignin-related proteins. Furthermore, while both *CASPLP1B1* and *SGN3* are associated with the Casparian strip, they may have specialized tasks in different processes or conditions. For example, *CASPLP1B1* and *SGN3* may have different roles in lignification under cell wall stress and in response to perturbations in Casparian strip formation, respectively.

The *COBL7* gene is known to regulate root hair growth and development, including the biosynthesis and deposition of cellulose at the tips of growing root hairs (Z. Li et al. 2022). Disruption of *COBL7* function could result in impaired cellulose biosynthesis, which could trigger a compensatory increase in lignin biosynthesis and deposition. *COBL7* may play a more direct role in the regulation of lignin biosynthesis, such that its mutation could deregulate the lignin biosynthetic pathway leading to overproduction. The contrasting effects of different T-DNA alleles of THE1 receptor kinase on lignification have been reported in literature (Merz et al. 2017). Specifically, *the1-4* gain of function allele enhances lignification, whereas *the1-1* loss of function allele diminishes this process. The hypersensitivity of *the1-4* to isoxaben is due to the presence of a truncated version of the THE1 protein. It efficiently stimulates cell wall damage responses, highlighting the enhanced responsiveness of this allele to perturbations in cellulose synthesis (Merz et al. 2017). A similar situation may occur in the case of *COBL7*. However, without additional experimental data, it is challenging to distinguish between these possibilities definitively.

One approach to further validate these results would be to generate and analyze double mutants involving both *SUB* and the putative candidate. This could help to determine whether these proteins work together or have separate roles in the lignification process. Such studies could provide deeper insights into the complex network of interactions that orchestrate the plant's response to cell wall perturbations and may ultimately lead to a better understanding of plant stress response mechanisms. Using techniques such as yeast two-hybrid assays or FRET-FLIM analysis might also be beneficial to confirm and further investigate these protein-protein interactions.

Overall, while the involvement of the tested candidates in the callose production response to isoxaben treatment seems unlikely, their potential role in lignification offers promising routes

for further exploration. These results emphasize the importance of a nuanced understanding of the diverse and complex roles of different proteins in each response pathway and highlight the need for further in-depth studies.

4.4. Isoxaben-induced changes in lignin biosynthesis genes and the role of *SUB* in the transcriptome

The focus on the *SUB* and its role in reacting to isoxaben introduces a new layer to understanding plant molecular biology, particularly concerning lignin biosynthesis. Comparing the *SUB*-dependent transcriptome at various time intervals after isoxaben exposure was an effective approach for exploring the temporal dynamics of transcriptional response.

The differential induction of genes involved in monolignol biosynthesis at various time points suggests a time-dependent molecular response that can provide a more nuanced view of how plants respond to isoxaben. As observed in the study, the sequential activation of enzymes in the phenylpropanoid pathway shows that isoxaben treatment triggers a well-coordinated transcriptional response, producing key enzymes necessary for monolignol biosynthesis. This highlights the sophisticated nature of the plant reaction to external stimuli at the transcriptional level. The study investigated the role of *SUB* in modulating the expression of genes involved in monolignol biosynthesis under isoxaben stress. The observed fluctuations in the expression of key genes like *4CL8*, *CAD2*, and *CAD6* in the *sub-21* and *SUB-OE L1* seedlings in a time-dependent manner provides evidence that *SUB* activity may be intricately linked with reaction to isoxaben and monolignol biosynthesis.

The significant decrease in *CAD2* expression at the 30-minute mark in the *sub-21* sample suggests a potential regulatory influence of *SUB* on this gene. Similarly, the distinct patterns of expression of *CAD6*, *4CL8*, and *CAD2* across different time points in different genotypes provide valuable insights into the possible modulatory function of *SUB*. The probable role of *SUB* in these temporal gene expression patterns further reinforces its importance in plant development and response to external stimuli.

The detection of four *RBOH* genes and their differential expression patterns in the presence and absence of *SUB* provide new avenues to explore the significance of *SUB* in ROS production during oxidative lignin polymerization. The differential regulation of *RBOHA/B/D* in the presence of *SUB* again highlights the complexity and dynamism of plant transcriptional response to external stressors like isoxaben.

The evaluation of the 17 members of the Arabidopsis LAC gene family and their response to isoxaben treatment further expand the understanding of the lignin formation process. The

varying induction of *LAC5*, *LAC2*, *LAC10*, and *LAC12* and their responsiveness to isoxaben in the presence and absence of *SUB* provide evidence of the potential regulatory function of *SUB* in the context of lignin biosynthesis.

The analysis of the PRX gene family members illuminates the role of *SUB* in their isoxaben-triggered transcriptional response. The observed induction of *PRX4* at all time points recorded suggests a robust transcriptional response possibly orchestrated by *SUB*. This interpretation is further supported by the reduced upregulation in *sub-21* and heightened induction in *SUB-OE L1* at 5 and 8 hours compared to the wild-type. Furthermore, the variable expression of other *PRX* genes, including *PRX28* and *PRX01*, in response to isoxaben treatment and their dependence on *SUB* shed light on its versatile role in plant development. Interestingly, while the expression of various other *PRX* genes was induced, this induction was largely independent of *SUB*, indicating additional regulatory mechanisms in the isoxaben-induced lignification process. These findings highlight the complex landscape of plant transcriptional responses, with *SUB* acting as a pivotal yet not exclusive regulator.

In summary, the effects of modulated *SUB* activity on lignin biosynthetic gene expression show temporal variations. This may be due to the analysis of whole seedlings subjected to isoxaben treatment. To obtain even more precise results, it would be necessary to perform a single-cell RNAseq experiment. The findings of this study show that the presence of ectopic lignification is mainly in root endodermal cells, making this cell layer a good sample to investigate in future studies. The variability of results may also suggest a complex, time-dependent transcriptional response to isoxaben treatment under the regulatory influence of *SUB*.

5. Conclusions

Cell walls play a critical role in several cellular processes, including growth, development, and defense mechanisms. This is facilitated by plasma membrane-based sensors and pattern recognition receptors that convey signals to downstream pathways. The Arabidopsis receptor kinase STRUBBELIG regulates cell wall remodeling in response to cell wall perturbations. It contributes to increased intracellular ROS, stress gene activation, and ectopic lignin and callose production in seedlings exposed to isoxaben, a cellulose biosynthesis inhibitor (Chaudhary et al. 2020, Chaudhary et al. 2021). The data presented in this study shows that *SUB* is required for pectinase-induced cell wall damage leading to increased callose production. In contrast, the protective response to cellulose and hemicellulose degradation can occur independently of *SUB*. Meaning that *SUB* is not involved in responses to all types of cell wall damage, but it only responds to specific signals.

The study hypothesized the existence of different types of *SUB*-containing cell surface receptor complexes that could be uniquely influenced by other stress agents. Application of cell wall degrading enzymes led to a decrease in *SUB*:EGFP abundance at the plasma membrane. However, the activity of these treatments resulted in non-uniform responses, suggesting that different *SUB* complexes are affected.

Many candidate genes were identified as putative members of the *SUB* signaling pathway. Using lignin accumulation assay as a proxy, it was possible to find genes potentially involved in this CBI-induced stress response. Uncovered candidates such as *CASPLIB1* could be part of the complex regulatory network for this biological process.

Both developmental signals and various stresses can stimulate lignin production. Under stress, lignin can be deposited in cells that do not typically undergo this process. Data imply that isoxaben-induced lignin formation begins in the endodermis and extends to the cortex. Preliminary results indicate a potential role of *SUB* in lignin propagation. However, the regulation of this initial specificity towards the endodermis needs to be better understood.

6. Supplement

Table S1. List of genotyping primers

No.	Primer name	Sequence
1	RP_CASPL	GTTGGACGAGACGAGAGATTG
2	LP_CASPL	GCGGTTTCAAAGCTTACATTG
3	RP_COBRA	GGGATCTACTAGATACTTCAT
4	LP_COBRA	GCCAACAAAAAATGCTCCAGG
5	RP_HERK2	CTTACCAAACCCTCCA ACTCC
6	LP_HERK2	ACTGGTCACAATGCTACTGCC
7	RP_LMK1	CTCACACTTTAATGACAACCA
8	LP_LMK1	CGCAGAAGTGATACCATATTTT
9	RP_PMR4	CATTCTTGCATATTTTGTGCCA
10	LP_PMR4	TGAAGCTAAGGTATGGATTTGG
11	RP_RUL1	GTAACATTCCTTCCGGTCTCC
12	LP_RUL1	GAGTTAAAGGTGCTTCCCGG
13	SAIL_LB2	GCTTCCTATTATATCTTCCCAAATTACCAATACA
14	SALK_LBb1.3	ATTTTGCCGATTCGGAAC
15	RP_SIMP1	GATAAATTCGGGGCTGAAAG
16	LP_SIMP1	TCTTTTACGGGTCCAATACC
17	RP_SRF3	GCTAACAGAGTTTTCAAACCGC
18	LP_SRF3	TTCAGCTAATCAGTTCACCGG
19	RP_SRF7	TCGGTTACATGAAGCAGATC
20	LP_SRF7	GGCTCTTGGATTCTTACTTATAG

