Understanding the Root Xylem Plasticity for Designing Resilient Crops

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Abstract

Xylem is a main road in plant long-distance communication. Through xylem plants transport water, minerals and myriad of signaling molecules. With the onset during early embryogenesis, the development of xylem tissues relays on hormone gradients, activity of unique transcription factors, distribution of mobile miRNAs and receptor-ligand pathways. These regulatory mechanisms are often interconnected and all together contribute to the plasticity of water conducting tissue. Remarkably, root xylem carries water to all above-ground organs and therefore influences all aspects of plant growth. Because of the global warming and increasing water deficit, we need to come up with solutions for the crops of the future. It is clear that structure of water conducting elements directly impacts water transport within the plant. Among plant pathogens- vascular wilts attacking xylem -are the most harmful. Our knowledge about xylem anatomy and rewiring ability could bring the solutions against these diseases. In this review we summarize the recent findings on the molecular mechanisms of xylem formation with a special attention to the cellular changes, and cell wall rearrangements that are necessary to create functional capillaries. We emphasize the impact of abiotic factors and pathogens on xylem plasticity and discuss multidisciplinary approach to model xylem in crops.

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Root Phenotypes for the FutureUnderstanding the Root Xylem Plasticity for Designing Resilient Crops

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Abstract Xylem is a main road in plant long-distance communication. Through xylem plants transport water, minerals and myriad of signaling molecules. With the onset during early embryogenesis, the development of xylem tissues relays on hormone gradients, activity of unique transcription factors, distribution of mobile miRNAs and receptor-ligand pathways. These regulatory mechanisms are often interconnected and all together contribute to the plasticity of water conducting tissue. Remarkably, root xylem carries water to all above-ground organs and therefore influences all aspects of plant growth. Because of the global warming and increasing water deficit, we need to come up with solutions for the crops of the future. It is clear that structure of water conducting elements directly impacts water transport within the plant. Among plant pathogens- vascular wilts attacking xylem -are the most harmful. Our knowledge about xylem anatomy and rewiring ability could bring the solutions against these diseases. In this review we summarize the recent findings on the molecular mechanisms of xylem formation with a special attention to the cellular changes, and cell wall rearrangements that are necessary to create functional capillaries. We emphasize the impact of abiotic factors and pathogens on xylem plasticity and discuss multidisciplinary approach to model xylem in crops.

Highlights

- Making xylem vessels: differentiation of xylem cells happens gradually from the precursors to fully functional hollow lignified capillaries
- Molecular genetic framework of xylem formation: from hormones and transcription factors to small regulatory RNAs and peptide-receptor pathways
- Abiotic stresses influence xylem patterning
- To fight xylem pathogens, plants produce polymers that block the infection and create new xylem elements to overcome the contaminated vessels
- The multidisciplinary approach to model the plant water use in different plant species and crop varieties will help in designing resilient crops

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

The process of xylem development is fascinating and has been attracting developmental biologists for more than a century. The early studies on xylem formation demonstrated wounding-induced xylogenesis as well as formation of xylem from callus and trans-differentiation of the cell tissue culture cells into the xylem cells (Vöchting 1892, Simon 1908, Jacobs 1952, Wetmore and Rier 1963, Fukuda and Komamine 1980). In this review we summarize the recent advances in root xylem development research with a special attention to environmental inputs that contribute to the patterning of this tissue. Our goal is to highlight the importance of xylem research in the light of global warming and increasing risk of vascular wilt diseases. First, we introduce the developmental steps that xylem initials undergo to become functional conducting elements. Later we discuss the molecular mechanisms and the key players in formation of this tissue. Afterwards, we focus on the abiotic stresses that modify xylem and vascular wilt pathogens and the ways plants fight against them. Finally, we summarize and discuss a multidisciplinary approach to phenotype and model xylem capacity.

1. Making xylem vessels: from xylem precursors to fully functional capillaries

Development of xylem tissue can be divided into early steps, when the totipotent cells commit to create xylem, and to later steps- when the xylem precursors transform to functional capillaries. Because the fully differentiated xylem cells are not alive, the developmental plasticity can be rather observed in the early steps. However, after the cell clearing, the surrounding procambium and later parenchyma cells contribute to plasticity when non-cell autonomously acting in final steps in xylem maturation (Bollhöner, Zhang et al. 2013, Pesquet, Zhang et al. 2013) and in case of pathogen infection- with secreting polymers to block the propagation (reviewed in (Yadeta and Thomma 2013, Kashyap, Planas-Marques et al. 2021).

In Arabidopsis the first provascular cells originate in the early embryogenesis, when at the 16-cell stage the inner lower cells divide periclinally to give rise to the ground and vascular tissue initials (Scheres, Wolkenfelt et al. 1994) (Figure 1A). Later on, the provascular initials through formative divisions create initials for pericycle and procambium. Remarkably, while the pericycle initials will only divide anticlinally from now on, the procambium initials will continue periclinal divisions giving rise to the future xylem, phloem and procambium tissues. At the early globular stage, the most apical suspensor cell becomes a hypophysis that will later divide asymmetrically giving rise to QC (quiescent center) and columella stem cell. In the fully mature embryo, the quiescent center (QC) is surrounded by the stem cells giving rise to all types of root tissues: 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, later towards the hypophysis (Friml, Vieten et al. 2003).

Upon seed germination, the embryonic root meristem rapidly produces new tissues, the root tip emerges and rapidly elongates. Within hours root tissues undergo differentiation, including functional root hairs and xylem capillaries to provide with the uptake of water and minerals (Figure 1B). In the growing primary root, as well as in the growing lateral root, developing leaf and flower the only primary xylem is found; this early

conducting tissue will be functionally replaced by metaxylem and later secondary xylem.

