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Review article

Root cell wall solutions for crop plants in saline soils

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ABSTRACT

The root growth of most crop plants is inhibited by soil salinity. Roots respond by modulating metabolism, gene expression and protein activity, which results in changes in cell wall composition, transport processes, cell size and shape, and root architecture. Here, we focus on the effects of salt stress on cell wall modifying enzymes, cellulose microfibril orientation and non-cellulosic polysaccharide deposition in root elongation zones, as important determinants of inhibition of root elongation, and highlight cell wall changes linked to tolerance to salt stressed and water limited roots. Salt stress induces changes in the wall composition of specific root cell types, including the increased deposition of lignin and suberin in endodermal and exodermal cells. These changes can benefit the plant by preventing water loss and altering ion transport pathways. We suggest that binding of Na^+ ions to cell wall components might influence the passage of Na^+ and that Na^+ can influence the binding of other ions and hinder the function of pectin during cell growth. Naturally occurring differences in cell wall structure may provide new resources for breeding crops that are more salt tolerant.

1. Introduction

Plant evolution has resulted in a large array of mechanisms to tolerate the stresses associated with increased soil salinity. However, for most cereal crops the growth of roots is disrupted when soil salinity exceeds 4 dS/m, equivalent to about 40 mM NaCl. Increased soil salinity exposes plants to ionic sodium (Na^+) and chloride (Cl^-), which leads to a cascade of responses in the plant due to the ionic and osmotic components of salt stress [1,2].

Salt stress can indirectly affect cell wall properties by causing changes in gene expression, but Na^+ can also physically interact with the cell wall components directly, and change their chemical properties [3]. An increase in soil salinity results in accumulation of Na^+ in the apoplast, which can lead to an increase in interactions between Na^+ and negatively charged sites within cell wall polymers, and also influence apoplastic pH. Salinity causes transient alkalinisation of the apoplast, and this could limit growth in the context of the acid growth theory [4,5]: Auxin activates plasma membrane H^+-ATPases and protons are extruded into the apoplast, apoplastic acidification induces cell wall loosening by activating expansins and other remodelling enzymes resulting in loosening of the cell wall. Hence, growth could be limited by a decrease in free apoplastic protons causing a shift in the apoplastic pH away from the range that favors cell-wall loosening [4], although in maize the inhibition of growth as a result of salinity was not associated with the capacity of the epidermal cells to acidify their walls [6].

Transcriptional analyses of many plant species to a variety of salt treatments show that the transcript levels of many cell wall related genes consistently change in response to salt stress. Transcriptional changes occur, for example, for genes linked to cell wall extensibility, lignin and suberin synthesis and genes associated with the modification of cell wall polysaccharides, such as xyloglucan endotransglycosylases [7–9]. One consequence of salt stress is often a reduction in the rate of cell elongation. Therefore, it is of no surprise that differential transcription of genes regulating the rate of cell wall loosening and stiffening is consistently observed. In this review, we concentrate upon the how, where and why of the affects of salt on cell wall modifications in response to a rise in soil salinity.

2. Na^+ can bind to cell wall constituents

Cell walls are negatively charged and reversibly bind cations such as Ca^{2+} and Na^+ [3,10]. There are examples where the ion binding is physiologically important. For example, the cross-linking of some pectin molecules is dependent on, and co-ordinated by, binding of Ca^{2+}

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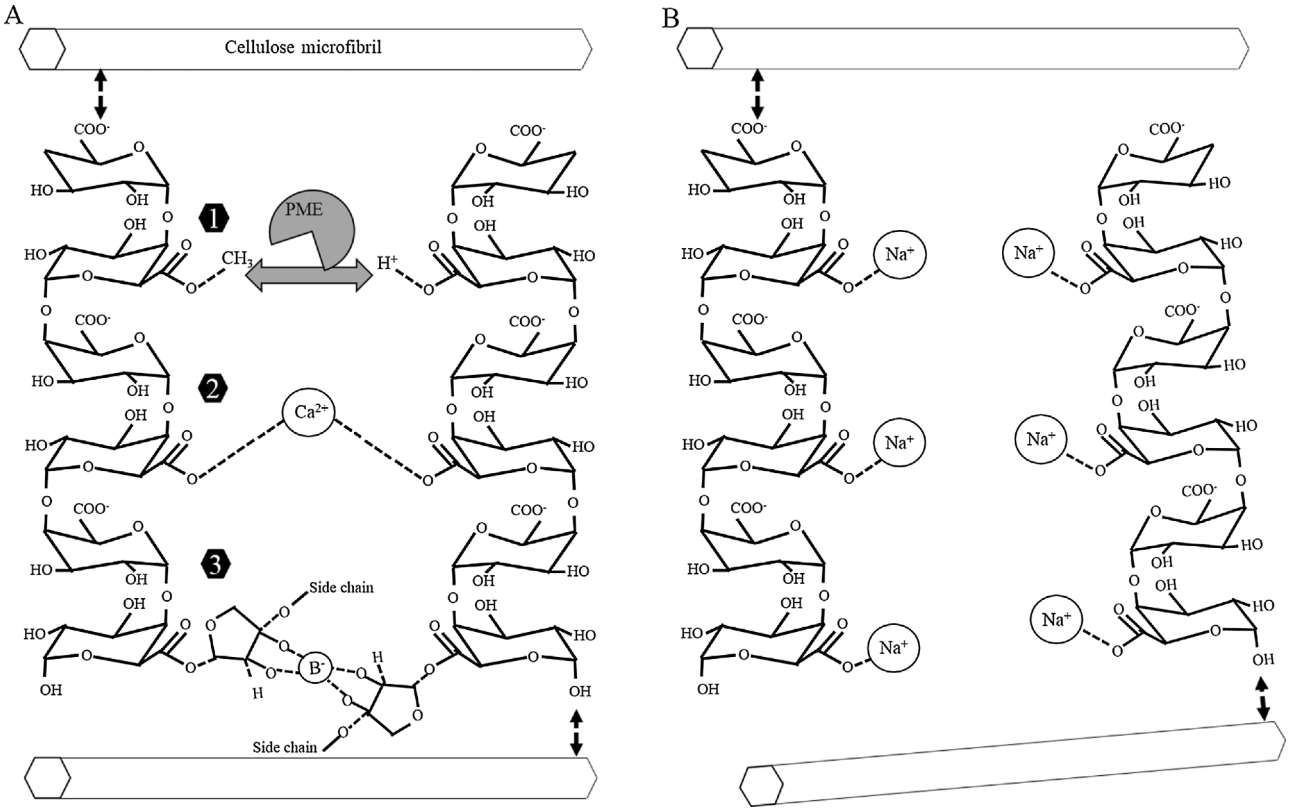