7. References

- Albenne, Cécile, Hervé Canut, Laurent Hoffmann, and Elisabeth Jamet. 2014. “Plant Cell Wall Proteins: A Large Body of Data, but What about Runaways?” *Proteomes* 2 (2): 224–42. <https://doi.org/10.3390/proteomes2020224>.
- Anderson, Catherine M., Tanya A. Wagner, Mireille Perret, Zheng Hui He, Deze He, and Bruce D. Kohorn. 2001. “WAKs: Cell Wall-Associated Kinases Linking the Cytoplasm to the Extracellular Matrix.” *Plant Molecular Biology* 47 (1–2): 197–206. <https://doi.org/10.1023/A:1010691701578>.
- Arnaud, Dominique, Seungchul Lee, Yumiko Takebayashi, Daeseok Choi, Jaemyung Choi, Hitoshi Sakakibara, and Ildoo Hwang. 2017. “Cytokinin-Mediated Regulation of Reactive Oxygen Species Homeostasis Modulates Stomatal Immunity in Arabidopsis.” *Plant Cell* 29 (3): 543–59. <https://doi.org/10.1105/tpc.16.00583>.
- Attacha, Safira, David Solbach, Krisztina Bela, Anna Moseler, Stephan Wagner, Markus Schwarzländer, Isabel Aller, Stefanie J. Müller, and Andreas J. Meyer. 2017. “Glutathione Peroxidase-like Enzymes Cover Five Distinct Cell Compartments and Membrane Surfaces in Arabidopsis Thaliana.” *Plant Cell and Environment* 40 (8): 1281–95. <https://doi.org/10.1111/pce.12919>.
- Azevedo Souza, Clarice de, Shundai Li, Andrew Z. Lin, Freddy Boutrot, Guido Grossmann, Cyril Zipfel, and Shauna C. Somerville. 2017. *Cellulose-Derived Oligomers Act as Damage-Associated Molecular Patterns and Trigger Defense-like Responses*. *Plant Physiology*. Vol. 173. <https://doi.org/10.1104/pp.16.01680>.
- Bacete, Laura, and Thorsten Hamann. 2020. “The Role of Mechanoperception in Plant Cell Wall Integrity Maintenance.” *Plants* 9 (5). <https://doi.org/10.3390/plants9050574>.
- Bacete, Laura, Hugo Mérida, Eva Miedes, and Antonio Molina. 2018. “Plant Cell Wall-Mediated Immunity: Cell Wall Changes Trigger Disease Resistance Responses.” *Plant Journal* 93 (4): 614–36. <https://doi.org/10.1111/tpj.13807>.
- Bacete, Laura, Julia Schulz, Timo Engelsdorf, Zdenka Bartosova, Lauri Vaahtera, and Guqi Yan. 2022. “THESEUS1 Modulates Cell Wall Stiffness and Abscisic Acid Production in Arabidopsis Thaliana” 119 (1). <https://doi.org/10.1073/pnas.2119258119/-/DCSupplemental>. Published.
- Bai, Yang, Prasad Vaddepalli, Lynette Fulton, Hemal Bhasin, Martin Hülskamp, and Kay Schneitz. 2013. “ANGUSTIFOLIA Is a Central Component of Tissue Morphogenesis Mediated by the Atypical Receptor-like Kinase STRUBBELIG.” *BMC Plant Biology* 13: 16. <https://doi.org/10.1186/1471-2229-13-16>.
- Barbosa, Inês Catarina Ramos, Nelson Rojas-Murcia, and Niko Geldner. 2019. “The Casparian Strip—One Ring to Bring Cell Biology to Lignification?” *Current Opinion in Biotechnology* 56: 121–29. <https://doi.org/10.1016/j.copbio.2018.10.004>.
- Barros, Jaime, Henrik Serk, Irene Granlund, and Edouard Pesquet. 2015. “The Cell Biology of Lignification in Higher Plants.” *Annals of Botany* 115 (7): 1053–74. <https://doi.org/10.1093/aob/mcv046>.
- Bashline, Logan, Lei Lei, Shundai Li, and Ying Gu. 2014. “Cell Wall , Cytoskeleton , and Cell Expansion in Higher Plants CELLULOSE AND NON-CELLULOSIC.” *Molecular Plant* 7 (4): 586–600. <https://doi.org/10.1093/mp/ssu018>.

- Basu, Debarati, Lu Tian, Tayler DeBrosse, Emily Poirier, Kirk Emch, Hayley Herock, Andrew Travers, and Allan M. Showalter. 2016. “Glycosylation of a Fasciclin-Like Arabinogalactan-Protein (SOS5) Mediates Root Growth and Seed Mucilage Adherence via a Cell Wall Receptor-like Kinase (FEI1/ FEI2) Pathway in Arabidopsis.” *PLoS ONE* 11 (1): 1–27. <https://doi.org/10.1371/journal.pone.0145092>.
- Bayer, Edward A., Henri Chanzy, Raphael Lamed, and Yuval Shoham. 1998. “Cellulose, Cellulases and Cellulosomes.” *Current Opinion in Structural Biology* 8 (5): 548–57. [https://doi.org/10.1016/S0959-440X\(98\)80143-7](https://doi.org/10.1016/S0959-440X(98)80143-7).
- Blaschek, Leonard, Emiko Murozuka, Henrik Serk, Delphine Ménard, and Edouard Pesquet. 2023. “Different Combinations of Laccase Paralogs Nonredundantly Control the Amount and Composition of Lignin in Specific Cell Types and Cell Wall Layers in Arabidopsis.” *The Plant Cell* 35 (2): 889–909. <https://doi.org/10.1093/plcell/koac344>.
- Blaschek, Leonard, Nuoendagula, Zoltán Bacsik, Shinya Kajita, and Edouard Pesquet. 2020. “Determining the Genetic Regulation and Coordination of Lignification in Stem Tissues of Arabidopsis Using Semiquantitative Raman Microspectroscopy.” *ACS Sustainable Chemistry & Engineering* 8 (12): 4900–4909. <https://doi.org/10.1021/acssuschemeng.0c00194>.
- Blaschek, Leonard, and Edouard Pesquet. 2021. “Phenoloxidases in Plants—How Structural Diversity Enables Functional Specificity.” *Frontiers in Plant Science* 12 (October): 1–24. <https://doi.org/10.3389/fpls.2021.754601>.
- Boerjan, Wout, John Ralph, and Marie Baucher. 2003. “Lignin Biosynthesis.” *Annual Review of Plant Biology* 54: 519–46. <https://doi.org/10.1146/annurev.arplant.54.031902.134938>.
- Brutus, Alexandre, Francesca Sicilia, Alberto Macone, Felice Cervone, and Giulia De Lorenzo. 2010. “A Domain Swap Approach Reveals a Role of the Plant Wall-Associated Kinase 1 (WAK1) as a Receptor of Oligogalacturonides.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (20): 9452–57. <https://doi.org/10.1073/pnas.1000675107>.
- Chaudhary, Ajeet, Xia Chen, Jin Gao, Barbara Leśniewska, Richard Hammerl, Corinna Dawid, and Kay Schneitz. 2020. “The Arabidopsis Receptor Kinase STRUBBELIG Regulates the Response to Cellulose Deficiency.” *PLOS Genetics* 16(1): e1008433. <https://doi.org/10.1371/journal.pgen.1008433>
- Chaudhary, Ajeet, Xia Chen, Barbara Lesniewska, Rodion Boikine, Jin Gao, Sebastian Wolf, and Kay Schneitz. 2021. “Cell Wall Damage Attenuates Root Hair Patterning and Tissue Morphogenesis Mediated by the Receptor Kinase STRUBBELIG.” *Development (Cambridge)* 148 (14). <https://doi.org/10.1242/DEV.199425>.
- Cheung, Alice Y., and Hen Ming Wu. 2011. “THESEUS 1, FERONIA and Relatives: A Family of Cell Wall-Sensing Receptor Kinases?” *Current Opinion in Plant Biology* 14 (6): 632–41. <https://doi.org/10.1016/j.pbi.2011.09.001>.
- Chevalier, D., M. Batoux, L. Fulton, K. Pfister, R. K. Yadav, M. Schellenberg, and K. Schneitz. 2005. “STRUBBELIG Defines a Receptor Kinase-Mediated Signaling Pathway Regulating Organ Development in Arabidopsis.” *Proceedings of the National Academy of Sciences* 102 (25): 9074–79. <https://doi.org/10.1073/pnas.0503526102>.
- Choudhury, Feroza K., Rosa M. Rivero, Eduardo Blumwald, and Ron Mittler. 2017. “Reactive Oxygen Species, Abiotic Stress and Stress Combination.” *Plant Journal* 90 (5): 856–67. <https://doi.org/10.1111/tbj.13299>.

