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REVIEW

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Understanding the root xylem plasticity for designing resilient crops

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Abstract

Xylem is the main route for transporting water, minerals and a myriad of signalling molecules within the plant. With its onset during early embryogenesis, the development of the xylem relies on hormone gradients, the activity of unique transcription factors, the distribution of mobile microRNAs, and receptor–ligand pathways. These regulatory mechanisms are often interconnected and together contribute to the plasticity of this water-conducting tissue. Environmental stresses, such as drought and salinity, have a great impact on xylem patterning. A better understanding of how the structural properties of the xylem are regulated in normal and stress conditions will be instrumental in developing crops of the future. In addition, vascular wilt pathogens that attack the xylem are becoming increasingly problematic. Further knowledge of xylem development in response to these pathogens will bring new solutions against these diseases. In this review, we summarize recent findings on the molecular mechanisms of xylem formation that largely come from Arabidopsis research with additional insights from tomato and monocot species. We emphasize the impact of abiotic factors and pathogens on xylem plasticity and the urgent need to uncover the underlying mechanisms. Finally, we discuss the multidisciplinary approach to model xylem capacities in crops.

KEYWORDS
abiotic stresses, eudicots and monocots, phenotypic and modelling, wilt pathogens, xylem development

1 | INTRODUCTION

Water is arguably the most important component of life. Vascular plants transport water and dissolved nutrients efficiently from the roots to all above-ground parts using a specialized tissue called the xylem. This tissue is composed of lignified conducting elements, fibres, and parenchyma cells.

The process of xylem development is fascinating and has attracted developmental biologists for more than a century. The early studies on xylem formation demonstrated wounding-induced xylogenesis as well as the formation of xylem from callus and transdifferentiation of the tissue culture cells into the xylem cells (Fukuda & Komamine, 1980; Jacobs, 1952; Simon, 1908; Vöchting, 1892; Wetmore & Rier, 1963). In this review, we summarize the recent advances in root xylem development research. We pay special attention to environmental inputs that contribute to the patterning of this tissue. Our goal is to highlight the importance of xylem research in light of global warming and the increasing risk of vascular wilt

diseases. Firstly, we introduce the developmental steps that xylem initials undergo to become functional conducting elements. We then discuss the molecular mechanisms and the key players in the formation of this tissue. Subsequently, we focus on the abiotic stresses that trigger xylem modification. We also discuss vascular wilt pathogens and the ways plants fight against them. Finally, we summarize and discuss a multidisciplinary approach to phenotype and model xylem capacity.

2 | MAKING XYLEM VESSELS: FROM XYLEM PRECURSORS TO FULLY FUNCTIONAL CAPILLARIES

In vascular plants, the xylem tissues are produced by procambium (for example, in monocot species and during formation of primary xylem found in the growing root tips, young leaves and flowers) or by vascular cambium in the eudicot and gymnosperm plants during secondary growth. The development of xylem tissue can be divided into early steps when totipotent cells commit to creating xylem, and to later steps when the xylem precursors transform to functional capillaries (Figure 2a). During differentiation, the water-conducting elements and fibres undergo cell death, while the surrounding procambium and later parenchyma cells still contribute to plasticity by noncell autonomously acting in the final steps in xylem maturation, more specifically, in postmortem lignification (Bollhöner et al., 2013; Pesquet et al., 2013).

In the Arabidopsis embryo, the first provascular cells originate at the 16-cell stage when the inner lower cells divide periclinally to give rise to the ground and vascular tissue initials (Scheres et al., 1994). Subsequently, the provascular initials will create initials for the pericycle and procambium through formative divisions. Remarkably, while the pericycle initials will only divide anticlinally from this point on, the procambium initials will continue periclinal divisions giving rise to the future xylem, phloem and procambium tissues. At the early globular stage of embryogenesis, the most apical suspensor cell becomes a hypophysis that will later divide asymmetrically giving rise to the quiescent centre (QC) and columella stem cells. In the fully mature embryo, the QC is surrounded by stem cells that give rise to all types of root tissues, such as initials for the vasculature, the ground tissues, the epidermis, and the lateral root cap and columella. These early developmental steps in embryogenesis require auxin accumulation towards the apical cell first, and later towards the hypophysis (Friml et al., 2003).

The recent transcriptional dissection of the embryogenesis in monocot species shows similarity to the eudicots in the early steps and in the late steps, however, the intermediate steps seem to be more divergent (Hao et al., 2021). It remains an open question to what extent the developmental programmes are conserved or divergent between the monocot and eudicot species and whether xylem development regulators uncovered in Arabidopsis have immediate orthologs that share the same function in monocots (Armenta-Medina et al., 2021). Interestingly, the first steps, including the formation of the zygote and first asymmetrical division, are quite similar, but, unlike the stereotypical cell divisions during later stages in Arabidopsis embryogenesis, in the monocot embryos, there is no clear stereotypical cell division pattern. However, at the later stages, the shoot apical meristem and root apical meristem is formed; unlike in Arabidopsis, the root meristem is located in the centre of the monocot embryo (Armenta-Medina et al., 2021; Hao et al., 2021). The ontogeny of xylem formation in monocot species needs to be carefully described. Using recent transcriptomics resources, it will be important to follow the expression of the candidate regulators of xylem development and to study their function.

Remarkably, at the cellular level, xylem elements in eudicots and monocots have similar structures, including the cell wall thickening and lignification pattern. In addition, some key transcription factors seem to be conserved (Zhang et al., 2018). However, vascular patterning shows major differences due to the loss of the cambial genes and, consequently, secondary growth in monocots (Figure 1) (Roodt et al., 2019).

