How Plants Sense and Respond to Stressful Environments[1][OPEN]

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Plants are exposed to an ever-changing environment to which they have to adjust accordingly. Their response is tightly regulated by complex signaling pathways that all start with stimulus perception. Here, we give an overview of the latest developments in the perception of various abiotic stresses, including drought, salinity, flooding, and temperature stress. We discuss whether proposed perception mechanisms are true sensors, which is well established for some abiotic factors but not yet fully elucidated for others. In addition, we review the downstream cellular responses, many of which are shared by various stresses but result in stress-specific physiological and developmental output. New sensing mechanisms have been identified, including heat sensing by the photoreceptor phytochrome B, salt sensing by glycosylinositol phosphorylceramide sphingolipids, and drought sensing by the specific calcium influx channel OSCA1. The simultaneous occurrence of multiple stress conditions shows characteristic downstream signaling signatures that were previously considered general signaling responses. The integration of sensing of multiple stress conditions and subsequent signaling responses is a promising venue for future research to improve the understanding of plant abiotic stress perception.

During its lifetime, a plant has to make numerous decisions in order to survive. These decisions are based on the input of cues from their environment. Extreme weather events such as suboptimal temperatures, altered water availability, and high soil ion content, result in abiotic stress which can lead to decreased growth. While the downstream cellular signaling and physiological responses to these abiotic stresses have been identified, the sensing mechanisms have long remained enigmatic. Now, several recent reports describe the possible perception mechanisms under different abiotic stress conditions.

Abiotic stresses are perceived by primary sensory mechanisms that translate the physical and chemical environment, such as water availability, ion concentration, and temperature, into a biological signal. For example, heat perception in mammals is mediated by both the TRANSIENT RECEPTOR POTENTIAL (TRP) cationic channel family and the TWIK-RELATED POTASSIUM (TREK) channel family present in neurons. Increasing temperatures led to depolarization and increased action potential firing of corresponding neurons (Voets et al., 2004; Viatchenko-Karpinski et al., 2018). In *Caenorhabditis elegans*, high sodium levels result in an influx of Ca^{2+} and membrane depolarization mediated by TRANSMEMBRANE CHANNEL LIKE1 (TMC1; Chatzigeorgiou et al., 2013). In both cases, stress conditions are sensed directly by transmembrane ion channels, which translate the environmental signal into a cellular response (i.e. a calcium spike and/or altered membrane potential).

In plants, abiotic stress perception mechanisms have been described for some stresses, whereas for others identification of the true primary sensing mechanism has proven challenging. Also, in the absence of clear ligands, the criteria for definition of "abiotic stress sensors" have been unclear. Recently,

ADVANCES

- High temperature perception is mediated by light receptors.
- Salinity sensing is mediated by GIPC sphingolipids, which act as monovalent cation sensors.
- Membrane fluidity during temperature stress is not a temperature sensor, but does mediate the acclimation response.
- Two homologous calcium channels regulate osmotic stress-dependent calcium influxes.
- Oxygen is sensed by oxidation of the N-terminus of ERFVIIs and the response is primed by ethylene.

[1]This work was supported by a European Research Council (ERC) consolidator grant ("Sense2SurviveSalt", 724321, to C.T.).

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[OPEN]Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.19.01464

Vu et al. (2019) proposed three criteria to determine whether a cellular component is a true heat sensor: (1) the abiotic stress condition must directly result in the alteration of structural properties or activity of a component; (2) these properties affect the signal transduction pathway; and (3) alterations of the properties or activity result in physiological and morphological adaptations of the plant in response to the stress condition.

In a broader perspective, these criteria can also be applied to characterize primary sensors of other abiotic stresses, yet we would like to add that it must be the external environment that is sensed (even if perception occurs intracellularly), to distinguish primary stress perception from intracellular signaling processes. The latter can also perceive chemicals, e.g. internal Ca^{2+} concentration, or can be temperature dependent, without necessarily sensing the external environment.

Thus, our definition of a true primary abiotic stress sensor requires it to perceive the stress through sensing of suboptimal environmental conditions and to set in motion a cellular signaling pathway to coordinate responses for acclimation. We discuss the progress and proposed sensing mechanisms that have been reported recently for the main abiotic stress conditions: drought/osmotic stress, flooding, high salinity, and extreme temperatures (Fig. 1). We summarize the primary sensing mechanisms, followed by the downstream cellular and physiological responses and their often overlapping components. The recent identification of several abiotic stress sensors has been instrumental in the unraveling of downstream signaling responses during abiotic stress, and ultimately stress resilience, of plants.

PERCEPTION/SENSING

Temperature

Sensing of the ambient temperature is essential for plants to mediate the timing of development and growth during different seasons as well as to respond to extreme short-term temperature fluctuations. Temperature influences many aspects of plant life, including root and shoot growth, seed yield, flowering, and sensitivity to pests (Quint et al., 2016). Temperature changes the physical properties of all molecules in the cell, thereby altering enzyme kinetics, protein binding, membrane fluidity, and protein folding. Hence, it is essential to discriminate the physical effects of temperature from the actual sensing mechanisms (Vu et al., 2019).