- Claverie, Justine, Suzanne Balacey, Christelle Lemaître-Guillier, Daphnée Brulé, Annick Chiltz, Lucie Granet, Elodie Noirot, et al. 2018. “The Cell Wall-Derived Xyloglucan Is a New DAMP Triggering Plant Immunity in *Vitis Vinifera* and *Arabidopsis Thaliana*.” *Frontiers in Plant Science* 871 (November): 1–14. <https://doi.org/10.3389/fpls.2018.01725>.
- Colcombet, Jean, Aurélien Boisson-Dernier, Roc Ros-Palau, Carlos E. Vera, and Julian I. Schroeder. 2005. “*Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASES1 and 2 Are Essential for Tapetum Development and Microspore Maturation.” *Plant Cell* 17 (12): 3350–61. <https://doi.org/10.1105/tpc.105.036731>.
- Cosgrove, Daniel J. 2016. “Plant Cell Wall Extensibility: Connecting Plant Cell Growth with Cell Wall Structure, Mechanics, and the Action of Wall-Modifying Enzymes.” *Journal of Experimental Botany* 67 (2): 463–76. <https://doi.org/10.1093/jxb/erv511>.
- Cosgrove, Daniel J. 2022. “Building an Extensible Cell Wall.” *Plant Physiology* 189 (3): 1246–77. <https://doi.org/10.1093/plphys/kiac184>.
- Cosgrove, Daniel J., and Michael C. Jarvis. 2012. “Comparative Structure and Biomechanics of Plant Primary and Secondary Cell Walls.” *Frontiers in Plant Science* 3 (AUG): 1–6. <https://doi.org/10.3389/fpls.2012.00204>.
- Daher, Firas Bou, and Siobhan A. Braybrook. 2015. “How to Let Go: Pectin and Plant Cell Adhesion.” *Frontiers in Plant Science* 6 (JULY): 1–8. <https://doi.org/10.3389/fpls.2015.00523>.
- Daudi, Arsalan, and Jose A O'brien. 2012. “Detection of Hydrogen Peroxide by DAB Staining in *Arabidopsis* Leaves HHS Public Access Author Manuscript.” *Bio Protoc* 2 (18): 4–7.
- Deitch, AD, H Law, and R deVere White. 1982. “A Stable Propidium Iodide Staining Procedure for Flow Cytometry.” *J Histochem Cytochem.* <https://doi.org/10.1177/33.1.2578146>.
- Denness, Lucinda, Joseph Francis McKenna, Cecile Segonzac, Alexandra Wormit, Priya Madhou, Mark Bennett, John Mansfield, Cyril Zipfel, and Thorsten Hamann. 2011. “Cell Wall Damage-Induced Lignin Biosynthesis Is Regulated by a Reactive Oxygen Species- and Jasmonic Acid-Dependent Process in *Arabidopsis*.” *Plant Physiology* 156 (3): 1364–74. <https://doi.org/10.1104/pp.111.175737>.
- Desprez, Thierry, Samantha Vernhettes, Mathilde Fagard, Guislaine Refre, Thierry Desnos, Nicolas Py, Sandra Pelletier, and Herman Ho. 2002. “Resistance against Herbicide Isoxaben and Cellulose Deficiency Caused by Distinct Mutations in Same Cellulose Synthase Isoform CESA6 1” 128 (February): 482–90. <https://doi.org/10.1104/pp.010822.sion>.
- Dixon, Richard A., and Jaime Barros. 2019. “Lignin Biosynthesis: Old Roads Revisited and New Roads Explored.” *Open Biology* 9 (12). <https://doi.org/10.1098/rsob.190215>.
- Doblas, Verónica G., Elwira Smakowska-Luzan, Satoshi Fujita, Julien Alassimone, Marie Barberon, Mathias Madalinski, Youssef Belkhadir, and Niko Geldner. 2017. “Root Diffusion Barrier Control by a Vasculature-Derived Peptide Binding to the SGN3 Receptor.” *Science* 355 (6322): 280–84. <https://doi.org/10.1126/science.aaj1562>.
- Does, Dieuwertje Van der, Freddy Boutrot, Timo Engelsdorf, Jack Rhodes, Joseph F. McKenna, Samantha Vernhettes, Iko Koevoets, et al. 2017. “The *Arabidopsis* Leucine-

- Rich Repeat Receptor Kinase MIK2/LRR-KISS Connects Cell Wall Integrity Sensing, Root Growth and Response to Abiotic and Biotic Stresses.” *PLoS Genetics* 13 (6): 1–27. <https://doi.org/10.1371/journal.pgen.1006832>.
- Dünser, Kai, Shibu Gupta, Aline Herger, Mugurel I Feraru, Christoph Ringli, and Jürgen Kleine-Vehn. 2019. “ Extracellular Matrix Sensing by FERONIA and Leucine-Rich Repeat Extensins Controls Vacuolar Expansion during Cellular Elongation in Arabidopsis Thaliana .” *The EMBO Journal* 38 (7): 1–12. <https://doi.org/10.15252/embj.2018100353>.
- Duroux, Laurent, and Karen G. Welinder. 2003. “The Peroxidase Gene Family in Plants: A Phylogenetic Overview.” *Journal of Molecular Evolution* 57 (4): 397–407. <https://doi.org/10.1007/s00239-003-2489-3>.
- Ellinger, Dorothea, and Christian A Voigt. 2014. “Callose Biosynthesis in Arabidopsis with a Focus on Pathogen Response : What We Have Learned within the Last Decade,” 1349–58. <https://doi.org/10.1093/aob/mcu120>.
- Endler, Anne, Christopher Kesten, René Schneider, Yi Zhang, Alexander Ivakov, Anja Froehlich, Norma Funke, and Staffan Persson. 2015. “A Mechanism for Sustained Cellulose Synthesis during Salt Stress.” *Cell* 162 (6): 1353–64. <https://doi.org/10.1016/j.cell.2015.08.028>.
- Engelsdorf, Timo, Nora Gigli-Bisceglia, Manikandan Veerabagu, Joseph F. McKenna, Lauri Vaahtera, Frauke Augstein, Dieuwertje Van der Does, Cyril Zipfel, and Thorsten Hamann. 2018. “The Plant Cell Wall Integrity Maintenance and Immune Signaling Systems Cooperate to Control Stress Responses in Arabidopsis Thaliana.” *Science Signaling* 11 (536). <https://doi.org/10.1126/scisignal.aao3070>.
- Enns, Linda C., Masahiro M. Kanaoka, Keiko U. Torii, Luca Comai, Kiyotaka Okada, and Robert E. Cleland. 2005. “Two Callose Synthases, GSL1 and GSL5, Play an Essential and Redundant Role in Plant and Pollen Development and in Fertility.” *Plant Molecular Biology* 58 (3): 333–49. <https://doi.org/10.1007/s11103-005-4526-7>.
- Escocard de Azevedo Manhães, Ana Marcia, Fausto Andres Ortiz-Morea, Ping He, and Libo Shan. 2021. “Plant Plasma Membrane-Resident Receptors: Surveillance for Infections and Coordination for Growth and Development.” *Journal of Integrative Plant Biology* 63 (1): 79–101. <https://doi.org/10.1111/jipb.13051>.
- Eyüboğlu, Banu, Karen Pfister, Georg Haberer, David Chevalier, Angelika Fuchs, Klaus F.X. Mayer, and Kay Schneitz. 2007. “Molecular Characterisation of the STRUBBELIG-RECEPTOR FAMILY of Genes Encoding Putative Leucine-Rich Repeat Receptor-like Kinases in Arabidopsis Thaliana.” *BMC Plant Biology* 7: 1–24. <https://doi.org/10.1186/1471-2229-7-16>.
- Feng, Wei, Daniel Kita, Alexis Peaucelle, Heather N. Cartwright, Vinh Doan, Qiaohong Duan, Ming Che Liu, et al. 2018. “The FERONIA Receptor Kinase Maintains Cell-Wall Integrity during Salt Stress through Ca²⁺ Signaling.” *Current Biology* 28 (5): 666–675.e5. <https://doi.org/10.1016/j.cub.2018.01.023>.
- Ferrari, Simone, Daniel V Savatin, Francesca Sicilia, Giovanna Gramegna, Felice Cervone, and Giulia De Lorenzo. 2013. “Oligogalacturonides : Plant Damage-Associated Molecular Patterns and Regulators of Growth and Development” 4 (March): 1–9. <https://doi.org/10.3389/fpls.2013.00049>.
- Franková, Lenka, and Stephen C. Fry. 2013. “Biochemistry and Physiological Roles of

- Enzymes That ‘cut and Paste’ Plant Cell-Wall Polysaccharides.” *Journal of Experimental Botany* 64 (12): 3519–50. <https://doi.org/10.1093/jxb/ert201>.
- Fujita, Satoshi, Damien De Bellis, Kai H Edel, Philipp Köster, Tonni Grube Andersen, Emanuel Schmid-siegert, Valérie Dénervaud Tendon, et al. 2020. “SCHENGEN Receptor Module Drives Localized ROS Production and Lignification in Plant Roots,” 1–18. <https://doi.org/10.15252/embj.2019103894>.
- Fujita, Satoshi, Damien De Bellis, Kai H Edel, Philipp Köster, Tonni Grube Andersen, Emanuel Schmid-Siegert, Valérie Dénervaud Tendon, et al. 2020. “SCHENGEN Receptor Module Drives Localized ROS Production and Lignification in Plant Roots.” *The EMBO Journal* 39 (9): 1–18. <https://doi.org/10.15252/embj.2019103894>.
- Fulton, Lynette, Martine Batoux, Prasad Vaddepalli, Ram Kishor Yadav, Wolfgang Busch, Stig U. Andersen, Sangho Jeong, Jan U. Lohmann, and Kay Schneitz. 2009. “DETORQUEO, QUIRKY, and ZERZAUST Represent Novel Components Involved in Organ Development Mediated by the Receptor-like Kinase STRUBBELIG in Arabidopsis Thaliana.” *PLoS Genetics* 5 (1). <https://doi.org/10.1371/journal.pgen.1000355>.
- Gall, Hyacinthe Le, Florian Philippe, Jean Marc Domon, Françoise Gillet, Jérôme Pelloux, and Catherine Rayon. 2015. “Cell Wall Metabolism in Response to Abiotic Stress.” *Plants* 4 (1): 112–66. <https://doi.org/10.3390/plants4010112>.
- Galletti, Roberta, Carine Denoux, Stefano Gambetta, Julia Dewdney, Frederick M Ausubel, Giulia De Lorenzo, and Simone Ferrari. 2008. “The AtrbohD-Mediated Oxidative Burst Elicited by Oligogalacturonides in Arabidopsis Is Dispensable for the Activation of Defense Responses Effective against Botrytis Cinerea.” *Plant Physiology* 148 (3): 1695–1706. <https://doi.org/10.1104/pp.108.127845>.
- Galletti, Roberta, Simone Ferrari, Giulia De Lorenzo, Istituto Pasteur-fondazione Cenci Bolognetti, and Biotecnologie Charles. 2011. “Arabidopsis MPK3 and MPK6 Play Different Roles in Basal and Oligogalacturonide- or Flagellin-Induced Resistance against Botrytis Cinerea 1 [W]” 157 (October): 804–14. <https://doi.org/10.1104/pp.111.174003>.
- Gao, Jin, Ajeet Chaudhary, Prasad Vaddepalli, Marie Kristin Nagel, Erika Isono, and Kay Schneitz. 2019. “The Arabidopsis Receptor Kinase STRUBBELIG Undergoes Clathrin-Dependent Endocytosis.” *Journal of Experimental Botany* 70 (15): 3881–94. <https://doi.org/10.1093/jxb/erz190>.
- Geng, Pan, Su Zhang, Jinyue Liu, Cuihuan Zhao, Jie Wu, Yingping Cao, Chunxiang Fu, Xue Han, Hang He, and Qiao Zhao. 2020. “MYB20, MYB42, MYB43, and MYB85 Regulate Phenylalanine and Lignin Biosynthesis during Secondary Cell Wall Formation1[OPEN].” *Plant Physiology* 182 (3): 1272–83. <https://doi.org/10.1104/PP.19.01070>.
- Gigli, Nora, Bisceglia Timo, and Engelsdorf Thorsten. 2019. “Plant Cell Wall Integrity Maintenance in Model Plants and Crop Species - Relevant Cell Wall Components and Underlying Guiding Principles.” *Cellular and Molecular Life Sciences*, no. 0123456789. <https://doi.org/10.1007/s00018-019-03388-8>.
- Gonneau, Martine, Thierry Desprez, Alain Guillot, Samantha Vernhettes, and Herman Höfte. 2014. “Catalytic Subunit Stoichiometry within the Cellulose Synthase Complex.” *Plant Physiology* 166 (4): 1709–12. <https://doi.org/10.1104/pp.114.250159>.
- Gonneau, Martine, Thierry Desprez, Marjolaine Martin, Verónica G. Doblas, Laura Bacete,