In Arabidopsis primary root, the xylem pole is located in the center of the vascular cylinder and consists of two outermost protoxylem (PX) cell files and three metaxylem cell files: one inner metaxylem (IMX) and two outer metaxylem (OMX) cell files (Figure 1B, Figure 2A, D). Interestingly, that from the very beginning protoxylem and metaxylem initials have distinct gene expression profiles (Kubo, Udagawa et al. 2005) even though they undergo similar cellular changes and have very similar function in transporting water. In Tomato primary root (M-82) the organization of the primary xylem tissue is similar, including two protoxylem cell files on each side of the xylem pole and double amount of the metaxylem cell files (six instead of three) (Figure 1B). Not only on the morphological level, but also on the molecular level xylem development regulators seem to be conserved between Arabidopsis and Tomato (Kajala, Gouran et al. 2021). Later in the development of dicotyledon plant, primary growth will be replaced with secondary growth and cambium will give rise to all secondary vascular tissues.

Remarkably, xylem differentiation happens gradually- first, the outermost protoxylem cell files show thickening of the cell wall and lignification, later on the outermost metaxylem develops thick cell wall and lignin (Figure 1B), whereas the central metaxylem gets lignified and fully functional much later.

On the cellular level, protoxylem and metaxylem undergo similar processes: starting with multiple anticlinal divisions of the xylem precursor, then elongation, thickening of the cell wall, accumulating lytic enzymes in the vacuole, secreting lignin monomers to the apoplast (Schuetz, Benske et al. 2014) and lignification, that will continue after the cell death (Pesquet, Zhang et al. 2013) (Figure 2B).

The nature of helical pattern the protoxylem cell wall thickening and later lignification versus metaxylem cell wall thickening and lignification is not yet fully clear. What are the molecular determinants that define the pattern of the thickening in the cell wall? How are they recruited there? The work on LACCASE4/17 elegantly demonstrated that these enzymes localize to the secondary cell walls of the developing protoxylem and metaxylem cells (Schuetz, Benske et al. 2014), but what brings them exactly to the sites where the lignin biosynthesis should take place? Similar to 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, De Rybel et al. 2011, Roppolo and Geldner 2012). Interestingly, in the metaxylem patterning Rho GTPAses have been demonstrated to play a key role in establishing specific membrane domains where the microtubules assembly is inhibited (Oda and Fukuda 2013). The same research group later nicely showed that the size of piths is regulated in a quantitative manner by microtubule- binding proteins CORD1 and CORD2 (Sasaki, Fukuda et al. 2017). Another question is how the diameter and the length of the xylem capillaries are controlled? Answering these questions could help to modify xylem networks in crops to adapt better to environmental conditions.

Remarkably, while the primary xylem is playing a key role in the water uptake in the root tips, the major part of the xylem tissues in plants originate from cambium during secondary growth (De Rybel, Mähönen et al. 2016). Recent advances in our understanding of secondary growth allowed to trace the origin of cambium. The team led by Mahonnen used an elegant approach of lineage tracing and molecular genetic studies to show that xylem-adjacent procambium cell gives rise to the cambium tissue (Smetana, Mäkilä et al. 2019).

Understanding the secondary xylem developmental switches and better phenotyping of this tissue will contribute to the further advances in optimizing xylem networks.

2. The molecular genetic framework of xylem formation

The process of xylem formation can be divided into three steps: first, the commitment of the totipotent cell to become a precursor of vascular tissue and later xylem tissue (can be observed in embryo development or trans-differentiation of xylem parenchyma or mesophyll or callus cells); second, the differentiation of xylem precursor into conducting element with the last step of clearing the cytoplasm and organelles; third, so-called post-mortem differentiation, when the adjacent cells contribute to the last steps of formation conducting element. Here we briefly discuss the key players in every step.

2.1 Pre-patterning

The early studies on xylem formation showed that auxin and cytokinin play a central role in differentiation of this tissue (Jacobs 1952). With establishing of Arabidopsis as a plant model, the molecular research on vascular development has been significantly advanced. The large genetic screens in Arabidopsis could identify additional components including cytokinin signaling, transcription factors and micro RNAs that control xylem development (Benfey, Linstead et al. 1993, Turner and Somerville 1997, Cano-Delgado, Metzlaff et al. 2000, Yokoyama and Nishitani 2006). Developing molecular tools allowing to visualize the distribution of the hormone signaling in addition to mutant approach could demonstrate that auxin is the first signal required for xylem initiation and differentiation (Ulmasov, Murfett et al. 1997, Friml, Vieten et al. 2003, Weijers, Schlereth et al. 2006, Herud-Sikimić, Stiel et al. 2021). The molecular mechanism of auxin- induced xylem formation has been studied in details. The auxin-dependent transcription factor MONOPTERUS (MP) expressed in embryo, targets TMO5 and TMO7 (TARGET OF MONOPTERUS 5 and 7)(Schlereth, Möller et al. 2010), two bHLH transcription factors. TMO5 creates a complex with LHW (LONESOME HIGHWAY)- another bHLH transcription factor (Katayama, Iwamoto et al. 2015, Ohashi-Ito, Iwamoto et al. 2019)- to upregulate the expression of cytokinin biosynthetic enzymes genes LONELY GUY3 and 4 (LOG3 and 4) and a cytokinin signaling inhibitor ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN6 (AHP6) in protoxylem cells (Mähönen, Bishopp et al. 2006). The increase in cytokinin signaling due to up-regulation of LOG3 and LOG4 promotes provascular cell divisions. Interestingly, the overexpression of TMO5/LHW led to the ectopic xylem vessel differentiation (Katayama, Iwamoto et al. 2015). In conclusion, the complexes of TMO5/LHW are key players in production of vascular initial cells.