Heat

Increased temperatures lead to water loss due to increased evaporation. In addition, high temperatures inhibit proper protein folding and induce formation of protein aggregates at the cellular level. Currently, two different high-temperature perception mechanisms have been described in plants, namely, heat shock proteins (HSPs) for heat and phytochromes for milder changes in ambient temperature. HSPs sense misfolded and denatured proteins and this protein family is highly conserved among all organisms (Fig. 1; Schlesinger, 1990). When water-soluble proteins denature, for example, during high temperatures, their hydrophobic core is exposed. These hydrophobic regions of multiple proteins aggregate together, causing protein precipitation. HSPs also contain a hydrophobic region that is used to interact with such unfolded proteins (Yamamoto et al., 1991; Benarroch, 2011; Oroz et al., 2017). When HSPs bind to aggregated proteins, Heat Shock Factor (HSF) transcription factors are released and bind to Heat Shock Elements (HSEs) to regulate transcription (Åkerfelt et al., 2010; Al-Whaibi, 2011). HSPs are also upregulated by other stresses, including drought and salt (Liu et al., 2011). However, for these stresses, HSPs do not have a role in sensing; rather, they are upregulated through downstream signaling pathways (Yoshida et al., 2008).

Sensing of their ambient temperature allows plants to regulate germination and flowering time and to adjust their architecture. The signaling pathways used by plants to transduce ambient temperature information overlap with those that signal light cues (Gray et al., 1998; Huq and Quail, 2002; Balasubramanian et al., 2006; Tao et al., 2008; Koini et al., 2009). A central regulator in the convergence of light and temperature cues is the transcription factor PHYTOCHROME INTERACTING FACTOR4 (PIF4; Delker et al., 2014; Johansson et al., 2014; Ma et al., 2016; Hayes et al., 2017). PIF4 promotes hypocotyl elongation in response to both shade and warm temperatures and its activity is negatively regulated by phytochrome B (phyB; Lorrain et al., 2008; Koini et al., 2009).

It was shown that phyB functions as both a light and temperature sensor (Fig. 1; Jung et al., 2016; Legris et al., 2016). phyB exists in either an active Pfr (far-red absorbing) state or inactive Pr (red absorbing) state. Absorption of red light promotes the conversion of Pr to the active Pfr state, whereas absorption of far-red light promotes the reversion of Pfr back to the inactive Pr state. An effect of this is that phyB becomes less active in shade (where there is a high proportion of far-red light; Lorrain et al., 2008). The conversion from Pr to Pfr also occurs spontaneously in a process known as thermal reversion. The speed of thermal reversion is positively correlated with temperature, with the effect that warm temperatures promote the inactivation of phyB (Jung et al., 2016; Legris et al., 2016). As a result of this, PIF4 is able to accumulate and promote cell elongation at warm temperatures (Casal and Balasubramanian, 2019).

Recent findings in Arabidopsis (*Arabidopsis thaliana*) show that temperature sensing in the cotyledons is required for the high-temperature response in the hypocotyl, but that roots are able to respond independently

of other organs (Bellstaedt et al., 2019). Temperature responses in the root also show little similarity with those in the shoot on the transcriptome level. Together these observations lead to the hypothesis that roots have an alternative, unidentified temperature-sensing mechanism (Bellstaedt et al., 2019). Given that phyB function as a thermo-sensor requires activation by light (Legris et al., 2016), it is reasonable to speculate that a different, novel thermosensing mechanism occurs underground.