- Fabien Miart, Rodnay Sormani, et al. 2018. "Receptor Kinase THESEUS1 Is a Rapid Alkalinization Factor 34 Receptor in Arabidopsis." *Current Biology* 28 (15): 2452-2458.e4. <https://doi.org/10.1016/j.cub.2018.05.075>.
- Hamann, Thorsten, Mark Bennett, John Mansfield, and Christopher Somerville. 2009. "Identification of Cell-Wall Stress as a Hexose-Dependent and Osmosensitive Regulator of Plant Responses." *Plant Journal* 57 (6): 1015–26. <https://doi.org/10.1111/j.1365-313X.2008.03744.x>.
- Han, Xiao, Tae Kyung Hyun, Minhua Zhang, Ritesh Kumar, Eun ji Koh, Byung Ho Kang, William J. Lucas, and Jae Yean Kim. 2014. "Auxin-Callose-Mediated Plasmodesmal Gating Is Essential for Tropic Auxin Gradient Formation and Signaling." *Developmental Cell* 28 (2): 132–46. <https://doi.org/10.1016/j.devcel.2013.12.008>.
- Harholt, Jesper, Anongpat Suttangkakul, and Henrik Vibe Scheller. 2010. "Biosynthesis of Pectin." *Plant Physiology* 153 (2): 384–95. <https://doi.org/10.1104/pp.110.156588>.
- He, Zheng Hui, Iain Cheeseman, Deze He, and Bruce D. Kohorn. 1999. "A Cluster of Five Cell Wall-Associated Receptor Kinase Genes, Wak1-5, Are Expressed in Specific Organs of Arabidopsis." *Plant Molecular Biology* 39 (6): 1189–96. <https://doi.org/10.1023/A:1006197318246>.
- Heim, Dale R., John R. Skomp, Edward E. Tschabold, and Ignacio M. Larrinua. 1990. "Isoxaben Inhibits the Synthesis of Acid Insoluble Cell Wall Materials in Arabidopsis Thaliana." *Plant Physiology* 93 (2): 695–700. <https://doi.org/10.1104/pp.93.2.695>.
- Hématy, Kian, Pierre Etienne Sado, Ageeth Van Tuinen, Soizic Rochange, Thierry Desnos, Sandrine Balzergue, Sandra Pelletier, Jean Pierre Renou, and Herman Höfte. 2007. "A Receptor-like Kinase Mediates the Response of Arabidopsis Cells to the Inhibition of Cellulose Synthesis." *Current Biology* 17 (11): 922–31. <https://doi.org/10.1016/j.cub.2007.05.018>.
- Hessler, Giuliana, Stephan M Porthene, Eva-Maria Gerlach, Tim Lienemann, Gerald Koch, Christian A Voigt, and Stefan Hoth. 2021. "POWDERY MILDEW RESISTENT4 - Dependent Cell Wall Deposition Is a Consequence but Not the Cause of Temperature-Induced Autoimmunity ." *Journal of Experimental Botany* 72 (21): 7549–63. <https://doi.org/10.1093/jxb/erab423>.
- Hill, Joseph L., Mustafa B. Hammudi, and Ming Tien. 2014. "The Arabidopsis Cellulose Synthase Complex: A Proposed Hexamer of Cesa Trimers in an Equimolar Stoichiometryw." *Plant Cell* 26 (12): 4834–42. <https://doi.org/10.1105/tpc.114.131193>.
- Hoffmann, Natalie, Anika Benske, Heather Betz, Mathias Schuetz, and A. Lacey Samuels. 2020. "Laccases and Peroxidases Co-Localize in Lignified Secondary Cell Walls throughout Stem Development." *Plant Physiology* 184 (2): 806–22. <https://doi.org/10.1104/pp.20.00473>.
- Hong, Z., A. J. Delauney, and D. P.S. Verma. 2001. "A Cell Plate-Specific Callose Synthase and Its Interaction with Phragmoplastin." *Plant Cell* 13 (4): 755–68. <https://doi.org/10.1105/tpc.13.4.755>.
- Hong, Z., Z. Zhang, J. M. Olson, and D. P.S. Verma. 2001. "A Novel UDP-Glucose Transferase Is Part of the Callose Synthase Complex and Interacts with Phragmoplastin at the Forming Cell Plate." *Plant Cell* 13 (4): 769–79. <https://doi.org/10.1105/tpc.13.4.769>.

- Hu, Huizhen, Ran Zhang, Zhangsheng Tao, Xukai Li, Yuyang Li, Jiangfeng Huang, Xinxin Li, et al. 2018. “Cellulose Synthase Mutants Distinctively Affect Cell Growth and Cell Wall Integrity for Plant Biomass Production in Arabidopsis.” *Plant and Cell Physiology* 59 (6): 1144–57. <https://doi.org/10.1093/pcp/pcy050>.
- Ito, Emi, Masaru Fujimoto, Kazuo Ebine, Tomohiro Uemura, Takashi Ueda, and Akihiko Nakano. 2012. “Dynamic Behavior of Clathrin in Arabidopsis Thaliana Unveiled by Live Imaging.” *Plant Journal* 69 (2): 204–16. <https://doi.org/10.1111/j.1365-313X.2011.04782.x>.
- Jacobs, Andrew K., Volker Lipka, Rachel A. Burton, Ralph Panstruga, Nicolai Strizhov, Paul Schulze-Lefert, and Geoffrey B. Fincher. 2003. “An Arabidopsis Callose Synthase, GSL5, Is Required for Wound and Papillary Callose Formation.” *Plant Cell* 15 (11): 2503–13. <https://doi.org/10.1105/tpc.016097>.
- Jemmat, Achraf M, Philippe Ranocha, Aurélie Le Ru, Maxime Neel, Sara Raggi, Simone Ferrari, Vincent Burlat, et al. 2021. “Coordination of Five Class III Peroxidase Encoding Genes for Early Germination Events of Arabidopsis Thaliana To Cite This Version : HAL Id : Hal-03372633.”
- Johnson, Joy M., Johannes Thürich, Elena K. Petutschnig, Lothar Altschmied, Doreen Meichsner, Irena Sherameti, Julian Dindas, et al. 2018. “A Poly(A) Ribonuclease Controls the Cellotriose-Based Interaction between Piriformospora Indica and Its Host Arabidopsis.” *Plant Physiology* 176 (3): 2496–2514. <https://doi.org/10.1104/pp.17.01423>.
- Jonsson, Kristoffer, Olivier Hamant, and Rishikesh P. Bhalerao. 2022. “Plant Cell Walls as Mechanical Signaling Hubs for Morphogenesis.” *Current Biology* 32 (7): R334–40. <https://doi.org/10.1016/j.cub.2022.02.036>.
- Kachroo, Pradeep, and Aardra Kachroo. 2012. *The Roles of Salicylic Acid and Jasmonic Acid in Plant Immunity. Molecular Plant Immunity*. <https://doi.org/10.1002/9781118481431.ch4>.
- Kámán-Tóth, Evelin, Tamás Dankó, Gábor Gullner, Zoltán Bozsó, László Palkovics, and Miklós Pogány. 2019. “Contribution of Cell Wall Peroxidase- and NADPH Oxidase-Derived Reactive Oxygen Species to Alternaria Brassicicola-Induced Oxidative Burst in Arabidopsis.” *Molecular Plant Pathology* 20 (4): 485–99. <https://doi.org/10.1111/mpp.12769>.
- Kamiya, Takehiro, Monica Borghi, Peng Wang, John M.C. Danku, Lothar Kalmbach, Prashant S. Hosmani, Sadaf Naseer, Toru Fujiwara, Niko Geldner, and David E. Salt. 2015. “The MYB36 Transcription Factor Orchestrates Casparian Strip Formation.” *Proceedings of the National Academy of Sciences of the United States of America* 112 (33): 10533–38. <https://doi.org/10.1073/pnas.1507691112>.
- Kessler, Sharon A, Heike Lindner, Daniel S Jones, and Ueli Grossniklaus. 2015. “Functional Analysis of Related Cr RLK 1L Receptor-like Kinases in Pollen Tube Reception .” *EMBO Reports* 16 (1): 107–15. <https://doi.org/10.15252/embr.201438801>.
- Khandal, Hitaishi, Amar Pal Singh, and Debasis Chattopadhyay. 2020. “The MicroRNA397b-LaCCASE2 Module Regulates Root Lignification under Water and Phosphate Deficiency.” *Plant Physiology* 182 (3): 1387–1403. <https://doi.org/10.1104/PP.19.00921>.
- Kluge, Stefanie, Benjamin Bonhage, Jörn Viell, Mari Granström, Alois Kindler, and Antje C. Spiess. 2019. “Enzymatic Production of Cello-Oligomers with Endoglucanases.”

