Cell wall integrity maintenance during plant development and interaction with the environment

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Abstract

Cell walls are highly dynamic structures that provide mechanical support for plant cells during growth, development, and adaptation to a changing environment. Thus, it is important for the plant to monitor the state of the walls and ensure their functional integrity at all times. This monitoring involves perception of physical forces at the cell wall–plasma membrane interphase. These forces are altered during cell division, morphogenesis and in response to various abiotic and biotic stresses. Mechanisms responsible for perception of physical stimuli involved in these processes have been difficult to separate from other regulatory mechanisms perceiving chemical signals like hormones, peptides or cell wall fragments. However, recently developed technologies in combination with more established genetic and biochemical approaches are beginning to open up this exciting field of study. Here we will review our current knowledge of plant cell wall integrity signaling using selected recent findings and highlight how the plasma membrane-cell wall interphase can act as venue for sensing changes in the physical forces affecting plant development and stress responses. More importantly, we discuss how these signals may be integrated with chemical signals derived from established signaling cascades to control specific adaptive responses during exposure to biotic and abiotic stresses.

Introduction

Plant cell walls contain the turgor pressure prevalent in plant cells and provide structural support during growth as well as protection from biotic and abiotic stresses. They perform these different functions by changing their composition and structure in response to internal and external stimuli. The ability to change dynamically has been summarily described as cell wall plasticity and identified as one of the reasons for the limited success of past attempts to facilitate energy and food production from plants by genetically manipulating cell wall composition and structure [1,2]. A key element of plasticity seems to involve a mechanism monitoring functional integrity of the cell wall and initiating compensatory responses when cell wall integrity (CWI) is impaired. CWI impairment is caused by cell wall damage (CWD), which can arise during exposure to biotic or abiotic stress, or development[3–5]. The extent of CWD can vary from mild strain (leading to reduced CWI) to severe mechanical distortion (lost CWI), and depending on the cause, may also be accompanied by release of cell wall fragments.

Here we summarize our knowledge regarding the cell wall components directly relevant for CWI maintenance in primary cell walls, followed by a short overview of recent advances in analytical technologies. These should enable us to characterize the processes responsible for the dynamic changes in cell wall composition and structure in a non-invasive manner with improved levels of temporal and spatial resolution. We will discuss both the possible involvement of CWI signaling in shoot apical meristem (SAM) patterning and regulation of cell cycle progression, summarize the contributions of CWI maintenance to biotic and abiotic stress responses and present concepts describing how the maintenance mechanism could interact with pattern triggered immunity (PTI) and contribute to turgor level maintenance.

Plant cell wall metabolism

In order to understand the mode of action of the CWI maintenance mechanism, it is necessary to consider certain cell wall metabolic processes relevant for cell wall (CW) formation,

58 modification and integrity maintenance. These include production of cellulose, pectins and lignin,
59 which form complex, dynamic and strong cell wall matrices permitting expansion during
60 development and adaptive responses to CWD arising [6]. Figure 1 provides a simplified overview of
61 the main components of primary plant cell walls. More importantly it also highlights chemical and
62 physical stimuli, which are detected at the plasma membrane (PM) – CW interface and initiate
63 responses by the plant cell. Cellulose is the main load-bearing component of both primary and
64 secondary cell walls and produced by CELLULOSE SYNTHASES (CESAs) [6]. Different CESAs
65 are active during primary and secondary cell wall formation with their localization at the PM being
66 tightly regulated [7]. Transport processes and components responsible for the dynamics of CESA
67 trafficking are exemplified by adaptor protein complexes like the TPLATE complex [8]. It consists of
68 proteins such as TPLATE, which recognizes CESAs and initiates clathrin-mediated endocytosis.
69 Previously it was shown that CESA movement during primary and secondary cell wall formation is
70 guided by microtubules, which contribute to microfibril orientation [9]. Interactions between CESAs
71 and microtubules also influence CESA complex velocity while the COMPANION OF
72 CELLULOSE SYNTHASE proteins 1 and 2 (CC1/2) and CELLULOSE SYNTHASE
73 INTERACTING protein 1 (CSI1) interact with both microtubules and CESAs to facilitate
74 intracellular CESA complex trafficking, stabilize the microtubules and prevent displacement due to
75 cellulose synthase complex movement [10,11]. Intriguingly once the microtubule-based patterns are
76 imprinted, secondary cell wall synthesis can progress even if microtubules are not present [12].
77 Microtubule organization and thereby cellulose microfibril orientation can be influenced by e.g.
78 light, hormones, and mechanical stimuli, illustrating how cellulose deposition can be reorganized in
79 response to mechanical and chemical stimuli [13]. They activate downstream responses, including
80 enzymes modifying availability of metabolites and carbon levels [14,15]. The tight integration of
81 energy and cellulose metabolism is further illustrated by the isolation of mutants causing resistance
82 to the CESA inhibitor C17, which affect cytochrome C maturation and activate mitochondrial
83 retrograde signaling [16].

Another class of cell wall components encompasses pectins, which consist mainly of the polysaccharides homogalacturonan (HG), rhamnogalacturonan-I and -II (RG-I/-II). The precursors are synthesized in the Golgi and transported to the PM, where they are processed and incorporated into existing cell wall structures [17,18]. Despite the recent progress in understanding pectin domain synthesis and modification, most of the processes required for pectin formation, deposition, processing and regulation are still not well understood. Pectic polysaccharides influence wall porosity and extensibility during cell morphogenesis [19]. Partial de-methylesterification of HG by pectin methylesterase inhibitors (like PECTIN METHYLESTERASE INHIBITOR6) is also an important modification responsible for the localization of cell wall modifying enzymes (like PEROXIDASE36), formation of cross-linked, gel-like networks in cell walls (including water binding capacity of seeds) and for cell-cell adhesion [20–22]. Pectin or pectin-derived degradation products (Oligogalacturonides, OGs) arising from cell wall degradation during pathogen infection have been implicated in responses to biotic and abiotic stress responses and can activate changes in calcium (Ca^{2+}) and reactive oxygen species (ROS)- levels [23–25]. Two different types of plasma membrane localized kinases have been shown to bind to pectic polysaccharides or OGs. Wall associated kinases (WAKs) can bind pectins in a calcium-dependent manner through their extracellular domains, which exhibit similarities to fibronectin domains in animals [26,27]. The second one is the receptor kinase FERONIA (FER), which belongs to the *Catharanthus roseus*-LIKE RECEPTOR-LIKE KINASE1-LIKE family (*Cr*RLK1L) [28,29]. Pectic polysaccharides could be of particular interest in the context of CWI maintenance, since they are more sensitive to mechanical deformation than other cell wall components like cellulose [4]. In parallel they are also chemically more accessible, allowing cross-linking with possible sensors to facilitate detection of mechanical deformation and release of signaling molecules like OGs, capable of activating defense responses.

