Cell wall signalling in flooded plants

Unravelling potential mechanisms in response to flooding stress – a comprehensive literature review

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Date: 1/6/2023

Layman summary

You may have noticed that giving your houseplants too much water is harmful to them. It limits their ability to take up oxygen from the air, which causes them to suffocate. The symptoms are that their leaves will hang down and decay of the roots. Excessive watering in the form of floods can destroy crops in the same way that it can hurt your houseplant. Floods have major consequences for the quantity and quality of agricultural food production. According to the Food and Agriculture Organisation of the United Nations (FAO), floods contributed to 65% of crop damage and loss induced by natural disasters between 2006 and 2016. Heavy rainfalls, causing severe floods, are more likely to happen in the future due to the impacts of climate change. Our current and future food supply is seriously threatened by the impacts of floods. Therefore, we must develop innovative agricultural technologies to combat crop yield loss caused by water stress.

One area of research that can help with this goal is the development of new varieties of crops that are more resistant to long-term floods. When plants experience floods, they develop all kinds of adaptations that help them to survive. For example, plants develop new organs that help them prevent suffocation. Understanding how plants perceive flooding stress and how this perception results in the development of these adaptations is necessary knowledge to develop flood-resistant crops. For example, this knowledge would allow opportunities to arise for future researchers to develop new crops that respond faster and more intensely to floods.

One mechanism that plants use to become aware of their surroundings is by determining the status of their cell walls. It uses the cell wall as a sensing tool to detect harmful growth conditions, such as hot and cold temperatures and drought conditions. Thanks to these mechanisms, plants can respond quickly to these stresses, allowing them to survive these harmful conditions. Although the cell wall status plays a significant role in the perception of several damaging growth conditions, its role during flooding stress is hardly investigated. Therefore, we asked the question: “Are cell walls also involved in the perception of flooding stress in plants?”

To find an answer to this question, we searched the literature for potential cell wall sensing mechanisms that are active during floods. Here, we merged the expertise of two distinct research fields by combining the knowledge of (1) plant mechanisms and adaptations during floods and (2) plant cell wall sensing mechanisms. In this review, we concluded that there is enough evidence to believe that sensing the cell wall status is needed for plants to undergo adaptations that help them plants to survive floods. The most prominent role is reserved for the cell wall component, pectin. We believe that during floods, the pectin changes in form. Consequently, we believe that this is perceived by the plant, and they undergo adaptions that protect them against floods. With this report, we contributed to the field of science by providing credible theories of potential explanations of how plants trigger adaptations during floods, which eventually contributes to the efforts to limit food loss caused by floods.

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Introduction

According to the Intergovernmental Panel on Climate Change (IPCC) reports, temperature rise remains the current general pattern of the global climate[1]. This phenomenon brings an increasingly difficult environmental challenge due to more intense rainfall, causing rising flooding risks. Prolonged flooding of agricultural fields has a detrimental impact on nearly all phases of crop development, ultimately resulting in yield loss[2]. Food and Agriculture Organization of the United Nations (FAO) estimated that flooding contributed 65% of all crop damages and losses caused by natural disasters from 2006 to 2016[3]. Preventing crop yield loss due to floods is vital to feed the predicted 9.7 billion people by 2050[4]. Flooding presents a significant issue for plants since it reduces the gas diffusion upon submergence by a factor of 10,000 in comparison to an aerated environment, limiting the plant's exchange of oxygen (O_2) and carbon dioxide (CO_2)[5]. Due to the decrease in oxygen levels, plants will suffer from O_2 deprivation (hypoxia), causing the plant to shift from mitochondrial respiration to anaerobic fermentation as a source of ATP production[6]. As a result, hypoxic cells rapidly exhaust their energy reserves, depleting their energy reserves. In addition, photosynthesis levels are reduced upon flooding due to the insufficiency of carbon dioxide levels and the blocking of light by turbid floodwater[5]. The combination of these flooding-induced stresses causes plants to face an energy crisis, leading to an excessive build-up of toxic compounds that are harmful in high concentrations. Perception and signalling of flooding are vital processes for plants, which induce plant adaptations aiding against challenges posed by submergence.

Plant cell walls are among the first factors influenced by physical stimuli induced by abiotic stresses[7,8]. Plants have evolved cell wall-monitoring mechanisms to transduce these cell wall changes to intracellular signals. Changes in temperature, cell wall composition, turgor pressure or mechanical forces, which affect the spatial relationship between cell walls and plasma membrane, could be indicative of abiotic stress[9]. These signals are incorporated into cellular decision-making upon abiotic stresses. Among the best described are the roles of cell wall signalling in acclimation strategy pathways of drought stress, heat stress, cold stress, osmotic stress, and the overabundance of heavy metals[10,11]. For example, several plasma membrane-located receptor-like kinases recognise saline-stress-induced turgor loss, and trigger a series of downstream proteins, resulting in an increased tolerance against abiotic osmotic stress[12].

Despite the reported role of cell walls in abiotic stress signalling, cell wall-mediated signalling upon flooding stress has received little to no attention in the literature. It remains obscure to which extent plant cell walls contribute to the perception and signalling of flooding stress. In this review, we first provide a general overview of flooding stress, followed by highlighting the insights of the cell wall signalling mechanisms that are pertinent to abiotic stresses, and finally, we will hypothesise about potential cell wall signalling mechanisms of plants involved in flooding stress signalling.

Responses of plant flooding stress

Next to alterations of O_2 and CO_2 dynamics, the volatile plant hormone ethylene accumulates quickly in plant tissue as a response to submergence[5]. Plants utilise the information provided by the alternations of these factors as signals to detect flooding stress. This chapter will cover hypoxia- and ethylene-mediated plant responses to coping with challenges resulting from flooding stress.

Plant responses to waterlogging

To survive flooding stress, plants have developed a wide variety of strategies, depending on the species, depth of the flooding and its duration[13] (Fig. 1). One of these strategies is improving the efficiency of longitudinal oxygen transport from the shoot to the root tips in response to waterlogging (soil flooding) stress. For instance, plants form radial oxygen loss (ROL) barriers by fortifying their cell walls with lignin and suberin[5,14]. These barriers prevent oxygen loss to the rhizosphere, resulting in an increased O_2 delivery to the root tip. Additionally, under flooding stress, plants develop branches with gas-filled, thin-walled cells that can improve the delivery of oxygen from the shoot to the root, called aerenchyma[14]. Lysigenous aerenchyma is the best-described aerenchyma type in literature, which is formed when cortex root cells undergo ROS-signalling-induced programmed cell death (PCD). Next, plants develop specialised organs named adventitious roots (AR), which are filled with aerenchyma and ROL barriers[13,14]. Air-contacting AR increases gas diffusion and transport towards the root apex, allowing the plant to withstand flood-induced low oxygen levels. Flooding-induced AR formation is mediated by the elongation of basal cells in pre-existing root primordia[5,15]. Studies in rice have revealed that the elongating basal cell (AR root primordia) exerts a mechanical force on the overlying epidermal cells, leading to epidermal cell PCD[16]. Like lysigenous aerenchyma, this epidermal cell PCD is triggered by flooding-induced ROS signalling[5]. However, it remains unclear how epidermal cell sense the exerted mechanical force and how this perception is linked with ROS signalling to trigger PCD. Furthermore, reduced root growth is another characteristic of plants that are affected by waterlogging[17]. In anoxic or hypoxic conditions, the energy reserves of plants are quickly depleted, causing further root growth to be limited under flooding stress. Finally, plants experience hypo-osmotic stress during flooding stress. When exposed to prolonged or repeated flooding of soils, plant cells swell due to an increased influx of water into the cell plasma membrane, leading to a variety of problems[18]. Although plant responses to hypo-osmotic stress are relatively unknown, plants have been reported to modify their cell wall structure to prevent cell elongation and plasma membrane rupture[19].