- Cellulose* 4: 4279–90. <https://doi.org/10.1007/s10570-019-02390-4>.
- Kohorn, Bruce D. 2016. “Cell Wall-Associated Kinases and Pectin Perception.” *Journal of Experimental Botany* 67 (2): 489–94. <https://doi.org/10.1093/jxb/erv467>.
- Kohorn, Bruce D., Susan Johansen, Akira Shishido, Tanya Todorova, Rhysly Martinez, Elita Defeo, and Pablo Obregon. 2009. “Pectin Activation of MAP Kinase and Gene Expression Is WAK2 Dependent.” *Plant Journal* 60 (6): 974–82. <https://doi.org/10.1111/j.1365-313X.2009.04016.x>.
- Kohorn, Bruce D., and Susan L. Kohorn. 2012. “The Cell Wall-Associated Kinases, WAKs, as Pectin Receptors.” *Frontiers in Plant Science* 3 (May): 1–5. <https://doi.org/10.3389/fpls.2012.00088>.
- Kohorn, Bruce D., Susan L. Kohorn, Tanya Todorova, Gillian Baptiste, Kevin Stansky, and Meghan McCullough. 2012. “A Dominant Allele of Arabidopsis Pectin-Binding Wall-Associated Kinase Induces a Stress Response Suppressed by MPK6 but Not MPK3 Mutations.” *Molecular Plant* 5 (4): 841–51. <https://doi.org/10.1093/mp/ssr096>.
- Kong, Yanqiong, Jia Chen, Lingli Jiang, Hong Chen, Yanan Shen, Lifeng Wang, Yujie Yan, et al. 2023. “Structural and Biochemical Basis of Arabidopsis FERONIA Receptor Kinase-Mediated Early Signaling Initiation.” *Plant Communications*, 100559. <https://doi.org/10.1016/j.xplc.2023.100559>.
- Kopischke, Michaela, Lore Westphal, Korbinian Schneeberger, Richard Clark, Stephan Ossowski, Vera Wewer, René Fuchs, et al. 2013. “Impaired Sterol Ester Synthesis Alters the Response of Arabidopsis Thaliana to Phytophthora Infestans.” *Plant Journal* 73 (3): 456–68. <https://doi.org/10.1111/tpj.12046>.
- Kubicek, Christian P., Trevor L. Starr, and N. Louise Glass. 2014. “Plant Cell Wall-Degrading Enzymes and Their Secretion in Plant-Pathogenic Fungi.” *Annual Review of Phytopathology* 52: 427–51. <https://doi.org/10.1146/annurev-phyto-102313-045831>.
- Kubo, Minoru, Makiko Udagawa, Nobuyuki Nishikubo, Gorou Horiguchi, Masatoshi Yamaguchi, Jun Ito, Tetsuro Mimura, Hiroo Fukuda, and Taku Demura. 2005. “Transcription Switches for Protoxylem and Metaxylem Vessel Formation,” 1855–60. <https://doi.org/10.1101/gad.1331305.GENES>.
- Kuki, Hiroaki, Ryusuke Yokoyama, Takeshi Kuroha, and Kazuhiko Nishitani. 2020. “Xyloglucan Is Not Essential for the Formation and Integrity of the Cellulose Network in the Primary Cell Wall Regenerated from Arabidopsis Protoplasts.” *Plants* 9 (5): 1–14. <https://doi.org/10.3390/plants9050629>.
- Kurihara, Daisuke, Yoko Mizuta, Yoshikatsu Sato, and Tetsuya Higashiyama. 2015. “ClearSee: A Rapid Optical Clearing Reagent for Whole-Plant Fluorescence Imaging.” *Development (Cambridge)* 142 (23): 4168–79. <https://doi.org/10.1242/dev.127613>.
- Kwak, Su Hwan, and John Schiefelbein. 2007. “The Role of the SCRAMBLED Receptor-like Kinase in Patterning the Arabidopsis Root Epidermis.” *Developmental Biology* 302 (1): 118–31. <https://doi.org/10.1016/j.ydbio.2006.09.009>.
- Kwak, Su Hwan, Ronglai Shen, and John Schiefelbein. 2005. “Positional Signaling Mediated by a Receptor-like Kinase in Arabidopsis.” *Science* 307 (5712): 1111–13. <https://doi.org/10.1126/science.1105373>.
- Kwak, Su Hwan, Sooah Woo, Myeong Min Lee, and John Schiefelbein. 2014. “Distinct Signaling Mechanisms in Multiple Developmental Pathways by the SCRAMBLED

- Receptor of Arabidopsis.” *Plant Physiology* 166 (2): 976–87.
<https://doi.org/10.1104/pp.114.247288>.
- Lampugnani, Edwin R., Ghazanfar Abbas Khan, Marc Somssich, and Staffan Persson. 2018. “Building a Plant Cell Wall at a Glance.” *Journal of Cell Science* 131 (2): jcs207373.
<https://doi.org/10.1242/jcs.207373>.
- Lee, Yuree, Maria C. Rubio, Julien Alassimone, and Niko Geldner. 2013. “A Mechanism for Localized Lignin Deposition in the Endodermis.” *Cell* 153 (2): 402–12.
<https://doi.org/10.1016/j.cell.2013.02.045>.
- Lei, Lei, Abhishek Singh, Logan Bashline, Shundai Li, Yaroslava G. Yingling, and Ying Gua. 2015. “Cellulose Synthase Interactive1 Is Required for Fast Recycling of Cellulose Synthase Complexes to the Plasma Membrane in Arabidopsis.” *Plant Cell* 27 (10): 2926–40. <https://doi.org/10.1105/tpc.15.00442>.
- Li, Jing, Rachel A. Burton, Andrew J. Harvey, Maria Hrmova, Ahmad Z. Wardak, Bruce A. Stone, and Geoffrey B. Fincher. 2003. “Biochemical Evidence Linking a Putative Callose Synthase Gene with (1→3)-β-D-Glucan Biosynthesis in Barley.” *Plant Molecular Biology* 53 (1–2): 213–25.
<https://doi.org/10.1023/B:PLAN.0000009289.50285.52>.
- Li, Zhengzheng, Tao Zhou, Pengyue Sun, Xinhang Chen, Luping Gong, Peng Sun, Shengchao Ge, and Yun Kuan Liang. 2022. “COBL9 and COBL7 Synergistically Regulate Root Hair Tip Growth via Controlling Apical Cellulose Deposition.” *Biochemical and Biophysical Research Communications* 596: 6–13.
<https://doi.org/10.1016/j.bbrc.2022.01.096>.
- Lieberman, Louisa M., Erin E. Sparks, Miguel A. Moreno-Risueno, Jalean J. Petricka, and Philip N. Benfey. 2015. “MYB36 Regulates the Transition from Proliferation to Differentiation in the Arabidopsis Root.” *Proceedings of the National Academy of Sciences of the United States of America* 112 (39): 12099–104.
<https://doi.org/10.1073/pnas.1515576112>.
- Lin, Lin, Si Hui Zhong, Xiao Feng Cui, Jianming Li, and Zu Hua He. 2012. “Characterization of Temperature-Sensitive Mutants Reveals a Role for Receptor-like Kinase SCRAMBLED/STRUBBELIG in Coordinating Cell Proliferation and Differentiation during Arabidopsis Leaf Development.” *Plant Journal* 72 (5): 707–20.
<https://doi.org/10.1111/j.1365-313X.2012.05109.x>.
- Liu, Jun, Jia Li, and Libo Shan. 2020. “SERKs.” *Current Biology* 30 (7): R293–94.
<https://doi.org/10.1016/j.cub.2020.01.043>.
- Lorenzo, Giulia De, Simone Ferrari, Moira Giovannoni, Benedetta Mattei, and Felice Cervone. 2019. “Cell Wall Traits That Influence Plant Development, Immunity, and Bioconversion.” *Plant Journal* 97 (1): 134–47. <https://doi.org/10.1111/tpj.14196>.
- Luna, Estrella, Victoria Pastor, Jérôme Robert, Victor Flors, Brigitte Mauch-mani, and Jurriaan Ton. 2011. “Callose Deposition : A Multifaceted Plant Defense Response” 24 (2): 183–93.
- Ma, Yi, Yichen Zhao, Robin K. Walker, and Gerald A. Berkowitz. 2013. “Molecular Steps in the Immune Signaling Pathway Evoked by Plant Elicitor Peptides: Ca²⁺-Dependent Protein Kinases, Nitric Oxide, and Reactive Oxygen Species Are Downstream from the Early Ca²⁺ Signal.” *Plant Physiology* 163 (3): 1459–71.
<https://doi.org/10.1104/pp.113.226068>.