Upon seed germination, the embryonic root meristem rapidly produces new tissues, the root tip emerges and rapidly elongates. Within hours the root tissues undergo differentiation, including the formation of functional root hairs and xylem capillaries to provide the uptake of water and minerals (Figure 1). In the growing primary root, as well as in the growing lateral roots, developing leaves and flowers only the primary xylem is found which will be functionally replaced by the secondary xylem later. In Arabidopsis, the switch from primary growth to secondary growth happens gradually (Ye et al., 2021). A recent study showed that this transition is induced by cytokinin (CK) that specifically activates LATERAL ORGAN BOUNDARIES DOMAIN transcription factors (Ye et al., 2021).

In the Arabidopsis primary root, the xylem pole is located in the centre of the vascular cylinder and consists of two outermost protoxylem cell files and three inner metaxylem cell files (Figures 1 and 2). Interestingly, from the very beginning, protoxylem and metaxylem initials have distinct gene expression profiles even though they undergo similar cellular changes and have a very similar function in transporting water (Kubo et al., 2005).

The organization of primary xylem tissue in the tomato primary root is quite similar to Arabidopsis, with two protoxylem cell files on each side of the xylem pole and six-cell files in the centre for the metaxylem (Figure 1).

The organization of the vascular tissues in the monocot primary root shows radial patterning that varies between species. For example, six to seven vascular bundles are distributed in radial symmetry around the large central metaxylem cell file in the wheat primary root (Figure 1).

Remarkably, xylem differentiation in both eudicots and monocots happens gradually. In eudicots, firstly, the outermost protoxylem cell files show thickening of the cell wall and lignification. Subsequently, the outermost metaxylem undergoes the same process, and, finally, also the inner metaxylem is lignified and becomes functional (Figure 1). In monocots, firstly, the protoxylem files within each of the vascular bundles are lignified and become functional; later, the newly

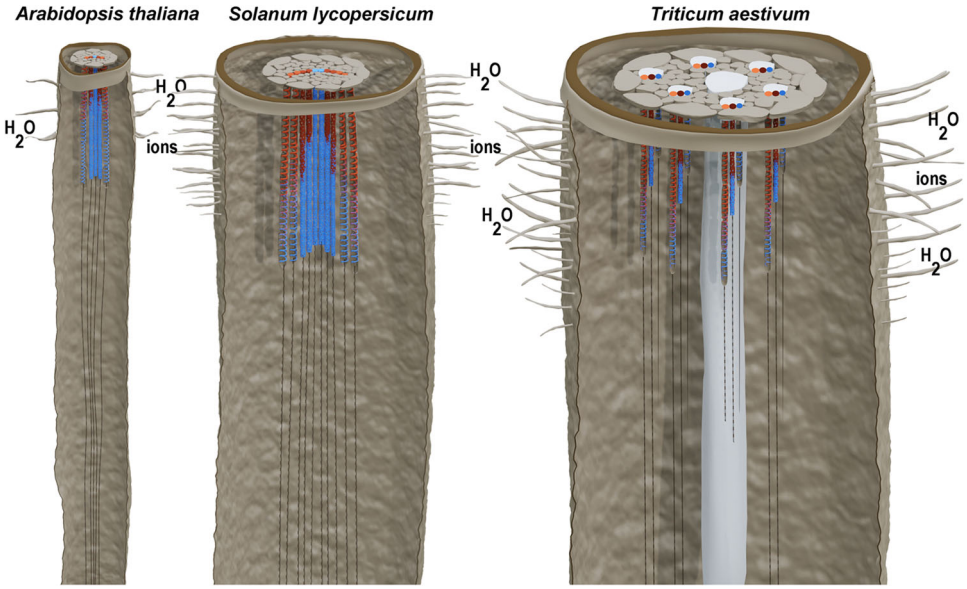

FIGURE 1 Xylem differentiation in Arabidopsis, tomato and wheat roots. The gradual lignification (in red) is observed first in protoxylem, and later, metaxylem cells

formed additional metaxylem and protoxylem undergo similar processes and then the central metaxylem will undergo terminal maturation. It is important to note that the anatomy and maturation of the xylem system of monocot species varies a great deal, depending on the environmental conditions. As an example, a study comparing the anatomy of the rice root (well-adapted to flooding conditions) and the wheat root (well-adapted to the water-deficit conditions) showed better xylem plasticity in wheat under drought conditions. The number of central metaxylem files and their diameters were upregulated in the wheat roots (measured near the shoot junction) under water deficit conditions. Another powerful resource for the evidence of xylem plasticity in monocots comes from the book and the review by botanist Sherwin Carlquist (Carlquist, 2012, 2020). Analysing the anatomy of the plant species adapted to the different environments, the author draws clear evidence of xylem anatomy specialized for the given water availability conditions given.

3 | THE MOLECULAR GENETIC FRAMEWORK OF XYLEM FORMATION

During xylem formation, firstly, totipotent cells commit to become precursors of vascular tissue and subsequently, xylem tissue (which can be observed in embryo development, transdifferentiation of xylem parenchyma, or mesophyll or callus cells); secondly, the xylem precursor cells differentiate into a hollow conducting element by clearing the cytoplasm, degrading the nucleus and organelles; thirdly, so-called postmortem differentiation, the adjacent cells will contribute to the last steps of the formation of the conducting element. Here, we briefly discuss the key players in each step.