In addition to MP-mediated pathway, auxin promotes the expression of Homeodomain-Leucin Zipper III (HD-ZIP III) transcription factors, that will specify the protoxylem versus metaxylem in a dosage-dependent manner (Carlsbecker, Lee et al. 2010, Ohashi-Ito, Matsukawa et al. 2013). On the one side, these transcription factors are induced by auxin and on the other side, they are downregulated by miRNA165/166 diffusing into the vascular cylinder from endodermis (Carlsbecker, Lee et al. 2010) (Figure 2D). More recently, it has been nicely demonstrated, that the receptor-like kinases BARELY ANY MERISTEM (BAM1and BAM2) control the movement of these small regulatory RNAs toward the vasculature and therefore regulate xylem development. Indeed, in the *bam1bam2*double mutant the levels of miRNA165/166 in the stele are reduced, while the expression of HD-ZIP III transcription factors is increased. These mutants show irregular xylem development: the metaxylem files develop instead of protoxylem files (Fan, Aguilar et al. 2021). BAM1 and BAM2 are receptor like kinases that bind small signaling peptides from CLE (**CL**AVATA3-**E**MBRYO-surrounding region-related) family. It is still not fully clear, if the role of BAMs in movement of small RNAs depends on the peptide-activation mechanism or not. Interestingly, the first receptor-like kinases shown to play a role in embryo radial pattern formation (including provascular tissue formation) were RPK1(TOAD1) and RPK2(TOAD2) (Nodine, Yadegari et al. 2007). These two receptor-like kinases are expressed in the early globular embryo and contribute to periclinal cell divisions forming radial pattern. Later study demonstrated that these receptors are implicated in CLE peptide perception and in addition to their role in embryo, are essential in root apical meristem maintenance (Racolta, Nodine 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 the vascular patterning.

2.2 Executors

Following early specification, xylem initials undergo differentiation including series of anticlinal divisions, elongation, cell wall thickening, accumulation of the lytic enzymes and monolignons in the vacuole, lignification in the secondary cell wall and vacuole disruption that will end in the cell death (Figure 2B). To initiate this differentiation program, VASCULAR-RELATED NAC-DOMAIN (VND) 6 and 7 transcription factors act in metaxylem and protoxylem respectively (Kubo, Udagawa et al. 2005) to induce transcription factors that activate genes related to secondary cell wall formation and cell death. To comprehensively characterize the transcriptional regulation of the enzymes acting in secondary cell wall formation, the regulatory network has been established (Taylor-Teeples, Lin et al. 2015). Here, using a combination of gene expression data,

the authors isolated 467 xylem transcription factors that have been used in a yeast-one-hybrid experiments with promoters of secondary cell wall genes. Interestingly, this regulatory network identified an upstream regulator of VNDs, could confirm the known transcription factors like MYB46, and hundreds of novel regulators (Taylor-Teeples, Lin et al. 2015). The multiple transcription factors act redundantly to initiate cell wall thickening, lignification and cell death (Figure 2C). It has been shown, that the enzyme METACASPASE9 (AtMC9) acts in the degradation of the cell content after the bursting of the central vacuole leading to the cell death (Bollhöner, Zhang et al. 2013). Remarkably, the switch to the last step in xylem differentiation might be strictly controlled by the environment, because, on the one hand, postponing the death can provide with more plasticity; on the other hand, it will delay the functionality of the tissue.

3. Effects of abiotic stresses on xylem patterning

Plants sense the environment and adjust the physiological response to survive. Among abiotic conditions that impact plant growth are water availability, light, temperature, soil salinity, nutrients abundance, soil pH. Remarkably, the molecular mechanisms of sensing the environment and adaptations have developed on the timeframe of more than 450 million years, since first plants colonized the land. The key hormone mediating abiotic stress responses is abscisic acid (ABA), that was already produced in the algae species, but became a key stress signal only in the common ancestor of the land plants that developed an ABA-dependent modulation of PYRABACTIN RESISTANCE 1-like (PYL) protein (Sun, Harpazi et al. 2019). Here we discuss the effects of abiotic stresses on the xylem patterning described in the literature. Our better understanding of how plants react and modify their xylem to drought, salt, or temperatures will help to better understand how to exploit these traits and develop resilient crops in the future.

3.1 Drought

The molecular mechanisms of the drought stress have been extensively studied in the last decades (reviewed in (Yu, Wu et al. 2015, Martignago, Rico-Medina et al. 2019, Ramachandran, Augstein et al. 2020)). Recent studies could nicely pinpoint a mechanism of ABA-mediated response to water deficit that involves developmental switch in xylem cell identity ((Ramachandran, Wang et al. 2018, Bloch, Puli et al. 2019)). Interestingly, that under drought conditions or following ABA treatment the outer metaxylem cells develop into protoxylem cells with helical pattern of the secondary cell walls. Morphological changes in xylem are already visible after a four-hour treatment, and are reversible showcasing the plasticity of the xylem tissue (Ramachandran, Augstein et al. 2021). In the most recent study Ramachandran et al show that differentiation measured by lignification onset of the inner metaxylem is enhanced by drought and direct application of ABA (Ramachandran, Augstein et al. 2021). Simultaneously, the fate of the outer metaxylem is transformed into that of protoxylem (Figure 3). Conforming with these results, disturbing ABA biosynthesis or its perception either through application of biosynthesis inhibitor fluridone, 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 (Ramachandran 2018, Bloch 2019). This morphological response to ABA could be explained as protoxylem cells are less vulnerable to embolism compared to metaxylem and also may have increased lateral water movement for embolism repair. The study conducted in maize could show this tendency (Hwang, Ryu et al. 2016).