- Malinovsky, Frederikke G., Jonatan U. Fangel, and William G.T. Willats. 2014. "The Role of the Cell Wall in Plant Immunity." *Frontiers in Plant Science* 5 (MAY): 1–12. <https://doi.org/10.3389/fpls.2014.00178>.
- Marconi, Marco, and Krzysztof Wabnick. 2021. "Shaping the Organ: A Biologist Guide to Quantitative Models of Plant Morphogenesis." *Frontiers in Plant Science* 12 (1). <https://doi.org/10.3389/fpls.2021.746183>.
- Mareri, Lavinia, Luigi Parrotta, and Giampiero Cai. 2022. "Environmental Stress and Plants." *International Journal of Molecular Sciences* 23 (10): 1–9. <https://doi.org/10.3390/ijms23105416>.
- Marzol, Eliana, Cecilia Borassi, Jose José Estevez, Philippe Ranocha, Ariel Aptekman, Mauro Bringas, Janice Pennington, et al. 2022. "Class III Peroxidases PRX01, PRX44, and PRX73 Potentially Target Extensins during Root Hair Growth in Arabidopsis Thaliana." *International Journal of Molecular Sciences* 23 (10): 5375. <https://doi.org/10.3390/ijms23105375>.
- Masucci, James D., William G. Rerie, Daphne R. Foreman, Meng Zhang, Moira E. Galway, M. David Marks, and John W. Schiefelbein. 1996. "The Homeobox Gene GLABRA 2 Is Required for Position-Dependent Cell Differentiation in the Root Epidermis of Arabidopsis Thaliana." *Development* 122 (4): 1253–60. <https://doi.org/10.1242/dev.122.4.1253>.
- Meents, Miranda J., Yoichiro Watanabe, and A. Lacey Samuels. 2018. "The Cell Biology of Secondary Cell Wall Biosynthesis." *Annals of Botany* 121 (6): 1107–25. <https://doi.org/10.1093/aob/mcy005>.
- Ménard, Delphine, Leonard Blaschek, Konstantin Kriechbaum, Cheng Choo Lee, Henrik Serk, Chuantao Zhu, Alexander Lyubartsev, et al. 2022. "Plant Biomechanics and Resilience to Environmental Changes Are Controlled by Specific Lignin Chemistries in Each Vascular Cell Type and Morphotype." *The Plant Cell* 34 (12): 4877–96. <https://doi.org/10.1093/plcell/koac284>.
- Merz, David, Julia Richter, Martine Gonneau, Clara Sanchez-Rodriguez, Tobias Eder, Rodnay Sormani, Marjolaine Martin, Kian Hématy, Herman Höfte, and Marie Theres Hauser. 2017. "T-DNA Alleles of the Receptor Kinase THESEUS1 with Opposing Effects on Cell Wall Integrity Signaling." *Journal of Experimental Botany* 68 (16): 4583–93. <https://doi.org/10.1093/jxb/erx263>.
- Mitsuda, Nobutaka, Motoaki Seki, Kazuo Shinozaki, and Masaru Ohme-Takagi. 2005. "The NAC Transcription Factors NST1 and NST2 of Arabidopsis Regulate Secondary Wall Thickenings and Are Required for Anther Dehiscence." *Plant Cell* 17 (11): 2993–3006. <https://doi.org/10.1105/tpc.105.036004>.
- Moussu, Steven, Sebastian Augustin, Andra Octavia Roman, Caroline Broyart, and Julia Santiago. 2018. "Crystal Structures of Two Tandem Malectin-like Receptor Kinases Involved in Plant Reproduction." *Acta Crystallographica Section D: Structural Biology* 74 (7): 671–80. <https://doi.org/10.1107/S205979831800774X>.
- Nakano, Yoshimi, Masatoshi Yamaguchi, Hitoshi Endo, Nur Ardiyana Rejab, and Misato Ohtani. 2015. "NAC-MYB-Based Transcriptional Regulation of Secondary Cell Wall Biosynthesis in Land Plants." *Frontiers in Plant Science* 6 (MAY): 1–18. <https://doi.org/10.3389/fpls.2015.00288>.
- Nam, Kyoung Hee, and Jianming Li. 2002. "BRI1/BAK1, a Receptor Kinase Pair Mediating

- Brassinosteroid Signaling.” *Cell* 110 (2): 203–12. [https://doi.org/10.1016/S0092-8674\(02\)00814-0](https://doi.org/10.1016/S0092-8674(02)00814-0).
- Nixon, B. Tracy, Katayoun Mansouri, Abhishek Singh, Juan Du, Jonathan K. Davis, Jung Goo Lee, Erin Slabaugh, et al. 2016. “Comparative Structural and Computational Analysis Supports Eighteen Cellulose Synthases in the Plant Cellulose Synthesis Complex.” *Scientific Reports* 6 (June): 1–14. <https://doi.org/10.1038/srep28696>.
- Osakabe, Yuriko, Kazuko Yamaguchi-Shinozaki, Kazuo Shinozaki, and Lam Son Phan Tran. 2013. “Sensing the Environment: Key Roles of Membrane-Localized Kinases in Plant Perception and Response to Abiotic Stress.” *Journal of Experimental Botany* 64 (2): 445–58. <https://doi.org/10.1093/jxb/ers354>.
- Palin, Robert, and Anja Geitmann. 2012. “The Role of Pectin in Plant Morphogenesis.” *BioSystems* 109 (3): 397–402. <https://doi.org/10.1016/j.biosystems.2012.04.006>.
- Paredez, Alexander R., Christopher R. Somerville, and David W. Ehrhardt. 2006. “Visualization of Cellulose Synthase Demonstrates Functional Association with Microtubules.” *Science* 312 (5779): 1491–95. <https://doi.org/10.1126/science.1126551>.
- Park, Yong Bum, and Daniel J. Cosgrove. 2015. “Xyloglucan and Its Interactions with Other Components of the Growing Cell Wall.” *Plant and Cell Physiology* 56 (2): 180–94. <https://doi.org/10.1093/pcp/pcu204>.
- Paul Barratt, D. H., Katharina Kölling, Alexander Graf, Marilyn Pike, Grant Calder, Kim Findlay, Samuel C. Zeeman, and Alison M. Smith. 2011. “Callose Synthase GSL7 Is Necessary for Normal Phloem Transport and Inflorescence Growth in Arabidopsis.” *Plant Physiology* 155 (1): 328–41. <https://doi.org/10.1104/pp.110.166330>.
- Payne, Christina M., Brandon C. Knott, Heather B. Mayes, Henrik Hansson, Michael E. Himmel, Mats Sandgren, Jerry Ståhlberg, and Gregg T. Beckham. 2015. “Fungal Cellulases.” *Chemical Reviews* 115 (3): 1308–1448. <https://doi.org/10.1021/cr500351c>.
- Pedersen, Gustav B, Leonard Blaschek, Kristian E.H. Frandsen, Lise C Noack, and Staffan Persson. 2023. “Cellulose Synthesis in Land Plants.” *Molecular Plant*. The Author. <https://doi.org/10.1016/j.molp.2022.12.015>.
- Persson, Staffan, Alexander Paredez, Andrew Carroll, Hildur Palsdottir, Monika Doblin, Patricia Poindexter, Natalie Khitrov, Manfred Auer, and Chris R. Somerville. 2007. “Genetic Evidence for Three Unique Components in Primary Cell-Wall Cellulose Synthase Complexes in Arabidopsis.” *Proceedings of the National Academy of Sciences of the United States of America* 104 (39): 15566–71. <https://doi.org/10.1073/pnas.0706592104>.
- Pfister, Alexandre, Marie Barberon, Julien Alassimone, Lothar Kalmbach, Yuree Lee, Joop E.M. Vermeer, Misako Yamazaki, et al. 2014. “A Receptor-like Kinase Mutant with Absent Endodermal Diffusion Barrier Displays Selective Nutrient Homeostasis Defects.” *ELife* 3: e03115. <https://doi.org/10.7554/eLife.03115>.
- Piršelová, B., and I. Matušíková. 2013. “Callose: The Plant Cell Wall Polysaccharide with Multiple Biological Functions.” *Acta Physiologiae Plantarum* 35 (3): 635–44. <https://doi.org/10.1007/s11738-012-1103-y>.
- Polko, Joanna K., and Joseph J. Kieber. 2019. “The Regulation of Cellulose Biosynthesis in Plants.” *Plant Cell* 31 (2): 282–96. <https://doi.org/10.1105/tpc.18.00760>.
- Pradhan Mitra, Prajakta, and Dominique Loqué. 2014. “Histochemical Staining of