Fig. 1. A model for how excess Na^+ might influence cell wall pectin properties.
A. Cellulose microfibrils are linked by pectins (rhamnogalacturonan I, II and homogalacturonan), and also by xyloglucan (not shown). Pectin links are important for cell wall strength. Pectin can contain up to 17 different monosaccharides, it has a backbone of 1,4-linked α-D-GalpA residues, these residues can be methyl-esterified and where there are unesterified sections Ca^{2+} cross-links occur (reviewed by O'Neill, Ishii, Albersheim and Darvill [10], Vincken, et al. [116]. Black arrows represent complex layers of pectin molecules and black dashed lines represent ionic bonds (not to scale). The distribution of side chains remains to be established. Two rhamnogalacturonan II molecules can complex with boron forming a borate-diol ester and Ca^{2+} promotes this dimer formation. Apiofuranosyl residues of 2-*O*-methyl-D-Xyl-containing side chains participate in the cross-linking. Excess Na^+ may displace the Ca^{2+} and hinder dimerization. Newly deposited pectins are methyl esterified: (1) Pectin methylesterases (PMEs) regulate the removal of methyl esters. Carboxyl groups on de-methesterified pectin interact with ions. (2) Interaction of Ca^{2+} with the carboxyl groups (Ca^{2+} bridges) is important for stabilising pectin. (3) Borate diol ester cross links can form between two rhamnogalacturonan molecules via apiofuranosyl residues, this is thought to require more than nine Ca^{2+} bridges. B. Na^+ binds polygalacturonic acid [117]. When there is excess salt in the apoplasm Na^+ can interfere with PME function, the interaction of Ca^{2+} with the carboxyl groups and the formation of borate diol ester cross links.

to the negatively charged residues (Fig. 1A) [11]. If the ratio of Na^+: to Ca^{2+} is high, Na^+ could displace Ca^{2+} from these binding sites, so reducing pectin crosslinking (Fig. 1B) and subsequently slowing down cell elongation [12]. We suggest that this may contribute to how root elongation is reduced in saline soil. Na^+ interference in pectin cross-linking could reduce the stabilising influence of pectin in the cell wall. Detection of the initial loss in stability is likely to trigger mechanisms to rigidify the cell wall. Proteins such as wall associated kinases (WAKs), Feronia and arabinogalactan proteins are possible candidates for recognising these changes [13,14].

The cell wall changes due to the physical interaction of Na^+ with cell wall components as a result of salt stress are less well documented than the changes in gene expression. Yet a physical interaction between Na^+ and the cation exchange sites on the cell wall might be a key reason why the chemical composition of the cell wall changes when Na^+ is present. There are notable differences in cell wall composition between different cell types and different species which we propose might reflect different strategies to cope with excess salt. The strategy used might depend on the developmental stage of the cell. For example, in the growing regions there may be changes in cell wall components that result in a tighter binding of Ca^{2+} that helps to maintain growth; or in the fully expanded regions there may be upregulation of negatively charged cell wall components that "trap" Na^+ and restrict its movement to the stele and ultimately to the shoots. The ability of the cell wall components to bind Na^+ ions might greatly influence the passage of Na^+ [15]. For example, it has been suggested that cell walls in the stele of citrus plants might influence root Na^+ transport by acting as Na^+ traps [16]. Genetic variation for Na^+ binding of root cell walls has been reported in barley (*Hordeum vulgare* L.), where a two-fold greater Na^+ adsorption was observed for a salt tolerant variety relative to a salt sensitive variety [17]; however, it is currently unknown how much Na^+ could be trapped in the apoplast or whether this would make a significant contribution to sequestering Na^+ in root tissue or affect accumulation in the shoot. At present there are few reports detailing how compositional variation in different cell wall polymers influences Na^+ binding, or how this would influence potassium (K^+) passage or the binding and passage of Ca^{2+} in roots.

3. Changes in the chemical composition of root cell walls in response to salt treatments

It is difficult to measure the changes in cell wall composition that occur in response to salt treatments. This is because the chemical composition of root tissues already varies significantly across many cell layers, and changes during development [18]. Additionally, the growing zones of the root contract with salinity, complicating direct comparison when tissues are measured as distance from the root tip [19,20]. All plant cells are surrounded by an extensible primary cell wall, which contains cellulose, pectin and non-cellulosic polysaccharides. Secondary cell walls are produced when the cell stops expanding, and are more rigid and thicker than the primary wall [21]. They generally contain more cellulose and can accumulate lignin and

suberin [22]. The cell wall composition is different in monocots and dicots and even between plant varieties [23]. Depending on plant species, and root tissue, cell wall components can include cellulose and varying amounts of xyloglucan, arabinoxylan, glucuronarabinoxylan, pectins, callose and (1;3,1;4)-β-glucan. For example, wheat roots contain approximately 23–29 mol% xyloglucan, 38–48 mol% arabinoxylan and a low amount of pectin and (1;3,1;4)-β-D-glucan [24]. The proportion of different cell wall components can change in response to exposure to salt. Therefore, measurements of changes in root cell wall components relating to growth rate in response to salt stress need to be assayed spatially and temporally to obtain meaningful data, and they need to be high resolution to resolve any differences in the intricate matrix of components.