On a molecular level, it has been shown that ABA signaling in endodermis upregulates the expression of mobile miRNA165/166 and a reduction of miRNA165/166 repressor ZLL/AGO10 (Bloch, Puli et al. 2019). The elevated levels of miRNAs suppress the HD-ZIPIII transcription factors that control the metaxylem cell fate and therefore outer metaxylem cells often change their identity to protoxylem (Carlsbecker, Lee et al. 2010, Ramachandran, Wang et al. 2018). The mutant *phb1-d* which is resistant to miRNA165/166, and mutants *scr and shr* which have a strongly reduced overall levels of miRNA165/166 do not show a xylem phenotype in response to ABA (Bloch, Puli et al. 2019). Additionally, a selection of the master regulators of xylem differentiation, VND1-3&7, are necessary to properly regulate these changes in xylem structure (Ramachandran, Augstein et al. 2021). It has been shown, that VND1-3 are key regulators in the final lignification 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 no differences on this trait, but instead show a reduction of the morphological changes seen in the outer metaxylem. These findings altogether reinforce a mechanism of direct relation between the water conducting tissue formation and the water deficit signaling.

Interestingly, that this xylem response seems to be quite conserved across eudicots both on a molecular and a morphological level, as a shift from metaxylem to protoxylem can be observed in members of the*Brassica, Nicotiana, Phtheirospermum,* and *Solanum* family and a similar increase in expression in VND homologs can be seen in tomato compared to Arabidopsis (Bloch, Puli et al. 2019, Ramachandran, Augstein et al. 2021). Furthermore, evidence to the relation of VNDs and miRNA165/166 to water deficiency in monocots has given proof that this regulatory pathway could be quite well conserved among plant species. Mutant lines of a VND homolog, *nut1,* in maize shows drought stress in normal conditions caused by an underdeveloped protoxylem in mature plants (Dong, Xu et al. 2020). Additionally, a knockdown of miRNA166 in rice caused leaf rolling, a typical drought stress response, when grown normally, and a reduced transpiration rate, which was caused by a reduced diameter of xylem cells (Zhang, Zhang et al. 2018).

Besides ABA, jasmonic acid (JA) and cytokinin (CK) have been shown to play a role in xylem regulation in response to drought. CK is a known inhibitor of protoxylem differentiation by regulating pseudophosphotransfer protein AHP6 (Mähönen, Bishopp et al. 2006). It has been demonstrated that drought lowers the levels of CK while simultaneously increasing levels of JA (Nishiyama, Watanabe et al. 2011) (Jang, Chang et al. 2017, Jang and Choi 2018). Interestingly, lower levels of CK have also been linked to an increased sensitivity to ABA, suggesting a possible crosstalk between these pathways. However, the specific roles of ABA and the JA/CK balance seem to be different. While ABA mostly regulate the differentiation and cell fate of the different xylem through VNDs and miRNA165/166, the JA/CK balance is regulating the amount of xylem vessels within the axis (Jang, Chang et al. 2017, Jang and Choi 2018, Jang, Lee et al. 2018). It has been shown that drought stress leads to an increase of number of xylem vessels which are originating 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.

3.2 Salt stress

Another common abiotic stress that is experienced by plants is osmotic stress due to a saline environment. Comparably to water deficiency, ABA biosynthesis and signaling are activated by salt stress. However, the differentiation switch of xylem observed in drought conditions mediated by ABA is not replicated during the NaCl treatment (Bloch, Puli et al. 2019), showing 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 Tomato plants(Sánchez-Aguayo, Rodríguez-Galán et al. 2004) and other plant species (Neves, Marchiosi et al. 2010, FERNANDEZ-GARCIA, HERNANDEZ et al. 2011, Oliveira, Mota et al. 2020, Kong, Mostafa et al. 2021). However, not much is known on the molecular mechanism of salt-induced over-lignification.

ACAULIS 5 (ACL5) gene is expressed in the procambium cells and it encodes a putative spermine synthase that has been isolated in the mutant screen. Loss-of function *acl5* mutant has an increased xylem vessel diameter (Shinohara, Okamoto et al. 2019), impaired xylem patterning and salt hypersensitivity. On the cellular level, *acl5*mutant shows severe inhibition of the secondary cell wall formation; the vessels have helical pattern of the cell wall, but not the pitted pattern and xylem fibers are not produced. The role of ACL5 has been demonstrated in inhibition of the final step of xylem differentiation- cell death (Muñiz, Minguet et al. 2008). In this mutant the developing xylem cells undergo early cell death, therefore the elongation and secondary cell wall formation are prematurely terminated (Muñiz, Minguet et al. 2008). Several works showed that salt-tolerant plants have reduced xylem vessel diameter; mutant *acl5* and quadruple mutant of the *SAC51* gene family have increased xylem diameter and show hypersensitivity to salt (Shinohara, Okamoto et al. 2019). Taken together, the abiotic stresses impact on xylem patterning through modulating hormonal signaling and affecting the structure of conducting elements- from patterning the secondary cell wall to defining the thickness of the cell wall and vessel diameter. The molecular mechanisms of stress-regulated xylem patterning is an exceptionally important topic especially in the light of global climate change and we

hope near future will bring more discoveries in this field.

4. Wilt pathogens and their impact on xylem

On top of abiotic factors that affect plant growth, multiple pathogens and pests interfere with plant development and often cause plant death. Vascular system is an attractive target for many pathogens while wilt pathogens that attack xylem tissues are among the most harmful for plant health (Yadeta and Thomma 2013). This group of pathogens are represented by microscopic fungi (for instance, *Fusarium oxysporum*) or bacteria (for instance, *Xylella fastidiosa*) and can lead to wilting and even death of the plant. The invasion of these pathogens often happens through the root system, where they enter the epidermis, continue through cortex and endodermis reaching the xylem. In xylem these pathogens proliferate and spread to the above ground organs (Bae, Han et al. 2015). Another strategy is used by vascular bacteria, for instance, *Xylella fastidiosa*, that directly inoculated into the xylem tissue by insects that attack the plant (Wang, Lee et al. 2017). Remarkably, the strategies to resist wilt pathogens are often associated with xylem capacity to recognize the pathogen and to block the infection by producing polymers (lignin, suberin), or secreting gels and formation tyloses (reviewed in (Kashyap, Planas-Marques et al. 2021)) (Figure 3). While pathogen recognition is a highly advanced field today, our understanding of the xylem responses to compartmentalize the pathogen mainly relays on the anatomical studies of the infected tissues and some recent proteomics and transcriptomics studies (Hu, Puri et al. 2019, Xiong, Sun et al. 2021).