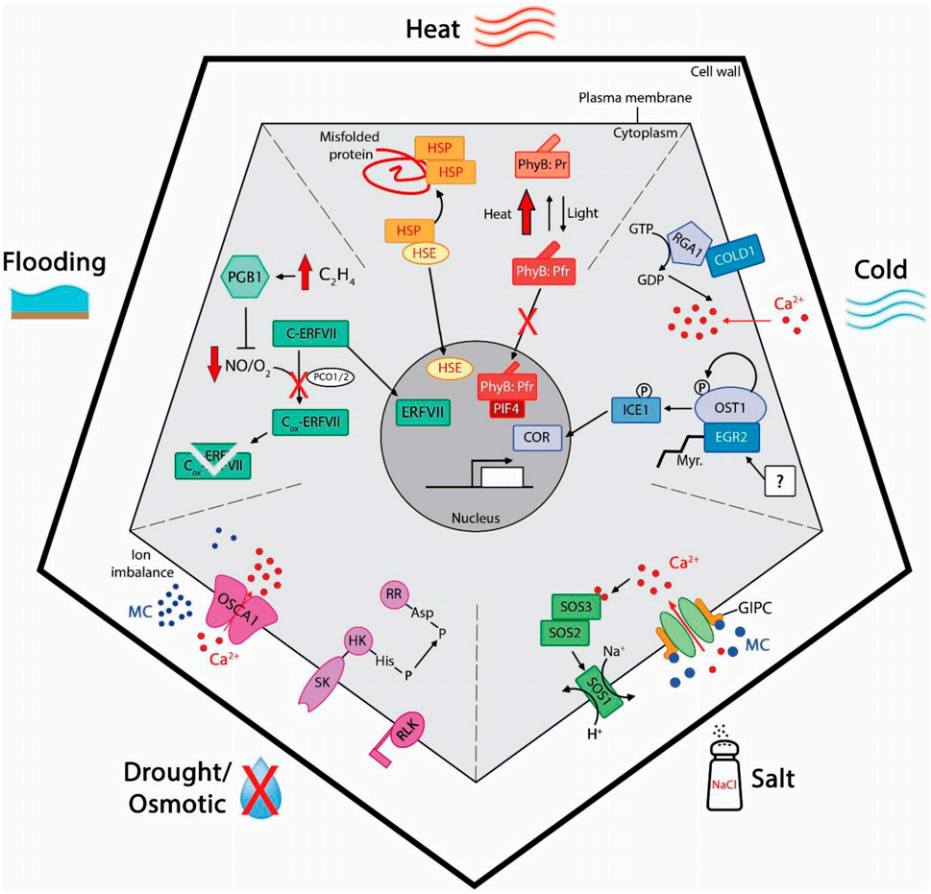

Figure 1. Sensing mechanisms for abiotic stress in plants. The five major abiotic stress conditions described in this article are sensed by separate sensing mechanisms. Key proteins in cold temperature sensing are COLD1 in rice (*Oryza sativa*) and the OST1 pathway in Arabidopsis. COLD1 interacts with RGA1 during cold temperatures, resulting in increased GTPase activity, which activates an unknown calcium influx channel. OST1 is activated upon NMT1-mediated myristoylation of its interactor EGR2 in response to cold temperatures. OST1 is first autophosphorylated, after which it phosphorylates ICE1, leading to the induction of COR gene expression. High temperatures are sensed through recognition of denatured proteins by HSPs, which subsequently release HSE to induce heat-related gene expression. Alternatively, temperature is perceived by the known red/far-red light receptor phyB. High temperatures increase the speed of conversion of activated phyB (Pfr) into inactive phyB (Pr), resulting in increased PIF4 stabilization and hence repression of light-induced gene expression. Flooding sensing is mediated mainly by ethylene accumulation and decreased NO/oxygen levels. Reduced oxygen levels lead to reduced N-terminal Cys oxidation of ERFVII by PCO1/2. This prevents degradation of C-ERFVII, which can relocalize to the nucleus to mediate expression of genes containing hypoxia-responsive elements to mediate flooding tolerance. Drought is likely sensed by a number of transmembrane proteins. OSCA1 is hypothesized to be activated by increased membrane tension occurring during osmotic stress, resulting in the influx of Ca^{2+} to mediate downstream signaling responses. A homolog of OSCA1, called CSC1, is also described as an osmotic stress-regulated calcium channel. The TSC contains a membrane-localized signaling kinase (SK) domain attached to a His kinase (HK) domain in which the His residue is phosphorylated upon osmotic stress. The His kinase subsequently phosphorylates the Asp of a regulator domain, which initiates the signaling cascade in response to drought and/or osmotic stress. Osmotic stress leads to dissociation of the plasma membrane from the cell wall, which is sensed by RLKs localized in the plasma membrane that contain a sensory domain protruding into the cell wall. High salinity is sensed by binding of monovalent cations to the negatively charged GlcA of the GIPC sphingolipids. Upon Na^+ binding, an unknown calcium influx channel is activated, which results in the activation of the SOS pathway to exclude excess Na^+ from the cell. P, Phosphate; Myr, Myristoylation; C_2H_4, ethylene; PGB1, phytoglobin1; ERFVII, ethylene response factor7; C_{ox}-ERFVII, ERF7 containing the oxidized Cys; MC: monovalent cations.

Cold

Low temperatures delay many developmental processes and vegetative growth in plants. Extremely low temperatures, i.e. freezing temperatures, result in the formation of ice crystals which can permanently damage cells if not dealt with appropriately. The SNF1-related protein kinases2 (SnRK2) protein kinase family member Open Stomata1 (OST1), which was earlier identified for its role in stomatal closure (Mustilli et al., 2002; Ding et al., 2015), is the central regulator in the Arabidopsis cold signaling pathway. Increased myristoylation of clade-E Growth-Regulating2 (EGR2) after 30 min of cold treatment, by N-myristoyltransferase1 (NMT1), induces OST1 phosphorylation activity during cold temperatures (Fig. 1; Ding et al., 2019). In response to cold, OST1 phosphorylates the transcription factors Inducer of CBF expression1 (ICE1) and Basic Transcription Factor3 (BTF3), peaking after 2 h of cold treatment, leading to Cold-Regulated (COR) gene expression (Ding et al., 2015, 2018). Low-temperature-induced OST1 phosphorylation activity is upregulated independently of the plant hormone abscisic acid (ABA), a known inducer of OST1 activity in response to osmotic stress (Merlot et al., 2002; Ding et al., 2015). Thus, while EGR2 or NMT1 might be involved in initial sensing of cold, OST1 is a downstream target in the signaling cascade. Whether NMT1 or EGR2 activity is directly regulated by cold temperatures, and hence these proteins can be considered cold sensors, is currently unknown.

In rice (*Oryza sativa*), the quantitative trait locus Chilling-Tolerance Divergence1 (COLD1) contains SNPs associated with chilling tolerance in *japonica* rice varieties (Ma et al., 2015). *Os*COLD1^{jap} encodes a transmembrane protein that interacts with the G-protein α-subunit1 (RGA1). This interaction results in enhanced GTPase activity and an influx of Ca^{2+} into the cell, leading to the activation of the cold tolerance response. It has been proposed that COLD1 promotes Ca^{2+} influx within minutes after the cold temperatures are applied through interaction with an unidentified Ca^{2+} channel or, alternatively, that COLD1 may itself function as a Ca^{2+} channel (Ma et al., 2015). The proposed mode of action of COLD1 during cold temperatures shows similarities with the mammalian RGA1 (Wang and Chong, 2010). Two orthologs of COLD1 in Arabidopsis, GTG1 and GTG2, are plasma membrane-localized G-protein-coupled receptor-type G-proteins important for plant development (Fig. 1; Jaffé et al., 2012). However, although both orthologs regulate pollen formation and perhaps Suc-dependent root growth (Alvarez et al., 2013), to date no involvement in cold sensing or tolerance has been reported.

