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REVIEW PAPER

Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress

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Abstract

Adverse environmental conditions have negative effects on plant growth and development. Receptor proteins on the plasma membrane sense various environmental stimuli and transduce them to downstream intra- and intercellular signalling networks. Receptor-like kinases (RLKs) play important roles in perceiving the extracellular ligands and activating the downstream pathway via phosphorylation of intracellular serine/threonine kinase domains. The *Arabidopsis* genome possesses >600 RLK-encoding genes, some of which are implicated in the perception of environmental signals during the life cycle of the sessile plants. Histidine kinases are also membrane-localized kinases and perceive osmotic stress and plant hormones. In this review, we focus on the RLKs and histidine kinases that play a role in plant response to abiotic stresses. We summarize our recent understanding of their specific roles in stress responses and absicisic acid (ABA) regulation. Elucidation of the functions of these kinases in the osmotic stress response will provide a better understanding of stress-sensing mechanisms in plants and help to identify potential candidate genes for genetic engineering of improved stress-tolerant crops.

Key words: Abiotic stress, histidine kinase, hormone signalling, phosphorylation, receptor-like kinase, signal transduction, stress signalling.

Introduction

Plants have evolved adaptive mechanisms that allow them to survive in an ever-changing environment. Since plants are sessile, they must be able to sense their natural environment and undergo changes in their physiology and development in response to those environmental cues whether they are adverse or beneficial. External stimuli activate the receptor molecules and initiate complex downstream signalling networks that exhibit cross-talk in order to respond to various environmental and developmental cues in an appropriate and integrated manner. Abiotic and biotic stresses often result in significant decreases in the yield of native plants and economically important crop plants. Due to the negative impact on growth and yield, the complex mechanisms involved in stress response and adaptation, such as stress signalling and the regulation of gene expression, have been widely studied with the aim of improving stress tolerance (Tran *et al.*, 2007*a*, 2010*a*; Baena-Gonzalez and Sheen, 2008; Manavalan *et al.*, 2009; Tran and Mochida, 2010; Hadiarto and Tran, 2011; Osakabe *et al.*, 2011, 2012; Jogaiah *et al.*, 2012; Ma *et al.*, 2012).

Plants need to allocate energy to reprogramme stress signalling networks that initiate adaptive responses to stress conditions (Ahuja *et al.*, 2010; Skirycz and Inze, 2010; Thao and Tran, 2012). Endogenous stimuli, such as plant hormones, coordinate and modulate the molecular and biochemical

mechanisms that provide increased stress tolerance and adjust overall plant growth and development for greater survival (Peleg and Blumwald, 2011; Choudhary *et al.*, 2012*a*, *b*, *c*; Ha *et al.*, 2012; Le *et al.*, 2012*a*). The external signals involved in cell–cell signalling are also able to control molecular processes by activation of coordinated intercellular signalling during growth and development and stress responses. The pivotal role of cell–cell communication has been identified in cell fate determination and organ development (Van Norman *et al.*, 2011). Signalling molecules involved in the cell–cell communication include small organic molecules, small peptides, ions, and physical stimuli. These signals are initially received by receptor proteins and sequentially transmitted to target signalling elements.