While cellulose and pectin are highly abundant in primary cell walls, lignin is normally only found in secondary cell walls [2]. Our understanding of the processes giving rise to lignin production has improved profoundly during recent years [2]. Lignin protects plant cells from environmental

stress, provides structural support, and acts as diffusion barrier. The latter function is illustrated by the Casparian strip separating cortex and endodermis in the roots of plants [30]. Strip formation requires ROS provided by NADPH oxidases such as RESPIRATORY BURST OXIDASE HOMOLOG F (RBOHF) and ROS-metabolizing peroxidases, which catalyze oxidation of monolignols [31]. Both enzyme classes are guided to the strip domain by CASPARIAN STRIP DOMAIN PROTEINs (CASPs) as illustrated by the co-localization of PEROXIDASE64 and CASP1 [31,32]. Intriguingly, CWD-induced by cellulose biosynthesis inhibition triggers RBOHD-dependent lignin deposition in the root elongation zone [33]. This exemplifies an important function of lignin, namely reinforcement of cell walls in response to CWD, which occurs during enzymatic cell wall degradation, pathogen infection and exposure to abiotic stress [34,35]. More importantly, it suggests that lignin deposition is dynamic and adaptive, i.e. it can occur also in primary cell walls if required.

Technology development is creating new opportunities for plant cell wall research

Advances in the dissection of processes mediating CW formation are facilitated by dramatic improvements in analytical technologies available. In this context, particularly interesting are all-optical, non-contact methods such as fluorescence emission Brillouin micro-spectroscopy or automated indentation / deformation methods such as cellular force microscopy automated confocal extensometers in combination with (fluorescence) microscopy imaging [36–38]. These methodologies have several benefits over established methods, which often involved in vitro measurements and were limited to tissue surfaces. The techniques allow real-time, in vivo measurements of both static and dynamic systems in a fully automated manner with high resolution across several levels of size to investigate non-invasively the mechanical characteristics of individual cells and tissues both at the surface and in sub-surface tissue layers. Automated confocal extensometers allow the analysis of mechanical properties by applying (and measuring) forces ranging from 1 to 10mN to samples smaller than 2mm in a scanning mode with step sizes smaller than 50nm [38]. Cellular force

136 microscopy covers an even greater range up to centimeters with step sizes down to 2µm and
137 applicable forces up to two times the regular levels of turgor pressure, providing complementary
138 options for manipulation and analysis [37]. The automated, user-defined scanning protocols increase
139 user-friendliness and should facilitate uptake of the methods in the research community. However,
140 the most important difference is the ability to combine measurements of stress / strain at different
141 levels with simultaneous high-resolution imaging of subcellular processes.

142 While the previously described methods involve classical deformation experiments, acoustic-
143 optical Brillouin microspectroscopy in combination with fluorescence measurements elegantly
144 assesses mechanical information by detecting the changing interaction of light with thermal
145 vibrations arising from sample inhomogeneity [36]. This method can therefore be used for
146 characterizing the mechanical characteristics of sub-epidermal cell walls and mapping of root
147 mechanical characteristics where conventional deformation-based methodologies failed. The main
148 advantages made possible by the non-contact and label-free working mode are the feasibility of non-
149 invasive studies and the possibility to characterize the mechanical characteristics of sub-epidermal
150 cell walls and mapping of mechanical characteristics where conventional deformation-based
151 methodologies failed. Importantly, the user can simultaneously characterize the extracellular matrix
152 of a cell and cytoplasm stiffness in 3D with high resolution. While these novel analytical methods
153 provide higher resolution and more accurate information than ever before, combining them with
154 specialized software tools (like MorphoGraphX) will have the most profound effects [39]. This is
155 because such tools allow integration and analysis of data originating from magnetic resonance
156 imaging, scanning electron-, cellular force and laser confocal microscopy while automatically
157 correcting for technical errors (arising from sample curvature). Additionally, such tools will facilitate
158 the integration of data into simulation tools, which can be used to characterize growth direction,
159 anisotropy and local curvature in plant tissues [39]. Development of these hardware and software tools
160 is complemented by generation of other tools like highly selective oligosaccharide-based probes [40].
161 These form a complementary alternative to monoclonal antibodies, which have been used in the past

to study modifications of cell wall components like homogalacturonan during pectin synthesis. In parallel established experimental protocols have been modified to permit live-cell imaging of fast-moving organelles and cell components using spinning disc or epifluorescence microscopes [41]. These new methods will allow non-invasive, real-time, high resolution imaging of cell wall enzymes and the cytoskeleton. However, even modified protocols cannot address shortcomings related to optimal focal distance, making the continued development of new approaches for live-cell imaging essential. The recent technical developments outlined here will allow us to investigate the processes modifying cell walls in novel ways. They will enable us to simultaneously characterize the changes in mechanical forces prevalent and chemical signaling processes driving cellular events during development and plant environment interactions.

Plant cell wall mechanics and integrity signaling during development

Organ initiation in the shoot apical meristems (SAMs) of plants is controlled by a combination of different regulatory processes involving peptide-based signaling processes, phytohormones and is modified in response to mechanical forces [42–44]. Expression of SHOOT MERISTEM LESS (STM), a key regulator of SAM maintenance, is induced by micro-mechanical manipulation [45]. STM in turn inhibits cell differentiation in the SAM through changes in cytokinin levels and the cytokinin-regulated cell-cycle regulator CYCLIN D3;1 (CYCD3;1) [46]. Here we will focus on the interplay between mechano-perception and the phytohormone auxin since it allows us to illustrate in detail how exposure to mechanical forces and chemical signaling can lead to modifications of the cell walls surrounding SAM cells and long-term adaptation of SAM patterning. Changes in auxin distribution and responsiveness of cells to the phytohormone are major drivers in regulating organ initiation in the SAM [43]. The importance of auxin transport in particular is illustrated by loss of organ primordia (formation of pin-like shoots) in *pinformed1* (*pin1*) *Arabidopsis* plants [47]. *PIN1* encodes a plasma membrane-localized, polarly distributed auxin transporter, which is essentially required in plants. Mechanical deformation of cell walls causes a