Thus, while several key components of the cold signaling pathway have been studied in great detail, the actual primary sensing mechanism is still to be identified. A number of promising candidates have been proposed but the true nature of their function in cold tolerance has to be confirmed. In addition to Arabidopsis phyB, it has been shown that the activity of *Marchantia polymorpha* phototropin is temperature dependent. Blue light activates phototropin, and the speed of thermal reversion is positively correlated with temperature, allowing the liverwort to respond to low temperatures (Fujii et al., 2017). It may be that temperature-dependent thermal reversion is a general property of photoreceptors in plants.

Membrane Fluidity during Extreme Temperatures

Membranes are dynamic structures in which lipids and proteins are in constant motion. Altering the fluidity can have profound effects on tolerance against stress conditions (Nicolson, 2014). Cellular membrane composition, i.e. the fractions of phospholipids, sphingolipids, galactolipids, and sterols, and the degree of fatty acid saturation determine the viscosity of the membrane. High temperatures lead to increased fluidity of the membrane. During chilling temperatures the opposite occurs, where the rigidity of the membrane increases, leading to loss of the bilayer structure and membrane leakiness (Thomashow, 1999). Additionally, extreme temperatures lead to altered lipid composition of membranes. High temperatures result in the accumulation of unsaturated triacylglycerols (TAGs) through PHOSPHOLIPID:DIACYLGLYCEROL ACYLTRANSFERASE1 (PDAT1), which likely function as storage lipids to counteract increased membrane fluidity during heat stress (Higashi et al., 2015; Mueller et al., 2017). On the other hand, changes in TAG content and degree of saturation are also associated with cold tolerance of Arabidopsis accessions (Degenkolbe et al., 2012; Arisz et al., 2018; Tan et al., 2018). This suggests that the degree of fatty acid saturation, which is decreased during cold temperatures to maintain membrane viscosity is a determining factor for survival (Fig. 2). Yet the (de)saturation of lipids is a response to extreme temperatures to preserve cell integrity rather than the sensing mechanism. Genetic studies show that overexpression of *DIACYLGLYCEROL ACYLTRANSFERASE1* (*DGAT1*), producing unsaturated TAG, induces cold tolerance, likely in coordination with SENSITIVE TO FREEZING2 (SFR2) activity (Arisz et al., 2018). *DGAT1* expression peaks ~10 h after cold temperatures are applied, and *SFR2* activity is additionally increased during freezing temperatures (Moellering et al., 2010). Whether the bona fide temperature sensors described in the first paragraphs of this section could activate lipid modifying enzymes that adjust the membrane composition, or whether other mechanisms are responsible for initiating the lipid remodeling response, remains to be established. Finally, it has been proposed that increased membrane fluidity causes heat-induced Ca^{2+} influx by plasma membrane-localized Ca^{2+} channels, which may in turn affect downstream thermotolerance (Saidi et al., 2009; Finka et al., 2012).

Drought

Drought induces a complex array of responses in plants, including stomatal closure, reduced turgor

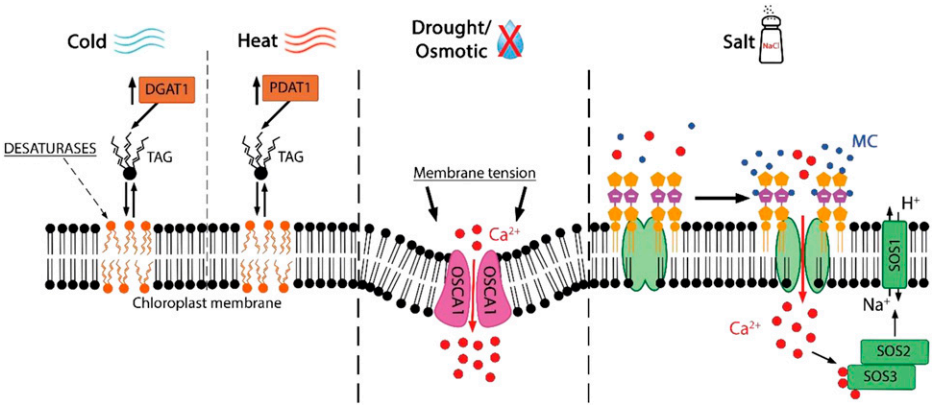

Figure 2. Membrane composition-related sensing mechanisms. During both heat and cold, PDAT1 and DGAT1 expression is upregulated respectively, increasing the TAG content serving as a lipid storage of polyunsaturated lipids. Desaturase activity is increased during cold conditions to increase desaturation of lipids in the lipid bilayer of chloroplast and other membranes to maintain membrane fluidity. During heat, unsaturated TAG content in the cytosol increases to maintain membrane fluidity. Drought and/or osmotic stress lead to membrane tension, which is likely sensed by the hyperosmolality-gated calcium-permeable channel OSCA1 (pink). Upon osmotic stress, OSCA1 is activated, facilitating the influx of Ca^{2+}, resulting in activation of a drought tolerance signaling cascade. Monovalent cations are likely sensed by GIPCs (orange lipids). The negatively charged GlcA (purple pentagons) in GIPC binds Na^+ ions, leading to opening of an unidentified calcium channel facilitating the influx of Ca^{2+} in the presence of high extracellular Na^+ levels. The intracellular Ca^{2+} binds SOS3, which forms a complex with SOS2 to activate the Na^+/H^+ antiporter SOS1. MC, Monovalent cations.