Receptor-like kinases (RLKs), which form a large gene family in plants, contain Ser/Thr kinase as a cytosolic domain while having structural elements similar to animal receptor tyrosine kinases (RTKs). RLKs convey signal to their target proteins in the cytoplasm by catalytic processes of protein kinase activity. In *Arabidopsis*, the RLK family includes >600 members, with the leucine-rich repeat RLKs (LRR-RLKs) constituting the largest group among the RLKs (Shiu and Bleecker, 2001*a*, *b*, 2003; Gish and Clark, 2011). These RLKs regulate various plant processes of plant growth and development as well as homeostatic mechanisms underlying the abiotic stress response. In addition, RLKs have been known to have a major role in integrating environmental and plant hormone signalling (Shiu and Bleecker, 2001*b*; Dievart and Clark, 2004) (Fig. 1). Recent studies suggested that the rapid evolution and diversification of RLK-encoding genes in the plant genome might be enhanced by various environmental associations (Alcazar *et al.*, 2010; Smith *et al.*, 2011). Natural
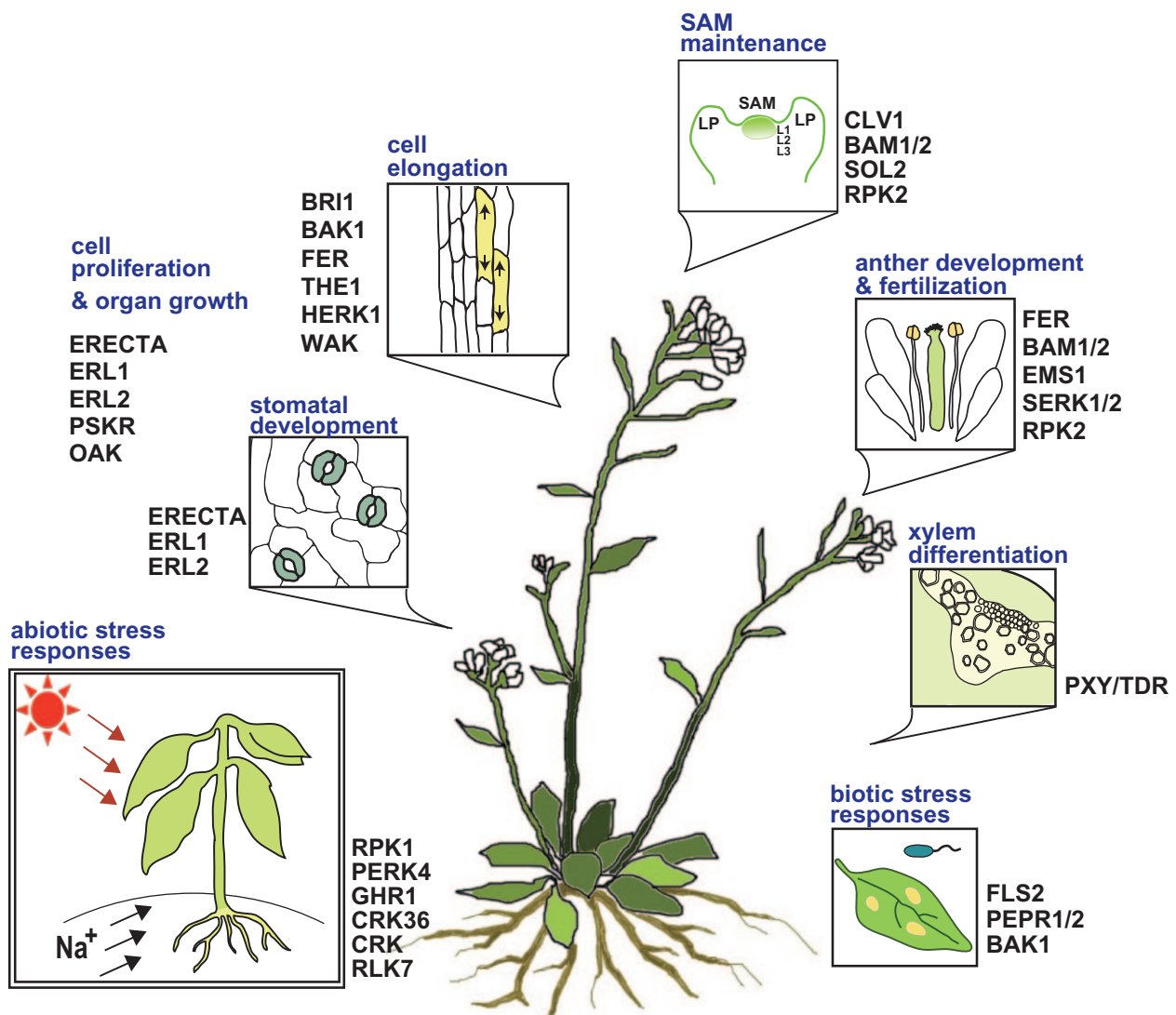

Fig. 1. Overview of plant receptor-like kinases (RLKs) and their functions. The RLKs form a large gene family in plants and regulate various plant processes, including growth and development and responses to biotic and abiotic stresses. The typical well-known RLKs shown in the figure are also listed in Supplementary Table S1 at *JXB* online. The RLKs that control abiotic stress signallings are also listed in Table 1.