After successful blocking the infected region of xylem, plants often need to re-connect the network by creating new conducting elements from the adjacent parenchyma cells. Remarkably, the induction of physical barriers and re-wiring require a highly coordinated and timely response in xylem tissue; often the susceptible plants also produce polymers or gels and tyloses to stop the propagation of the pathogen, but not in a local or rapid manner and then the infection takes over (Planas-Marquès, Kressin et al. 2020). Studying the molecular mechanisms and the genetic basis of re-connecting 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.

5. Perspectives in Xylem phenotyping and water use modeling

Plants consume large amounts of water for their metabolism and even more water they lose during transpiration. Therefore, plant water management, including the uptake, the hydraulics within the xylem system and transpiration through stomata impact the global water cycle, the agriculture and our ecosystems (Schlesinger and Jasechko 2014). Xylem system in plants exploits a passive mechanism of water transport from the root to the shoot. Because this most essential for plants process relays on water movement within dead capillaries, two large areas of research deal with how the water conducting tissue is formed and can be modified (vascular development) and how water moves inside the xylem network (plant hydraulics). While the developmental biologists look into gene regulatory networks, anatomy and cell biology, the plant hydraulics focuses on biophysics of water flow, xylem embolism, hydraulic conductance and vulnerability (Venturas, Sperry 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. We believe, such collaborations 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 for example with "Root Xylem Index" that could be used to assess the capability of root xylem network of the specific plant species and varieties. This index can be used later by farmers to select the best cultivars or varieties for a given field and conditions. In the recent study on maize roots five cultivars of maize were grown in pots and later used for anatomical analysis and modelling. The authors created a high-resolution root system hydraulic atlases for maize plant (Heymans, Couvreur et al.). In this study among the other parameters, five were related to the vasculature. This work is a nice example of modelling water transport combining the anatomical traits and mathematics and this protocol for assessment of root hydraulic capability can be used with many other species (Heymans, Couvreur et al.). The microscopic differences in the structure of xylem cells can be later used to estimate global water use properties of the plant. This knowledge will be essential in designing crops with optimal xylem system.

Figure legends

Figure 1. Xylem formation: from the precursors to functional capillaries. A. The ontogeny of xylem in early Arabidopsis embryo. B. The on-set of primary xylem differentiation in Arabidopsis and Tomato roots. The illustration shows the gradual lignification of the protoxylem and later metaxylem cells and below the confocal images show a region where the protoxylem is already lignified. The confocal images were done with Zeiss780, the Calcofluor staining decorates cell walls and in red- Basic Fuchsin staining shows the lignification. The scale bar corresponds to 50mm.

Figure 2. Xylem pattern formation. A. In the root tip the xylem pole is located in the center of the vascular cylinder, with three metaxylem cells flanked by protoxylem. B. Cellular changes during proto- and metaxylem differentiation. C. The key transcription factors mediating xylem differentiation. D. Radial patterning in xylem mediated by HD-ZIP III transcription factors and mobile miRNAs.

Figure 3. Environmental stresses impact on xylem patterning. Xylem pathogens infect functional vessels, that can lead to tyloses (cell outgrowth of the xylem parenchyma), production of polymers for vascular coating and induction of new xylem vessels in adjacent tissue to reconnect the vascular system. Under drought conditions, ABA induces miRNAs production that represses HD-ZIP III transcription factors leading to developing more protoxylem cell files replacing metaxylem. In addition, ABA signaling induces early lignification of the inner metaxylem. Salt stress leads to increased lignification and decreased diameter of xylem vessels.

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References

Bae, C., S. W. Han, Y. R. Song, B. Y. Kim, H. J. Lee, J. M. Lee, I. Yeam, S. Heu and C. S. Oh (2015). "Infection processes of xylem-colonizing pathogenic bacteria: possible explanations for the scarcity of qualitative disease resistance genes against them in crops." *Theor Appl Genet* **128** (7): 1219-1229.

Benfey, P. N., P. J. Linstead, K. Roberts, J. W. Schiefelbein, M. T. Hauser and R. A. Aeschbacher (1993). "Root development in Arabidopsis: four mutants with dramatically altered root morphogenesis." *Development* **119** (1): 57-70.

Bloch, D., M. R. Puli, A. Mosquna and S. Yalovsky (2019). "Abiotic stress modulates root patterning via ABA-regulated microRNA expression in the endodermis initials." *Development* **146** (17).

Bollhöner, B., B. Zhang, S. Stael, N. Denancé, K. Overmyer, D. Goffner, F. Van Breusegem and H. Tuominen (2013). "Post mortem function of AtMC9 in xylem vessel elements." *New Phytologist* **200** (2): 498-510.

Cano-Delgado, A. I., K. Metzlaff and M. W. Bevan (2000). "The eli1 mutation reveals a link between cell expansion and secondary cell wall formation in Arabidopsis thaliana." *Development* **127** (15): 3395-3405.

Carlsbecker, A., J.-Y. Lee, C. J. Roberts, J. Dettmer, S. Lehesranta, J. Zhou, O. Lindgren, M. A. Moreno-Risueno, A. Vatén, S. Thitamadee, A. Campilho, J. Sebastian, J. L. Bowman, Y. Helariutta and P. N. Benfey (2010). "Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate." *Nature* **465** (7296): 316-321.

De Rybel, B., A. P. Mähönen, Y. Helariutta and D. Weijers (2016). "Plant vascular development: from early specification to differentiation." *Nature Reviews Molecular Cell Biology* **17** (1): 30-40.