pressure, altered leaf gas composition, and reduced photosynthesis rates, leading to reduced growth and crop yield (Farooq et al., 2012). Water deficit results in osmotic stress for plants, and thus water availability is likely first sensed as a decrease in osmotic potential. Therefore, drought sensors are also referred to as osmosensors. The complexity of plant responses to water-limiting conditions make it challenging to find true sensors of water deficit, although several primary sensing mechanisms have been proposed.

The first potential osmosensor was identified in a screen using EMS-mutagenized aequorin-expressing Arabidopsis seedlings to study the influx of Ca^{2+} in response to sorbitol. This resulted in the isolation of *reduced hyperosmolality-induced* $[Ca^{2+}]_i$ *increase1* (*osca1*; Yuan et al., 2014). This mutant showed a decrease in Ca^{2+} accumulation when exposed to sorbitol but not in response to H_2O_2 or ABA. A reduction in primary root length and leaf area was observed in *osca1* seedlings grown under osmotic stress conditions, revealing an increased sensitivity to osmotic stress. *OSCA1* encodes a hyperosmolality-gated calcium channel located at the plasma membrane (Yuan et al., 2014). High extracellular osmotic potential (Fig. 1) or plasma membrane tension (Fig. 2) caused by water deficit likely triggers the opening of the pore and allows Ca^{2+} influx within seconds after the stress condition is perceived (Liu et al., 2018). Given the subtle phenotypic differences during hyperosmotic growth conditions in *osca1* mutants, it is likely that multiple redundant osmosensors are present in plants. Phylogenetic analysis showed that Arabidopsis contains 15 homologs of OSCA1, suggesting that indeed sensing of hyperosmotic conditions could be mediated by a redundant family of calcium channels (Liu et al., 2018).

A parallel study identified CALCIUM PERMEABLE STRESS-GATED CATION CHANNEL1 (*At*CSC1A/*At*OSCA1.2) as a hyperosmotic stress-induced Ca^{2+} channel in response to mannitol (Hou et al., 2014). CSC1A shows high sequence similarity to OSCA1. Both contain a transmembrane domain homologous to the Domain of Unknown Function221 (DUF221) domain, which is also present in the drought-responsive protein Early Responsive to Dehydration4 (ERD4; Ganie et al., 2017). However, the exact function and subcellular location of CSC1A/OSCA1.2 in plants is currently unknown.

In the search for osmosensors, other sensory systems have been characterized that show homology to the two-component phosphorelay system (TCS) present in bacteria and yeast (Singh et al., 2015). The TCS contains a His kinase with an extracellular stress-sensing domain, resulting in autophosphorylation of the His. The corresponding Response Regulator protein is subsequently phosphorylated by the His kinase, initiating the signaling cascade (Fig. 1). Typical bacterial TCSs sensing alterations in osmotic pressure have been identified (Yuan et al., 2017). TCSs have also been identified in both Arabidopsis and rice, but no direct phosphorylation activity has been found in response to drought or osmotic stress. Although ARABIDOPSIS HIS KINASE1 (AHK1) does seem to play a role in transcriptional regulation in response to drought, no reduction in ABA levels or defects in stomatal closure, two key drought responses, have been observed in the *ahk1* mutant (Sussmilch et al., 2017), making it less likely that AHK1 is an osmosensor.

A cellular hallmark of drought and osmotic stress is the loss of turgor pressure, which in severe, prolonged drought conditions will result in plasmolysis and detachment of the plasma membrane from the cell wall. The plasma membrane contains several receptor-like kinases (RLKs) that monitor the integrity of the cell wall (Fig. 1). Hence, dissociation of the plasma membrane from the cell wall leads to increased phosphorylation of downstream target proteins (Feng et al., 2016). The Arabidopsis genome encodes over 600 RLKs, of which 17 belong to the *Catharanthus roseus* RLK family. Within this family, FERONIA (FER) activity is increased during salt stress, a treatment that also results in osmotic stress and eventually plasmolysis. It is, however, likely that this is the result of distorted pectin filament organization, monitored by FER, rather than direct sensing of turgor or plasmolysis (Feng et al., 2018), which classifies *Cr*RLKs as cell wall integrity sensors rather than as specific osmo- or sodium sensors.

Salt Stress

Soil salinization is one of the major abiotic threats for agriculture. Similar to drought, it quickly limits water uptake and causes osmotic stress, with later accumulation of ions leading to ionic stress (Munns and Tester, 2008). As a result, plant growth on saline soils is limited by reduced turgor pressure, reduced photosynthesis, and changes in development, which are required for survival (Julkowska and Testerink, 2015; van Zelm et al., 2020).