3.1 | Prepatterning

Early studies on xylem formation showed that auxin and CK play a central role in the differentiation of this tissue (Jacobs, 1952). Molecular research on vascular development has been significantly advanced with the establishment of Arabidopsis as a plant model. Large forward genetic screens in Arabidopsis could identify additional components, such as transcription factors and microRNAs (miRNAs) that also control xylem development (Benfey et al., 1993; Cano-Delgado et al., 2000; Mayer et al., 1991; Turner & Somerville, 1997; Yokoyama & Nishitani, 2006). Molecular tools and the analysis of mutant lines demonstrated that auxin is the first signal required for xylem initiation and differentiation (Friml et al., 2003; Herud-Sikimić et al., 2021; Ulmasov et al., 1997; Weijers et al., 2006). The molecular mechanism of auxin-induced xylem formation has been studied in detail in Arabidopsis, showing that the auxin-dependent transcription factor MONOPTEROS (MP) expressed in the embryo targets TARGET OF MONOPTEROS 5 and 7 (TMO5 and TMO7) (Schlereth et al., 2010), two basic helix-loop-helix (bHLH) transcription factors. TMO5 creates a complex with LONESOME HIGHWAY, another bHLH transcription factor (Katayama et al., 2015; Ohashi-Ito et al., 2019), to upregulate the expression of CK biosynthetic enzyme genes LONELY GUY 3 and 4 (LOG3 and LOG4) and a CK signalling inhibitor ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6) in protoxylem cells (Mähönen et al., 2006). This increase in CK signalling promotes periclinal cell divisions to establish the provascular tissue and procambium. In the absence of CK signalling, for example in the *wol* mutant (that lacks the CK receptor WOODEN LEG, a two-component histidine kinase also known as ARABIDOPSIS HISTIDINE KINASE 4), the number of vascular cell files is significantly reduced. This results from fewer periclinal cell

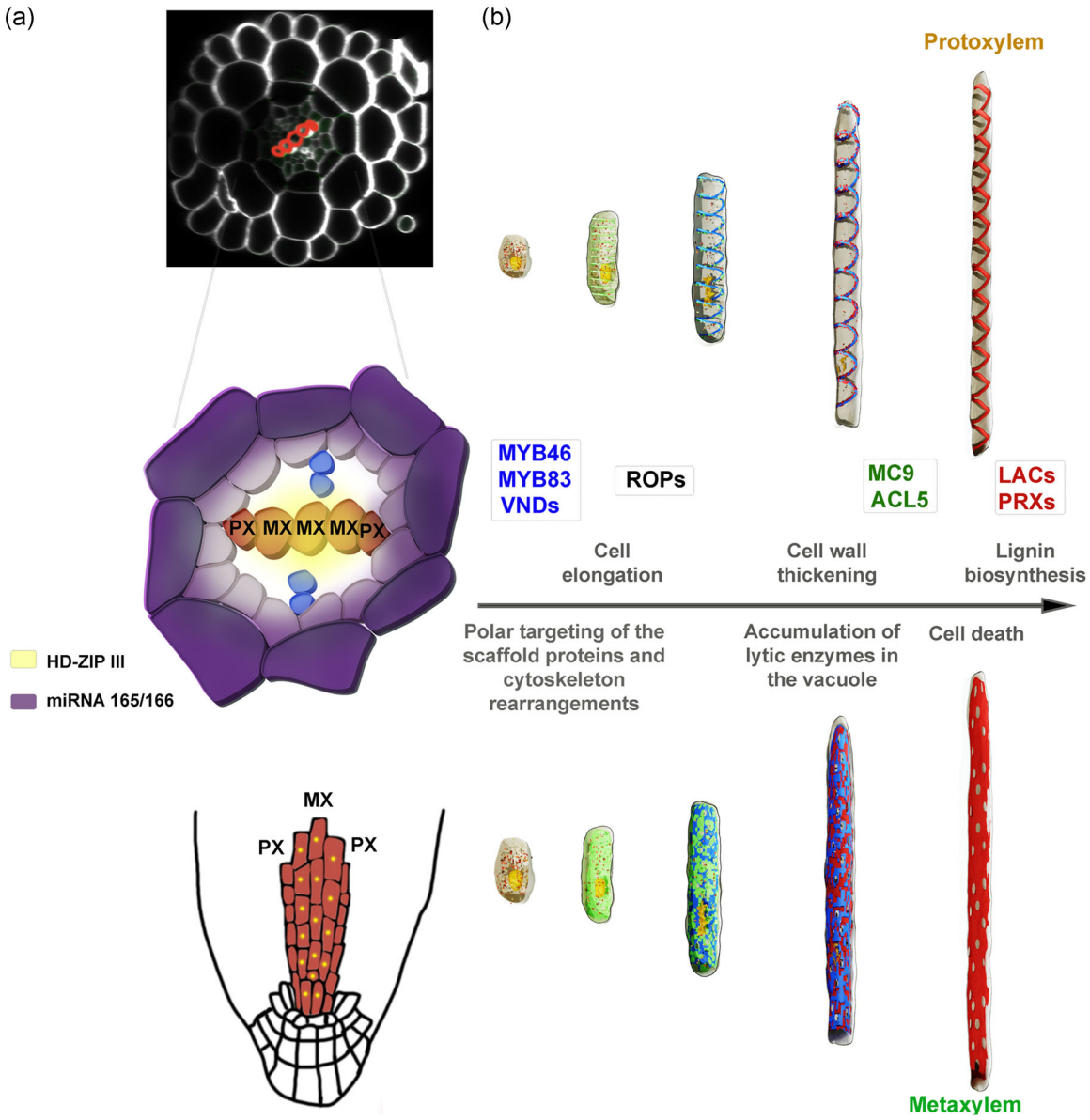

FIGURE 2 Xylem pattern formation in Arabidopsis. (a) Radial patterning in the xylem is mediated by the gradients of HD-ZIP III transcription factors and mobile microRNAs that originate in the endodermis and diffuse into the vascular cylinder. (b) Cellular changes during proto- and metaxylem differentiation and key regulators, mediating xylem differentiation [Color figure can be viewed at wileyonlinelibrary.com]

divisions of the procambium cells; at the same time, the vascular cells differentiate into protoxylem (Mayer et al., 1991).