- Arabidopsis Thaliana Secondary Cell Wall Elements.” *Journal of Visualized Experiments*, no. 87: 1–11. <https://doi.org/10.3791/51381>.
- Qu, Shaofeng, Xi Zhang, Yutong Song, Jinxing Lin, and Xiaoyi Shan. 2017. “THESEUS1 Positively Modulates Plant Defense Responses against Botrytis Cinerea through GUANINE EXCHANGE FACTOR4 Signaling.” *Journal of Integrative Plant Biology* 59 (11): 797–804. <https://doi.org/10.1111/jipb.12565>.
- Rasul, S., C. Dubreuil-Maurizi, O. Lamotte, E. Koen, B. Poinssot, G. Alcaraz, D. Wendehenne, and S. Jeandroz. 2012. “Nitric Oxide Production Mediates Oligogalacturonide-Triggered Immunity and Resistance to Botrytis Cinerea in Arabidopsis Thaliana.” *Plant, Cell and Environment* 35 (8): 1483–99. <https://doi.org/10.1111/j.1365-3040.2012.02505.x>.
- Rasul, S, O Lamotte, E Koen, B Poinssot, G Alcaraz, D Wendehenne, and S Jeandroz. 2012. “Nitric Oxide Production Mediates Oligogalacturonide- Triggered Immunity and Resistance to Botrytis Cinerea in Arabidopsis Thaliana” 1: 1483–99. <https://doi.org/10.1111/j.1365-3040.2012.02505.x>.
- Reyt, Guilhem, Priya Ramakrishna, Isai Salas-González, Satoshi Fujita, Ashley Love, David Tiemessen, Catherine Lapierre, et al. 2021. “Two Chemically Distinct Root Lignin Barriers Control Solute and Water Balance.” *Nature Communications* 12 (1). <https://doi.org/10.1038/s41467-021-22550-0>.
- Richmond, Todd A, and Chris R Somerville. 2000. “The Cellulose Synthase Superfamily” 124 (October): 495–98.
- Rogers, Louisa A., and Malcolm M. Campbell. 2004. “The Genetic Control of Lignin Deposition during Plant Growth and Development.” *New Phytologist* 164 (1): 17–30. <https://doi.org/10.1111/j.1469-8137.2004.01143.x>.
- Rohde, Antje, Kris Morreel, John Ralph, Geert Goeminne, Vanessa Hostyn, Riet De Rycke, Sergej Kushnir, et al. 2004. “Molecular Phenotyping of the Pal1 and Pal2 Mutants of Arabidopsis Thaliana Reveals Far-Reaching Consequences on Phenylpropanoid, Amino Acid, and Carbohydrate Metabolism.” *Plant Cell* 16 (10): 2749–71. <https://doi.org/10.1105/tpc.104.023705>.
- Roppolo, Daniele, Brigitte Boeckmann, Alexandre Pfister, Emmanuel Boutet, Maria C. Rubio, Valérie Dénervaud-Tendon, Joop E.M. Vermeer, Jacqueline Gheyselinck, Ioannis Xenarios, and Niko Geldner. 2014. “Functional and Evolutionary Analysis of the CASPARIAN STRIP MEMBRANE DOMAIN PROTEIN Family.” *Plant Physiology* 165 (4): 1709–22. <https://doi.org/10.1104/pp.114.239137>.
- Sanmartín, Neus, Victoria Pastor, Julia Pastor-Fernández, Victor Flors, Maria Jose Pozo, and Paloma Sánchez-Bel. 2021. “Role and Mechanisms of Callose Priming in Mycorrhiza-Induced Resistance.” *Journal of Experimental Botany* 71 (9): 2769–81. <https://doi.org/10.1093/JXB/ERAA030>.
- Schallus Thomas et al. 2009. “Malectin: A Novel Carbohydrate-Binding Protein of the Endoplasmic Reticulum and a Candidate Player in the Early Steps of Protein N-Glycosylation.” *Molecular Biology of the Cell* 20 (August): 2673–83. <https://doi.org/10.1091/mbc.E08>.
- Scheible, Wolf Rüdiger, Ravit Eshed, Todd Richmond, Deborah Delmer, and Chris Somerville. 2001. “Modifications of Cellulose Synthase Confer Resistance to Isoxaben and Thiazolidinone Herbicides in Arabidopsis Ixr1 Mutants.” *Proceedings of the*

- National Academy of Sciences of the United States of America* 98 (18): 10079–84.
<https://doi.org/10.1073/pnas.191361598>.
- Scheller, Henrik Vibe, and Peter Ulvskov. 2010. “Hemicelluloses.” *Annual Review of Plant Biology* 61: 263–89. <https://doi.org/10.1146/annurev-arplant-042809-112315>.
- Schneitz, Kay, Martin Hülskamp, Steven D. Kopczak, and Robert E. Pruitt. 1997. “Dissection of Sexual Organ Ontogenesis: A Genetic Analysis of Ovule Development in *Arabidopsis Thaliana*.” *Development* 124 (7): 1367–76. <https://doi.org/10.1242/dev.124.7.1367>.
- Schuetz, Mathias, Anika Benske, Rebecca A. Smith, Yoichiro Watanabe, Yuki Tobimatsu, John Ralph, Taku Demura, Brian Ellis, and A. Lacey Samuels. 2014. “Laccases Direct Lignification in the Discrete Secondary Cell Wall Domains of Protoxylem.” *Plant Physiology* 166 (2): 798–807. <https://doi.org/10.1104/pp.114.245597>.
- Sevilem, Iris, Shunsuke Miyashima, and Ykä Helariutta. 2013. “Cell-to-Cell Communication via Plasmodesmata in Vascular Plants.” *Cell Adhesion and Migration* 7 (1): 27–32. <https://doi.org/10.4161/cam.22126>.
- Shin, Yesol, Andrea Chane, Minjung Jung, and Yuree Lee. 2021. “Recent Advances in Understanding the Roles of Pectin as an Active Participant in Plant Signaling Networks.” *Plants* 10 (8): 1–22. <https://doi.org/10.3390/plants10081712>.
- Shiu, S.-H., and A. B. Bleecker. 2001. “Plant Receptor-Like Kinase Gene Family: Diversity, Function, and Signaling.” *Science Signaling* 2001 (113): re22–re22. <https://doi.org/10.1126/stke.2001.113.re22>.
- Somerville, Chris, Stefan Bauer, Ginger Brininstool, Michelle Facette, Thorsten Hamann, Jennifer Milne, Erin Osborne, et al. 2004. “Toward a Systems Approach to Understanding Plant Cell Walls.” *Science* 306 (5705): 2206–11. <https://doi.org/10.1126/science.1102765>.
- Son, Yong, Yong-Kwan Cheong, Nam-Ho Kim, Hun-Taeg Chung, Dae Gill Kang, and Hyun-Ock Pae. 2011. “Mitogen-Activated Protein Kinases and Reactive Oxygen Species: How Can ROS Activate MAPK Pathways?” *Journal of Signal Transduction* 2011: 1–6. <https://doi.org/10.1155/2011/792639>.
- Song, Jae Hyo, Su Hwan Kwak, Kyoung Hee Nam, John Schiefelbein, and Myeong Min Lee. 2019. “QUIRKY Regulates Root Epidermal Cell Patterning through Stabilizing SCRAMBLED to Control CAPRICE Movement in *Arabidopsis*.” *Nature Communications* 10 (1). <https://doi.org/10.1038/s41467-019-09715-8>.
- Srivastava, Vaibhav, Lauren S McKee, and Vincent Bulone. 2017. “Plant Cell Walls.” *ELS*, no. July 2018: 1–17. <https://doi.org/10.1002/9780470015902.a0001682.pub3>.
- Stegmann, Martin, Jacqueline Monaghan, Elwira Smakowska-Luzan, Hanna Rovenich, Anita Lehner, Nicholas Holton, Youssef Belkhadir, and Cyril Zipfel. 2017. “The Receptor Kinase FER Is a RALF-Regulated Scaffold Controlling Plant Immune Signaling.” *Science* 355 (6322): 287–89. <https://doi.org/10.1126/science.aal2541>.
- Taylor-Teeple, M., L. Lin, M. De Lucas, G. Turco, T. W. Toal, A. Gaudinier, N. F. Young, et al. 2015a. “An *Arabidopsis* Gene Regulatory Network for Secondary Cell Wall Synthesis.” *Nature* 517 (7536): 571–75. <https://doi.org/10.1038/nature14099>.
- Tokunaga, Naohito, Tsuyoshi Kaneta, Seiichi Sato, and Yasushi Sato. 2009. “Analysis of Expression Profiles of Three Peroxidase Genes Associated with Lignification in *Arabidopsis Thaliana*.” *Physiologia Plantarum* 136 (2): 237–49.

<https://doi.org/10.1111/j.1399-3054.2009.01233.x>.

- Torres, Miguel Angel, and Jeffery L. Dangl. 2005. "Functions of the Respiratory Burst Oxidase in Biotic Interactions, Abiotic Stress and Development." *Current Opinion in Plant Biology* 8 (4): 397–403. <https://doi.org/10.1016/j.pbi.2005.05.014>.
- Turlapati, Phanikanth V., Kye Won Kim, Laurence B. Davin, and Norman G. Lewis. 2011. "The Laccase Multigene Family in Arabidopsis Thaliana: Towards Addressing the Mystery of Their Gene Function(S)." *Planta* 233 (3): 439–70. <https://doi.org/10.1007/s00425-010-1298-3>.
- Ursache, Robertas, Tonni Grube Andersen, Peter Marhavý, and Niko Geldner. 2018. "A Protocol for Combining Fluorescent Proteins with Histological Stains for Diverse Cell Wall Components." *Plant Journal* 93 (2): 399–412. <https://doi.org/10.1111/tpj.13784>.
- Vaddepalli, Prasad, Lynette Fulton, Martine Batoux, Ram Kishor Yadav, and Kay Schneitz. 2011. "Structure-Function Analysis of Strubbelig, an Arabidopsis Atypical Receptor-like Kinase Involved in Tissue Morphogenesis." *PLoS ONE* 6 (5). <https://doi.org/10.1371/journal.pone.0019730>.
- Vaddepalli, Prasad, Lynette Fulton, Jennifer Wieland, Katrin Wassmer, Milena Schaeffer, Stefanie Ranf, and Kay Schneitz. 2017. "The Cell Wall-Localized Atypical β -1,3 Glucanase ZERZAUST Controls Tissue Morphogenesis in *Arabidopsis Thaliana*." *Development* 144 (12): 2259–69. <https://doi.org/10.1242/dev.152231>.
- Vaddepalli, Prasad, Anja Herrmann, Lynette Fulton, Maxi Oelschner, Stefan Hillmer, Thomas F Stratil, Astrid Fastner, et al. 2014. "The C2-Domain Protein QUIRKY and the Receptor-like Kinase STRUBBELIG Localize to Plasmodesmata and Mediate Tissue Morphogenesis in Arabidopsis Thaliana," 4139–48. <https://doi.org/10.1242/dev.113878>.
- Vanholme, Ruben, Brecht Demedts, Kris Morreel, John Ralph, and Wout Boerjan. 2010. "Lignin Biosynthesis and Structure." *Plant Physiology* 153 (3): 895–905. <https://doi.org/10.1104/pp.110.155119>.
- Vatén, Anne, Jan Dettmer, Shuang Wu, York Dieter Stierhof, Shunsuke Miyashima, Shri Ram Yadav, Christina J. Roberts, et al. 2011. "Callose Biosynthesis Regulates Symplastic Trafficking during Root Development." *Developmental Cell* 21 (6): 1144–55. <https://doi.org/10.1016/j.devcel.2011.10.006>.
- Verma, Desh Pal S., and Zonglie Hong. 2001. "Plant Callose Synthase Complexes." *Plant Molecular Biology* 47 (6): 693–701. <https://doi.org/10.1023/A:1013679111111>.
- Wagner, T. A. 2001. "Wall-Associated Kinases Are Expressed throughout Plant Development and Are Required for Cell Expansion." *The Plant Cell Online* 13 (2): 303–18. <https://doi.org/10.1105/tpc.13.2.303>.
- Wang, Hongli, Xi Cheng, Dongmei Yin, Dongliang Chen, Chang Luo, Hua Liu, and Conglin Huang. 2023. "Advances in the Research on Plant WRKY Transcription Factors Responsive to External Stresses." *Current Issues in Molecular Biology* 45 (4): 2861–80. <https://doi.org/10.3390/cimb45040187>.
- Wang, Xiaopei, Yanli Niu, and Yuan Zheng. 2021. "Multiple Functions of Myb Transcription Factors in Abiotic Stress Responses." *International Journal of Molecular Sciences* 22 (11). <https://doi.org/10.3390/ijms22116125>.
- Wang, Ying, Xifeng Li, Baofang Fan, Cheng Zhu, and Zhixiang Chen. 2021. "Regulation and Function of Defense-Related Callose Deposition in Plants." *International Journal of*