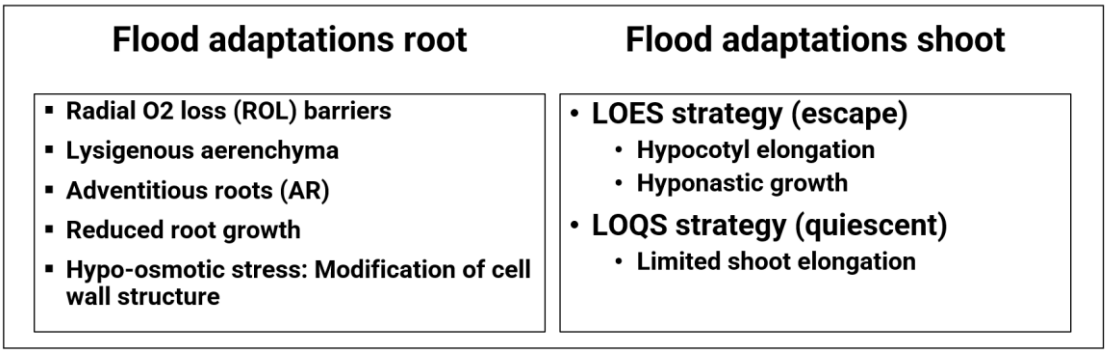

Figure 1. Overview of flood-induced adaptations of plants in roots and shoots.

Physiological strategies upon submergence

Submergence is the (partial) immersion of the shoot, cutting off contact with the aerial environment and is even more detrimental than waterlogging for plants[5]. There are two strategies known across

plant species to overcome submergence stress: the low-O2 escape strategy (LOES), which involves growth stimulation, and the low-O2 quiescent syndrome (LOQS), which is characterised by growth inhibition. Lowland rice is an example of a plant that uses the LOQS strategy. Plants with this strategy slow down their metabolism and preserve energy until the flood subsides, which helps them recover once the flood fades[20]. On the other hand, plants that use the LOES strategy (like deepwater rice) promote the bending of leaves above water (hyponasty) and rapid elongation of aerial organs to aerate the plant as quickly as possible. Both strategies are activated by ethylene signalling. However, separate hormonal signalling pathways downstream of ethylene distinguish these pathways. In *Arabidopsis*, brassinosteroid (BR) signalling is downstream of ethylene, promoting hyponastic growth[21] (Fig. 2A). It was revealed that chemical disruption of BR biosynthesis led to plants being unable to induce hyponasty. Similarly, the BR biosynthesis mutant *rotundifolia3* lost the ability to initiate ethylene-mediated hyponasty. Next to ethylene's impact on BR signalling, ethylene was also reported to deplete the abscisic acid (ABA) pool in *Rumex palustris*, negating the inhibitory effect of ABA on hyponastic growth[22]. Like hyponastic growth, flooding-induced stem elongation relies on ethylene-mediated depletion of ABA, which enables internode cells to be responsive to gibberellic acid (GA)-mediated elongation[5,23].

On the other hand, in lowland rice, SUBMERGENCE 1A (SUB1A) is a key gene, controlling the LOQS strategy by limiting high-energy processes (e.g., growth)[24]. *SUB1A* transcription levels were observed to be upregulated by ethylene and submergence conditions[25]. In rice, SUB1A-1 was reported to increase BR hormone levels after submergence by transcriptional activation of BR biosynthesis genes. As a result, the enhanced BR levels restrict GA activation by upregulation of GA-inhibiting enzymes, causing a inhibition of shoot growth[24] (Fig. 2B).

The growth-affecting responses of both LOES and LOQS plants are ultimately dependent on the level of cell wall flexibility. Plants evolved tightly controlled mechanisms to influence the cell wall fluidity via a variety of mechanisms, for example, wall loosening or wall stiffening enzymes[5]. In addition, plants can alter the biochemical composition of their cell walls, which will be discussed in-depth in the following chapter (See; *Cell wall biochemistry modification upon flood stress*).

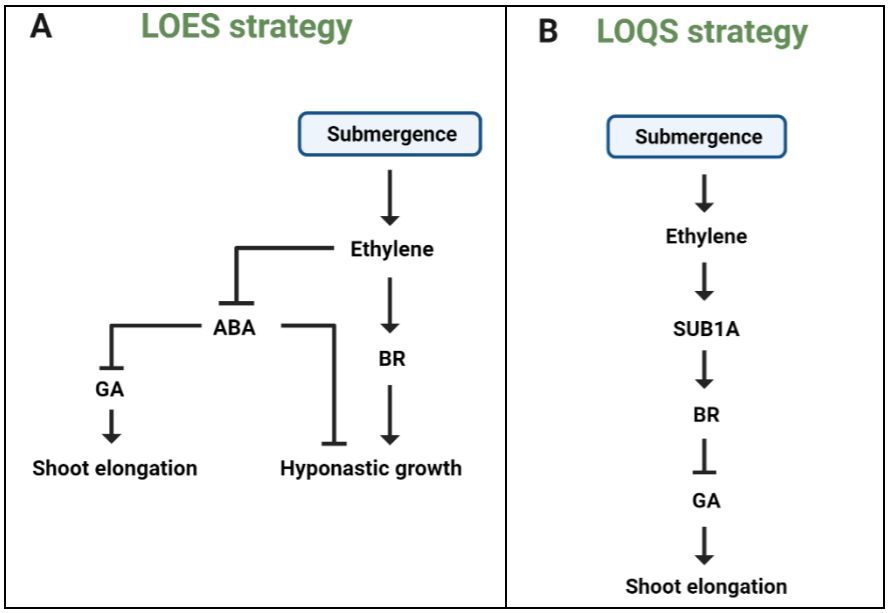

Figure 2. Submergence-induced shoot architecture modification pathway. Submergence stress elevates ethylene hormone levels in plant tissues, which in LOES strategy plants negatively regulate abscisic acid (ABA) signalling and positively regulates brassinosteroid (BR) signalling. Consequently, ABA cannot exert its inhibitory effect on hyponastic growth, while BR signalling promotes it. Additionally, ABA is unable to inhibit GA signalling in submergence conditions, causing the promotion of GA-mediated shoot elongation. **(B)** Submergence also accumulates ethylene in LOQS strategy plants, which activates SUB1A expression levels. By acting as a promoter of BR biosynthesis, SUB1A limits the accumulation of GA, causing GA-mediated shoot elongation to be restricted upon the submergence of these plants.

Cell wall biochemistry modification upon flood stress

As indicated in the previous chapter, cell wall composition is one of the factors determining cell size and shape, directing the plant organ morphology. Since growth inhibition/promotion of plant roots and shoots is vital for the plant's survival upon submergence, changes in the cell wall composition are essential in response to flooding stress[8,26]. Here, we will highlight alterations in cell wall biochemical properties induced by flooding stress.

Cellulose

Cell walls are intricate structures created from cellulose microfibrils covered by pectin, hemicellulose, and lignin working together as an adhesive matrix[8,27] (Fig. 3). The spatial arrangement of cellulose microfibrils has a significant impact on the orientation of plant cell walls, providing them strength and allowing turgor-based growth[28]. Flooding stress was reported to downregulate the cellulose biosynthesis mechanisms in various plant species[29–33]. For instance, soybean proteomic and transcription analyses revealed that flooding stress significantly reduced the activity and abundance of proteins involved in cellulose biosynthesis[29,30]. Similar trends were seen in grey poplar and wheat plants, where flooding downregulated several genes involved in cellulose production[31,32]. Furthermore, the cellulosic polysaccharide content of Azuki bean (*Vigna angularis*) and wheat seedlings was observed to be reduced in the epicotyl/hypocotyl upon water stress[32,33]. Comparable to terrestrial

plants, the cellulose content of hydrophytes, also known as aquatic plants, was also reduced when exposed to flooding stress. For instance, the cellulose content of flooded *Ludwigia repens* shoots was observed to be decreased by 1.5 times compared to those in aerial environments[34]. The downregulation of cellulose biosynthesis genes in flooded plants is hypothesised to be correlated with the inhibition of cell elongation under flooding stress conditions by plants using the LOQS strategy[8,27]. In contrast to plants with the LOQS strategy, the cellulose content of deepwater rice was unaffected between submerged and aerial-contacting internodes[35]. This finding hints that cell wall cellulose reduction upon flooding stress is a quiescent-specific trait. However, the studies available on the biochemical properties of the cell wall in flood stress conditions are limited and, therefore, still too early to draw hard conclusions.