3.2 | Radial patterning

In addition to the MP-mediated pathway, auxin promotes the expression of CLASS III *HOMEODOMAIN LEUCINE ZIPPER* (*HD-ZIP III*) transcription factors that specify protoxylem versus metaxylem fate in a dose-dependent manner (Carlsbecker et al., 2010; Ohashi-Ito et al., 2013). There are five HD-ZIP III genes in Arabidopsis: REVOLUTA (REV)/AMPHIVASAL VASCULAR BUNDLE1 (AVB1), PHABULOSA (PHB), PHAVOLUTA (PHV), CORONA (CNA)/INCURVATA4 (ICU4), and ATHB8 and these genes have a conserved function in eudicots and monocots in the shoot apical meristem maintenance and leaf formation (McConnell et al., 2001). Loss-of-function of all five HD-ZIP III in Arabidopsis abolishes completely the xylem differentiation. On the one hand, these transcription factors are induced by auxin and on the other hand, they are downregulated by short regulatory RNAs, miRNA165/166 diffusing into the vascular cylinder from the endodermis (Carlsbecker et al., 2010) (Figure 2). The expression of these regulatory RNAs is induced by complexes of SHORTROOT-SCARECROW transcription factors acting in the endodermis. Because the signal diffuses from the endodermis towards the vasculature, and, thus, creates a gradient, this dictates the radial patterning of the xylem tissue. Remarkably, these studies provided a

starting point for understanding xylem formation in trees (Robischon et al., 2010). The HD-ZIP III transcription factors in Populus affect the rate of the differentiation of the secondary xylem and phloem (Du et al., 2011). Interestingly, the miRNA166 in rice has a similar function of targeting an HD-ZIP III transcription factor OsHB4. The loss of function of miRNA166 resulted in the reduced diameter of the xylem, leaf rolling and overall better drought tolerance (Zhang et al., 2018). The HD-ZIP III transcription factors in both maize and Arabidopsis are targeted by miRNA165/166 to control the adaxial-abaxial leaf pattern formation (McConnell et al., 2001). In addition, it has been shown, that the HD-ZIP III transcription factors strongly repress periclinal cell divisions in the root vasculature. In the *phb phv cna athb8* quadruple mutant of HD-ZIP III genes in Arabidopsis the number of vascular cells is twice as high as in the wild-type (Miyashima et al., 2019).

Not only do classical plant hormones and transcription factors play a key role in establishing the xylem, but also cell-surface localized receptors and peptide ligands. Remarkably, plants possess a large number of LEUCINE-RICH REPEAT RECEPTOR-LIKE KINASES encoding genes in comparison with other living organisms (Torii, 2004). These proteins have an extracellular domain that can bind small ligands secreted to the apoplast, a transmembrane domain and a cytoplasmic (kinase) domain. It has been proposed recently that the receptor-like kinases BARELY ANY MERISTEM (BAM) 1 and 2 control the movement of miRNA165/166 towards the vasculature and, therefore, regulate xylem patterning (Fan et al., 2021). BAM1 and BAM2 bind small signalling peptides from the CLAVATA3/EMBRYO SURROUNDING REGION RELATED (CLE) family of peptides. However, it is still not clear if the role of BAMs in the movement of small RNAs depends on the peptide activation mechanism. Additional evidence supporting the role of BAMs in xylem formation comes from the recently published single-cell RNAseq dataset from the Arabidopsis embryo where BAM1 expression was shown to be enriched in vascular initials (Kao et al., 2021). It remains to be uncovered how BAM receptors contribute to vascular and xylem development and which CLE peptide ligands play a role in these pathways.

Another group of receptor-like kinases shown to play a role in embryo provascular tissue are RECEPTOR-LIKE PROTEIN KINASE 1 and 2 (Nodine et al., 2007). These kinases are expressed in the early globular embryo and contribute to periclinal cell divisions forming a radial pattern. These receptors are also implicated in CLE peptide perception and in addition to their role in embryo development, are essential in root apical meristem maintenance (Racolta et al., 2018). In conclusion, in addition to the key hormones establishing vascular tissues, downstream transcription factors, regulatory RNAs, and receptor-like kinases dependent pathways contribute to vascular patterning.

3.3 | Executors

Fully functional xylem conduits show an extreme example of cell differentiation. Final differentiation includes elongation, cell wall thickening, accumulation of lytic enzymes in the vacuole, lignification, and vacuole disruption that will result in cell death (Figure 2). What are the factors that switch on the terminal transition of xylem differentiation? Secondary cell wall biosynthesis is positively regulated by NAC and MYB transcription factors in both eudicots and monocots. To initiate the differentiation process, the master transcription regulators VASCULAR-RELATED NAC-DOMAIN (VND) 6 and 7 act in metaxylem and protoxylem, respectively, to activate genes related to secondary cell wall formation and cell death (Kubo et al., 2005; Taylor-Teeples et al., 2015; Turco et al., 2019) (Figure 2). A recent study by Turco et al. (2019) aimed to identify the genes and the networks involved in the developmental switch of the xylem cells using the single-cell sequencing approach. This study showed nicely that VND7 initiates this switch and the downstream targets of this pathway have been identified. MYB46 and MYB83 are the additional transcription factors driving the differentiation of the xylem, acting in parallel to VND7. MYB83 expression is sufficient to induce lignin biosynthesis in the secondary cell wall of xylem cells (McCarthy et al., 2009). Additional MYB transcription factors contribute to xylem differentiation, some of them redundantly and some of them in a tissue-specific manner (Figure 2). In addition to VND6 and VND7, the additional NAC transcription factors act in inducing the secondary cell wall regulatory network. The SECONDARY WALL ASSOCIATED NAC domain protein 1 (SND1), for example, was shown to act specifically in the differentiation of the fibre cells in the Arabidopsis inflorescence stems (Zhong et al., 2006). It was demonstrated recently that SND transcription factors regulate secondary cell wall biosynthesis both in Arabidopsis and Populus (Zhong et al., 2021).

The final steps of xylem differentiation are regulated by proteolytic enzymes, nucleases, lignin biosynthesis enzymes and the machinery bringing monolignols to the site of lignin formation. The tracheary elements of the xylem undergo programmed cell death that occurs after the deposition of the secondary cell walls when tonoplast rupture is induced. It has been demonstrated, that in Arabidopsis, the enzyme METACASPASE9 acts in the degradation of the cell content after the bursting of the central vacuole leading to cell death (Bollhöner et al., 2013). At the same time, ACAULIS5 prevents premature death during xylem differentiation (Muñiz et al., 2008). The enzyme that is responsible for the nuclear degradation in the *Zinnia elegans* xylem cell culture is ZINNIA ENDONUCLEASE 1 (ZEN1) (Ito & Fukuda, 2002). The homologue of this enzyme in Arabidopsis is BIFUNCTIONAL NUCLEASE 1 (BFN1), but its role in xylem differentiation has not been demonstrated and the expression of this gene seems to be specific to the flowers, and not to the roots.