variations at the *Strubbelig Receptor Family 3* (*SRF3*) in *Arabidopsis* were able to change the early immune responses and control immune-triggered incompatibilities between the variations (Alcazar *et al.*, 2010). *OUTGROWTH-ASSOCIATED PROTEIN KINASE* (*OAK*) alleles from *Arabidopsis* accessions have been shown to cause a variety of abnormal growth phenotypes and cell death responses (Smith *et al.*, 2011). The *OAK* gene belongs to a subgroup of similar *RLK* genes which are composed of a tandem cluster in the *Arabidopsis* genome and vary between the natural accessions. Furthermore, this gene was the causal gene for the incompatibility of the accessions (Smith *et al.*, 2011). These RLK studies on plant self-incompatibility and innate immunity indicated that the molecular evolution and divergence of RLKs play a major role in defining the diversity of environmental responses in the natural variations.

Typical molecular approaches, such as transcriptome analyses, also suggested that the RLKs can be considered as key regulators in growth and developmental processes as well as in various environmental stress responses (Chae *et al.*, 2009; Hwang *et al.*, 2011; Marshall *et al.*, 2012). The changes in transcriptome in response to abiotic and biotic stresses have been widely characterized, and the results suggested that general regulatory pathways are involved in the processes of gene expression during stresses (Kilian *et al.*, 2007; Matsui *et al.*, 2008; Osakabe *et al.*, 2011, 2012; Le *et al.*, 2012*b*; Ma *et al.*, 2012). The transcription of various *RLK* genes is also regulated and modulated in response to stresses at the levels of individual genes and/or subfamilies (Chae *et al.*, 2009; Tanaka *et al.*, 2012).

Histidine kinases (HKs) localized to cellular membranes or the endoplasmic reticulum were originally identified as the first member of two-component systems that mediate bacterial environmental signals to downstream members, the response regulators (RRs) (Mizuno, 2005; Ha *et al.*, 2012). In plants, two-component systems or histidine-to-aspartate (His–Asp) phosphorelays are involved in the regulation of various biological processes, including the perception of plant hormones, such as ethylene and cytokinins (CKs), and responses to environmental stimuli. A typical, multistep His–Asp phosphorelay consists of hybrid HKs, histidine phosphotransfer proteins (HPs), and RRs (Mochida *et al.*, 2010; Le *et al.*, 2011; Schaller *et al.*, 2011; Ha *et al.*, 2012). Recently, CK receptor AHKs were shown to act as negative regulators in abiotic stress responses via a CK-mediated signalling pathway (Tran *et al.*, 2007*b*, 2010*b*; Jeon *et al.*, 2010; Nishiyama *et al.*, 2011, 2012). On the other hand, ATHK1/AHK1, a non-CK receptor kinase, was identified as an osmosensor acting as a positive regulator of osmotic stress signal transduction (Tran *et al.*, 2007*b*; Wohlbach *et al.*, 2008).

In this review, we will discuss the current knowledge of membrane-bound receptor proteins that act as key regulators in the perception of environmental signals in plants. The focus will be on RLKs and HKs, which perceive plant hormones and small peptides and are involved in the regulation of plant abiotic stress responses. The elucidation of how these membrane elements form a molecular connection between an environmental stress response and the regulation of plant growth will greatly enhance our understanding of how plants control their adaptive response to adverse environmental conditions.