Dong, Z., Z. Xu, L. Xu, M. Galli, A. Gallavotti, H. K. Dooner and G. Chuck (2020). "Necrotic upper tips1 mimics heat and drought stress and encodes a protoxylem-specific transcription factor in maize." *Proc Natl Acad Sci U S A* **117** (34): 20908-20919.

Fan, P., E. Aguilar, M. Bradai, H. Xue, H. Wang, T. Rosas-Diaz, W. Tang, S. Wolf, H. Zhang, L. Xu and R. Lozano-Duran (2021). "The receptor-like kinases BAM1 and BAM2 are required for root xylem patterning."*Proc Natl Acad Sci U S A* **118** (12).

FERNANDEZ-GARCIA, N., M. HERNANDEZ, J. CASADO-VELA, R. BRU, F. ELORTZA, P. HEDDEN and E. OLMOS (2011). "Changes to the proteome and targeted metabolites of xylem sap in Brassica oleracea in response to salt stress." *Plant, Cell & Environment* **34** (5): 821-836.

Friml, J., A. Vieten, M. Sauer, D. Weijers, H. Schwarz, T. Hamann, R. Offringa and G. Jürgens (2003). "Efflux-dependent auxin gradients establish the apical–basal axis of Arabidopsis." *Nature***426** (6963): 147-153.

Fukuda, H. and A. Komamine (1980). "Establishment of an Experimental System for the Study of Tracheary Element Differentiation from Single Cells Isolated from the Mesophyll of Zinnia elegans." *Plant Physiol* **65** (1): 57-60.

Herud-Sikimić, O., A. C. Stiel, M. Kolb, S. Shanmugaratnam, K. W. Berendzen, C. Feldhaus, B. Höcker and G. Jürgens (2021). "A biosensor for the direct visualization of auxin." *Nature***592** (7856): 768-772.

Heymans, A., V. Couvreur and G. Lobet "Combining cross-section images and modeling tools to create high-resolution root system hydraulic atlases in Zea mays." *Plant Direct* **n/a** (n/a): e334.

Hu, X., K. D. Puri, S. Gurung, S. J. Klosterman, C. M. Wallis, M. Britton, B. Durbin-Johnson, B. Phinney, M. Salemi, D. P. G. Short and K. V. Subbarao (2019). "Proteome and metabolome analyses reveal differential responses in tomato -Verticillium dahliae-interactions."*J Proteomics* **207** : 103449.

Hwang, B. G., J. Ryu and S. J. Lee (2016). "Vulnerability of Protoxylem and Metaxylem Vessels to Embolisms and Radial Refilling in a Vascular Bundle of Maize Leaves." *Frontiers in Plant Science***7** (941).

Jacobs, W. P. (1952). "THE ROLE OF AUXIN IN DIFFERENTIATION OF XYLEM AROUND A WOUND." *American Journal of Botany* **39** (5): 301-309.

Jang, G., S. H. Chang, T. Y. Um, S. Lee, J. K. Kim and Y. D. Choi (2017). "Antagonistic interaction between jasmonic acid and cytokinin in xylem development." *Sci Rep* **7** (1): 10212.

Jang, G. and Y. D. Choi (2018). "Drought stress promotes xylem differentiation by modulating the interaction between cytokinin and jasmonic acid." *Plant Signal Behav* **13** (3): e1451707.

Jang, G., S. Lee, S. H. Chang, J.-K. Kim and Y. D. Choi (2018). "Jasmonic acid modulates xylem development by controlling polar auxin transport in vascular tissues." *Plant Biotechnology Reports***12** (4): 265-271.

Kajala, K., M. Gouran, L. Shaar-Moshe, G. A. Mason, J. Rodriguez-Medina, D. Kawa, G. Pauluzzi, M. Reynoso, A. Canto-Pastor, C. Manzano, V. Lau, M. A. S. Artur, D. A. West, S. B. Gray, A. T. Borowsky, B. P. Moore, A. I. Yao, K. W. Morimoto, M. Bajic, E. Formentin, N. A. Nirmal, A. Rodriguez, A. Pasha, R. B. Deal, D. J. Kliebenstein, T. R. Hvidsten, N. J. Provart, N. R. Sinha, D. E. Runcie, J. Bailey-Serres and S. M. Brady (2021). "Innovation, conservation, and repurposing of gene function in root cell type development." *Cell* **184** (12): 3333-3348.e3319.

Kashyap, A., M. Planas-Marques, M. Capellades, M. Valls and N. S. Coll (2021). "Blocking intruders: inducible physico-chemical barriers against plant vascular wilt pathogens." *J Exp Bot* **72** (2): 184-198.

Katayama, H., K. Iwamoto, Y. Kariya, T. Asakawa, T. Kan, H. Fukuda and K. Ohashi-Ito (2015). "A Negative Feedback Loop Controlling bHLH Complexes Is Involved in Vascular Cell Division and Differentiation in the Root Apical Meristem." *Curr Biol* **25** (23): 3144-3150.

Kong, Q., H. H. A. Mostafa, W. Yang, J. Wang, M. Nuerawuti, Y. Wang, J. Song, X. Zhang, L. Ma, H. Wang and X. Li (2021). "Comparative transcriptome profiling reveals that brassinosteroid-mediated lignification plays an important role in garlic adaption to salt stress." *Plant Physiology and Biochemistry* **158** : 34-42.

Kubo, M., M. Udagawa, N. Nishikubo, G. Horiguchi, M. Yamaguchi, J. Ito, T. Mimura, H. Fukuda and T. Demura (2005). "Transcription switches for protoxylem and metaxylem vessel formation." *Genes Dev***19** (16): 1855-1860.