Pectin

Plant pectin levels under flooding stress have been found to change in contradictory manners, depending on the species. For example, the pectin-degrading genes pectate lyase and polygalacturonase homologues in soybean were downregulated during flooding stress[30]. The authors suggested that the activation of these enzymes caused growth to terminate upon flooding[27]. In contrast, the pectin content of maize and azuki bean seedlings (*Vigna angularis)* under water stress conditions was considerably lower, triggering cell wall thinning and increasing the cell wall's viscoelastic extensibility, implying a potential enhanced growth promotion[33,36]. Azuki bean, however, is a plant whose growth responses to submergence are known to be restricted. It was suggested that the reduction in cell wall pectin levels has little impact on the growth decline due to strongly reduced cellular osmotic concentrations upon flooding[33]. Therefore, doubts remain surrounding how changes to the pectic cell wall affect growth under flooding stress.

Next to alterations to the quantity of pectin, pectin can also be chemically modified by cell wall-located proteins when plants experience flooding stress. The best-studied modification is de-methyl-esterification of the homogalacturonan domains of pectin polysaccharides, which are catalysed by a family of enzymes called pectin methylesterases (PMEs)[37]. As a result of PME activity, negatively charged carboxyl groups of the pectin backbone are exposed, which take part in cross-linking interactions with calcium cations, allowing susceptibility to pectin-degrading enzymes. In addition to pectin degradation, PME activity is also linked to the regulation of cell wall-mediated growth by restricting cell elongation, although its growth-restricting role is not always clear-cut[38]. For instance, it was seen that deepwater rice had increased PME transcription levels upon submergence, suggesting that PMEs contribute to the rapid internode elongation of flood-escaping plants during submersion by loosening the cell wall[39]. In addition, several studies with submerged soybean roots reported an increase in protein abundance and enzyme activity of pectin methyl-esterase inhibitor (PMEI) proteins, which cover the putative active site of PME and, therefore, is known to restrict PME activity[40,41]. Given that the root growth restriction was seen during flooding stress[17], the enhanced PMEI activity contradicts the theory that PME limits cell elongation. The researchers proposed a cell wall modifying mechanism of PMEI contributing to tolerance against flooding stress, although the exact mechanism was not further explored[40]. Next, involvement in the development of flood-induced aerenchyma in the roots of three legume species (pea, chickpea, and runner bean) have been attributed to elevated pectin de-methyl-esterification levels[42]. Immunolabeling studies revealed that upon flooding, the cell wall pectin backbones of one to three cell layers adjacent to the aerenchyma cavity were rapidly de-methyl-esterified, preparing cell-specific tissue for PCD and enabling aerenchyma development. Additionally, aerenchyma development may be accelerated by additional cell wall composition modification, according to the researchers, who found that eliminating cellulose carbohydrates unmasks additional pectin de-methyl-esterification binding sites. The authors hypothesised that cellulases

degrade cellulose polysaccharides in preparation for the de-methyl-esterification of pectin by PME enzymes and the subsequent pectin degradation.

Lignin

Lignin is a complex phenolic polymer found in the cell walls of plants. In addition to the role of lignin in preventing ROL in flooded roots, lignin is also directly linked to mechanical strength and resistance to lodging[32]. Like cellulose and pectin, lignin biosynthesis genes were observed to downregulate upon flooding stress in various plant species. Lignin precursor biosynthesis enzymes, such as *PHE AMMONIA LYASE 1* (*PAL*) isozymes and *CHALCONE SYNTHASE* (*CHS*), were suppressed in grey poplar as a response to flooding stress, suggesting a reduction in lignin levels[31]. Furthermore, phloroglucinol staining experiments provided additional evidence for reduced lignin content in the cell walls of soybean roots and hypocotyls as a response to flooding stress[43]. In addition, waterlogging stress decreased the internode lignin levels of multiple wheat cultivars, presumably due to the effective repression of lignin biosynthesis genes[32]. Because the production of structural carbohydrate components like lignin requires high levels of energy, the authors suggested that the reduction of lignin content could be a mechanism to save energy and, therefore, a method to survive prolonged floods. In LOES strategy plants, such as deepwater rice, a lignin reduction was also revealed. Numerous *CONIFERYL ALCOHOL DEHYDROGENASE* (CAD) genes were downregulated upon submergence, resulting in a decreased lignin content in newly elongated internodes[39]. Given that lignin deposition is known to limit cell elongation, it was hypothesised that deepwater rice's lower lignin content aids the internode elongation under submerged conditions as a mechanism to escape the water. On the contrary, no variation in lignin content was found in the submerged internodes of lowland rice, suggesting that lignin reduction upon flooding is not a universal mechanism in plants[39]. Recently, it was observed that waterlogging of waterlogging-tolerant hot pepper (*Capsicum annuum*) resulted in differential expression (primarily upregulation) of various genes of the phenylpropanoid biosynthesis pathways, which are responsible for the biosynthesis of several lignins[44]. As a result, the researchers hypothesised that enhanced lignin deposition aids hot pepper in developing resistance against waterlogging, which is in contrast to the lignin-reducing phenotype observed in other plant species[26,32,45]. It remains complicated to determine what function lignin exactly plays concerning flooding stress, given the variety of functions that have been associated with it.

Cell wall integrity sensing and signalling of non-flood-induced abiotic stress

One of the first plant factors affected by abiotic stresses are the mechanical characteristics of cell walls, as well as the disintegration of contact between the wall and plasma membrane[10]. Over the last two decades, accumulating evidence has emerged that plants have evolved devoted mechanisms which monitor the integrity of the cell wall and can induce adaptations in response to modifications of the cell wall integrity (CWI). CWI sensors, which are plasma-membrane-bound proteins that detect changes in the mechanical characteristics of the cell wall, play significant roles in these processes. For instance, alterations in biochemistry or mechanical forces are signals for CWI sensors to trigger downstream signalling pathways. In this chapter, we will provide an overview of literature-described CWI sensors and associated CWI sensing components in primarily responses to abiotic stress.

Scaffolding function of RLP44 in pectin-mediated cell wall modification

BR signalling induced by cell wall modifications has been reported to activate wall-modifying proteins, leading to cell wall remodelling[46]. For instance, disruption of the pectin de-methyl-esterification levels

via overexpression of PMEI enhanced the activation of BR signalling pathways, resulting in cell elongation in *Arabidopsis*. The phosphorylation levels of the plasma-membrane localised receptor-like protein RLP44 were reduced in the PMEI overexpression background, causing RLP44 to form direct interactions with BR receptor BRASSINOSTEROID INSENSITIVE1 (BRI1) and its co-receptor BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) (Fig. 3). What followed was the activation of BRI1 signalling, promoting transcriptional upregulation of cell wall-loosening enzymes such as PMEs and expansins.