Several proteases were identified among the targets of VND6, including the XYLEM CYSTEINE/SERINE PEPTIDASES (XCP) 1 and 2 (Avci et al., 2008) in Arabidopsis. Interestingly, these peptidases act in the micro-autolysis of the xylem element, just before the tonoplast implosion happens (Avci et al., 2008).

Among the lignin biosynthesis genes—secreted enzymes LACCASES (LACs) and PEROXIDASES (PRXs) facilitate lignin polymerization through oxidizing the monomers—monolignols (Wang et al., 2013). The oxidative activity of PRXs, but not LACs, requires

the peroxide as a cosubstrate. This can be produced by superoxide dismutase proteins and NADPH oxidases (Wang et al., 2013). In Arabidopsis, the LAC4 and PRX64 localize to the lignified domains in the cell wall in fibres, whereas LAC4, LAC17, and PRX72 were localized to the lignified cell walls in the xylem vessel elements (Hoffmann et al., 2020; Yi Chou et al., 2018). We still do not fully understand how these enzymes are regulated at the transcriptional and cell biology level; the new insights into the biology of these lignin-maker machines will be instrumental for the understanding of xylem maturation. Furthermore, looking at the cellular level, mature protoxylem and metaxylem are hollow lignified capillaries with a helical- or pitted-patterned cell wall. Both cell types originate from the procambium cells through elongation, thickening of the cell wall, accumulation of lytic enzymes in the vacuole, secretion of lignin monomers to the apoplast, lignification, and the programmed cell death that will occur through a burst of the vacuole (Pesquet et al., 2013; Schuetz et al., 2014) (Figure 2). The nature of the mechanisms underlying the helical patterning of the protoxylem cell wall thickening compared to the solid tube with pits of the metaxylem are not yet fully clear. Nevertheless, lignin biosynthesis proteins LACCASE 4 and 17 localize to the secondary cell walls of developing proto- and metaxylem cells (Schuetz et al., 2014). What brings them exactly to the sites where the lignin biosynthesis should take place? We are still missing many details in the machinery of xylem differentiation. Similar to the lignification of the Casparian strips, there might be CASP-like proteins that recruit the components of this machinery to establish domains of future secondary cell wall formation (Roppolo & Geldner, 2012; Roppolo et al., 2011). Interestingly, it has been shown, that in the metaxylem patterning Rho GTPases play a key role in the metaxylem patterning by establishing specific membrane domains where microtubule assembly is inhibited (Oda & Fukuda, 2013). Moreover, the size of the pits of the metaxylem is regulated in a quantitative manner by microtubule-binding proteins CORTICAL MICROTUBULE DISORDERING 1 and 2 (Sasaki et al., 2017). Another aspect of the xylem that is unknown is how the diameter and the length of the capillaries are controlled. Answering these questions could help to modify xylem networks in crops to adapt better to environmental conditions.

Remarkably, the switch to the last step in xylem differentiation is strictly controlled by developmental and environmental cues. However, the nature of the signals and the molecular mechanism of inducing final maturation has not yet been fully described. On the one hand, postponing cell death can enhance plasticity; on the other hand, this will delay the functionality of the tissue. Remarkably, the lignification of the tracheary elements in the xylem starts before the rupture of the vacuole but continues after the cell clearing. It has been demonstrated in the study in *Z. elegans* that after the cell death, the secondary cell wall lignification still takes place thanks to the adjacent cells that supply monolignols and peroxide (Pesquet et al., 2013). That is, cell-to-cell communication between the differentiating xylem and the parenchyma cells is essential for this noncell autonomous lignification. It will be highly important to uncover the mechanisms of this cell-to-cell communication and to study the mechanism of xylem lignification in grass species because cell wall thickness defines the mechanical strength of the stem and can improve the agronomic potential of crop species.

4 | EFFECT OF ABIOTIC STRESSES ON XYLEM PATTERNING

Plants sense the environment and adjust their physiological response to survive. Water availability, light, temperature, soil salinity, the abundance of nutrients and soil pH are among the abiotic conditions that impact plant growth. Remarkably, the molecular mechanisms of sensing the environment and adapting to it have developed for more than 450 million years, ever since the first plants colonized the land. A key hormone mediating abiotic stress responses is abscisic acid (ABA), which had already been produced in algae species, but only became a key stress signal in early land plants that developed an ABA-dependent modulation of PYRABACTIN RESISTANCE 1 (PYR1)-like (PYL) proteins (Sun et al., 2019). We discuss here the effects of abiotic stresses on the xylem patterning described in the literature. Our better understanding of how model plants react and modify their xylem to drought, salt, or temperatures in the controlled laboratory settings will help to better understand and point research into a direction on how to exploit these traits and develop more resilient crops in the future.