188 transient influx of calcium (Ca^{2+}) into the cytoplasm of SAM cells from the apoplast [48]. Dependent
189 on the direction of cell wall deformation (either expansion or shrinkage), the Ca^{2+} influx patterns
190 seem to differ slightly and induce apparently PIN1 redistribution in the SAM. Interestingly, auxin
191 application can activate two RHO GTPases: RHO IN PLANTS2 (ROP2) and 6 [49,50]. ROP6
192 regulates the activity of RHO INTERACTING CRIB CONTAINING PROTEIN 1 (RIC1), which
193 interacts with and controls KATANIN, an enzyme modulating microtubule organization [51,52]. Such
194 auxin-dependent reorganization of microtubules leads to changes in the expression of genes
195 encoding enzymes modifying pectin (pectin methylesterase, PME) or loosening cell walls
196 (expansin, EXP; xyloglucan endo-transglucosylase / hydrolase, XTH) [53]. These alterations in gene
197 expression are correlated with changes in the pattern of organ initiation in the SAM. Local
198 application of active PME and EXP enzyme preparations as well as local modification of pectins *in*
199 *vivo* lead also to changes in the mechanical characteristics of the cell wall and initiation of
200 primordia growth [54,55]. Such modifications of cell wall composition and structure lead probably to
201 permanent changes in the mechanical forces prevalent in the SAM resulting in long-term adaptation
202 of organ patterning to mechanical forces. This limited overview illustrates how SAM exposure to
203 mechanical forces could lead to adaptive changes in SAM patterning and illustrates how advanced
204 our knowledge is regarding molecular mechanisms regulating adaptation.
205 By contrast we know very little about the mechanisms perceiving the mechanical forces or
206 detecting mechanical deformation of the cell walls leading to signal generation exemplified by the
207 initial transient Ca^{2+} influx [4]. The Ca^{2+} influx could be mediated by proteins belonging to families
208 implicated in hypo- (MID1-COMPLEMENTING ACTIVITY1, MCA1), hyper- (REDUCED
209 HYPEROSMOLALITY-INDUCED $[Ca^{2+}]_i$ INCREASE, OSCA) osmotic stress or wound
210 perception (CYCLIC NUCLEOTIDE GATED CHANNEL, CNGC; GLUTAMATE LIKE
211 RECEPTOR, GLR) [56–59]. MCA1 is of particular interest here since loss of the *Zea Mays* MCA1
212 homolog affects organogenesis and leaf formation and the protein is required for mechano- and
213 hypo-osmotic stress perception, i.e. processes underlying perception of mechanical deformation

[60,61]. Simultaneously, MCA1 is also an important component of the CWI maintenance mechanism, which detects CWD (arising also in response to mechanical deformation) and initiates adaptive responses [33,35]. Functional studies found that intact Ca^{2+}- signaling is required for CWI maintenance in *Arabidopsis* seedlings and that responses are sensitive to turgor manipulation while a genetic analysis found that MCA1 is dependent on THESEUS1 (THE1) [33,35]. THE1 was originally identified through its requirement for arresting cell elongation in response to CWD caused by reduced cellulose production [62]. THE1 (like FER) belongs to the *Cr*RLK1L family with 17 members in *Arabidopsis* (including ERULUS, CURVY1, HERKULES1 and HERKULES2) [63]. Several *Cr*RLK1L family members have been implicated in developmental processes and biotic as well as abiotic defence responses with FER being the best-characterized member [28,64]. It binds pectins in vitro, is required for salt stress resistance as well as mechano-perception in *Arabidopsis* roots and seems to act as a scaffold for signaling processes required during PTI and development, with the switching between the two mediated through binding by RAPID ALKALINIZATION FACTORs (RALFs) [28,64,65]. RALFs were originally identified through their ability to alkalinize the pH in tissue cultures, which is interesting here since binding of THE1 to RALF34 is pH dependent[66]. Intriguingly, several RALFs are regulated by cleavage by SITE-1 PROTEASE (S1P), similarly like PMEs [67,68] . Thus, S1P activity could regulate CWI and immune signaling as well as CW properties simultaneously, which suggests that S1P activity has to be tightly controlled [64]. Developmental activities of FER include gametophytic development, regulation of root hair formation through interactions with ROP2 in response to changes in auxin levels and general growth regulation as evidenced by *fer* plants, which are significantly smaller than wildtype controls [69]. While it remains to be determined if *Cr*RLK1L family members have an active role in SAM patterning, the available data suggest that *Cr*RLK1Ls have relevant functionalities and interact with molecular components (already implicated in SAM activity) in other developmental contexts. This raises the possibility that CWI monitoring components may be responsible for perception of

239 mechanical forces in the SAM and generation of signals feeding into mechano-sensitive SAM
240 patterning processes co-controlled by phytohormones like auxin or cytokinin.
241 The plant cell cycle is essential for growth and development in all plants [70]. Its activity is
242 tightly regulated through close interactions between dedicated signaling processes involving
243 phytohormones and cell-cycle specific regulatory elements [71]. The signaling processes allow
244 adaptation to changes in the metabolic state of cells to biotic or abiotic stress [72–74]. Phytohormones
245 (such as auxin and cytokinin) modulate cell cycle progression at different transition points and act
246 also in a tissue specific manner ensuring regulation with a high degree of temporal and spatial
247 resolution [71]. The cell cycle is simultaneously also controlling a large number of biological
248 processes, namely those required for cell expansion to ensure successful coordination of cell
249 division with growth and development [70,75]. This also includes *de novo* formation of cell walls
250 during cytokinesis and modification during cell elongation [75,76]. In animal and yeast cells, regulation
251 works also in the opposite direction. Cell cycle progression is influenced by the extracellular matrix.
252 Integrins and piezo channels act as mechano-sensors in animals, which connect the matrix with the
253 PM and regulate cell cycle progression [77,78]. In yeast, osmo-sensing, mechano-perception and CWI
254 monitoring cooperate to regulate cell cycle progression [79,80]. In elongating yeast cells CWI
255 monitoring also modifies cell expansion in response to changes in the mechanical characteristics of
256 the walls, implicating CWI maintenance both in regulation of the cell cycle and cell elongation [81].
257 In plants, knowledge regarding modification of cell cycle activity in response to mechanical forces
258 or CWI impairment is very limited [82]. Inhibition of cellulose biosynthesis by mutations in *CESA*s
259 leads to redistribution of PIN1 in the SAM and reduction in the mitotic index of SAM cells [83].
260 Similarly, cellulose biosynthesis inhibition in *Arabidopsis* seedlings causes cell cycle arrest in an
261 osmo-sensitive manner [84]. The arrest seems to occur at the G1-S-transition since it involves
262 CYCD3;1 [84,85]. Cellulose biosynthesis inhibition induces osmo-sensitive modifications of cytokinin
263 homeostasis, with the changes in cytokinin levels apparently being brought about by enhanced
264 expression of genes encoding cytokinin degrading enzymes [84]. In *nitrate reductase 1 nitrate*