RLKs control plant growth during environmental stresses

The membrane-localized RLKs have been shown to control diverse signalling events (Fig. 1), and these RLKs constitute the largest gene family in various plant genomes, with >600 members in *Arabidopsis* and 1100 members in rice, and are classified based on their extracellular structures (Supplementary Table S1 available at *JXB* online) (Shiu and Bleecker, 2001*a*, *b*, 2003; Morillo and Tax, 2006; Gish and Clark, 2011). The LRR-RLKs form a major RLK family, which has >200 members and is classified into 15 subfamilies (LRR I–LRR XV) in *Arabidopsis* (Shiu and Bleecker, 2001*a*, *b*, 2003). These LRR-RLKs are implicated in diverse signalling events (Figs 1, 2; Supplementary Table S1), such as brassinosteroid (BR) perception by the BR INTENSITIVE 1 (BRI1) and BR1-ASSOCIATED RECEPTOR KINASE1 (BAK1) (Clouse, 2011; Choudhary *et al.*, 2012*a*), meristem size regulation as shown by the functions of CLAVATA1 (CLV1) involved in perception of the peptide hormone CLV3 (Deyoung and Clark, 2008; Muller *et al.*, 2008; Ogawa *et al.*, 2008; Kinoshita *et al.*, 2010), and in organ growth and stomatal cell differentiation by the ERECTA family and the EPF (EPIDERMAL PATTERNING FACTOR) peptide receptors (Kim *et al.*, 2012).

Increasing evidence suggests that RLKs regulate the environmental stress response and play an essential role in the resulting adaptive mechanisms. The RLKs regulate the homeostatic mechanisms underlying abiotic and biotic stress responses and have a major role in integrating environmental and plant hormone signallings. In the first two sections, we focus on the RLK families that have important roles to control water stress signalling and the adaptive responses in plants. RLKs, such as RPK1, CYSTEINE-RICH RLK (CRK36), PROLINE-RICH-EXTENSIN-LIKE RLK4 (PERK4), and GHR1 (GUARD CELL HYDROGEN PEROXIDE-RESISTANT1), have been reported to control water stress signalling directly in *Arabidopsis* (Osakabe *et al.*, 2005; Bai *et al.*, 2009; Y. Osakabe *et al.*, 2010; Lee *et al.*, 2011; Hua *et al.*, 2012; Tanaka *et al.*, 2012) (Fig. 2, Table 1). Many aspects of stomatal morphology and vascular tissue structure have evolved to optimize gas exchange, photosynthesis, and water use efficiency (WUE) under various degrees of drought stress (Bergmann and Sack, 2007; Casson and Hetherington, 2010; Yoo *et al.*, 2010). The ERECTA (ER) family controls stomatal differentiation and affects WUE in plants. Their functions and the signal transduction pathways are also summarized in this section.