Changes occur in the chemical composition of root cell walls in response to salt treatments, and these have been noted in both model and crop plants. For instance, cell-wall peroxidase activity increased in wheat and with it lignification [25]. The structural arrangement of cellulose microfibrils in sorghum (*Sorghum bicolor* L.) epidermal cell walls changed in response to salt treatments, becoming less parallel, and invagination in these walls increases [26]. In cotton (*Gossypium hirsutum* L.) the amount of cellulose decreased and the amount of uronic acid increased in response to salt treatments [27]. Root tip pectin content in soya bean (*Glyxine max*) decreased [28] and Casparian bands in the maixe (*Zea mays* L.) endodermis developed closer to the root tip in response to salt treatments [29]. All of the above noted cell wall changes can be broadly classified into either general changes in the overall chemical composition of the cell wall, or into localised changes (Fig. 2). Localised changes include cell wall changes in particular cell types, like cell layers with a Casparian strip, such as the endodermis and exodermis and other specialised cells which are modified in response to salt stress.

The regulation of many cell wall remodelling genes changes in response to salt and osmotic stress. Cell expansion is generally achieved by a co-ordinated increase in turgor and an increase, often directionally, in cell wall extensibility. Hence, the regulation of cell wall modifying proteins alters cell wall structure allowing it to yield to turgor and drive cellular expansion [30]. Adaptation to osmotic changes therefore requires changes in cell wall extensibility which involves the function of endo-β-1,4-glucanases, expansins, xyloglucan endotransglucosylase/hydrolase (XTH) proteins and pectin methylesterases.

Endo-1,4-glucanases are needed for cell elongation and normal cell wall assembly and variation in the sensitivity of these enzymes to high concentrations of NaCl has been observed [31,32]. In maize roots experienceing water-deficit region-specific changes in the amounts of different cell wall glucanases, hydrolases and peroxidase proteins have been observed [20]. In monocots (1,3-1,4)-β-endoglucanase degrades (1,3-1,4)-β-glucan, and β-glucosidases may be involved in releasing ABA from ABA-Glc esters and/or in lignin biosynthesis whilst peroxidases might be involved in cross-linking of phenolics or in generating hydroxyl radicals for polysaccharide cleavage [20]. Water limitation caused a decrease in the abundance of five β-glucosidases and an (1,3-1,4)-β-endoglucanase specifically 0–3 mm from the maize root tip, not in the 3–12 mm region, and the abundance of different peroxidases significantly changed in different root regions. The role of (1,3-1,4)-β-glucan deposited in the walls of cells in growing root tips in general, and the relative importance of glucanase activity and (1,3-1,4)-β-glucan content in cell wall adjustment to salt and osmotic changes in roots has not been reported.

In barley roots it has been observed that salt stress resulted in an increase in a different type of glucanase, 1,3-β-glucanase [33]. This enzyme is involved in degrading callose (1,3-β-glucan), and accordingly an increase in 1,3-β-glucanase might be expected to cause a decrease of callose. However, in sorghum, salinity was reported to induce and increase the deposition of callose in the cell walls of the outer cortex [26]. Changes in callose are important in regulating the opening and closing

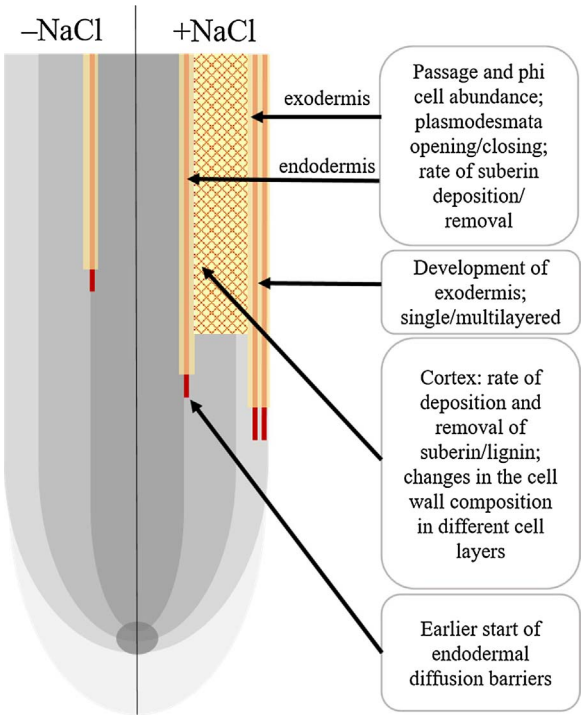

Fig. 2. Changes in soil salinity trigger cell wall modifications in monocot roots that influence ion and water transport.
Modifications are possible in monocot roots that are not possible in the model plant Arabidopsis due to increased numbers of cell layers, such as development of a multilayered exodermis and differentiation in the cell wall composition of cortex cell layers. Features observed in wild grasses, like salt tolerant *Hordeum marinum*, such as presence of an exodermis which is not observed in cultivated cereal crops could be useful for increasing salinity tolerance of future crop varieties. Differences in the spatial (cell specific) and temporal changes in cell wall composition are expected depending on whether there is a sudden increase or decrease in salinity or whether salinity gradually changes over a growing season. An increase in salinity causes Casparian bands (diffusion barriers) to develop closer to the root tip. The secondary cell wall is synthesised at the stage where cessation of cell elongation ceases, shown here as the start of endodermal diffusion barriers.

of plasmodesmata which in turn influences cell-to-cell communication and movement of nutrients; which might be of particular importance in the heavily suberised cells that are highly restricted in direct nutrient uptake and export [34]. This assumption is supported by the observation that the heavily suberised cells of the fully developed endodermis show a large number of plasmodesmata to most likely facilitate ion and water flux [35]. It would be of interest to test whether salt stress triggers changes in plasmodesmatal opening and closing in different root cell types, and how this fast and dynamic adaptation might contribute to tolerating rapid changes in salinity.

Expansins are associated with increasing cell wall extensibility and maintaining root elongation under a great variety of stimuli and stresses, including salt and water deficit stress [36]. The role of expansins in cell wall loosening is well established, but no enzymatic activity has been assigned to them and their direct working mechanism remains elusive [37]. It is therefore unknown if Na^+ or Cl^- have a direct effect on these proteins or on the components of the cell wall they might interact with. Despite our lack of knowledge in the precise working mechanisms of expansins, they have been shown many times to play crucial roles during adaptation to stresses, including salinity. For example, overexpression of a rose expansin RhEXPA4 in Arabidopsis (*Arabidopsis thanliana*) conferred greater drought and salt tolerance [38], and loss of the AtEXLA2 expansin led to greater sensitivity to abiotic stress, including salt stress [39]. Over expression of a wheat (*Triticum aestivum* L.) expansin, TaEXPB23 in tobacco (*Nicotiana tabacum*) conferred tolerance to salt, in terms of biomass produced in

saline conditions, by enhancing water retention ability and decreasing osmotic potential [9,40].