265 *reductase 2* (*nia1 nia2)* seedlings, the inhibitor treatment effects on expression of *CYCD3;1* and
266 cytokinin degradation genes were absent [84]. *nia1 nia2* seedlings are frequently used as genetic tools
267 to study nitric oxide signaling in vivo since nitrate metabolism is impaired and nitric oxide
268 production reduced [86]. Genetic studies revealed that THE1-mediated CWI signaling is not required
269 for the effect on *CYCD3;1* expression and that NIA1 NIA2-mediated processes are downstream
270 from THE1 [84]. These results suggest that in plants mechano- and osmo-sensitive processes at the
271 CW-PM interface also influence cell cycle activity, similar to what has been observed in animals
272 and yeast [77,80]. While THE1-mediated signaling is not required for the regulation of cell cycle,
273 NIA1 NIA2 -mediated processes seem to have a key role, implying that THE1 is not the only CWI
274 sensor and that nitric-oxide mediated processes are essential [84]. The available knowledge suggests
275 that cell cycle progression in plants is modified in response to CWI impairment through a poorly
276 understood cytokinin-based process. In the SAM CWI signaling may also be active based on the
277 observed phenotypes and effects of *ZmMCA1* loss on organogenesis, but significant more data
278 needs to be compiled to gain mechanistic insights.
279
280 *Plant cell wall integrity signaling during plant environment interactions*
281 Plant pathogens frequently degrade plant cell walls during infection to gain access to the
282 nutrients in the cytosol [64,66,87–89]. The chemical signals elicited during this process are relatively well
283 known: plants have various receptors for sensing pathogen-associated molecular patterns (PAMPs)
284 and damage-associated molecular patterns (DAMPs) [4,23]. DAMPs are signals originating from
285 damaged plant cells and are capable of inducing defenses in unharmed cells [90]. DAMPs elicited
286 during pathogen attack include fragments of degraded CW polymers, extracellular ATP, and
287 endogenous peptides like elicitor peptide Pep1. Several cell wall-derived DAMPs, exemplified by
288 cellobiose and xyloglucan, have been identified [90,91]. Another type is represented by Pep1, which is
289 converted into the active form by the damage-induced, Ca^{2+}-dependent METACASPASE4 [92]. Pep1
290 is a core component of PTI, required for enhancing pathogen defense responses while also

291 influencing root growth together with auxin [93,94]. In parallel to chemical signaling, the pathogen
292 attack modifies also the physical forces prevalent at the PM-CW interphase; as the cell wall is
293 degraded, mechanical support needed to maintain plant cell shape is diminished. This may provide
294 additional information for the plant cell regarding the type of infection (e.g. compatible vs. non-
295 compatible, necrotrophic vs. biotrophic). However, the importance of this physical stimulus is
296 difficult to assess, is undoubtedly pathogen-specific, and our knowledge of molecular components
297 involved is limited. Loss of THE1 affects resistance to *Botrytis cinerea* while FER influences
298 resistance to *Pseudomonas syringae*, implicating CWI signaling in biotic stress responses [88,95].
299 However, in the case of FER, the results are challenging to interpret as FER mutant plants exhibit
300 altered cell wall composition under non-stress growth conditions and FER affects several hormone
301 signaling pathways [96]. Recent findings show that loss of PTI leads to enhanced CWI signaling while
302 intact PTI limits CWI-controlled responses via the DAMPs Pep1 and 3 (Figure 2) [35]. This implies
303 that PTI and CWI maintenance are coordinated during pathogen infection. It suggests also that CWI
304 monitoring could form a back-up system for compromised PTI by inducing defense responses in the
305 absence of functional PAMP/DAMP signaling. FER is emerging as a promising candidate for
306 mediating this CWI-PTI coordination since it seems to act as scaffold in two different complexes
307 found at the PM [64]. One complex contains both FER and the central PTI component BRI1-
308 ASSOCIATED KINASE1 (BAK1), while the other complex is devoid of BAK1 [64]. When
309 complexed with BAK1, FER facilitates PAMP-Pattern recognition receptor interactions, thus
310 directing the plant towards full activation of PTI (involving probably PEP1-based signaling). FER
311 switching between the two different complexes seems to be regulated by the apoplastic peptide
312 RALF23 [64]. RALF23 influences simultaneously FER-dependent inhibition of MYC2, which
313 modulates salicylic acid (SA) and jasmonic acid (JA) signaling [88]. In parallel, the interaction
314 between FER and RALF1 controls the activity of ABA INSENSITIVE2 (ABI2), a key regulator of
315 abscisic acid (ABA)-based signaling processes (Figure 2). FER inhibits also the activity of the PM-
316 localized H^+-ATPase PLASMA MEMBRANE PROTON ATPASE 2 (AHA2) in a RALF-

dependent manner, thereby increasing the apoplastic pH and inhibiting growth[97]. This change in the apoplastic pH may also modulate the function of other *Cr*RLK1L family members, since THE1 appears to be incapable of binding to RALF34 at low pH but capable of binding at high pH[66]. Thus, if FER-RALF interaction indeed increases the apoplastic pH, THE1 interaction with RALF34 would be enabled, possibly modifying THE1 activity in the apoplast. This supports the notion that FER forms the scaffold in complexes coordinating CWI signaling and PTI activation with specificity generated through interactions between FER and different RALFs. This implies also that availability of RALFs is an important determinant for activation of PTI or CWI signaling. The concept of RALFs having a modulating activity is further supported by results from the plant pathogen *Fusarium oxysporum*, which secretes a RALF-homolog as virulence factor, apparently to weaken plant defense responses by modulating defense signaling directly[98].