The ERECTA family

Stomatal differentiation in the epidermis of plants is initiated by a series of asymmetric cell divisions and involves cell–cell
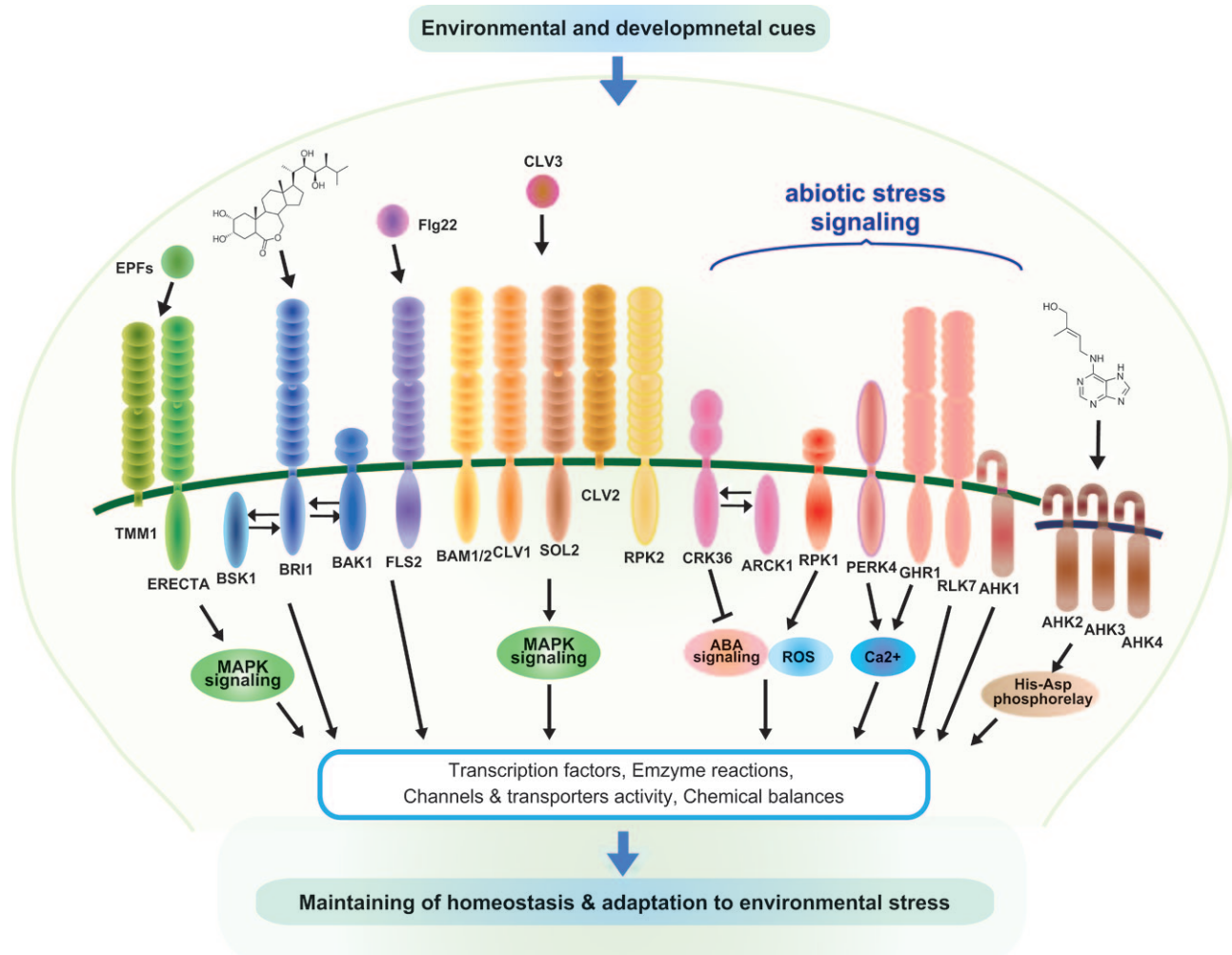
Fig. 2. The signaling networks of RLKs and histidine kinases (HKs) in plant stress responses. RLKs and HKs perceive peptidic ligands or phytohormones to trigger the signalling cascades, leading to an adaptation to the adverse environmental changes. Downstream pathways of several receptors have been studied in more detail. MAPK signalling cascades, ABA signalling pathway, reactive oxygen species (ROS), and calcium ions are involved in the RLK downstream pathways and affect the various molecules and cellular events. The region downstream of cytokinin HKs contains the His–Asp phosphorelay pathway composed of phosphotransfer proteins (HPs) and response regulators (RRs).

communication to establish their number and arrangement (Peterson *et al.*, 2010). The ERECTA family of LRR-RLKs, consisting of ERECTA (*ER*), *ERECTA-LIKE1* (*ERL1*) and *ERL2*, mediate various plant developmental processes, such as cell fate specification including stomatal development (Fig. 3) (Pillitteri and Torii, 2012). Disruption of the *ER* family produced a phenotype characterized by a high density of mispatterned stomata (Shpak *et al.*, 2005). The ER family together with the LRR receptor-like protein TOO MANY MOUTHS (TMM) control stomatal patterning in a synergistic manner (Guseman *et al.*, 2010). Recent findings suggest that the different types of receptor–ligand pairs between ER/TMM and EPFs may specify the different steps of stomatal development (Hara *et al.*, 2007, 2009; Ohki *et al.*, 2011; Lee *et al.*, 2012).

Mitogen-activated protein kinase (MAPK) cascades, which include a MAPKKK YODA, MAPK kinases MKK4, MKK5, MKK7, and MKK9, and MAPKs MPK3 and MPK6, act as a potential downstream pathway for ER signalling (Wang *et al.*, 2007). Basic helix–loop–helix (bHLH) transcription factors control stomatal development by serving as targets of MAPKs. Three paralogous bHLHs, SPEECHLESS (SPCH), MUTE, and FAMA, are key factors regulating stomatal development (Peterson *et al.*, 2010). These bHLHs control the progression of the stomatal lineage to generate a pair of guard cells that are sequentially differentiated from a protodermal cell, meristemoid mother cell, meristemoid cell, and guard mother cell. Other bHLHs involved in modulation of stomatal development are ICE1/SCRM1 and SCRM2, which physically interact with SPCH, MUTE, and FAMA (Peterson *et al.*, 2010).