Several transcriptomic studies have shown differential regulation of different gene members within the large gene families that are associated with cell wall extensibility. The gene families of interest here include expansins, xyloglucan endotransglucosylase (XTH/XET)/hydrolases, arabinogalactan proteins (AGPs) and peroxidases. For example in salt treated rice (*Oryza sativa* L.), maize, bermudagrass (*Cynodon dactylon*) or tomato (*Solanum lycopersicum*) roots, within the *XTH/XET*, *AGP* or peroxidase multigene families, some members are up-regulated whilst other members are down regulated in response to salt treatment [7–9,41]. However, the resulting cell wall changes that occur as a result of changing the regulation of different genes within these families is not known. Over expression of an XTH from *Capsicum annuum* in Arabidopsis, and in tomato, was associated with increased salt tolerance and maintenance of root growth in saline conditions and this was linked to reduced transpirational water loss [42,43]. RNA-seq analysis in bermudagrass genotypes differing in salt tolerance identified candidate genes encoding transcription factors involved in the regulation of lignin synthesis, reactive oxygen species (ROS) homeostasis controlled by peroxidases, and the regulation of phytohormone signalling that promotes cell wall loosening, and therefore root growth under salinity [41]. These observations indicate that crop plants and their wild relatives are likely to have genetic variation in expansin, XTH and peroxidase activity. Genetic variation in root expansins, glucosylase, hydrolase and peroxidase activity could be used to develop segregating plant populations for testing the roles of these enzymes in ROS control, water transport and salt tolerance.

4. Changes in cell wall function due to salinity

Changes in pectin content are observed in response to water deficit, and there are associations between higher cell wall pectin content and increased tolerance to salinity, but salt stress has also been observed to inhibit the accumulation of cell wall pectin [44,45]. Pectin demethylesterification is an important part of remodelling of pectins that is needed during cell elongation [11]. Pectins are galacturonic acid-rich polysaccharides and they include homogalacturonan, rhamnogalacturonan I and II and xylogalacuronan [46]. Pectins bind a large amount of water molecules and might contribute to a hydrated environment around cell membranes. However, many pectins do not bind water molecules very strongly. This is evident, for example, when using pectins as gelling agents to make jam. The sucrose present in the mixture is able to strip the pectins of their bound water molecules (solvation shell), forcing them to interact with each other and form a gel. Ions like Na^+ and Cl^- bind water molecules even more strongly than sucrose. Moreover, Na^+ can occupy the initiation sites of the substrate of pectin esterases (Fig. 1), leading subsequently to a decrease in pectin esterase activity [47]. However, replacing pectins with other cell wall components might cause a reduced flow of water into cells, and slow down processes such as cell elongation and transpiration rates and ultimately restrict energy production.

The pectin rhamnogalacturonan II in particular is important in root cell elongation in higher plants [37,48]. Efficient root cell elongation requires rhamnogalacturonan II cross-linking, which in turn requires the correct spatial orientation of pectin molecules to each other. This spatial orientation requires several steps and involves specific ions (Fig. 1A). Boron is important in pectin dimerization and rhamnogalaturonan II is the main boron binding site in plant cell walls [10,49]. Divalent cations, in particular Ca^{2+}, are important in stabilizing the borate cross link [50]. De-esterification of pectin enables formation of Ca^{2+} crosslinked gels affecting pit membrane porosity and water flow [51]. It is possible that excess Na^+ in the apoplast interferes with this process and this might lead to suboptimal spatial orientation of pectin and subsequently cellulose microfibrils to each other in the cell wall (Fig. 1B). Observation that salt treatment reduced incorporation of [14C] glucose into cell wall polysaccharides of cotton roots and that this can be alleviated by supplemental Ca^{2+} is consistent with binding site competition of Na^+ and Ca^{2+} [52]. The free Ca^{2+} displaced from pectin by Na^+ might also be a trigger in salt signalling and future research may reveal cell wall localised receptors that detect this displacement. Silicon, in addition to Ca^{2+} and boron, may also interact with pectins, and application of silicon can partially alleviate salt stress in grasses [53]. The underlying mechanism is not known and could be related to involvement of silicon in antioxidant systems or toxic metal ion complexation, immobilization or compartmentation [54], but it is also possible that silicon could function in stabilising pectin polymers in saline conditions.

In wheat, tolerance to stress due to water limitation is linked with greater glycosyl residue incorporation in pectins and altered pectin side chain structure [24,55]. The way in which greater glycosyl residue incorporation influences the properties of these pectins and the cell wall overall is not known. In soya bean a higher pectin content was observed in the root tips of a salt tolerant cultivar relative to a sensitive cultivar [28], however, no detailed analysis of the pectin was conducted and it is unclear which pectin type was present and whether this changed Na^+ interference in the tolerant cultivar. Differences in pectin were also noted in a recent study where the salt tolerance of rice was manipulated by overexpressing genes associated with regulating programmed cell death. Transgenic rice overexpressing one of three different anti-apoptotic genes (*AtBAG4*, *Hsp70* and *p35*) from diverse origins exhibited similar chemical composition to non-stressed plants whereas control plants exhibited typical changes related to salt exposure, including pectin degradation [56]. Exogenous expression of these three genes in rice significantly improved the salinity tolerance relative to controls. The advantage to plants in reducing anti-apoptotic genes during salt stress is not clear; maybe there is a short-term benefit in expressing these genes but long-term disadvantages for overall plant productivity. These studies indicate that the link between pectin deposition and chemical structure and salinity tolerance warrants further study.