4.1 | Water deficit

Any disruption in xylem flow (embolism) that is caused by water deficit, cold stress or by vascular pathogen infection, creates huge physiological stress in vascular plants. On a physiological level, water deficit leads to an increase in tension in xylem capillaries, which causes cavitation and eventually embolism. Gas bubbles that fill xylem capillaries cause a water disconnect from the root and the aboveground tissues (Tyree & Sperry, 1989). To restore the conductivity, the embolized cell needs to be refilled with water by its neighbouring cells (Brodersen et al., 2010). While the processes leading to embolism and its recovery are relatively well understood, less is known about the molecular processes in the xylem that a plant undertakes to minimize the damage caused by it. Nevertheless, these processes have been extensively studied in the last few decades (reviewed in Martignago et al., 2019; Ramachandran et al., 2020; Yu et al., 2015). A mechanism for ABA-mediated response to water deficit that involves a developmental switch in xylem cell identity has recently been identified (Bloch et al., 2019; Ramachandran et al., 2018). Interestingly, when Arabidopsis is grown under drought conditions or following an ABA treatment, this changes the identity of its outer metaxylem cells. These cells develop into protoxylem cells with their typical helical patterned secondary cell walls. These morphological changes in the xylem are already visible after 4-h treatment. Moreover, they are also reversible, showcasing the plasticity of the xylem tissue (Ramachandran et al., 2021). Besides morphological changes,

also the differentiation as measured by the lignification onset of the inner metaxylem is accelerated simultaneously by drought and direct application of ABA (Ramachandran et al., 2021). Conforming with these results, disturbing ABA biosynthesis or its perception either through the application of the biosynthesis inhibitor fluridone, in biosynthesis mutants *aba2-1* and *aba3-1*, or the quadruple ABA receptor mutant *pyr1pyl1pyl2pyl4* show disturbed xylem or were unable to replicate the drought phenotype (Bloch et al., 2019; Ramachandran et al., 2018). These responses to ABA could be explained by the fact that protoxylem cells may be less vulnerable to embolism compared to metaxylem (Hwang et al., 2016), and an increase in xylem area through earlier differentiation could aid in the overall water conductance of the plant (Meinzer et al., 2010).

On a molecular level, ABA signalling in the endodermis upregulates the expression of miRNA165/166 and simultaneously reduces their repressor ZLL/AGO10 (Bloch et al., 2019). The elevated levels of miRNAs suppress the HD-ZIP III transcription factors that control the metaxylem cell fate and, therefore, the outer metaxylem cells change their identity to protoxylem (Carlsbecker et al., 2010; Ramachandran et al., 2018). The mutant *phb1-d*, which is resistant to miRNA165/166, and mutants *scr and shr* which have strongly reduced overall levels of miRNA165/166 do not show a xylem drought phenotype in response to ABA (Bloch et al., 2019). In addition, a selection of master regulators of xylem differentiation, VND1–3, and 7, are necessary to properly regulate the changes in the xylem structure or differentiation (Ramachandran et al., 2021). VND1–3 are key regulators in the differentiation of the inner metaxylem under stress conditions, as *vnd1–3* triple mutants fail to show early differentiation of the inner metaxylem mediated by ABA application. On the other hand, *vnd7* mutants show a reduction of the morphological changes seen in the outer metaxylem in response to ABA (Ramachandran et al., 2021). These findings altogether reinforce a mechanism involving a direct relationship between water-conducting tissue formation and water-deficit signalling.

Interestingly, this xylem response seems to be quite conserved across eudicots on both a molecular and a morphological level, as a shift from metaxylem to protoxylem can be observed in members of the *Brassicaceae*, *Phtheirospermum japonicum* (Orobanchaceae), and Solanaceae family. A similar increase in the expression of VND homologues can be seen in tomatoes compared to Arabidopsis as a result of drought stress (Bloch et al., 2019; Ramachandran et al., 2021). Furthermore, evidence for the relation of VNDs and miRNA165/166 and its targets to water deficiency in monocots has given proof that this regulatory pathway could be quite well conserved among plant species and was established early in evolution. Mutant lines of a VND homologue in maize, *nut1*, show drought stress symptoms when grown under normal conditions, which is caused by an underdeveloped protoxylem in mature plants (Dong et al., 2020). In addition, a knockdown of miRNA166, as well as overexpression of members of the HD-ZIP III family, in rice caused leaf rolling, a typical drought stress response, when grown normally. Besides this, also a reduced transpiration rate, which was caused by a reduced diameter of the xylem cells was seen here (Zhang et al., 2018).

Besides ABA, jasmonic acid (JA) and CK have been shown to play a role in xylem regulation in response to drought. CK is a known inhibitor of protoxylem differentiation that acts by regulating pseudo-phosphotransfer protein AHP6 (Mähönen et al., 2006). AHP6 is a key player in the specification of the protoxylem. Loss-of-function mutation *ahp6* leads to impaired protoxylem formation. Instead of gradual complete differentiation, the protoxylem differentiates occasionally in this mutant. Since under drought CK levels go down, the expression of AHP6 is affected and, as a consequence, protoxylem differentiation is disturbed (Mähönen et al., 2006).

It has been demonstrated that drought lowers the levels of CK while simultaneously increasing levels of JA (Jang et al., 2017; Jang & Choi, 2018; Nishiyama et al., 2011). Interestingly, lower levels of CK have also been linked to an increased sensitivity to ABA, suggesting possible crosstalk between these pathways. However, the specific roles of ABA and the JA/CK balance seem to be different. While ABA mostly regulates the differentiation and cell fate of the different xylem through VNDs and miRNA165/166, the JA/CK balance regulates the number of xylem vessels within the axis (Jang et al., 2017; Jang & Choi, 2018; Jang et al., 2018). It has been shown that drought stress leads to an increased number of xylem vessels that originate from previously undifferentiated procambium cells. Application of JA shows a similar increase in the number of xylem cells, while application of CK shows a reduction.