Through quantification of intracellular Ca^{2+} spikes, it first became apparent that plants can perceive both the osmotic and ionic component of salt stress, with higher spikes for salt stress than for osmotic stress at an equal level of osmolarity (Donaldson et al., 2004; Tracy et al., 2008). While the osmotic changes occurring during salt stress could be sensed via mechanisms similar to that described above for water deficit, an additional salt-sensing mechanism would be required to sense the ionic component. Ca^{2+} signaling is of main importance for the exclusion of Na^+ by the SALT OVERLY SENSITIVE (SOS) pathway. The core of this pathway is made up of the SOS3 and SCaBP8 Ca^{2+} sensors, the SOS2 and SOS2-LIKE PROTEIN KINASE5 (PKB5) protein kinases, and the SOS1 Na^+/H^+ antiporter (Liu and Zhu, 1997, 1998; Shi et al., 2002; Quan et al., 2007; Yang et al., 2019). Ca^{2+} spikes are observed within 10 s after sodium application and activate both SOS3 and SCaBP8, which in turn activate SOS2 (Quan et al., 2007). In addition, Ca^{2+} dissociates SOS2 inhibitors 14-3-3 proteins from SOS2 (Zhou et al., 2014). SOS2 then phosphorylates the plasma membrane-localized SOS1, which excludes Na^+ from the cytosol (Fig. 1). This activation is thought to be initiated by salt stress-specific Ca^{2+} spikes (Liu and Zhu, 1997, 1998; Halfter et al., 2000). SOS1 exchanger activity is observed within 20 s of sodium application (Qiu et al., 2002; Martínez-Atienza et al., 2007).

Most recently, Ca^{2+} spikes induced by ionic stress were exploited to unravel the salt stress-sensing mechanism (Jiang et al., 2019), leading to the identification of the *monocation-induced* $[Ca^{2+}]_i$ *increases1* (*moca1*) mutant. This mutant lacks the Ca^{2+} spike initiated by monovalent cations (Na^+ as well as Li^+ and K+), while spikes initiated by reactive oxygen species (ROS), cold stress, osmotic stress, or multivalent cations were unaffected. In addition, in the *moca1* mutant, downstream signaling through activation of the SOS pathway was impaired. Tolerance to KCl or LiCl was not affected in *moca1*, raising the question about the function of MOCA1-dependent Ca^{2+} signaling in response to these cations. Furthermore, it remains unknown how this monovalent cation sensor initiates Na^+-specific responses. MOCA1 is a glucuronosyltransferase that transfers a negatively charged GlcA to inositol phosphorylceramide to form glycosylinositol phosphorylceramide (GIPC; Rennie et al., 2014). Mutations in MOCA1 cause an increased IPC:GIPC ratio in the lipid microdomains on the plasma membrane (Rennie et al., 2014; Jiang et al., 2019). As inositol phosphorylceramide does not contain the negatively charged head, *moca1* membranes have fewer binding sites for monovalent cations compared to the wild type, consistent with the strong Na^+-binding properties of GIPCs described previously (Markham et al., 2006). Interestingly, the *tsc10A-1* loss-of-function sphingolipid biosynthesis mutant was found during a mutant screen for altered leaf ionomics (Lahner et al., 2003), suggesting that sphingolipids may also play a more general role in ion homeostasis. These mutants have higher intracellular Na^+, K^+, and Rb^+ content in the leaves, which was shown to be root driven (Chao et al., 2011).

In direct contrast to the case for animals, no direct role for lipids has been found in cation sensing in plants. In animals, where GIPCs are absent, all reported Na^+ sensors to date are selective ion channels (reviewed by Isayenkov and Maathuis, 2019), of which some are regulated by the composition of their membrane environment (Whorton and MacKinnon, 2011; Li et al., 2019). Besides plants, GIPCs are found in fungi and protozoans, but it has not been established whether these lipids have a conserved function in cation sensing. The exact mechanism of the GIPC-mediated Ca^{2+} influx remains unknown and will be elusive as long as the associated Ca^{2+} channel remains unidentified (Fig. 2; Steinhorst and Kudla, 2019).

Besides sensing of monovalent cations in general, plants are also able to sense the cation Na^+ specifically. This is observed during halotropism, a process in which root growth is redirected in order to avoid high sodium concentrations (Galvan-Ampudia et al., 2013). Halotropism is not observed for other ions and was shown to be mechanistically different from hydrotropism (Dietrich et al., 2017; Deolu-Ajayi et al., 2019). The Na^+-specific sensor that induces this response has not been discovered, but is expected to sense the intracellular

Na^+ concentration, because the *sos1* mutant, which has a higher intracellular Na^+ concentration (Shi et al., 2002), has an enhanced halotropic response (Galvan-Ampudia et al., 2013).