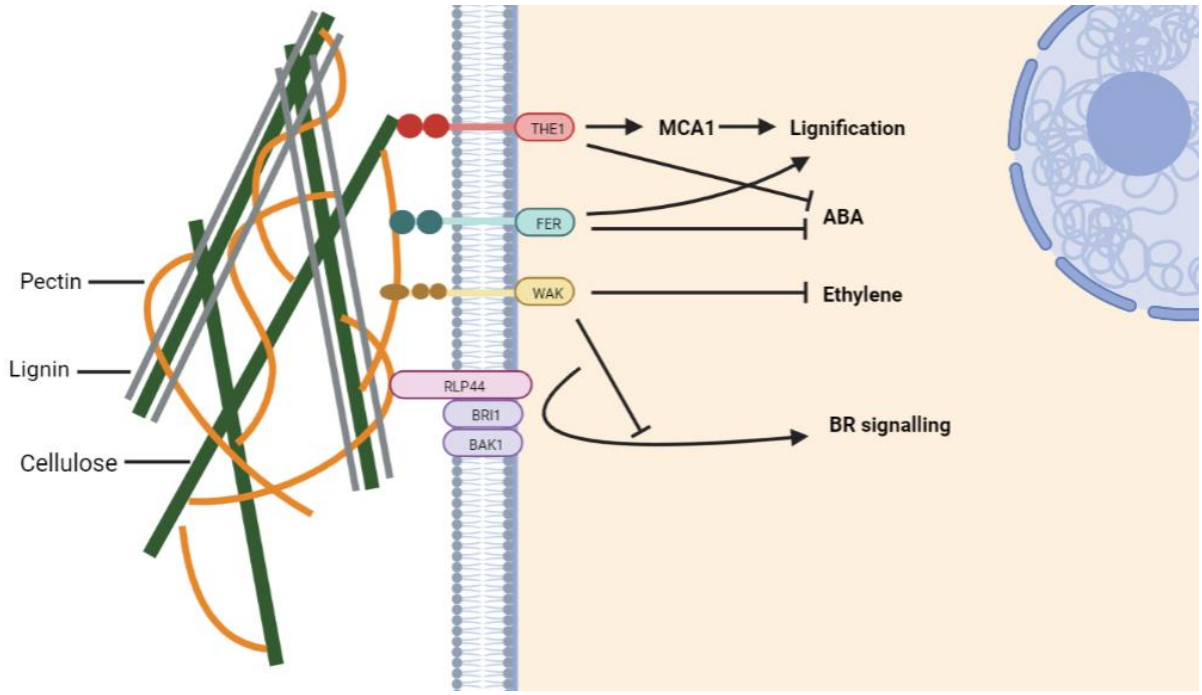

Figure 3. CWI monitor downstream signalling pathways of the plasma membrane-bound enzymes THESEUS1 (THE1), FERONIA (FER), wall-associated kinases (WAKs) and receptor-like protein 44 (RLP44). THE1 contains extracellular domains, which sense alterations in cell wall cellulose levels. When THE1 detects a drop in cellulose levels, it promotes MCA1-mediated lignification and inhibits ABA biosynthesis. FER, WAK and RLP44 are enzymes which sense de-methyl-esterified pectin levels in ratio to methyl-esterified pectin levels. In general, high de-methyl-esterified pectin levels activate the transduction pathways of these enzymes. Like THE1, FER promotes lignin deposition and restricts ABA signalling upon activation. Active WAK proteins limit ethylene biosynthesis and inhibit BR signalling via a BRI1/BAK1 mediated mechanism. RLP44 interacts with BRI1 and BAK1, promoting downstream BR signalling as a result.

Pectin integrity sensors: Wall-associated kinases (WAKs) and FERONIA (FER)

WAKs are receptor-like kinases with extracellular domains forming covalent bonds with pectins[10]. Several studies have revealed that the affinity of WAK proteins is greater for de-methyl-esterified pectin compared to methyl-esterified pectin[7]. Although not always unambiguous, as later findings revealed, it is believed that interaction with de-methyl-esterified pectin activates WAKs, triggering signal transduction pathways and eventually resulting in cell elongation promotion[10]. However, evidence recently presented for the rice enzyme OsWAK11 indicates a greater affinity for methyl-esterified pectin as opposed to de-methyl-esterified pectin, indicating organism-dependent specificity for WAKs' targets[47] (Fig. 4). WAKs have been linked to the perception of various abiotic stresses, for instance, heavy metal and high salinity stress. High soil concentrations of heavy metals (aluminium and copper) induced upregulation of genes encoding WAKs in *Arabidopsis* (*WAK1*) and rice (*OsWAK11*), indicating that WAKs are involved in sensing heavy metal stress, although the underlying mechanisms are still

uncovered[48,49]. In addition, Yue *et al.* observed that OsWAK11 acts as a mediator between BR signalling and alterations in the pectin methyl-esterification levels in rice cell walls[47]. The OsWAK11 protein levels stabilised in light conditions via a mechanism involving abundant de-methyl-esterified pectin levels. Once active, OsWAK11 directly phosphorylated the BR receptor OsBRI1, hindering OsBRI1 interaction with its co-receptor OsSERK1/OsBAK1 and inhibiting BR signalling, which resulted in the reduced growth of rice plants (Fig. 3). On the other hand, under low-light conditions, the ratio shifted toward a low abundance of de-methyl-esterified pectin, which caused OsWAK11 to phosphorylate and degrade, releasing the repression opposed on OsBRI1. Additionally, silencing of *OsWAK112* considerably increased the ability of plants to withstand salt stress, whereas heterologous *OsWAK112* expression in *Arabidopsis* caused a reduction of salt stress tolerance[50]. Results demonstrated that OsWAK112 negatively mediates plant responses to salt stress via inhibition of crucial processes in ethylene biosynthesis. It was revealed that OsWAK112 promotes the degradation of OsSAMS1, which synthesises S-adenosylmethionine (ethylene precursor) under high saline conditions. Supporting this, after salt treatment, overexpression of *OsWAK112* resulted in reduced ethylene and *OsSAMS* expression levels, eventually limiting the effectivity of ethylene to modulate salinity stress responses.

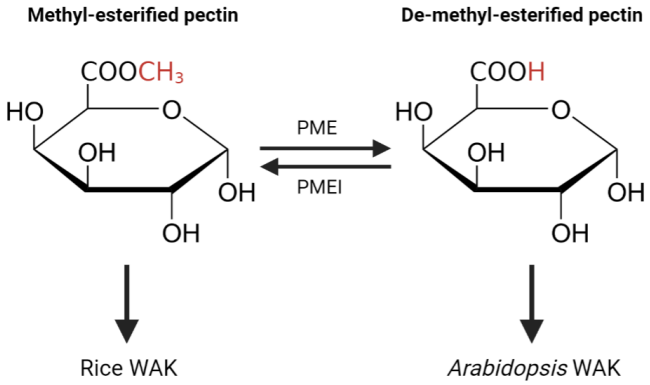

Figure 4. Variable pectin backbone configuration activates wall-associated kinases (WAKs), depending on the species. Pectin methylesterase (PME) activity generally promotes the activation of plant (*Arabidopsis*) WAK proteins by catalysing the formation of de-methyl-esterified pectin. Rice WAK proteins, on the other hand, are likely restricted by rice PME since they are activated by methyl-esterified pectin as opposed to de-methyl-esterified pectin. Pectin methylesterase inhibitor (PMEI) restricts PME activity. As a result, PMEI indirectly promotes methyl-esterified pectin and its ability to activate WAK proteins is reversed compared to PMEs.

FER is another CrRLK1L gene important to CWI sensing. Like WAKs, FER possesses extracellular domains that bind (de-methyl-esterified) pectin[10,51,52]. FER was indicated to be positively regulated by BR signalling since the expression levels of *FER* were constitutively active in BR-response mutants, and *FER* transcription levels were reduced in *bri1* mutants[53] (Fig. 5). In addition, responsiveness to 24-epibrassinolide was drastically decreased in *fer* mutants, providing additional evidence that FER mediates BR-responses[54]. Next to BR signalling, FER was also observed to be essential for ethylene-mediated hypocotyl growth reduction. This was indicated by loss of function *fer* mutants showing reduced growth rates compared to wildtype *Arabidopsis* seedlings when treated with ethylene[54]. However, according to the researchers, FER may not be a component of the ethylene signalling pathway. Instead, it was thought that FER is a critical promotor of the BR-induced responses, which alters the ratio between endogenous BRs and growth-inhibitory effects of ethylene on the hypocotyls. As a result, the FER-mediated enhanced BR signalling levels were proposed to modulate plants' ethylene responsiveness.