Mähönen, A. P., A. Bishopp, M. Higuchi, K. M. Nieminen, K. Kinoshita, K. Törmäkangas, Y. Ikeda, A. Oka, T. Kakimoto and Y. Helariutta (2006). "Cytokinin Signaling and Its Inhibitor AHP6 Regulate Cell Fate During Vascular Development." *Science* **311** (5757): 94-98.

Martignago, D., A. Rico-Medina, D. Blasco-Escámez, J. B. Fontanet-Manzaneque and A. I. Caño-Delgado (2019). "Drought Resistance by Engineering Plant Tissue-Specific Responses." *Front Plant Sci***10** : 1676.

Muñiz, L., E. G. Minguet, S. K. Singh, E. Pesquet, F. Vera-Sirera, C. L. Moreau-Courtois, J. Carbonell, M. A. Blázquez and H. Tuominen (2008). "ACAULIS5 controls Arabidopsis xylem specification through the prevention of premature cell death." *Development***135** (15): 2573-2582.

Neves, G. Y. S., R. Marchiosi, M. L. L. Ferrarese, R. C. Siqueira-Soares and O. Ferrarese-Filho (2010). "Root Growth Inhibition and Lignification Induced by Salt Stress in Soybean." *Journal of Agronomy and Crop Science* **196** (6): 467-473.

Nishiyama, R., Y. Watanabe, Y. Fujita, D. T. Le, M. Kojima, T. Werner, R. Vankova, K. Yamaguchi-Shinozaki, K. Shinozaki, T. Kakimoto, H. Sakakibara, T. Schmülling and L. S. Tran (2011). "Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis." *Plant Cell***23** (6): 2169-2183.

Nodine, M. D., R. Yadegari and F. E. Tax (2007). "RPK1 and TOAD2 Are Two Receptor-like Kinases Redundantly Required for Arabidopsis Embryonic Pattern Formation." *Developmental Cell* **12** (6): 943-956.

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

Ohashi-Ito, K., K. Iwamoto, Y. Nagashima, M. Kojima, H. Sakakibara and H. Fukuda (2019). "A Positive Feedback Loop Comprising LHW-TMO5 and Local Auxin Biosynthesis Regulates Initial Vascular Development in Arabidopsis Roots." *Plant Cell Physiol* **60** (12): 2684-2691.

Ohashi-Ito, K., M. Matsukawa and H. Fukuda (2013). "An atypical bHLH transcription factor regulates early xylem development downstream of auxin." *Plant Cell Physiol* **54** (3): 398-405.

Oliveira, D. M., T. R. Mota, F. V. Salatta, R. C. Sinzker, R. Končitíková, D. Kopečný, R. Simister, M. Silva, G. Goeminne, K. Morreel, J. Rencoret, A. Gutiérrez, T. Tryfona, R. Marchiosi, P. Dupree, J. C. del Río, W. Boerjan, S. J. McQueen-Mason, L. D. Gomez, O. Ferrarese-Filho and W. D. dos Santos (2020). "Cell wall remodeling under salt stress: Insights into changes in polysaccharides, feruloylation, lignification, and phenolic metabolism in maize." *Plant, Cell & Environment* **43** (9): 2172-2191.

Pesquet, E., B. Zhang, A. Gorzsás, T. Puhakainen, H. Serk, S. Escamez, O. Barbier, L. Gerber, C. Courtois-Moreau, E. Alatalo, L. Paulin, J. Kangasjärvi, B. Sundberg, D. Goffner and H. Tuominen (2013). "Non-cell-autonomous postmortem lignification of tracheary elements in Zinnia elegans." *Plant Cell* **25** (4): 1314-1328.

Planas-Marquès, M., J. P. Kressin, A. Kashyap, D. R. Panthee, F. J. Louws, N. S. Coll and M. Valls (2020). "Four bottlenecks restrict colonization and invasion by the pathogen Ralstonia solanacearum in resistant tomato." *J Exp Bot* **71** (6): 2157-2171.

Racolta, A., M. D. Nodine, K. Davies, C. Lee, S. Rowe, Y. Velazco, R. Wellington and F. E. Tax (2018). "A Common Pathway of Root Growth Control and Response to CLE Peptides Through Two Receptor Kinases in Arabidopsis." *Genetics* **208** (2): 687-704.

Ramachandran, P., F. Augstein, S. Mazumdar, T. V. Nguyen, E. A. Minina, C. W. Melnyk and A. Carlsbecker (2021). "Abscisic acid signaling activates distinct VND transcription factors to promote xylem differentiation in Arabidopsis." *Curr Biol* .

Ramachandran, P., F. Augstein, V. Nguyen and A. Carlsbecker (2020). "Coping With Water Limitation: Hormones That Modify Plant Root Xylem Development." *Front Plant Sci* **11** : 570.

Ramachandran, P., G. Wang, F. Augstein, J. de Vries and A. Carlsbecker (2018). "Continuous root xylem formation and vascular acclimation to water deficit involves endodermal ABA signalling via miR165."*Development* **145** (3).

Roppolo, D., B. De Rybel, V. Dénervaud Tendon, A. Pfister, J. Alassimone, J. E. Vermeer, M. Yamazaki, Y. D. Stierhof, T. Beeckman and N. Geldner (2011). "A novel protein family mediates Casparian strip formation in the endodermis." *Nature* **473** (7347): 380-383.

Roppolo, D. and N. Geldner (2012). "Membrane and walls: who is master, who is servant?" *Curr Opin Plant Biol* **15** (6): 608-617.

Sánchez-Aguayo, I., J. M. Rodríguez-Galán, R. García, J. Torreblanca and J. M. Pardo (2004). "Salt stress enhances xylem development and expression of S-adenosyl-L-methionine synthase in lignifying tissues of tomato plants." *Planta* **220** (2): 278-285.

Sasaki, T., H. Fukuda and Y. Oda (2017). "CORTICAL MICROTUBULE DISORDERING1 Is Required for Secondary Cell Wall Patterning in Xylem Vessels." *Plant Cell* **29** (12): 3123-3139.