Salt exposure also causes the synthesis and structure of cellulose to change. In sorghum salinity was reported to induce structural changes in cellulose microfibril arrangement in the root tips [26], and in Arabidopsis salt stress leads to microtubule depolymerisation and subsequently cellulose synthases are removed from the plasma membrane [57]. Not surprisingly plants deficient in cellulose are more sensitive to salt, for example the KORRIGAN/RADIALLY SWOLLEN Arabidopsis mutants [58]. It is possible that salt stress directly causes the depolymerisation of the microtubules in the cytoplasm, or this depolymerisation might be a consequence of a fully co-ordinated cellular process. The function of proteins involved in stabilising cellulose synthesis complexes have been revealed as being important in coping with salt and osmotic stresses by aiding microtubule reassembly [57]. Future research should include analysis of root zone specific changes in the chemical composition and structural relationships between each different cell wall polysaccharide in response to changes in salinity. This will require labelling of specific cell wall components and quantification of each component in each root zone in control and salt treated samples.

5. Root diffusion barriers and changes in salinity

The spatial distribution of cell wall characteristics change in response to salt stress along with the changes in chemical composition. Walls of specific cells can be modified, for example suberisation of the endodermis in Arabidopsis occurs earlier during salt stress [35], while many grass roots can develop a second diffusion barrier, called an exodermis or hypodermis, upon salt (and other abiotic) stress [59] (Fig. 3). These modifications presumably influence the movement of solutes and water, and help prevent water loss and the entry of excessive Na^+ and Cl^- into cells or the xylem. Root diffusion barriers can

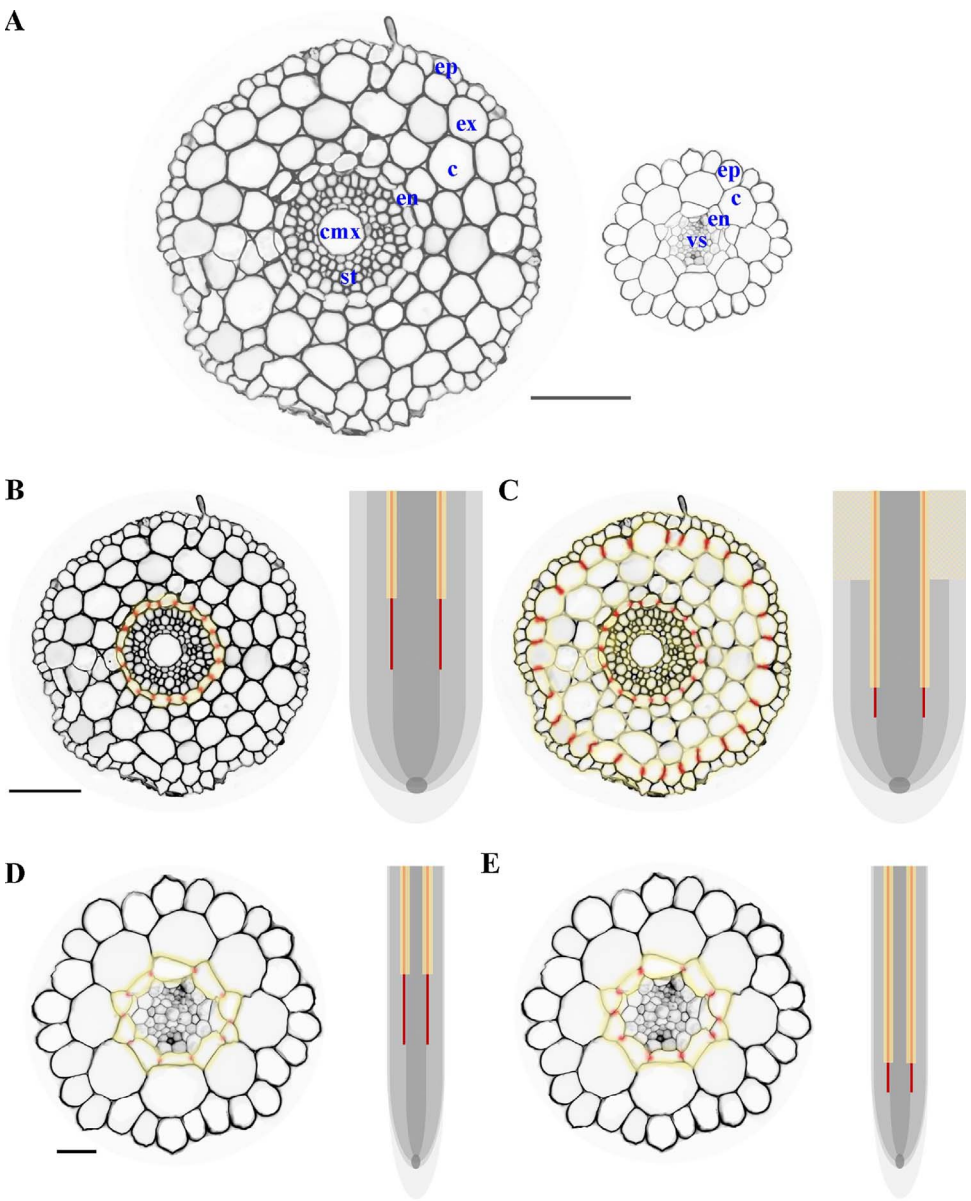

Fig. 3. A model for salt stress induced cereal root apoplastic barrier reinforcement in a cereal, relative to the model plant Arabidopsis.
(A) Transverse diagrams of typical root cell structures of a cereal such as wheat or barley, in comparison to Arabidopsis roots, representing an example of the differences in root and cell sizes. On the left is the cereal (based on wheat) and on the right is the Arabidopsis root (scale bar 100 µm); ep, epidermis, ex, exodermis/hypodermis; c, cortical cell layer(s), en, endodermis; cmx, central metaxylem vessel; st, cells in the stele (surrounded by en in A and B); vs, vascular system. (B-E) Transverse and longitudinal diagrams of cereal roots under (B) non-stressed growing conditions and (C) salt stressed conditions, scale bar is 100 µm. Close up of Arabidopsis roots in non-stressed (D) and salt stressed (E) growing conditions, scale bar is 20 µm. The cell walls where suberin is deposited are shaded yellow, the cell walls with Casparian strips have red shading. In many cereals, increased deposition of suberin or lignin are observed in the stele and outer cell layers of the cortex and the epidermis in response in salt treatments. Differences in apoplastic barrier development are observed in water deficit verses salt stressed conditions, and in different cereals there is variation in the number of cell layers where apoplastic barriers form in response to salt stress [29,68].

not only prevent ions from entering, but they can also act as barriers to prevent desirable ions, such as K^+, and water leaking out [60].