Environmental factors, such as light and carbon dioxide (CO_2), have roles in stomatal development (Casson and Hetherington, 2010). Stomatal density and the stomatal index are affected by the amount of light energy present during stomatal development. Casson *et al.* (2009) suggested that the

Table 1. Receptor-like kinases involved in regulation of plant responses to abiotic stresses.

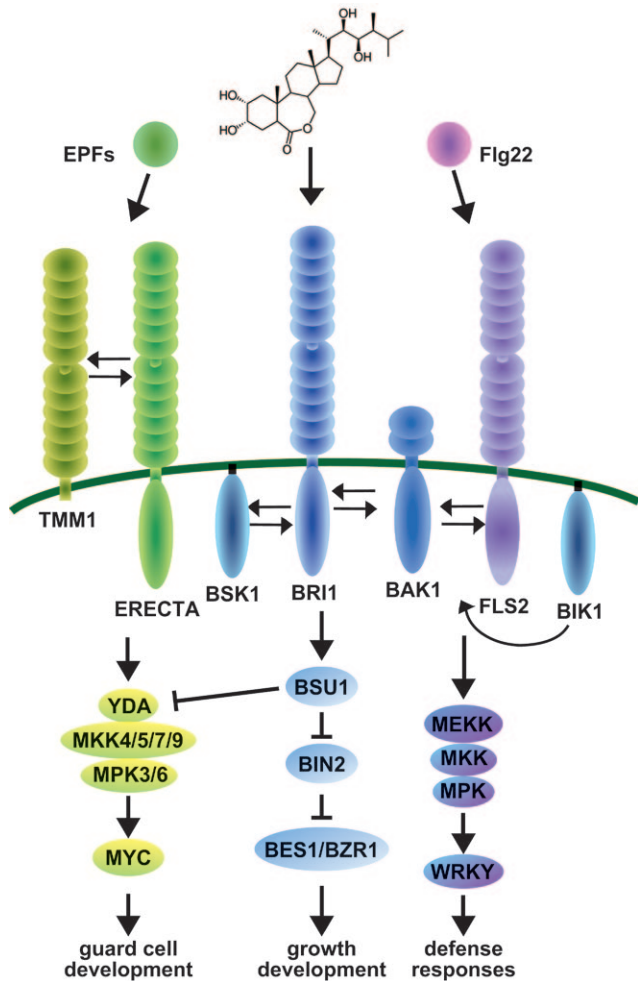| Type of RLK	Subfamily	Gene name	Full name	Organism	Homologues with known function	Expression	Function	References
LRR	LRR III	GHR1	GUARD CELL HYDROGEN PEROXIDE-RESISTANT1	Arabidopsis		Guard cells	Functions in ABA- and H_2O_2-regulated activation of S-type anion channels in guard cells.	Hua et al. (2012)
	LRRIX	SRLK	SALT-INDUCED RECEPTOR-LIKE KINASE	Medicago truncatula	BIR1	Abiotic stress-inducible	The RNAi prevents inhibition of root growth in Medicago under salt stress.	de Lorenzo et al. (2009)
	LRRXI	RLK7	RECEPTOR-LIKE KINASE 7	Arabidopsis		Abiotic stress-inducible	Involved in the control of germination speed and the tolerance to oxidant stress.	Pitorre et al. (2010)
	LRRXIII	OsSIK1	O. SATIVA STRESS-INDUCED PROTEIN KINASE GENE 1	Oryza sativa	ERECTA	Abiotic stress-inducible	Rice OX[a] plants exhibited tolerance to salt and drought stresses with increased levels of superoxide dismutase and decreased ROS accumulation.	Ouyang et al. (2010)
	LRRXV	RPK1	RECEPTOR-LIKE PROTEIN KINASE 1	Arabidopsis		Abiotic stress-inducible	Arabidopsis OXs showed enhanced ABA sensitivity and drought tolerance with increased superoxide dismutase level and decreased ROS accumulation.	Y. Osakabe et al. (2005, 2010)
PERK		PERK4	PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASE 4	Arabidopsis		Abiotic stress-inducible	Functions at early stage of ABA signalling to inhibit primary root cell elongation by perturbing Ca^{2+} homeostasis.	Bai et al. (2009)
CRK/DUF26		CRK36	CYSTEINE-RICH RECEPTOR-LIKE KINASE 36	Arabidopsis		Abiotic stress-inducible	Forms a complex with ARCK1 and negatively controls ABA and osmotic stress signal transduction.	Tanaka et al. (2012)
RLCK		ARCK1	ABA- AND OSMOTIC-STRESS-INDUCIBLE RECEPTOR-LIKE CYTOSOLIC KINASE 1	Arabidopsis		Abiotic stress-inducible	Forms a complex with CRK36 and negatively controls ABA and osmotic stress signal transduction.	Tanaka et al. (2012)
		OsRLCK253	O. SATIVA RECEPTOR-LIKE CYTOSOLIC KINASE 253	Oryza sativa		–	Arabidopsis OX plants have improved tolerance to drought and salt stresses.	Giri et al. (2011)
		PSTOL1	PHOSPHORUS-STARVATION TOLERANCE 1	Oryza sativa		Abiotic stress-inducible (P-deficient conditions)	Root growth and grain yield of rice OXs are enhanced in P-deficient soil with constitutive up-regulation of drought stress-responsive genes.	Gamuyao et al. (2012)
		GsCBRLK	G. soja calmodulin-binding receptor-like kinase	Glycine soja	CRLK1	Abiotic stress-inducible	Arabidopsis OX plants have enhanced tolerance to high salinity and ABA with increased expression levels of stress-responsive genes.	L. A. Yang et al. (2010)
		CRLK1	Ca^{2+}/CaM-regulated RLK	Arabidopsis		Abiotic stress-inducible	Knock-out mutants exhibited an increased sensitivity to chilling and freezing temperatures.	T. Yang et al. (2010a)