4.2 | Salt stress

Another common abiotic stress that is experienced by plants is osmotic stress due to a saline environment. This stress affects both plant metabolism and plant productivity negatively by suppressing photosynthesis. Plants can generally be divided into salt-sensitive (glycophytes) and salt-tolerant (halophytes) types. The majority of crop species are glycophytes and, therefore, the mechanisms of the salt stress adaptation is a very important topic. Comparable to water deficiency, ABA biosynthesis and signalling is activated by salt stress. However, the differentiation switch of xylem observed in drought conditions mediated by ABA is not replicated during a NaCl treatment (Bloch et al., 2019), showing that other unknown elements must play a part in the salt response. At the same time, it has been shown that high salinity stress induces an increased lignification of the xylem tissues in tomatoes and other plant species (Fernandez-Garcia et al., 2011; Kong et al., 2021; Neves et al., 2010; Oliveira et al., 2020; Sánchez-Aguayo et al., 2004). In addition, salt stress-induced acceleration in the differentiation of secondary xylem in soybean (Hilal et al., 1998). However, not much is known about the molecular mechanism of salt-induced overlignification. A link between the diameter of the xylem vessels and salt tolerance can be made. The spermine mutant *acl5*, which showed increased salt sensitivity, exhibited an increased xylem diameter and earlier cell death of the xylem leading to a shorter length of the xylem cells (Muñiz et al., 2008; Shinohara et al., 2019). A similar dependency can be observed in monocot species. The anatomical traits were examined in

the recent study of wheat watered with seawater. Following this salt stress, the number of vascular bundles was significantly reduced (Nassar et al., 2020). In conclusion, it seems that the reduced xylem network gives an advantage during salt stress.

4.3 | Temperature stress

Heat stress is defined as an elevated temperature over a period of time that is long enough to induce irreversible damage in the plant. Proteins often aggregate or change their conformation and become inactive; in addition, the fluidity of the lipids in the plasma membrane is affected. High temperatures are often associated with drought, as the plant increases its transpiration rate to cope with the increased temperatures thus depleting water sources faster. However, given an ample supply of water, the xylem seems to be mostly unaffected by heat as it will continue to transport water even if all living cells around it have been killed (Van de Wal et al., 2017), and the components making up the xylem are rather resistant to high temperatures (Yang et al., 2007). On the other side of the spectrum, freezing temperatures can, just like drought, cause the embolism of the xylem through freeze–thaw cycles because of the formation of ice in the xylem (Pittermann & Sperry, 2005). Interestingly, there is a high correlation between the diameter of individual xylem capillaries and the susceptibility to embolism caused by a freeze–thaw cycle (Davis et al., 1999). To this extent, perennial woody plants reduce the diameter of their xylem in the stem when they start experiencing freezing temperatures (Améglio et al., 2001; Lintunen et al., 2016; Zweifel & Häsler, 2000). Although these observations have only been observed in perennial woody plants which actually undergo seasonal changes, a link between xylem diameter and freezing resistance can still be drawn in Arabidopsis suggesting a conservation of this trait. In Arabidopsis *esk1* mutant lines, freezing tolerance was increased as measured by survival rate after a freezing shock (Xin & Browse, 1998). *esk1* mutants have a reduced xylem diameter which is likely caused by an impaired acetylation of xylan (Lefebvre et al., 2011), the main polysaccharide interacting with lignin polymers in the cell wall (Kang et al., 2019). Moreover, other xylan-related mutants, such as *irx9* and *parvus*, also show increased freezing tolerance (Ramírez & Pauly, 2019).

Taken together, abiotic stresses impact xylem patterning through modulating hormonal signalling and activity of the transcription factors which in turn affect the structure of the conducting elements—from patterning the secondary cell wall to vessel diameter. Moreover, these changes seem to be quite conserved among plant species as similar phenotypes in response to stresses are observed in a number of eudicots and monocots. Unfortunately, most studies on abiotic stresses in plants do not include the close examination of the xylem tissues, but rather different physiological parameters and plant hormone levels. The study of the molecular mechanisms of stress-regulated xylem patterning is an exceptionally important topic especially in light of global climate change and we hope that the near future will bring more discoveries in this field.

5 | WILT PATHOGENS AND THEIR IMPACT ON XYLEM

On top of the abiotic factors that affect plant growth, multiple pathogens and pests interfere with plant development and often cause plant death. The vascular system is an attractive target for many wilt pathogens that attack xylem tissues and are among the most harmful for plant health (Yadeta & Thomma, 2013). This group of pathogens is represented by microscopic fungi such as *Fusarium oxysporum* and *Verticillium longisporum* or bacteria, such as *Xylella fastidiosa*. The invasion of these pathogens often happens through the root system, where they enter the epidermis, continue through the cortex and endodermis, and finally reach the xylem where they proliferate and spread to the above-ground organs (Bae et al., 2015). Alternatively, also direct inoculation into the xylem through insect attacks is used by these pathogens to invade the host (Wang et al., 2017).

Remarkably, the plant's strategies to resist wilt pathogens are often associated with the capacity of the xylem to recognize the pathogen and to block the infection by producing polymers, such as lignin and suberin, or secreting gels and the formation of tyloses (reviewed in Kashyap et al., 2021) (Figure 3). In the case of *X. fastidiosa*, Gram-negative bacteria that specialize in the colonization of xylem vessels, the primary infection happens through insect vectors feeding on the plant. The plant responds by secreting gels and creating tyloses, limiting the spread of the bacteria (Petit et al., 2021). In a recent study on olive trees, the authors compared the xylem anatomy in two cultivars with different susceptibility to *Xylella* f. Interestingly, they found that the highly susceptible cultivar had larger xylem vessels and higher air-embolism vulnerability that possibly facilitate the infection (Petit et al., 2021).

While pathogen recognition by plant receptors is a highly advanced field today (recently nicely summarized in Ngou et al., 2021), our understanding of xylem responses to compartmentalize pathogens mainly relies on anatomical studies of infected tissues and some recent proteomics and transcriptomics studies (Hu et al., 2019; Xiong et al., 2021). Another interesting study on *V. longisporum* infecting Arabidopsis plants showed that this soil-borne fungal pathogen induces transdifferentiation of bundle sheath cells into functional xylem elements (Reusche et al., 2012). At the molecular level, de novo xylem formation correlates with the pathogen-induced expression of VND7. Moreover, the authors showed that the infected Arabidopsis plants with ectopic xylem hyperplasia were more tolerant to water deficit conditions (Reusche et al., 2012). This study is an excellent example of how pathogens can reprogramme the host plant tissue. The authors hypothesize, that this response is due to compensation for compromised water transport caused by infection.