The pathway of solute movement through roots can occur in three different ways: it may be apoplastic, symplasmic, transcellular or a combination of these [61,62]. Selective uptake of solutes into cells is achieved by transport proteins on the plasma membrane. The main cell layer preventing radial apoplastic flow in most plants is the endodermis. Some plants, like Arabidopsis or wheat develop only an endodermis, while other plants, including many crops like maize or grapevine (*Vitis*), develop an exodermis [63,64]. This second diffusion barrier is located beneath the epidermis and can consist of one or multiple cell layers, which prevent radial flow. Cell walls in these layers contain increasing amounts of lignin, suberin, cell wall proteins and carbohydrates depending on the plant species and variety, and the stage of diffusion barrier development. They in general resemble the endodermis, but can also have distinct features. For example, differentiation of the exodermis is observed in more mature parts of the root, lignification and suberisation can initiate at the same time and the lignin and suberin content in the exodermis is generally slightly lower compared to the endodermis [63,65].

Endodermis development has been generally classified into three stages. The first stage is the formation of the Casparian strip. The Capsarian strip consists of a ring of lignin deposited around the endodermal cells that acts as the diffusion barrier for water and solutes [66]. In this stage, the endodermis cells can still contribute in both the symplastic and the trans-cellular flow of nutrients and water. When the endodermis matures and reaches stage 2, suberin is deposited throughout the cell wall; direct uptake of nutrients or water into endodermal cells might be greatly slowed down, however, endodermal cells are highly symplastically connected to their neighbours through a large number of plasmodesmata [67]. Some cells remain in stage 1, without suberisation of the walls; these are called passage cells. The role of suberin is still under debate, and suberin might not stop apoplastic diffusion by itself, as a suberin layer does not prevent the diffusion of apoplastic tracers. Suberin might therefore have a different, not yet understood role [63]. In some dicot plant species, such as Arabidopsis, the endodermis remains in stage 2 until secondary growth of the root sets in and the cortex senesces [63]. In other plants, including most monocots like maize and wheat, the third and final stage of the endodermis is characterised by an extensive thickening of the cell wall, achieved by deposition of cellulose. The deposition of cellulose is particularly thick in the area of the cell wall oriented towards the stele

rather than the area oriented towards the cortex. The influence of this third stage on solute and water flow is currently unknown [63]. The exodermis has similar characteristics as the endodermis; however, Casparian strip development and suberin deposition appear to happen at the same developmental time point [65]. In standard conditions, rice exodermal and endodermal cell walls had six and thirty-four times more suberin, respectively, relative to the equivalent cell walls in maize, and this corresponded with substantially lower hydraulic conductivity [68]. Understanding the role, function and the mechanisms by which suberin influences solute and water uptake will be a crucial future step in our understanding of stress tolerance in plants, including crops.

Similar to the observations listed above for suberin deposition, the amount of lignin in root tissues has been reported to increase in response to salinity and this influences water and NaCl permeability. There are many reports of salt-induced lignification, for example in soya bean [69] and tomato [25], and greater lignification was observed in the stele of a salt tolerant relative of modern salt sensitive durum wheat (*Triticum turgidum*) [25]. In rice, loss of a nuclear factor that coordinates cell wall properties such as lignin deposition resulted in increased sensitivity to salinity [70]. However, the influence of the increased or decreased lignification on Na^+ or Cl^- permeability is rarely tested. There is an example for rice where stagnant conditions were shown to increase the deposition of lignified barriers to radial oxygen loss in the stele and exodermis and these barriers were shown to decrease NaCl permeability by 60% [71].

Salt stress can limit water availability and similar cell wall changes have been observed in roots stressed by either salinity or water limitation [14]. When root cells detect increasing salinity they reinforce diffusion barriers to limit water loss from the cells and the apoplasm, and possibly to limit salt entry into xylem vessels (Fig. 2). For instance, one study in maize directly compared the salt and osmotic (polyethylene glycol) stress effects on maize root cell walls. They observed that salt treatment induced suberisation of the exodermis before suberisation of the endodermis, whereas the osmotic stress intensified suberisation of exodermal and endodermal cell walls simultaneously [29]. The authors also report that the salt treatment induced suberisation of the cell wall in the entire cortex, whereas osmotic stress induced formation of an exodermis limited to three or four layers of the outermost cortical cells. It is clear that the accumulation of suberin influences water and oxygen flow, and waterlogging tolerance [72,73], but it is not entirely clear how important variation in suberin deposition may be in restricting Na^+ and Cl^- movement and in contributing to salt tolerance.

Although the function of some components of the diffusion barriers are not clear; there is no doubt that these barriers have a great influence on radial solute flow. Variation in the spatial development of endodermal and exodermal layers influences Na^+ transport, and in response to salinity the endodermis and exodermis develop closer to the root tip. In young plants there is an accelerated maturation of Casparian bands and suberin lamellae [65,74]. In addition to preventing entry of Na^+ into the stele, Casparian strips might also retain K^+ under these conditions and therefore maintain a higher K^+ to Na^+ ratio. This is supported by two observations: Firstly, Arabidopsis plants with a defective Casparian strip are sensitive to low K^+ and secondly, wildtype Arabidopsis plants grown under K^+ deficiency develop a Casparian strip early, similar to their reaction to salt stress [35,75].

The specialised cell layers of the endo- and exo-dermis in some plants, including members of the Brassicaeae family and maize, can develop phi cells. Phi cells are specialized cells, often occurring in layers of the cortex, with cell wall ingrowths in the inner cell walls that regulate the movement of ions from the cortex to the stele [76]. High salt conditions are thought to induce intensive wall thickenings in the inner wall of the endodermis in maize and phi thickenings in the rhizodermis in seminal roots (Fig. 2). The influence of these changes on Na^+, Cl^- and other solute transport has not been reported [77], and the exact role of phi cells as well as the chemical composition of the copious cell wall depositions associated with phi cells remains elusive.