Scheres, B., H. Wolkenfelt, V. Willemsen, M. Terlouw, E. Lawson, C. Dean and P. Weisbeek (1994). "Embryonic origin of the Arabidopsis primary root and root meristem initials." *Development* **120** (9): 2475-2487.

Schlereth, A., B. Möller, W. Liu, M. Kientz, J. Flipse, E. H. Rademacher, M. Schmid, G. Jürgens and D. Weijers (2010). "MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor." *Nature* **464** (7290): 913-916.

Schlesinger, W. H. and S. Jasechko (2014). "Transpiration in the global water cycle." *Agricultural and Forest Meteorology***189-190** : 115-117.

Schuetz, M., A. Benske, R. A. Smith, Y. Watanabe, Y. Tobimatsu, J. Ralph, T. Demura, B. Ellis and A. L. Samuels (2014). "Laccases Direct Lignification in the Discrete Secondary Cell Wall Domains of Protoxylem " *Plant Physiology* **166** (2): 798-807.

Shinohara, S., T. Okamoto, H. Motose and T. Takahashi (2019). "Salt hypersensitivity is associated with excessive xylem development in a thermospermine-deficient mutant of Arabidopsis thaliana." *Plant J***100** (2): 374-383.

Simon, S. (1908). "Experimenelle Untersuchungen uber die Entstehung von Gefassvergindungen." *Ber. Dtsch. Bot. Ges.* **26** : 364-396.

Smetana, O., R. Mäkilä, M. Lyu, A. Amiryousefi, F. Sánchez Rodríguez, M.-F. Wu, A. Solé-Gil, M. Leal Gavarrón, R. Siligato, S. Miyashima, P. Roszak, T. Blomster, J. W. Reed, S. Broholm and A. P. Mähönen (2019). "High levels of auxin signalling define the stem-cell organizer of the vascular cambium." *Nature* **565** (7740): 485-489.

Sun, Y., B. Harpazi, A. Wijerathna-Yapa, E. Merilo, J. de Vries, D. Michaeli, M. Gal, A. C. Cuming, H. Kollist and A. Mosquna (2019). "A ligand-independent origin of abscisic acid perception." *Proc Natl Acad Sci U S A* **116** (49): 24892-24899.

Taylor-Teeples, M., L. Lin, M. de Lucas, G. Turco, T. W. Toal, A. Gaudinier, N. F. Young, G. M. Trabucco, M. T. Veling, R. Lamothe, P. P. Handakumbura, G. Xiong, C. Wang, J. Corwin, A. Tsoukalas, L. Zhang, D. Ware, M. Pauly, D. J. Kliebenstein, K. Dehesh, I. Tagkopoulos, G. Breton, J. L. Pruneda-Paz, S. E. Ahnert,

S. A. Kay, S. P. Hazen and S. M. Brady (2015). "An Arabidopsis gene regulatory network for secondary cell wall synthesis." *Nature* **517** (7536): 571-575.

Turner, S. R. and C. R. Somerville (1997). "Collapsed xylem phenotype of Arabidopsis identifies mutants deficient in cellulose deposition in the secondary cell wall." *Plant Cell* **9** (5): 689-701.

Ulmasov, T., J. Murfett, G. Hagen and T. J. Guilfoyle (1997). "Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements." *The Plant Cell***9** (11): 1963-1971.

Venturas, M. D., J. S. Sperry and U. G. Hacke (2017). "Plant xylem hydraulics: What we understand, current research, and future challenges." *J Integr Plant Biol* **59** (6): 356-389.

Vöchting, H. (1892). *Über Transplantation am Pflanzenkörper: Untersuchungen zur Physiologie und Pathologie: Mit XI lithogr. Taf. u. 14 Fig. im Text* , Laupp.

Wang, P., Y. Lee, M. M. Igo and M. C. Roper (2017). "Tolerance to oxidative stress is required for maximal xylem colonization by the xylem-limited bacterial phytopathogen, Xylella fastidiosa." *Mol Plant Pathol* **18** (7): 990-1000.

Weijers, D., A. Schlereth, J. S. Ehrismann, G. Schwank, M. Kientz and G. Jürgens (2006). "Auxin Triggers Transient Local Signaling for Cell Specification in Arabidopsis Embryogenesis." *Developmental Cell***10** (2): 265-270.

Wetmore, R. H. and J. P. Rier (1963). "EXPERIMENTAL INDUCTION OF VASCULAR TISSUES IN CALLUS OF ANGIOSPERMS." *American Journal of Botany* **50** (5): 418-430.

Xiong, X. P., S. C. Sun, Q. H. Zhu, X. Y. Zhang, F. Liu, Y. J. Li, F. Xue and J. Sun (2021). "Transcriptome Analysis and RNA Interference Reveal GhGDH2 Regulating Cotton Resistance to Verticillium Wilt by JA and SA Signaling Pathways." *Front Plant Sci* **12** : 654676.

Yadeta, K. and B. Thomma (2013). "The xylem as battleground for plant hosts and vascular wilt pathogens." *Frontiers in Plant Science***4** (97).

Yokoyama, R. and K. Nishitani (2006). "Identification and characterization of Arabidopsis thaliana genes involved in xylem secondary cell walls." *J Plant Res* **119** (3): 189-194.

Yu, F., Y. Wu and Q. Xie (2015). "Precise protein post-translational modifications modulate ABI5 activity." *Trends Plant Sci***20** (9): 569-575.

Zhang, J., H. Zhang, A. K. Srivastava, Y. Pan, J. Bai, J. Fang, H. Shi and J. K. Zhu (2018). "Knockdown of Rice MicroRNA166 Confers Drought Resistance by Causing Leaf Rolling and Altering Stem Xylem Development."*Plant Physiol* **176** (3): 2082-2094.

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