Simultaneously, it is important to remember that FER can apparently bind to pectic polysaccharides in vitro via the Malectin domain [28,29]. It remains to be determined if the binding in vivo is also directly via the Malectin domain or involves LEUCINE-RICH REPEAT EXTENSIN (LRX) family proteins [99]. At least in the context of controlling vacuole size during cell expansion, the LRX proteins appear to be necessary linkers between FER and CW, enabling FER to sense the physical state of the CW and regulate vacuole size accordingly [99]. Additionally, LRXs can interact with RALF peptides in vegetative tissues and during pollen tube growth [99, 100]. This suggests that LRXs may be important partners of *Cr*RLK1Ls in sensing both chemical and physical stimuli. This creates the possibility that changes in the surface tension of the wall or displacement of CW versus PM lead to conformational changes in FER (Figure 2). Such conformational changes could open up domains for interactions with RALFs and activation of dedicated signaling cascades, which would be similar to mechano-perception in animal cells exemplified by TALLIN and WSC1 in *Saccharomyces cerevisiae* [101]. These results suggest that *Cr*RLKs could perceive simultaneously qualitatively different stimuli (pH, RALF abundance and mechanical deformation) and integrate them to activate downstream responses in a highly specialized and quantitative manner. This

concept raises the question if (and how) the pH levels in the apoplast may change during pathogen infection.

Several types of abiotic stress, such as drought, salinity and hypo-osmotic stress, affect the physical forces at the PM-CW interphase [102–104]. These changes are probably detected by the plant cell and function as indicators of abiotic stress. During hyperosmotic stress water escapes the protoplast leading to shrinkage and distortion of the PM-CW interphase since the stiff cell wall cannot shrink in a similar manner [105]. Hypo-osmotic stress would have the opposite effect, causing enhanced PM stretch by pushing the PM outwards. Stresses reducing the strength of the CW should have effects similar to hypo-osmotic stress, since the high levels of turgor pressure prevalent in plant cells in combination with a weakened CW would also lead to PM stretch. These phenomena can be studied in a relatively controlled manner in *Arabidopsis* seedling liquid culture, where cellulose biosynthesis inhibitors or cell-wall-degrading enzymes are used to weaken the CW (cause expansion), and turgor pressure levels can be manipulated through addition of osmotica like sorbitol to the growth medium (causing shrinkage) [106]. In this experimental system, stress responses including phytohormone accumulation, gene expression changes, and lignin deposition triggered by weakened cell walls are suppressed by co-treatment with osmoticum, indicating that CWI signaling induced by cell expansion is turgor sensitive [33, 35]. Identical effects have been reported for *S. cerevisiae*, implying that conditions may be comparable between yeast and plant cells [79]. This is further supported by WAK2 modulating invertase activity in Arabidopsis in response to changes in turgor pressure [107]. These observations would suggest a simple working model, where perturbations at the PM-CW interphase are sensed either as shrinkage (hyper-osmotic) or expansion (hypo-osmotic stress) of the protoplast (Figure 3). Perception of the hyper-osmotic stress involves probably ARABIDOPSIS HISTIDINE KINASEs (AHKs), ion channels like OSCA1, CNGC or GLRs and ion transporters like the K^+ exchange antiporters [56,57,59]. Hypo-osmotic stress sensitive channels like MCAs and chloroplast-localized MSLs would allow detection of expansion [58, 108]. However, a subset of CWI sensors detecting expansion could also be activated when the protoplast

369 shrinks due to hyperosmotic stress because parts of the PM, which remain connected to the CW
370 during plasmolysis (Hechtian strands) are probably being stretched [105]. Therefore, it is conceivable
371 that some of the sensors detecting PM stretch could be activated in response to both shrinkage and
372 expansion. Since their activity would be combined either with sensors detecting hypo-osmotic or
373 hyper-osmotic stress the cell receives much more detailed information about the state of the PM-
374 CW interface. *Cr*RLK1Ls like FER and THE1 could represent such sensors capable of monitoring
375 CW-PM perturbation in both directions since they reside in the PM, have an extracellular domain
376 possibly anchored in the CW and could thus undergo conformational changes in several directions.
377 This is supported by FER controlling gene expression in response to hypo-osmotic stress and
378 suppressing the response to ABA [65, 109]. Intriguingly, FER detects CWI impairment caused by salt
379 stress and prevents premature growth initiation, since *fer* seedling roots exhibit initial growth
380 recovery after exposure to salt stress followed by bursting cells possibly due to weakened cell walls
381 [28]. FER is not required for production of an initial Ca^{2+} influx in response to mechanical
382 perturbation but for a secondary, delayed Ca^{2+} peak, supporting the concept that FER mediates
383 delayed adaptive responses [28,65]. Regardless of FER's exact role in expansion signaling, the ABA-
384 FER connection allows coordination of cell shrinkage versus expansion by controlling the activity
385 of ABI2, which in turn regulates FER and ABA signaling [109]. By contrast, THE1 seems to control
386 responses like phytohormone production and lignin deposition only during challenging growth
387 conditions (like etiolation) or in response to drastic CWI perturbation (like inhibition of cellulose
388 biosynthesis or enzymatic cellulose digestion) [33,35,64]. This suggests that THE1 may function only as
389 expansion sensor during extreme conditions, which is supported by THE1 mutant seedlings and
390 plants exhibiting generally milder phenotypes than those of FER mutants and would imply that
391 *Cr*RLK1Ls may be involved in similar cellular processes with different molecular activities. This is
392 supported by the available literature, implicating *Cr*RLK1Ls in biological processes closely
393 associated with plant cell walls [96]. Seedlings carrying loss of function alleles for the *Cr*RLK1Ls
394 *THE1* and *ERULUS* exhibit attenuated responses to cellulose biosynthesis inhibition, while loss of

function alleles for *FER*, *CURVY1*, *HERKULES1* and *HERKULES2* lead to enhanced responses [35]. The results for *FER* appear surprising at first glance, since they seem to contradict the previously postulated requirement of FER in sensing the loss of CWI. However, these results actually support the concept that FER is required for recovery from loss of CWI. If FER is coordinating growth onset with CWI recovery after exposure to CWD then loss of FER will lead to breakdown of coordination. So, growth starts prematurely despite cell walls being not yet sufficiently reinforced. Too weak cell walls being exposed to regular plant growth or cellulose biosynthesis inhibition lead then to enhanced activation of CWI maintenance responses, because they are more stressed than the respective controls.