Indeed, after successfully blocking the infected region of the xylem, plants need to reconnect the xylem network by creating new conducting elements from the adjacent parenchyma cells. As such, the induction of physical barriers and rewiring requires a highly coordinated and timely response, as often a susceptible plant will still produce polymers or gels and tyloses to stop the propagation of the pathogen, but does not do this in a local or rapid manner and thus the

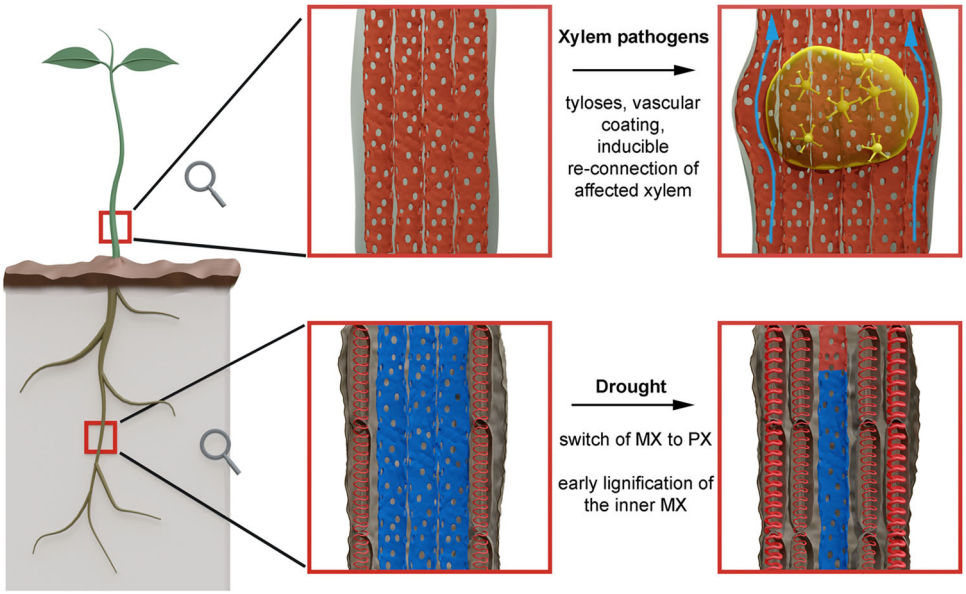

FIGURE 3 Pathogens and environmental stresses influence xylem patterning. The infection of the xylem with wilt pathogens can lead to tyloses (cell outgrowth of the xylem parenchyma that blocks the infected conduits), production of polymers for vascular coating and induction of new xylem vessels in adjacent tissue to reconnect the vascular system. Under drought conditions, abscisic acid (ABA) induces microRNAs production that represses HD-ZIP III transcription factors leading to the development of more protoxylem cell files replacing metaxylem. In addition, ABA signalling induces early lignification of the inner metaxylem [Color figure can be viewed at wileyonlinelibrary.com]

infection eventually takes over (Planas-Marquès et al., 2020). In rice, the vascular pathogen *Xanthomonas oryzae* pv. oryzae attacks xylem vessels. In resistant cultivars, the infected xylem secondary walls thicken within 48 h and the pit diameter decreases to reduce pathogen propagation (Hilaire et al., 2001). This response is associated with the accumulation of Peroxidase PO-C1 in xylem parenchyma cells.

Studying the molecular mechanisms and the genetic basis of blocking infection and reconnecting of xylem upon infection is essential and needs the combined effort of developmental biologists and plant pathologists. Developing strategies for crop plants that recognize and respond rapidly and locally to stop the pathogen will bring promising solutions for the future.

6 | PERSPECTIVES IN XYLEM PHENOTYPING AND WATER USE MODELLING

Plants consume large amounts of water for their metabolism and lose even more during transpiration. Therefore, plant water management, including water uptake, hydraulics within the xylem system, and transpiration through stomata impacts the global water cycle, agriculture, and our ecosystems (Schlesinger & Jasechko, 2014). The water moves within the xylem system by bulk flow from the root to the shoot driven by differences in the water potential. To fully understand the process of water transport and the responses of the plants to drought, there are many parameters to take into account: the soil-root hydraulics (Carminati & Javaux, 2020), the xylem conductance, the rhizosphere area, the transpiration flow through the stomata and additional measures that impact the water transport efficiency of the plant. Two large areas of research deal with how the water-conducting tissues are formed and can be modified (vascular development) and how water moves inside the xylem network (plant hydraulics). While developmental biologists look into gene regulatory networks, anatomy, and cell biology, plant hydraulics focuses on the biophysics of water flow, xylem embolism, hydraulic conductance, and vulnerability (Venturas et al., 2017). In our opinion, in the future, more collaboration between these two fields will bring valuable advances in the understanding of water transport. Such collaborations bringing together the genetics and biophysics of plant water transport will be essential for finding new solutions in developing better crops. Using the methodologies of both areas of research, it will be possible to come up with a "Root Xylem Index" that could be used to assess the capability of the root xylem network of a specific plant species and its varieties and even predict which genotypes will lead to the desired traits. The components contributing to this index can be anatomical traits, for example, xylem vessel diameters in the root and stem; pit size in the metaxylem, and the total xylem area in the cross-section and xylem functionality traits such as the conductivity of the root xylem at a specific age, embolism vulnerability; transpiration rates, and susceptibility to pathogens.

In a recent study on maize roots, five cultivars of maize were grown in pots and later used for anatomical analysis and modelling. Consequently, a high-resolution root system hydraulic atlas for maize plants was created (Heymans et al., 2021). This is a nice example of combining modelling water transport with anatomical traits and mathematics. Microscopic differences found in the structure of the

xylem can be used subsequently to estimate the global water use properties of the plant. Such efforts to quantify and model water transport in plants based on anatomical traits and tested functional parameters will provide knowledge that will be essential in designing crops with an optimal xylem system in the future. This "Root Xylem Index" can be used later by farmers to select the best cultivars for a given field and condition.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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