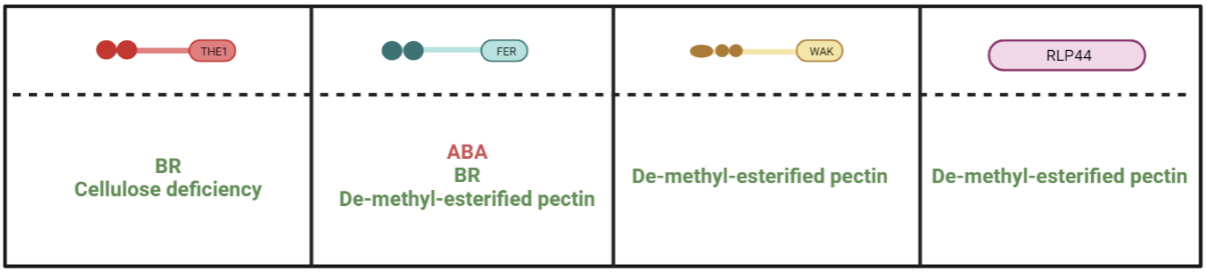

Figure 5. Overview of upstream regulators of cell wall integrity (CWI) sensors. The colour of the regulator indicates a positive (green) or negative (red) regulation on the CWI sensor.

FER is involved in detecting abiotic stresses and initiating responses to them[7]. For instance, mutant and transcriptional analyses indicated that FER is involved in sensing salt stress[52]. Wildtype *Arabidopsis* roots treated with saline conditions exhibit a pause in root growth, which was rapidly followed by a recovery of root growth. By contrast, *fer* mutants lack this growth recovery period. According to experiments, FER detected cell wall thinning brought on by saline stress situations. Once active, FER triggers downstream signal pathways via increasing cytosolic Ca^{2+} levels, promoting cell wall stiffening and root growth recovery. Furthermore, according to a recently accepted study, cold stress quickly activates the apple FERONIA receptor-like kinase gene *MdMRLK2*[55]. Transgenic plants overexpressing *MdMRLK2* increased apples' (*Malus domestica*) ability to withstand chilling stress. Increased cellulose, lignin, and hemicellulose levels were observed in transgenic plants with the *MdMRLK2* overexpression construct, indicating that FER contributes to the deposition of various cell wall molecules. This modification in the biochemical properties of the cell wall was thought to be one of the mechanisms by which *MdMRLK2* improves cold tolerance. Next to induction by cold stress, the expression and protein abundance of MdMRLK2 were also upregulated by ABA and drought treatments, which was reported to confer drought tolerance of apples[56]. *MdMRLK2* overexpression lines showed faster drought recovery periods and enhanced the photosynthetic rates when exposed to drought stress. Additionally, *MdMRLK2* overexpression increased the ABA hormone levels (1.51-fold) after seven days of drought treatment, which was suggested to contribute to drought tolerance of *MdMRLK2* overexpression lines (Fig. 3). Also, it was discovered that endogenous ABA levels promote the phosphorylation of FER, activating it as a result (Fig. 5). This finding implies that FER is negatively regulated by the ABA homeostasis levels, suggesting feedback loop between ABA and FER[57].

Cellulose integrity sensor: THESUS1

THESEUS1 (THE1) is among the better-characterised CWI sensors and one of the first CrRLK1L gene subfamily members identified[7,10,51]. THE1 was discovered in analyses of mutant screens as a suppressor of the enhanced hypocotyl length of the cellulose-biosynthesis-deficient mutant *procuste1-1* (*prc1-1*)[58]. Additionally, the hypocotyl growth of *the1* mutants, treated with the cellulose-synthase inhibitor isoxaben, was repressed. However, in a wildtype background and under non-stressed conditions, *the1* mutants did not exhibit this reduced growth phenotype, supporting that THE1 (partly) prevents hypocotyl growth reduction upon reduced cell wall cellulose content. In addition, in *the-1-4* (gain of function mutant) seedlings, isoxaben-induced wall stiffness was inhibited, providing additional evidence that THE1 negatively regulates cell wall stiffening upon cellulose deficiency[59]. Furthermore, *THE1* overexpression in a *prc1-1* mutant background increased the ectopic lignin deposition, indicating the lignin-depositing function of THE1[58]. Similarly, the lignin levels of *the1*/*prc1-1* double mutants were reduced in shoots and absent in roots. The combination of these findings revealed the involvement of THE1 in lignin deposition upon cellulose deficiency of the cell wall. According to genetic analysis experiments functional MID1-COMPLEMENTING ACTIVITY 1 (MCA1), a Ca^{2+} ion channel was required for THE1-mediated lignification of the cell wall induced by cellulose deficiency (isoxaben treatment),

revealing that MCA1 is downstream of THE1-mediated CWI regulation[60]. THE1 was additionally reported to contribute to drought stress tolerance. During drought stress, THE1 functions as a negative regulator of ABA biosynthesis and ABA-mediated processes[61]. Since enhanced ABA signalling is well-described to reduce turgor pressure, THE1 was suggested to raise turgor pressure in response to drought stress via an ABA-mediated mechanism. At last, mutant and overexpression analysis reported that THE1 was regulated by BR-mediated pathways in *Arabidopsis*[53], leading to cell elongation (Fig. 5).

Cell wall-mediated sensing mechanisms during hypo-osmotic stress

Cell wall signalling data under flooding stress are currently close to unavailable in the literature. Since hypo-osmotic stress is fundamentally linked to flooding stress[18], we will highlight reported cell wall signalling mechanisms as a response to hypo-osmotic stress.

Mechanical force sensors: Mechanosensitive channels

During hypo-osmotic stress, plants experience increased tension of the plasma membrane due to enhanced levels of turgor pressure[19]. Through plasma membrane-located mechanosensitive channels, cells can recognize these membrane tension changes. One of the best-studied families of mechanosensitive channels are members of the MscS-like (MSL) gene family[10]. MSL10 has been revealed to be involved in ROS-mediated PCD of *Arabidopsis* seedlings upon hypo-osmotic shock[62]. Cell swelling, induced by the combination of cellulose inhibitors and hypo-osmotic stress treatment, seemed to be sensed by MSL10. Consequently, MSL10 promotes Ca^{2+} influx in the cytosol, ultimately promoting PCD levels. Another mechanosensitive channel activated by hypo-osmotic stress is the plasma membrane-located MID1-COMPLEMENTING ACTIVITY1 (MCA1). Similar to MSL10, MCA1 is a mechanosensitive channel which senses increased pressure on the cell membrane, leading to activation[19]. Activation of MCA1 led to enhanced Ca^{2+} influx and ROS accumulation, triggering hypo-osmotic stress-induced signalling pathways. Interestingly, MCA1 and FER cooperate to regulate responses against hypo-osmotic stress. When roots were treated by hypo-osmotic shock, mutants lacking the receptor-like kinase FER displayed severely disrupted cytosolic Ca^{2+} signalling[63]. As a result, it was revealed that hypo-osmotic stress-induced transcription expression was significantly reduced in *fer* mutant backgrounds, indicating that FER is vital for the activation of hypo-osmotic stress responses.

Discussion: Potential cell wall signalling mechanisms in response to flooding stress

By monitoring alterations in the structural integrity of cell walls, plants can successfully acclimate to stressful abiotic conditions[8,10]. Alteration of the cell wall's mechanical properties and the subsequent sensing requires complex mechanisms. CWI sensing and signalling have been investigated for a variety of abiotic stresses, including drought, saline and cold stress[7,51,52]. Yet, our understanding of the function of CWI signalling in response to flooding stress is currently close to non-existent. Nonetheless, here, we present an overview of potential CWI maintenance signalling mechanisms upon flooding stress based on the available literature. It should be noted that these theories are speculated and yet to be experimentally validated.