- Molecular Sciences* 22 (5): 1–15. <https://doi.org/10.3390/ijms22052393>.
- Wolf, Sebastian. 2017. “Plant Cell Wall Signalling and Receptor-like Kinases.” *Biochemical Journal* 474 (4): 471–92. <https://doi.org/10.1042/BCJ20160238>.
- Wolf, Sebastian. 2022. “Cell Wall Signaling in Plant Development and Defense.” *Annual Review of Plant Biology* 73: 323–53. <https://doi.org/10.1146/annurev-arplant-102820-095312>.
- Wu, Yunzhe, Qingqing Xun, Yi Guo, Jinghua Zhang, Kaili Cheng, Tao Shi, Kai He, Suiwen Hou, Xiaoping Gou, and Jia Li. 2016. “Genome-Wide Expression Pattern Analyses of the Arabidopsis Leucine-Rich Repeat Receptor-Like Kinases.” *Molecular Plant* 9 (2): 289–300. <https://doi.org/10.1016/j.molp.2015.12.011>.
- Xi, Lin, Xu Na Wu, Max Gilbert, and Waltraud X. Schulze. 2019. “Classification and Interactions of LRR Receptors and Co-Receptors within the Arabidopsis Plasma Membrane – An Overview.” *Frontiers in Plant Science* 10 (April): 1–8. <https://doi.org/10.3389/fpls.2019.00472>.
- Xiao, Yu, Martin Stegmann, Zhifu Han, Thomas A. DeFalco, Katarzyna Parys, Li Xu, Youssef Belkhadir, Cyril Zipfel, and Jijie Chai. 2019. “Mechanisms of RALF Peptide Perception by a Heterotypic Receptor Complex.” *Nature* 572 (7768): 270–74. <https://doi.org/10.1038/s41586-019-1409-7>.
- Xie, Bo, Xiaomin Wang, and Zonglie Hong. 2010. “Precocious Pollen Germination in Arabidopsis Plants with Altered Callose Deposition during Microsporogenesis.” *Planta* 231 (4): 809–23. <https://doi.org/10.1007/s00425-009-1091-3>.
- Xie, Meng, Jin Zhang, Timothy J. Tschaplinski, Gerald A. Tuskan, Jin Gui Chen, and Wellington Muchero. 2018. “Regulation of Lignin Biosynthesis and Its Role in Growth-Defense Tradeoffs.” *Frontiers in Plant Science* 9 (September): 1–9. <https://doi.org/10.3389/fpls.2018.01427>.
- Xie, Meng, Jin Zhang, Tao Yao, Anthony C. Bryan, Yunqiao Pu, Jessy Labbé, Dale A. Pelletier, et al. 2020. “Arabidopsis C-Terminal Binding Protein ANGUSTIFOLIA Modulates Transcriptional Co-Regulation of MYB46 and WRKY33.” *New Phytologist* 228 (5): 1627–39. <https://doi.org/10.1111/nph.16826>.
- Yao, Tao, Kai Feng, Meng Xie, Jaime Barros, Timothy J. Tschaplinski, Gerald A. Tuskan, Wellington Muchero, and Jin Gui Chen. 2021. “Phylogenetic Occurrence of the Phenylpropanoid Pathway and Lignin Biosynthesis in Plants.” *Frontiers in Plant Science* 12 (August): 1–14. <https://doi.org/10.3389/fpls.2021.704697>.
- Yi Chou, Eva, Mathias Schuetz, Natalie Hoffmann, Yoichiro Watanabe, Richard Sibout, and A. Lacey Samuels. 2018. “Distribution, Mobility, and Anchoring of Lignin-Related Oxidative Enzymes in Arabidopsis Secondary Cell Walls.” *Journal of Experimental Botany* 69 (8): 1849–59. <https://doi.org/10.1093/jxb/ery067>.
- Yoon, Jinmi, Heebak Choi, and Gynheung An. 2015. “Roles of Lignin Biosynthesis and Regulatory Genes in Plant Development.” *Journal of Integrative Plant Biology* 57 (11): 902–12. <https://doi.org/10.1111/jipb.12422>.
- Zhang, Baocai, Yihong Gao, Lanjun Zhang, and Yihua Zhou. 2021. “The Plant Cell Wall: Biosynthesis, Construction, and Functions.” *Journal of Integrative Plant Biology* 63 (1): 251–72. <https://doi.org/10.1111/jipb.13055>.
- Zhang, Wenjuan, Wenqi Qin, Huiling Li, and Ai Min Wu. 2021. “Biosynthesis and Transport

- of Nucleotide Sugars for Plant Hemicellulose.” *Frontiers in Plant Science* 12 (November). <https://doi.org/10.3389/fpls.2021.723128>.
- Zhong, Ruiqin, Taku Demura, and Zhehg Hua Ye. 2006. “SND1, a NAC Domain Transcription Factor, Is a Key Regulator of Secondary Wall Synthesis in Fibers of Arabidopsis.” *Plant Cell* 18 (11): 3158–70. <https://doi.org/10.1105/tpc.106.047399>.
- Zhong, Ruiqin, Chanhui Lee, Ryan L. McCarthy, Cromwell K. Reeves, Evan G. Jones, and Zheng Hua Ye. 2011. “Transcriptional Activation of Secondary Wall Biosynthesis by Rice and Maize NAC and MYB Transcription Factors.” *Plant and Cell Physiology* 52 (10): 1856–71. <https://doi.org/10.1093/pcp/pcr123>.
- Zhong, Ruiqin, Elizabeth A. Richardson, and Zheng Hua Ye. 2007. “The MYB46 Transcription Factor Is a Direct Target of SND1 and Regulates Secondary Wall Biosynthesis in Arabidopsis.” *Plant Cell* 19 (9): 2776–92. <https://doi.org/10.1105/tpc.107.053678>.
- Zhong, Ruiqin, and Zheng-Hua Ye. 2009. “Secondary Cell Walls.” *ELS*, no. March. <https://doi.org/10.1002/9780470015902.a0021256>.
- Zhong, Silin, Hongying Li, Zsuzsanna Bodi, James Button, Laurent Vespa, Michel Herzog, and Rupert G. Fray. 2008. “MTA Is an Arabidopsis Messenger RNA Adenosine Methylase and Interacts with a Homolog of a Sex-Specific Splicing Factor.” *Plant Cell* 20 (5): 1278–88. <https://doi.org/10.1105/tpc.108.058883>.

8. Acknowledgments

First and foremost, I would like to thank my Ph.D. supervisor, Prof. Dr. Kay Schneitz. His constant guidance, encouragement, and patience have been invaluable throughout the entire process. From the early stages of my scientific journey to the final submission of my thesis, his expertise and thought-provoking discussions have been essential in shaping my academic growth.

Furthermore, I want to thank Prof. Dr. Ramon Torres and Dr. Klaus Michel of the CALM unit for their technical assistance during the extensive microscopy sessions and for their extraordinary patience in maintaining and managing the imaging equipment.

I would also like to express my gratitude to Prof. Dr. Brigitte Poppenberger for being my mentor and second examiner, and to Prof. Dr. Frank Johannes for chairing my doctoral examination.

I want to thank Minsoo Choi, Balaji Enugutti, and Rodion Boikine for their great help with the RNAseq experiment.

A special thank you goes to my lab mates, both past and present. To the old crew with whom I shared the struggles of my early Ph.D. life: Rachele, Athul, Xia, Annemarie, Jin, and Ajeet. To the new crew who have helped me get through the final years: Rodion, Ratula, Nicole, Tejasvinee, Chao, and Jiwon. The countless hours we spent together in the lab and the hectoliters of coffee we drank together made us not only colleagues but also friends.

I want to thank the administrative secretary, Susanna, for keeping the department running smoothly and always offering a helping hand and assistance when needed.

My thanks also go to Katrin and Heidi for their technical support and the maintenance of the lab.

I would also like to acknowledge the students who participated in the project and our student assistants: Jonas, Theodor, Sreelekha, Alina, Jasmin, Anna, Julia, and Selina.

I want to thank the members of the neighboring Chairs of Plant Sciences: Chair of Botany, Chair of Plant Systems Biology, Chair of Plant Genetics, and Chair of Phytopathology.

I am truly thankful to my family for their endless support and for asking me for almost five years when I was going to finish my Ph.D. To Mama and Tata, Szymon, Karolina and Wojtek, Bartek and Olga, Kasia and Piotr. Dziękuję za wszystko.

In addition, sincere thanks are due to my dear friends, especially Paula, Julia, Hania, and Magda. Thank you for keeping my spirits up.

Lastly, I would like to express my deepest gratitude to Jakob. I couldn't have done it without you.