[a]OX, overexpressor

Fig. 3. The model of ERECTA, BRI1, BAK1, and FLS signalling pathways. The guard cell development controlled by the ERECTA family (LRR-RLK) and its peptide ligands, EPFs, the brassinosteroid signalling mediated by BRI1 (LRR-RLK), BAK1 (LRR-RLK), and BSK1 (RLCK), and the defence responses controlled by flagellin receptor FLS2 (LRR-RLK), BAK1 (LRR-RLK), and BIK1 (RLCK), involve cross-talk at the early steps in the signalling cascades during various plant processes of growth and stress responses.

photoreceptor PhyB and a bHLH transcription factor, PIF4, might be the major factors involved in stomatal development (Casson *et al.*, 2009). Stomatal number is also affected by the atmospheric concentration of CO_2, and this association has been established over millenia. An analysis of stomatal characteristics and CO_2 over long geological time periods indicates that stomatal density negatively correlates with CO_2 levels, whereas stomatal size is positively correlated (Franks and Beerling, 2009; Lomax *et al.*, 2009). The phytohomone abscisic acid (ABA) mediates various physiological events during water stress, including stomatal closing and stomatal density. Lake and Woodward (2008) reported that *aba* mutant plants, which are defective in ABA biosynthesis, display higher stomatal densities (Lake and Woodward, 2008). The regulation of stomatal conductance and transpiration rates

is linked to stomatal development, and systemic signalling mediates stomatal development in response to environmental conditions (Casson and Hetherington, 2010). Although the above-mentioned regulatory factors control stomatal development under various environmental conditions, their molecular relationship to the signalling cascades controlled by EPFs, ER, MAPKs, and SPCH/MUTE/FAMA remain largely unknown. Knowledge of how environmental signals modulate these signalling pathways would help to clarify how this process impacts the ability of plants to adapt to adverse growth conditions.