There may be more subtle mechanisms that influence root solute flow, particularly in wild salt tolerant grass species. These traits may be useful in improving the salinity tolerance of cereal crops [78,79]. For example, inducible exodermis formation in wild wheat and barley relatives, or subtle changes in cell wall composition, which would allow roots to maintain water content and restrict binding of ions to the cell wall. Mangroves (*Rhizophora* L.) and other halophytes deposit high molecular weight viscous mucilage inside xylem vessels, which limits water flow and helps regulate internal salt concentrations [15,80]. In alga and seagrass the cell walls contain polyanonic low-methylated pectins and sulphated galactans and these differences in the pectin may be important in tolerating very saline conditions [81]. However, it is unlikely that cereal crops can be modified to introduce some of the more extreme mechanisms for coping with salinity observed in the roots of mangroves or seagrass, but investigating the chemical properties of cell wall components of these species might help us to understand and predict the chemical properties of components in other plant species.

6. Salt interactions with cell wall proteins

There are many types of proteins within root cell walls that potentially have roles in salt stress related signalling and maintenance of cell wall integrity in saline conditions. For example, phosphatase proteins, wall associated kinase (WAK) proteins, hydroxyproline-rich glycoproteins (HRGP), arabinogalactan proteins (AGP), glycine-rich proteins (GRPs), and proline-rich proteins (PRPs) [82–84]. Previous reports have suggested that phosphatase proteins and wall associated receptor-like kinases are important in maintaining cell wall integrity during salt stress [85,86]. For example, WAKs interact with pectin and Ca^{2+} and high salt could influence this interaction which could be important in signalling [13], and it has been proposed recently that AGPs might bind and carry Na^+ via a vesicle trafficking mechanism [87]. A role for AGPs in coping with salt is supported by observations that in saline conditions mutant Arabidopsis plants lacking fucose and xylose in root AGPs had shorter roots compared to wild type [88]. The arabinogalactan protein SALT-OVERSLY SENSITIVE5 (SOS5) is known to contribute to salt tolerance in Arabdiopsis; SOS5 is required for mucilage adherence and can affect the composition of pectin [89]. Recently, a role for AGPs in the transport of Na^+ via vesicle trafficking between the apoplast and the vacuole was suggested following observation that AGPs accumulate in salt-adapted tobacco BY-2 cell cultures [87]. Our knowledge about the salt induced protein signalling in the cell wall is continuously increasing, however, for many of these observations the mechanisms is still unclear [14].

Interest is growing in studying the coordination between cell wall changes and altered plasma membrane function in relation to coping with salt stress [90]. The cell wall has a role to play in processes to immobilize plasma membrane proteins, and cellulose deposition in the cell wall affects the trajectory and speed of plasma membrane protein diffusion [91]. In Arabidopsis, salt stress induces a specific clathrin-independent endocytic pathway that remodels endomembrane systems and internalizes transmembrane proteins in vacuoles [92]. It may be that specific salt stress induced vesicles form in roots in response to a rapid change in salinity. These vesicles could possibly store membrane proteins which are then returned to the membrane once the plant has adjusted to the osmotic change. Links have been observed between cell wall changes and endocytic responses triggered by salt stress. For example, mutants with phosphatidylinositol 5-phosphatase deficiency (*At5PTase9*) have defects in their endocytic response to salt stress, less ROS generation and calcium influx [93]; in a suppressor of actin *fragile fiber7* (*fra7*) mutant where the *AtSAC1* gene is disrupted it was found that At5PTase9 was mis-localised and this was associated with a decrease in the wall thickness of fibre cells and a weaker stem phenotype [94,95]. Components within the cell wall may function as an extension

of sensory microtubules, and we are yet to determine how this is co-ordinated in general, let alone in response to salt stress [96–98].

Ca^{2+} and reactive oxygen species (ROS) are implicated in rapid systemic signalling in response to stress, and they are linked with cell wall integrity during growth [99,100]. They are also involved in the relationship between cell wall changes and plasma membrane function in response to salt stress [101]. Ca^{2+} waves are propagated through the cortex and endodermal cell layers upon salt exposure [102]. The Ca^{2+} interactions with root cell walls are probably also important in the salt-stress induced Ca^{2+} waves, as the Na^+ might "free" the Ca^{2+} bound in the cell wall, which might contribute or even initiate the Ca^{2+} waves. Similarly salt stress induced root cell wall interactions with ROS are likely to be important. ROS production in the apoplast is required for lignin formation [103]. The reduced rate of cell elongation as a result of salt stress might lead to an increase in apoplasmic ROS that is implicated in signalling rather than lignin formation. Sugars, such as fructans, can scavenge ROS such as hydroxyl radicals, and it is possible that OH groups in cell wall polysaccharides can also scavenge ROS [104]. The roles of salt-induced changes in cell wall bound Ca^{2+} and ROS are not clear, and more research is required to study the spatio-temporal changes in the concentration of Ca^{2+} and ROS in different cellular compartments [105].

There are many salt-induced hormonal and metabolite changes in roots and some are likely to be linked to regulating cell wall modifications [106]. For example, the changes in auxin, ABA, ethylene, cytokinins and gibberellins are linked with responses to stresses such as salinity [107]. Gibberellins are involved in regulation of root growth and in salt adaptation through DELLA protein signalling in the endodermis [107,108]. Abscisic acid and auxin have long been implicated in root stress responses, but there have been recent developments reporting the function of other signalling related molecules, for example extracellular ATP (eATP) and gamma-aminobutyric acid (GABA) [85,109]. Salt-induced increase in eATP concentration is sensed by purinoceptors in the plasma membrane and this is linked to H_2O_2 induction and subsequently elevated cytoplasmic Ca^{2+} [110]. For example DORN1-like lectin receptor kinases elicit eATP responses, as can the Ca^{2+} regulatory protein annexin1 [111,112]. There may also be additional sensors or regulatory proteins in the cell wall associated with extracellular ATP. GABA is likely to be an important part of abiotic stress signalling in roots, particularly in relation to membrane transport [113]. Interestingly, deficiencies in GABA transaminase (GABA-T) have been linked to alterations in root cell wall composition. Under salt stress cell wall related genes were differentially expressed in an Arabidopsis *gaba-t* mutant and this plant had less methyl-esterified pectin than wild type plants [114]. We have limited information about the salt stress related modifications in the cell wall that are caused by changes in hormone and metabolite distribution and concentration. Yet, it can be assumed that it is part of a long term re-modelling strategy for the plant to adapt.