Flooding

While all of the abovementioned stress conditions share the characteristic of water depletion, flooding presents another extreme condition for plants. The effects of flooding vary depending on the turbidity of the water, but generally it leads to inhibition of gas exchange and reduction of photosynthesis. These limitations gradually lead to oxygen depletion (hypoxia), which restricts respiration and therefore causes energy imbalance. Initially, limited gas exchange leads to rapid accumulation of the gaseous hormone ethylene, which is not soluble in water and therefore accumulates in the membranes of the cells. Here it binds to the ethylene receptors, resulting in the stabilization of transcription factors ETHYLENE-INSENSITIVE3 (EIN3) and ETHYLENE-INSENSITIVE3-LIKE1 (EIL1). These regulate gene expression responsive for various adaptive responses, including shoot elongation, leaf hyponasty, and adventitious root formation (reviewed by Sasidharan and Voesenek, 2015). Hypoxia is sensed by the loss of oxidation of the N terminus of proteins belonging to group VII Ethylene Response Factors (ERFs; Fig. 1; Gibbs et al., 2011; Licausi et al., 2011). This mechanism is analogous to hypoxia-induced factor (HIF-1α)-mediated oxygen sensing in metazoans (reviewed by Kaelin and Ratcliffe, 2008). Hypoxia induces rapid ROS and nitric oxide (NO) bursts (reviewed by Sasidharan et al., 2018). All ERFVIIs in Arabidopsis contain an N-terminal MCGGAIIL sequence, which is highly conserved in other angiosperms. MET AMINO-PEPTIDASE (MetAP) removes the Met from this conserved sequence, exposing the destabilizing Cys at the N terminus (Graciet et al., 2010). During normoxia, the Cys is oxidized to Cys sulphuric acid by PLANT CYS OXIDASEs (PCOs), after which ARG-TRNA PROTEIN TRANSFERASE 1/2 (ATE1/2; White et al., 2017) targets the protein for degradation by PROTEOLYSIS1/6 (PRT1/6; Garzón et al., 2007). In the absence of NO/O_2, ERFVIIs are stabilized, after which they migrate to the nucleus and regulate hypoxia-responsive elements (Gasch et al., 2016). These transcriptional changes are quantifiable within 30 min (van Dongen et al., 2009). During this process, O_2 is the direct ligand of PCO1/2, and these oxidases are thus considered the O_2 sensors of plants (Weits et al., 2014; White et al., 2017). NO has been reported to mediate proteolysis of ERFVIIs as well, but this seems to be independent of oxidation by PCOs (Gibbs et al., 2014). Recently, cross talk between the ethylene signaling pathway and hypoxia sensing was described (Hartman et al., 2019). Initially, rapid ethylene signaling upon submergence enhances the NO-scavenging protein PHYTOGLOBIN1. The subsequent lower NO levels reduce ERFVII proteolysis and prime the plant's hypoxia response and survival.

Interestingly, roots grow agravitropically under hypoxia due to the asymmetrical localization of PIN2. It is hypothesized that this allows roots to avoid low-oxygen patches in the soil. However, in an ERFVII quintuple knockout mutant, roots show a stronger bending phenotype (Eysholdt-Derzsó and Sauter, 2017). As genes induced by ERFVIIs are important for hypoxia tolerance, it is hypothesized that root bending is not essential for survival in wild-type plants. This agravitropic growth in the absence of all known sensors suggests the existence of another oxygen perception mechanism.

DOWNSTREAM SIGNALING

Following sensing of specific abiotic stress cues, there is substantial overlap in the downstream signaling molecules utilized. These include Ca^{2+}, ROS, and mitogen-activated protein kinases (MAPKs), as well as the phytohormones auxin, ABA, gibberelins, ethylene, and brassinosteroids. Yet, plants are able to use these similar signaling components to induce stress-specific transcriptional and physiological responses.

Ca^{2+} Signaling

Calcium signaling plays a central role in abiotic stress signaling and is, among others, involved in the response to heat, cold, touch, salt, flooding, hypoxia, osmotic stress, and drought (Wilkins et al., 2016; Kudla et al., 2018). Ca^{2+} enters the cell via Ca^{2+}-permeable channels and then regulates downstream responses. These Ca^{2+} influxes contain a stress-specific fingerprint that varies in amplitude, timing, and frequency and could be formed by the activation of different Ca^{2+} channels by different stresses (Wang et al., 2019). The patterns activate specific intracellular Ca^{2+} sensors, which in turn regulate specific downstream responses like stress-responsive gene expression and protein interactions, illustrated by the aforementioned activation of the SOS pathway (Liu and Zhu, 1997, 1998; Whalley et al., 2011; Whalley and Knight, 2013). Also, the location of the Ca^{2+} influx has been shown to be stress-specific. For example, ionic stress in the roots results in transient Ca^{2+} waves through the whole plant (Choi et al., 2014).

ROS and Calcium Interplay

ROS are formed in almost every plant compartment during numerous enzymatic reactions and have long been considered solely detrimental to plant life. On the other hand, the large array of antioxidant molecules and enzymes keep ROS levels in balance, rendering ROS excellent signaling molecules on the single-cell level as well as for cell-to-cell communication (Choi et al., 2017; Mittler, 2017). ROS signals are mainly

produced at the cell wall and plasma membrane in response to stress conditions, and in chloroplasts due to damage to the photosynthetic apparatus. The proteins responsible for the largest production of ROS at the cell wall and plasma membrane are respiratory burst oxidase homologs (RBOHs), peroxidases, and to a lesser extent oxalate oxidases (Janků et al., 2019). Recent reports have shown that ROS accumulation and calcium production each enhance induction of the other during abiotic stress conditions. Superoxide produced by RBOH protein D activates calcium channels, which in turn activate the vacuolar calcium channel TWO PORE CHANNEL1 (TPC1). TPC1 transports vacuolar-stored Ca^{2+} resulting in the activation of RBOH protein D (Evans et al., 2016). This feedback loop is likely instrumental for propagation of the ROS and Ca^{2+} waves during salt stress and a proper acclimation response. Stress conditions including drought and high temperatures show similar calcium and ROS waves across the plasma membrane, but no in-depth mechanistic reports are present yet. The many different origins of ROS and the corresponding Ca^{2+} waves argue for a sophisticated signaling mechanism. Integrative studies could provide evidence for common ROS-calcium signaling pathways among different stress conditions.