Pectin methyl-esterification sensing via BR-mediated signalling in flooded plants

Pectin methyl-esterification: Potential regulator of root architecture modification

Numerous pectin methyl-esterification level sensing mechanisms were revealed to be mediated by BR signalling components. RLP44 and WAKs are enzymes activated by direct interaction with de-methyl-esterified pectin, causing them to promote and restrict BR signalling, respectively. BRs are plant hormones well described in the literature as enhancers of flooding tolerance in both LOES and LOQS strategy plants and are known to accumulate when exposed to flooding stress[10]. Modification of pectin de-methyl-esterification levels upon flooding stress has been reported in various plant species. However, inconsistencies exist regarding flooding stressed plants promoting or limiting pectin de-methyl-esterification levels. For instance, the upregulation of PMEs was reported in the shoots of submerged deepwater rice, indicating a reduction of de-methyl-esterified pectin[39]. By contrast, submerged soybean roots showed elevated PMEI protein abundance levels, which are enzymes widely recognised to inhibit the activity of PMEs[40]. Consequently, the authors believed that the de-methyl-esterified pectin level decreased in submerged soybean roots. The latter finding suggests that plants, upon flooding stress, like soybean, alter their pectin de-methyl-esterification levels as a signalling mechanism to convey flooding tolerance. We propose a de-methyl-esterified pectin-mediated mechanism which enhances flooding tolerance in the roots of flooded plants (Fig. 6C). In this hypothesised pathway, plants (soybean) promote PMEI protein levels in root tissue, decreasing the levels of de-methyl-esterified pectin as opposed to methyl-esterified pectin. Pectin-bound CWI sensors potentially sense these alterations and are activated or deactivated as a response. RLP44 was observed to be activated in PMEI overexpressing plants, triggering BR signalling responses and therefore is a plausible candidate to be involved in flood-mediated pectin sensing[46]. Another option would be the deactivation of WAKs in response to the decreased de-methyl-esterified pectin levels. In rice, OsWAK11 was revealed to restrict BR signalling by inactivating downstream responses of the BR receptor OsBRI1[48]. Both hypothesised pathways would result in enhanced BR signalling in flooded roots. It was revealed that BR signalling improves waterlogging stress in roots, supporting the theory for a PMEI-mediated promotion of flood tolerance in roots. Direct application of 24-epibrassinolide was shown to trigger waterlogging resistance via inhibition of soybean root growth, among others[64]. The authors believed that the waterlogging resistance of soybean was improved due to enhanced preservation of starch content. Since the hypothesised pathways lead eventually to increased BR signalling, this BR-mediated starch-preserving mechanism could be responsible for boosting flooding stress tolerance via a flood-induced PME-mediated signalling pathway.

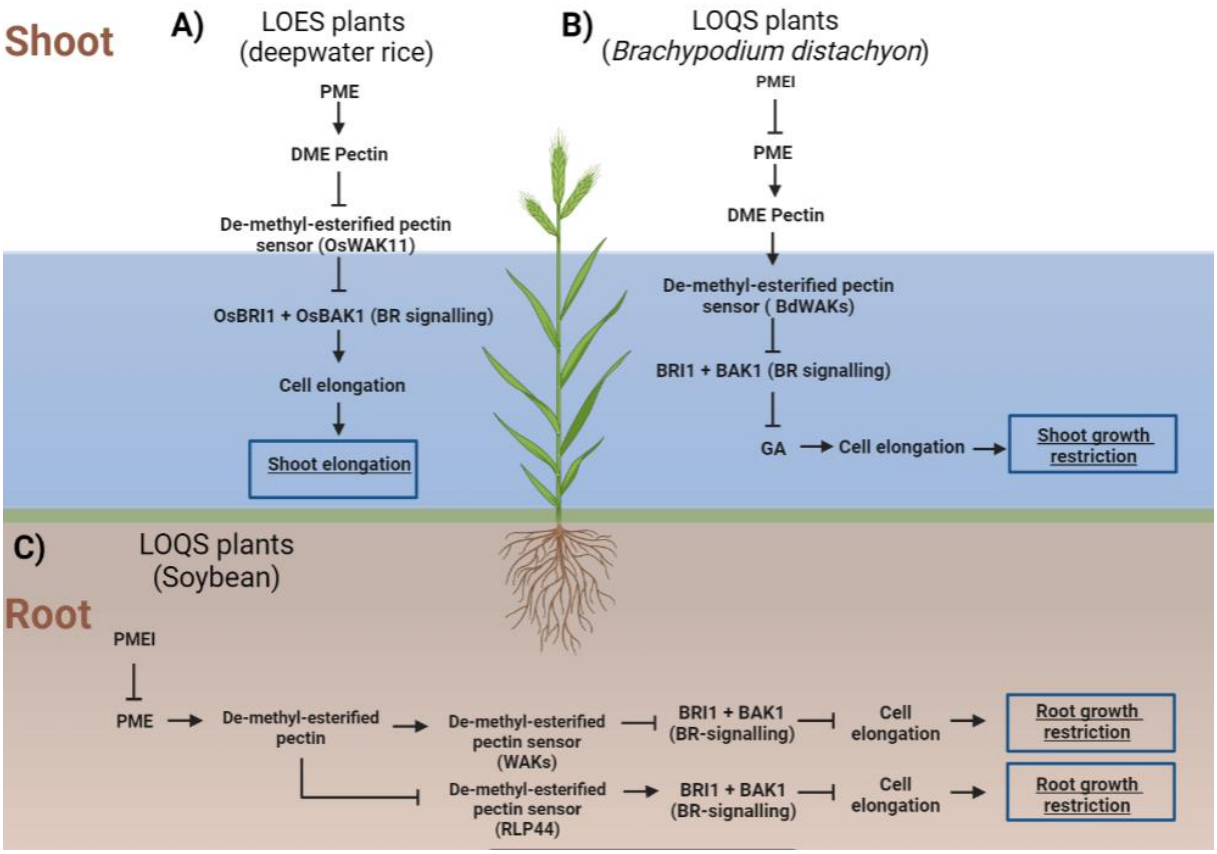

Figure 6. Proposed model of de-methyl-esterified pectin sensing in flooding stress conditions to promote/restrict root and shoot growth. (A) In this model, plants proactively modulate pectin de-methyl-esterification levels upon submergence by activating PME and PMEI transcription levels. The wall-associated kinase OsWAK11 degrades as DME activity rises in deepwater rice (LOES method), allowing the brassinosteroid (BR) receptor BRASSINOSTEROID INSENSITIVE1 (BRI1) and its co-receptor BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) to operate with greater efficiency. As a result, the increased BR signalling promotes BR-mediated shoot elongation. **(B)** On the other hand, we hypothesise that PMEI expression restricts PME activity in LOQS strategy plants, such as *Brachypodium distachyon*. As a result, BdWAK transcription levels are enhanced, which hinders interaction between BRI1 and BAK1, deactivating the downstream BR signalling pathway. In LOQS plants, BR signalling prevents the biosynthesis of the shoot elongation-promoting plant hormone gibberellic acid (GA). Eventually, this GA depletion restricts further shoot elongation during submergence, preventing exhaustion of energy reserves. **(C)** We speculate about a role for de-methyl-esterified pectin levels in the roots of flooded plants (soybean). In our proposed model, de-methyl-esterified pectin levels are restricted, via a PME-mediated mechanism in waterlogging stress conditions, as a result of elevated PMEI levels. Consequently, the modification in pectin structure is detected by WAK proteins or RLP44 or both, which promote the interaction between BRI1 and BAK1 to increase BR signalling. In the end, BR signalling conveys tolerance for waterlogging by, among other things, limiting root growth.

WAKs potentially regulate shoot elongation when submerged

Next to alterations to root growth, the literature provides indications for pectin de-methyl-esterification-mediated flood-induced growth responses in plant shoots. Deepwater rice are crops characterised by stem elongation as a mechanism to escape floods. Minami et al. observed enhanced PME expression levels in flooded shoots of deepwater rice, which was a counterintuitive finding given that PMEs promote pectin de-methyl-esterification, which typically limits cell elongation[39]. In light of

this discovery, the authors suggested that PMEs are linked to the cell wall-mediated hypocotyl elongation displayed by flooded deepwater rice. However, the underlying mechanisms were not further explored in this study. Understanding the reasoning behind the elevated PME transcription levels of submerged deepwater rice may be facilitated by WAK activity. WAKs are proteins that promote cell elongation by forming direct interactions with de-methyl-esterified pectin[10]. Recently, contradictory result regarding the pectin-mediated activation was revealed for OsWAK11 in rice. OsWAK11 was found to be stabilised by high levels of methyl-esterified pectin as opposed to WAK proteins in other plant species[47] (Fig. 4). Functional OsWAK11 restricted the activity of the BR-receptor OsBRI1, limiting the plants' downstream BR signalling pathway. This OsWAK11-mediated reduction of BR signalling was shown to be responsible for a reduced hypocotyl growth phenotype in rice. Due to the combination of these two findings regarding OsWAK11, we speculate the existence of a pectin-sensing and OsWAK11/BR-mediated mechanism that promotes shoot elongation in deepwater rice species. In this hypothesised pathway, deepwater rice upregulates the gene expression of PMEs, which enhances de-methyl-esterified pectin (reduces methyl-esterified pectin) levels. Following this pectin adaptation, WAK proteins, like OsWAK11, may detect these changes and are stimulated to be degraded as a response, eventually leading to the removal of BR signalling inhibition and, thus, restriction of BR-mediated shoot growth (Fig. 6A).