7. Future prospects

To understand the links between the transcriptional changes in cell wall genes and adaptations to salinity we need to identify or create genetic variation in the genes regulating cell wall properties and characterise how the variation influences cell wall chemistry in saline conditions. The measurement of variation in cell wall properties must capture spatial and temporal differences, following strategies such as those used for proteomic and metabolomics studies of root growth in saline soil where the root tip is divided into three regions representing active cell division, elongation and differentiation zones [19,20]. The genetic basis of the above ground growth response to salt stress in cereals, such as rice, has been tested by combining image-based phenotyping with genome-wide association methods [115]. The next step is to go underground with these methods, to phenotype diversity in cereal root salt stress responses and find the genetic loci regulating traits linked to salt stress adaptation. To date tolerance to salinity in cereals has been linked to traits that enable plants to rapidly alter their root growth in response to salt and traits that limit root-to-shoot transport of Na^+, such as better sequestration of Na^+ in root tissues. To complement this information we need to determine how differences in each apoplasmic component in root cells influences cell wall interactions with ions and the passage of ions. In summary, additional root cell wall mechanisms for adaptation to salinity may be revealed by research in the following areas:

- Screening salt tolerant wild cereal relatives for root cell wall traits that enable early and rapid responses to changes in salt, and support increased sequestration of Na^+ in mature root tissues.
- Characterisation and quantification of the Na^+ binding properties of different root cell wall constituents and the influence of Na^+ on cell wall binding of other ions.
- Exploration of the correlation between compositional differences in root cell wall layers, particularly the endo- and exo- dermal layers, and Na^+ transport properties. This will require Na^+ transport assays in mutant or transgenic plants with well characterised variations in cell wall composition in these cell layers.
- Identification of the full complement of root cell wall proteins that specifically register NaCl rather than general osmotic changes.
- Characterisation of the spatiotemporal changes in cell wall bound Ca^{2+} and ROS in root tissues in response to changes in salinity.

There are likely to be undiscovered changes in root cell wall biology that are advantageous in the presence of salt stress. If we can identify the genes responsible for these changes and provide molecular markers or high-throughput trait methodology we could deliver new material for salt tolerance to cereal breeding programs.

A. Cellulose microfibrils are linked by pectins (rhamnogalacturonan I, II and homogalacturonan), and also by xyloglucan (not shown). Pectin links are important for cell wall strength. Pectin can contain up to 17 different monosaccharides, it has a backbone of 1,4-linked α-D-Gal*p*A residues, these residues can be methyl-esterified and where there are unesterified sections Ca^{2+} cross-links occur (reviewed by O'Neill, Ishii, Albersheim and Darvill [10], Vincken, et al. [116]. Black arrows represent complex layers of pectin molecules and black dashed lines represent ionic bonds (not to scale). The distribution of side chains remains to be established. Two rhamnogalacturonan II molecules can complex with boron forming a borate-diol ester and Ca^{2+} promotes this dimer formation. Apiofuranosyl residues of 2-*O*-methyl-D-Xyl-containing side chains participate in the cross-linking. Excess Na^+ may displace the Ca^{2+} and hinder dimerization. Newly deposited pectins are methyl esterified: (1) Pectin methylesterases (PMEs) regulate the removal of methyl esters. Carboxyl groups on de-methesterified pectin interact with ions. (2) Interaction of Ca^{2+} with the carboxyl groups (Ca^{2+} bridges) is important for stabilising pectin. (3) Borate diol ester cross links can form between two rhamnogalacturonan molecules via apiofuranosyl residues, this is thought to require more than nine Ca^{2+} bridges. **B**. Na^+ binds polygalacturonic acid [117]. When there is excess salt in the apoplasm Na^+ can interfere with PME function, the interaction of Ca^{2+} with the carboxyl groups and the formation of borate diol ester cross links.

Modifications are possible in monocot roots that are not possible in the model plant Arabidopsis due to increased numbers of cell layers, such as development of a multilayered exodermis and differentiation in the cell wall composition of cortex cell layers. Features observed in wild grasses, like salt tolerant *Hordeum marinum*, such as presence of an exodermis which is not observed in cultivated cereal crops could be useful for increasing salinity tolerance of future crop varieties. Differences in the spatial (cell specific) and temporal changes in cell wall composition are expected depending on whether there is a sudden increase or decrease in salinity or whether salinity gradually changes over a growing season. An increase in salinity causes Casparian bands

(diffusion barriers) to develop closer to the root tip. The secondary cell wall is synthesised at the stage where cessation of cell elongation ceases, shown here as the start of endodermal diffusion barriers.

(A) Transverse diagrams of typical root cell structures of a cereal such as wheat or barley, in comparison to Arabidopsis roots, representing an example of the differences in root and cell sizes. On the left is the cereal (based on wheat) and on the right is the Arabidopsis root (scale bar 100 μm); ep, epidermis, ex, exodermis/hypodermis; c, cortical cell layer(s), en, endodermis; cmx, central metaxylem vessel; st, cells in the stele (surrounded by en in A and B); vs, vascular system. (B-E) Transverse and longitudinal diagrams of cereal roots under (B) non-stressed growing conditions and (C) salt stressed conditions, scale bar is 100 μm. Close up of Arabidopsis roots in non-stressed (D) and salt stressed (E) growing conditions, scale bar is 20 μm. The cell walls where suberin is deposited are shaded yellow, the cell walls with Casparian strips have red shading. In many cereals, increased deposition of suberin or lignin are observed in the stele and outer cell layers of the cortex and the epidermis in response to salt treatments. Differences in apoplastic barrier development are observed in water deficit verses salt stressed conditions, and in different cereals there is variation in the number of cell layers where apoplastic barriers form in response to salt stress [29,68].

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