Assuming that *Cr*RLK1Ls are involved in CWI monitoring, the available data regarding *Cr*RLK1Ls suggest that CWI monitoring is probably active in a large number of biological processes. We have decided to focus here on the adaptation to osmotic stress and cell expansion to provide a simplified model how CWI monitoring may enable plant cells to dynamically monitor the state of the PM–CW interface and integrate the resulting signals with those originating in other cellular processes (Figure 3). If hyperosmotic stress arises, it is detected initially by components like OSCA1 as well as KEAs and leads eventually to ABA accumulation. Increased ABA levels lead to osmolyte accumulation, repression of growth through direct inhibition of AHA2 and possibly regulation of FER [108]. In case the stress response mechanisms manage to normalize turgor levels, growth resumes. If the opposite situation arises, i.e. cell expansion due to hypo-osmotic stress, oscillatory growth or cell wall weakening, a slightly different set of molecular components is involved. These seem to include initially only FER, MSLs and MCA1, with the latter ones responsible for ion influx into the cytoplasm. In parallel, FER activates ABI2, leading to inhibition of ABA-based signaling processes. Activation of FER results in responses like growth arrest, metabolic changes to reduce the hypo-osmotic stress and/or stiffen cell walls. If these countermeasures are sufficient to neutralize hypo-osmotic / expansion stress, growth resumes. In case they are not sufficient, or the cell is actually exposed to cell wall distortion, detected by CWI

monitoring components like THE1, growth arrest is extended and more importantly lignin, JA and SA production are ramped up to activate general stress responses. In this scenario the coordination between CWI- monitoring and ABA-mediated osmotic stress responses could be mediated by FER acting as scaffold with situation specific signaling processes being controlled by RALFs, other *Cr*RLK1Ls, or a combination thereof. Importantly this coordination enables the cell to adapt to situations ranging from pronounced hyper-osmotic stress to hypo-osmotic stress situations and cell expansion due to weakened cell walls (highlighted in the model by the orange / purple and red / grey triangles) in a tightly controlled and integrated manner.

Perspective

Research into the regulatory processes controlling plant growth, development and interactions with the environment has been extremely productive over recent years. While our knowledge and understanding of these processes have increased dramatically, our appreciation how mechanical forces contribute to the same biological processes in parallel has grown as well. Simultaneously it has also become obvious that our knowledge of the molecular mechanisms perceiving mechanical forces and controlling adaptive responses is still extremely limited despite significant research efforts. The main reason for this has been, that it was challenging to investigate *in vivo* the relevant mechanical forces and establish their contributions to the ongoing chemical signaling processes. However, this situation should change with the increased usage of the new methods discussed above. Maintaining CWI is essential in many biological processes and the existence of *Cr*RLK1Ls in various plant species suggests that CWI signaling may be active throughout the plant kingdom [63, 110]. Importantly CWI maintenance involves perception of mechanical forces and generation of signals, which are then integrated with signals from other processes like PTI to generate specific adaptive responses. This suggests that dissecting the mode of action of the CWI maintenance mechanism represents an opportunity to understand how mechano-perception is integrated with other signaling processes in general. More importantly CWI

maintenance seems to be an essential element of cell wall plasticity, thus understanding its mode of action may help us to address problems caused by plasticity [1]. Here we have only discussed a few selected examples, which highlight areas where we already have information providing mechanistic insights into contributions of CWI signaling to biological processes (biotic, abiotic stress) or where we expect to see CWI signaling being implicated in the near future (SAM patterning, CW-cell cycle coordination). These serve to illustrate the exciting state of this research topic and its future potential to yield interesting novel insights. More importantly we are optimistic that the application of several of the recently developed technologies we mentioned here will ensure that the potential will be fulfilled.

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Author contributions

L.V., J.S. and T.H. contributed equally to concept, outline and writing of the manuscript, including generating the figures.

Competing interests

The authors declare that no conflicts of interest exist.

Figure Legends:

Figure 1: Simplified plant cell wall model depicting main cell wall components as well as physical and chemical stimuli relevant during interactions between plants and their environment

Left (magnifying glass): Schematic of cellulose microfibrils (green bars) with hemicellulose (light blue lines) and pectin (orange lines) in (primary) plant cell walls. Additional biologically or metabolically active components present in the matrix are calcium (red dots), proteins and other solutes (both light green). CESA complexes (purple) align with microtubules (brown) and catalyze cellulose microfibril formation along the plasma membrane (dark blue). Each deposited cellulose microfibril is composed of 12 to 36 glucan chains (black lines) of β-1,4-linked glucose (Glc) molecules. The middle lamellae (taupe) is separating adjacent cells while components shown with low-opacity coloring are illustrating the spatial dimensions.