Protein Kinases

MAPKs are a conserved protein family important for stress signaling and development (Ichimura et al., 2000; Xu and Zhang, 2015). MAPKs are activated in a phosphorylation cascade, which typically consists of three kinases, namely a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK), and a MAPK. In Arabidopsis, MAPKs are activated upon touch, cold, salt, drought, and wounding stress, for example (Ichimura et al., 2000), and regulate cellular responses such as gene expression (Colcombet and Hirt, 2008). Furthermore, MAPK pathways show cross talk with ethylene (Yoo et al., 2008; An et al., 2010), ROS (Chang et al., 2012), and ABA signaling pathways (Menges et al., 2008). An example of such cross talk is the regulation of ICE1, which is stabilized upon cold stress by phosphorylation at Ser-278 by OST1/SnRK2.6. However, in parallel, cold initiates a MAPK cascade via MPK3/6, which phosphorylates ICE1 at sites different from those targeted by OST1/SnRK2.6, marking ICE1 for degradation (Li et al., 2017). The exact reason for this interplay of stabilization and degradation is unknown, but it is hypothesized that this fine-tunes the stress responses. Like the other general stress responses, it is largely unknown how MAPK signaling cascades are able to initiate stress-specific responses (Krysan and Colcombet, 2018).

DISCUSSION/PERSPECTIVES

In the last decade, major breakthroughs have been made in the description of abiotic stimulus perception mechanisms, such as the ERFVIIs for hypoxia, phyB for ambient temperature perception, GIPCs as monovalent cation sensors, and potentially OSCA1 and CSC1A for osmotic stress. The downstream signaling pathways of some of these perception mechanisms are relatively well understood due to homology or analogy with other biological kingdoms (e.g. ERFVII and HSP) or signaling components shared with well-studied pathways (e.g. phyB). However, this is less understood for perception by GIPCs, OSCA1, or the potential cold-sensing mechanisms. Studies to unravel the mode of action of these perception mechanisms are complex, as interacting proteins are often unidentified and downstream signaling like ROS bursts and Ca^{2+} spikes are observed within 10 s after stress application. A great deal of cross talk is observed in downstream signaling pathways (see Outstanding Questions).

OUTSTANDING QUESTIONS

- Do sensing mechanisms for abiotic stress in roots and shoots overlap or do they act via different sensors?
- How can shared downstream signaling pathways lead to stress-specific responses?
- What are the sensing mechanisms for cold, drought, and Na^+ ions?
- How is a combination of stresses perceived and how is the downstream signaling integrated to induce an appropriate response in this case?

It is likely that more sensing mechanisms exist, because the known modes of action in abiotic stress perception cannot account for all observed physiological responses. In particular, roots are still able to respond to temperature and hypoxia in the absence of the known perception mechanisms for the corresponding stress (see Outstanding Questions). We hypothesize that this is also the case for sodium sensing leading to sodium-specific growth away from high salinity (Deolu-Ajayi et al., 2019). Interestingly, root directional growth is caused by asymmetrical PIN2 distribution during hypoxia and halotropism (Galvan-Ampudia et al., 2013; Eysholdt-Derzsó and Sauter, 2017). Therefore, these responses might share signaling pathways.

Most studies have focused on a single stress condition, while plants in the field are exposed to numerous combinations of stimuli, for example, simultaneous occurrence of (1) high temperatures, drought, and high salt concentrations; (2) flooding in coastal regions and high salt concentrations; (3) biotic and abiotic stresses. Interestingly, transcriptome analyses of plants exposed to combinations of stress conditions showed that more genes are differentially expressed than might be expected from the combination of the single stresses

(Rasmussen et al., 2013; Sewelam et al., 2014; Suzuki et al., 2016; Georgii et al., 2017). Similar results were found in genome-wide association mapping and microbial biodiversity studies in response to multiple stresses (Thoen et al., 2017; Rillig et al., 2019). Another layer of complexity is that when exposed to multiple stresses, plants prioritize one stress over the other and this prioritization is ecotype-dependent. This is observed, for example, during a combination of phosphate starvation and salt stress in Arabidopsis (Kawa et al., 2016). Currently, the response to combined stresses remains largely unpredictable (see Outstanding Questions), but emerging knowledge about signal integration of stress-signaling pathways is promising for future research (Im et al., 2014; Hayes et al., 2019).

In summary, major advances have been made in the elucidation of abiotic sensing mechanisms. Identification of bona fide sensing mechanisms is an important recent step in further elucidation of cellular signaling pathways and how these lead to appropriate responses in the context of a complex environment. Knowledge of how plant responses to stress are initiated will allow us to answer fundamental questions on how plant growth and development is shaped by the environment, and to understand what is required for a plant to tolerate a stressful environment.

ACKNOWLEDGMENTS

We thank Steven Arisz, Charlotte Gommers, Scott Hayes, and Sjon Hartman for their kind expert critical reads of our manuscript.

Received November 27, 2019; accepted February 26, 2020; published March 4, 2020.

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