Next, we speculate that a similar PME-mediated mechanism is present in LOQS strategy species to restrict shoot growth in flooding stress conditions. It was revealed that submergence enhanced the expression of several WAKs in the LOQS strategy grass species *Brachypodium distachyon*[65]. These findings suggest that WAKs potentially contribute to the submergence-mediated hypocotyl growth-restricting phenotype seen in *Brachypodium distachyon*. The literature is still lacking regarding the de-methyl-esterified pectin levels in shoots of flooded LOQS strategy plants. Nonetheless, it is easy to envision that PMEIs accumulate in shoots due to the increased PMEI transcription levels observed in flooded soybean roots[40]. We speculate that WAK proteins sense decreased levels of de-methyl-esterified pectin in LOQS strategy plants due to enhanced PMEI levels in flooded shoots, which eventually would lead to enhanced BR signalling. Since BR signalling was shown to restrict shoot growth of LOQS strategy plants[24], we speculate that WAKs are responsible for enhanced BR signalling, causing a restriction of shoot growth restriction in *Brachypodium distachyon* (Fig. 6B).

However, also contradictory data have been reported for this proposed flood -tolerance-enhancing mechanism mediated by de-methyl-esterified pectin. It was discovered that the rice WAK OsWAK112 limits ethylene biosynthesis under salt stress conditions[50]. Since ethylene is a plant hormone essential for the perception of flooding stress, the potential significance of flood tolerance-enhancing role WAKs is debatable. Plenty of unanswered questions regarding the role of WAKs in flooding stress remain. Which WAKs are active under flooding stress and whether they restrict ethylene biosynthesis in these circumstances are still unknown. In addition, it is important to note that not all de-methyl-esterified pectin-mediated downstream responses require the activation of pectin-bound CWI sensors. For instance, when multiple residues of the pectin backbone are de-methyl-esterified pectin, the negatively charged carboxyl groups can form calcium bonds with other pectin molecules[38]. This pectin crosslinking increased the cell wall hydration levels, which has been shown to impact the cell wall's biomechanical characteristics, such as increasing stiffness[66]. Considering this, alteration in PME activity during submergence does not necessarily correspond to CWI sensor activity.

The literature presents limited indications for the contribution of THE1 and FER in enabling flood-induced growth responses.

THESEUS1

In literature, the signalling pathways associated with THE1 activation resemble those seen in flood tolerance-enhancing pathways. For example, THE1 activates upon the perception of cellulose level reductions[7,58]. The literature indicates that the cellulose content of flooded plants typically decreases upon flooding, suggesting possible activation of THE1 in flooded plants[29–33]. In addition, THE1 was revealed to be a positive regulator of BR-mediated elongation and a negative regulator of ABA signalling responses, which are both coincidentally also shoot growth-promoting mechanisms characterised as enhancers of flood tolerance[5,53,61] (Fig. 2A & Fig. 5). THE1-downstream signalling pathway was also revealed to be mediated by the mechanosensitive channel MCA1, which is a protein involved in triggering hypo-osmotic stress-induced signalling pathways[19,60] (Fig. 3).

Despite some regulatory pathways of THE1 resembling those seen in flooded plants, THE1-related plant responses are substantially distinct from flood-induced responses. Several experiments revealed that cellulose-deficiency-mediated activation of THE1 led to the enhanced deposition of lignin in the cell wall[58,60]. These findings are contrary to the decreased lignin levels often observed in plants exposed to flooding stress[27,31,32,39,45]. Furthermore, it was found that THE1 contributes to growth promotion via BR-mediated cell elongation and prevention of hypocotyl growth reduction via stiffening of the cell wall[53,58]. However, not LOES strategy plans, but those using the LOQS strategy tended to reduce their cellulose content upon flooding[29–33]. Species that use shoot elongation as a mechanism to escape floods, like deepwater rice, showed no change in cellulose content[39]. The growth-inhibiting nature of LOQS strategy plants upon flooding stress conflicts with the cell-elongating activity of THE1. Together, these results indicate that a role for THE1 in conveying flood-mediated growth alterations is improbable. However, the contribution of THE1 initiating flood tolerance-enhancing adaptations cannot be ruled out due to the signs of THE1 activation in flood conditions and molecular factors downstream of THE1 not being studied prior in flooding stress conditions.

FERONIA

Like THE1, the activation pathway of FER overlaps (partly) with elements seen in plants experiencing flooding stress. For instance, the hormonal regulation of FER is consistent with flood-tolerance-inducing plant hormones. ABA and BR were revealed to impact FER activity respectively negatively and positively[53,57] (Fig. 5). Upon flooding, ABA functioned as an inhibitor of hyponastic growth, and its hormone levels were decreased[57,61]. In addition, the BR hormone levels were revealed to be enhanced upon flooding stress and contribute to flood tolerance in both LOES and LOQS strategy plants by promoting and restricting growth, respectively[5]. Another indication that FER aids in flooding stress signalling is its reported function in hypo-osmotic stress sensing. The upregulation of genes induced by hypo-osmotic shock was revealed to depend on the transmission of crucial intracellular signals by FER[63]. Since hypo-osmosis is a well-known frequent side effect of flooding stress[18], FER is hinted to be involved in sensing flooding stress. Finally, FER was discovered to activate in the presence of de-methyl-esterified pectin, which is a feature assumably present in submerged deepwater rice due to the upregulation of PME-coding genes[39].

Even though the regulation pathway of FER resembles signals that induce flood tolerance, it is unlikely to contribute to flood stress signalling due to its downstream responses not coinciding with flood-induced plant adaptations[52,55]. FER was revealed to be essential for root growth during saline-stressed plants by stiffening the cell wall[52]. In addition, overexpression of its homologue *MdMRLK2* in apple

trees enhanced the cellulose and lignin concentrations, boosting the resistance to cold stress[55]. These FER-induced responses are in contrast with the root growth restriction and cellulose and lignin reductions seen in plants experiencing flooding stress[27]. Similar to THE1, it is important to note that we cannot exclude the involvement of FER in the signalling of flood tolerance-enhancing mechanisms due to the literature lacking data on the molecular activity of FER during flood conditions. In the event of a flood, FER and THE1 may are activated, initiating mechanisms that are currently still unidentified. The activity levels of these proteins during flood conditions should be investigated to clarify this issue.

Cell wall modification as a facilitator of PCD in flooded roots.

When plant roots are under water stress, they stimulate the development of oxygen transport-enhancing organs. Two examples of oxygen transport-enhancing organs are adventitious roots and lysigenous aerenchyma. Studies revealed that the development of both organs requires some degree of root tissue PCD[5,13,14,16]. Cortex cell PCD and epidermal cell PCD are essential for lysigenous aerenchyma and adventitious root development, respectively (Fig. 7). In legume species, it was found that cell walls, specifically those closest to the starting point of aerenchyma formation (in the vascular stele), showed elevated levels of pectin de-methyl-esterification[42]. De-methyl-esterified pectin-mediated PCD may be caused by PME activity, which increases de-methyl-esterified pectin and increases susceptibility to pectin-degrading enzymes[37]. The authors believed that pectin de-methyl-esterification is a tightly regulated mechanism in legume roots, which locally facilitates enzymatic degradation of cell walls and PCD of aerenchyma-forming cells.