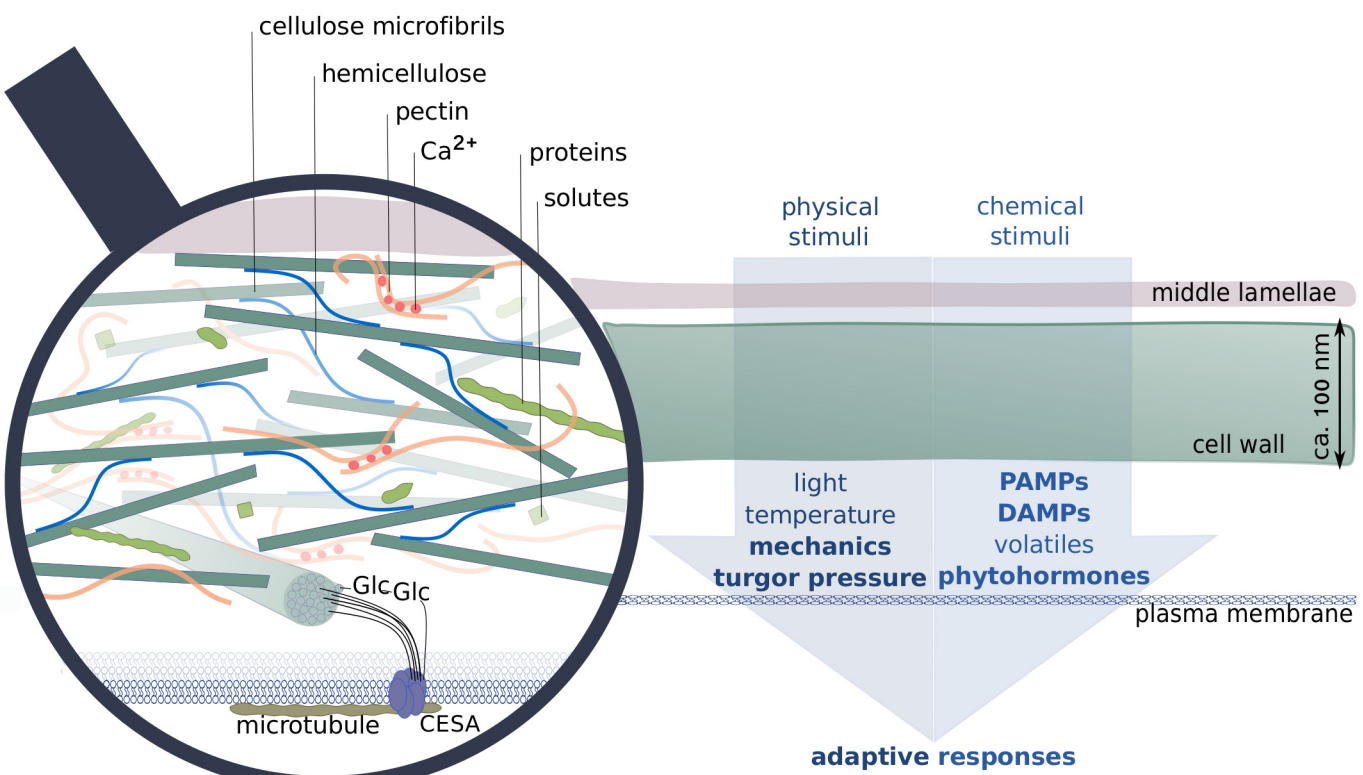
Right: Graphical summary of chemical and physical stimuli, which can affect the cell wall-plasma membrane continuum (indicated by two-colored arrow) and lead to CWI impairment activating the maintenance mechanism or activation of biotic and abiotic stress responses.

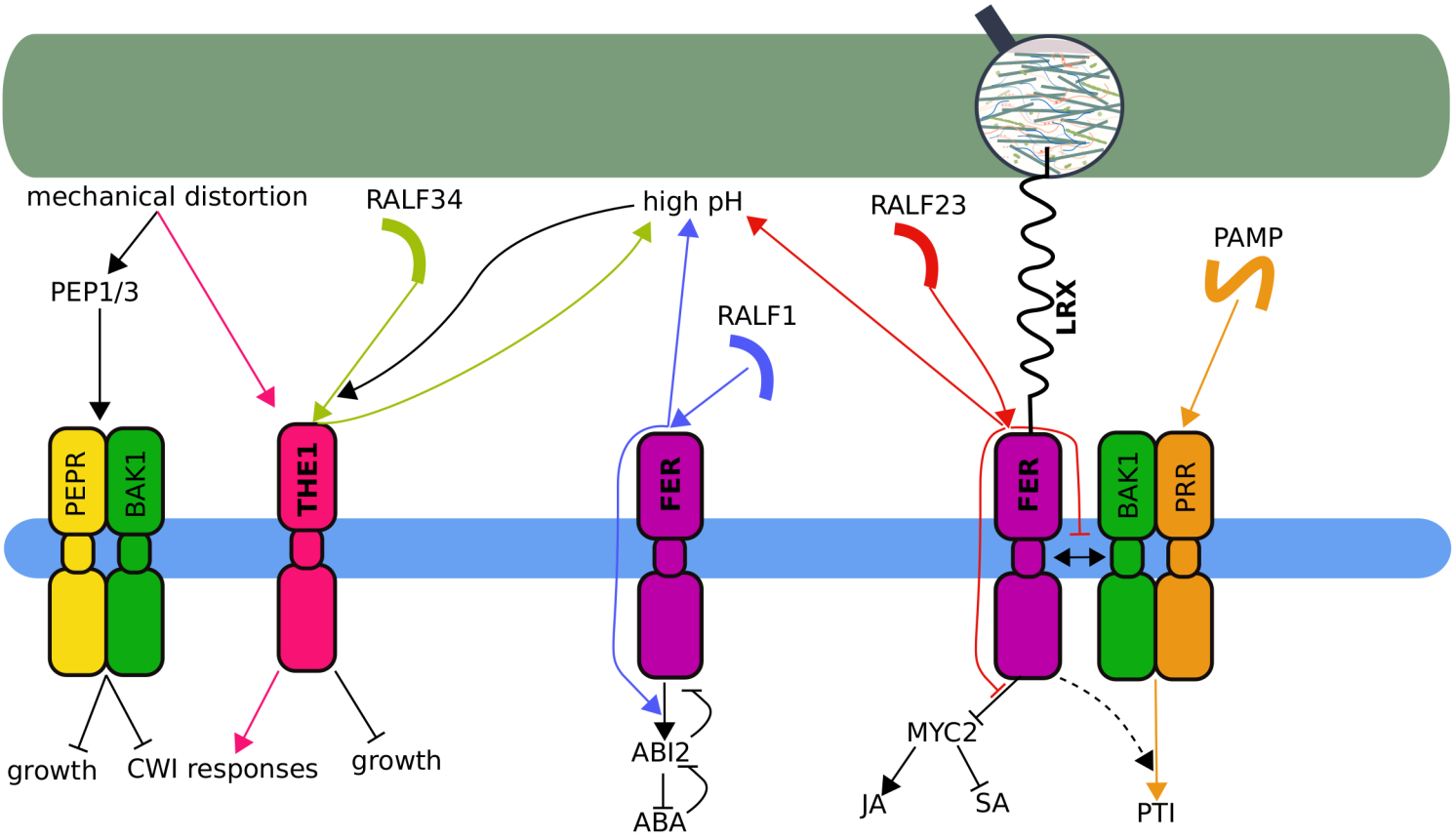
Figure 2 Functions of THE1 and FER at the intersection between chemical and physical signaling during biotic stress response