Another study revealed that the mechanosensitive channel MSL10 induced PCD via a ROS-mediated mechanism in *Arabidopsis* seedlings[62]. MSL10 perceived cell swelling signals in *Arabidopsis* when exposed to hypo-osmotic stress triggering downstream responses that eventually led to PCD. It has been indicated that ROS signalling and the perception of mechanical forces are essential factors triggering epidermal PCD during adventitious root development[16]. Therefore, we suspect there potentially is an adventitious root-developing mechanism in flooded plants, which is mediated by cell membrane force sensed by MSL10. Intriguingly, chemical disruption of cellulose led to enhanced efficiency of both MSL10-mediated and de-methyl-esterified pectin-mediated PCD by allowing cell expansion and unmasking of pectin de-methyl-esterified pectin binding sites, respectively[42,62]. Transcriptomic analysis of submerged soybean root tissue revealed a downregulation of cellulose biosynthesis genes and an upregulation of cellulose degradation genes, indicating that flood stress triggers cellulose reduction in plant roots[30]. It is, therefore, plausible to believe that plants actively reduce the root cellulose content in response to flooding stress as an approach to promote the development of AR and aerenchyma (Fig. 7).

In addition, it was revealed that plants deposit lignin to flood-induced AR and the outer cell layers of the aerenchyma (ROL barriers) to limit radial oxygen loss[5,14]. Interestingly, THE1 is a plant CWI sensor that enhances lignin deposition upon the perception of cellulose reduction in the cell wall[58,60]. These findings, along with the observation that the regulatory pathway of THE1 resembles flood signalling components (See*; THE1 and FER CWI sensing are likely insignificant for flood-induced growth responses*), it is tempting to speculate that THE1 is activated as a response to the cellulose reductions observed in flooded soybean roots. THE1 would subsequently promote the formation of lignin-mediated ROL barriers in AR and the outer cell layers of aerenchyma, enhancing oxygen transport to the root tip (Fig. 7).

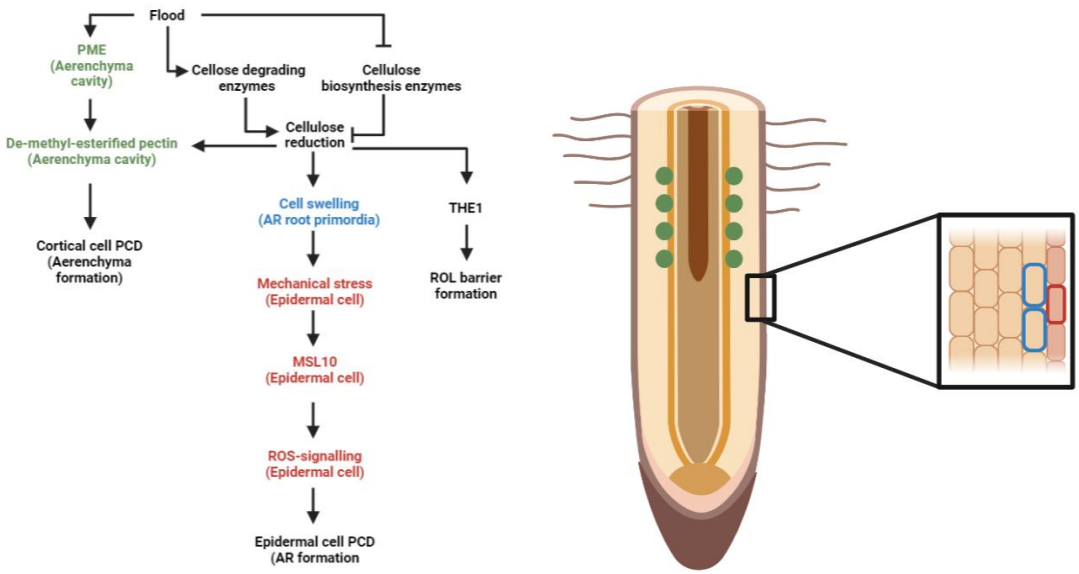

Figure 7. Proposed model for the role of cell wall integrity (CWI) sensors in flood-induced programmed cell death (PCD) in root tissue. The colours of the signalling components represent the cell type visualised (cell location is indicated by the corresponding colour in the schematic root). Aerenchyma cavity cells are marked by green dots, AR root primordia are marked by blue-outlined cells, and root epidermal cells are marked by red-outlined cells. In this model, PMEs are activated at cell specific locations in the aerenchyma cavity. Consequently, de-methyl-esterified pectin is promoted and followed by cortical PCD necessary for aerenchyma development. In addition, flooding stress reduces cellulose carbohydrate levels in root cell walls, which (1) promote pectin de-methyl-esterification in the aerenchyma cavity, (2) allow cell swelling of the adventitious root (AR) primordia, and (3) activate THE1 and THE1-mediated lignin fortification as a mechanism of radial oxygen loss (ROL) barrier development. Mechanical stress is exerted on the overlying epidermal cell by the swelling of adventitious root primordia as a result of the combination of hypo-osmotic shock and a decrease in the level of cellulose in the cell wall. In response to these signals, the membrane-bound protein MSCS-LIKE10 (MSL10) activates ROS-mediated PCD of the epidermal cell, enabling the AR root to expand beyond the epidermis.

Conclusion and future research suggestions

In conclusion, we believe the currently available literature provides adequate data to hypothesise that some degree of cell wall signalling occurs during flooding stress. In this thesis, we propose a model in which pectin-bound sensors, such as WAKs or RLP44, act as gatekeepers of flood-mediated growth responses by perceiving the PME-mediated pectin de-methyl-esterification alterations and triggering growth responses, which correlate with those seen in submerged plants and conveying flooding stress tolerance (Fig. 6). Next to the role of de-methyl-esterified pectin in growth responses, de-methyl-esterification of pectin in the aerenchyma cavity has been suggested to trigger essential signalling pathways, enabling aerenchyma development[42]. It appears probable that plants closely control the rate of de-methyl-esterification levels (in roots) as a means of communicating with systems that enhance flooding downstream. Future research should address whether and how de-methyl-esterified pectin impacts plants' ability to withstand flooding.

Our current understanding of signalling mediated by pectin de-methyl-esterification upon submergence is limited. Future research should first determine the impact of pectin de-methyl-esterification on the growth responses of roots and shoots during submergence and how these changes

affect the flood tolerance of the plants. The rice PME-coding genes *Os01g0880300* and *Os01g0312500* would be good candidates for knock-out or overexpression mutant analysis since they were found to be increased during submergence in deepwater varieties[39]. It would also be interesting to investigate soybean PMEI protein Glyma03g03460.1 because it showed a high increase in protein quantity and activity in roots after four days of the flooding stress treatment[40]. These mutant analyses can be verified and combined with immunostaining experiments to determine the spatial orientation and quantity of de-methyl-esterified pectin. To do this, we suggest adopting the immunostaining approach utilised by Pegg et al., who visualised different levels of pectin methyl-esterification using the monoclonal antibodies JIM7, JIM5, and LM19[40]. If the data acquired is promising, the putative pectin-sensing role of WAKs and RLP44 during flooding stress could potentially be determined afterwards.

Next, we propose a cell-wall mediated mechanism of flood-induced ROS, contributing to flooding stress tolerance (Fig. 7). We hypothesise that cell swelling of the AR primordia cell is creating mechanical stress on the epidermal cell, which is sensed by MSL10, which consequently trigger ROS-mediated PCD. Valuable knowledge can be gained by examining *msl10* mutants under waterlogging stress. Most notably, an answer would be provided as to whether MSL10 influences flood-induced AR root growth. We recommend future research using trypan blue staining tests, as described by Crowley et al.[67], to quickly assess PCD levels in wildtype and *msl10* knock-out mutants upon flooding. Interestingly, cellulose reduction of the cell wall increased both PCD mediated by MSL10 and mediated by pectin de-methyl-esterification[42,62]. In addition, a cellulose reduction in the roots of soybean plants was also proposed[30]. However, the correlation between cellulose reduction during flooding stress and flood-induced PCD has not been experimentally validated in the literature. To address this, future experiments should combine flooding stress and a cellulose-synthase-inhibitor isoxaben treatment in *Arabidopsis* or legume plant species and evaluate the development of oxygen enhancing organism.

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