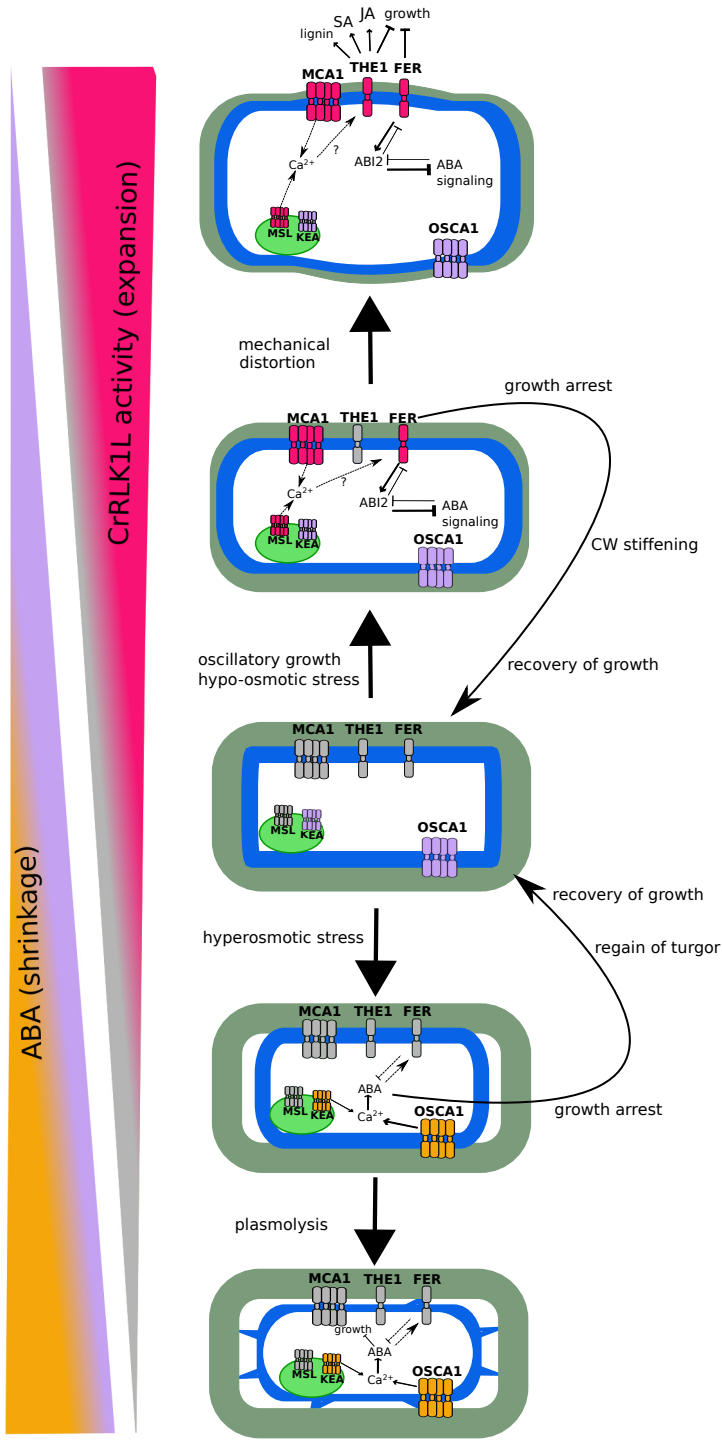
Mechanical distortion of the CW induces CWI responses through THE1 and leads to release of elicitor peptides PEP1 and PEP3, which suppress the CWI responses and growth in a PEPR1/2 dependent manner. In addition to mechanical distortion, the action of THE1 can be regulated by RALF34, which can bind to THE1 at high apoplastic pH and lead to further alkalinization of the apoplast. Both RALF1 and RALF23 can induce alkalinization of the apoplast in a FER dependent manner. Both of them also affect hormone signaling pathways through FER: RALF23 by inhibiting FER's de-stabilizing effect on the transcription factor MYC2, a master regulator of JA signaling; RALF1 by activating ABI2, a repressor of ABA signaling. In addition, RALF23 has been shown to inhibit FER's scaffold activity for pattern-recognition receptors (PRR) and their co-receptor BAK1, thus reducing sensitivity of the plant to respective PAMPs. In addition to being connected to chemical signaling through PAMPs, RALFs and phytohormones, FER is capable of sensing physical signals from the CW, possibly through LRX proteins linking FER to CW. These examples illustrate how THE1 and FER may be coordinating CWI signaling with DAMP/PAMP-induced PTI activation and phytohormone signaling. Green block represents the cell wall (CW), blue block represents the plasma membrane (PM). The white space in between the CW and PM represents the CW-PM interphase, where the solutes can diffuse freely and the changes in the mechanical forces are being sensed. The different colors of the arrows resemble different signaling pathways published.

Figure 3: A simplified model summarizing how responses to PM-CW perturbations may be coordinated in a plant cell.

In response to protoplast expansion, signaling through FER induces cessation of growth until CWI is restored. In response to protoplast shrinkage, ABA represses growth until turgor is restored to normal levels. Regulatory interactions between FER and ABA via ABI2 enable the plant cell to balance turgor pressure levels with the CW stability. If CWI is severely compromised leading to mechanical distortion, THE1-mediated signaling induces stress responses including growth arrest, accumulation of ectopic lignin and JA/SA production. PM-localized ion channels OSCA1 and MCA1, plastidial ion transporters (KEA1/2/3) and channels (MSL2/3), as well as the transcription factor MYC2 are representing examples of other signaling components involved in this signaling network. Coloured triangles on the side indicate relative contributions of ABA- and *Cr*RLK1L-based signaling cascades contributing to the responses.


cellulose microfibrils
hemicellulose
pectin
Ca2+
proteins
solutes
Glc
Glc
microtubule
CESA
physical stimuli
chemical stimuli
middle lamellae
ca. 100 nm
cell wall
light
temperature
mechanics
turgor pressure
PAMPs
DAMPs
volatiles
phytohormones
plasma membrane
adaptive responses


mechanical distortion
RALF34
high pH
RALF23
PAMP
PEP1/3
RALF1
LRX
PEPR
BAK1
THE1
FER
FER
BAK1
PRR
growth
CWI responses
growth
ABI2
ABA
MYC2
JA
SA
PTI


CrRLK1L activity (expansion)
ABA (shrinkage)
SA
JA
growth
lignin
THE1
FER
MCA1
ABI2
ABA
signaling
OSCA1
MSL
KEA
mechanical
distortion
growth arrest
CW stiffening
oscillatory growth
hypo-osmotic stress
recovery of growth
hyperosmotic stress
regain of turgor
plasmolysis