Recent studies have provided evidence that ER is a major genetic marker in various quantitative trait locus (QTL) and expression QTL (eQTL) studies of plant development and plant responses to environmental stresses. The molecular relationship between leaf production and leaf expansion was determined by a quantitative genetic analysis using a set of *Arabidopsis* recombinant inbred lines (RILs). One of the QTLs at ER was identified in a heterozygous inbred family as a control factor of both epidermal cell number and area (Tisne *et al.*, 2008). The QTLs for the different aspects of plant growth-related traits, sugar and starch content, and flowering time have been analysed using RILs derived from Landsberg *erecta* (L*er*) and Kondara (El-Lithy *et al.*, 2010). In their study, the authors detected co-locations of QTLs for these various aspects mainly at the *ER* locus (El-Lithy *et al.*, 2010). *ER* was found as the major factor of transcriptional regulation by conducting an eQTL analysis using the RILs of L*er*/Cape Verde Islands (Cvi). Additionally, candidate genes in the downstream signalling pathway were identified where ER has been linked to MAPK cascades and the WRKY transcription factor family (Terpstra *et al.*, 2010). Another QTL analysis using the RILs of L*er*/Cvi demonstrated that ER controls ethylene-induced petiole angles and ethylene-independent effects on initial angles (van Zanten *et al.*, 2010). Although it is not clear yet if ER is directly associated with the factors involved in the regulation of these diverse plant processes, these studies suggest that ER might be a key modulator of signalling pathways. This possibility, in which the ER RLK family responds to a variety of ligands, might explain the extensive diversity of their function.

The BRASSINOSTEROID INSENSITIVE 1 (BRI1) kinase

BRs are plant hormones involved in the regulation of various aspects of plant growth and development, as well as responses to both abiotic and biotic stresses (Divi and Krishna, 2009; Wolters and Jurgens, 2009; Choudhary *et al.*, 2012c; Wang, 2012). BRs negatively regulate stomatal development mediated by upstream signalling components including the BR receptor RLK BRI1, the protein phosphatase BRI1 SUPPRESSOR1 (BSU1), the BR SIGNALLING KINASE 1 (BSK1), and the GSK3-like kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2) (Tang *et al.*, 2008). When the BR level is low, BIN2 directly interacts with MAPKKK YODA and phosphorylates YODA, thereby inactivating it (Fig. 3). Inactivation of YODA by BIN2 in turn leads to de-repression of SPCH, allowing SPCH to initiate stomatal development.

When BR is high, the MAPKKK YODA pathway is activated by inactivation of BIN2 through BRI1, BSK1, and BSU1, thereby inhibiting stomatal development. BRs have also been shown to repress light-responsive gene expression and chloroplast development mainly through the BRASSINAZOLE RESISTANT 1 (BZR1)-mediated transcriptional network (Luo *et al.*, 2010; Sun *et al.*, 2010). Additionally, recent reports indicate that appropriate genetic engineering of BR signalling can lead to abiotic and/or biotic stress tolerance (Divi and Krishna, 2009; Wang, 2012). These reports indicate that BR and the EPF signalling systems work together to mediate signalling networks to optimize photosynthesis and WUE. The multifunctional roles of RLKs in various plant signalling pathways were shown through studies of BAK1 (Clouse, 2011). BRI1 requires BAK1 as a co-receptor to execute BR signalling. Another RLK, the flagellin receptor FLS2, was shown to act in concert with BAK1 in FLS2 signalling to regulate plant innate immunity (Wang, 2012) (Figs 2, 3). BAK1 belongs to the SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) family. Increasing evidence has demonstrated that the SERKs can act as co-receptors in multiple independent signalling pathways by their association with different RLKs (Li, 2010).

Function of RLKs in environmental stress responses

To explore the novel stress-responsive RLKs in plants, comprehensive studies of the transcriptional profiles of the *RLK* genes in environmental stresses have been performed (Chae *et al.*, 2009; Lehti-Shiu *et al.*, 2009; Hwang *et al.*, 2011; Marshall *et al.*, 2012). These studies indicated that the transcription of a number of *RLK* genes is controlled and modulated by various environmental cues, implying their important roles in stress responses.

Various RLKs that control biotic stress responses have been identified from several plant species (Supplementary Table S1 at *JXB* online). The bacterial flagellin fragment (Flg22) was recognized by an LRR-RLK, the FLAGELLIN SENSING2 (FLS2), which mediates defence responses in plants (Figs 2, 3) (Wang, 2012). FLS2 interacts with BAK1 and phosphorylates the cytoplasmic kinase BIK1 that is able to control the FLS2 signalling as a positive regulator (Lu *et al.*, 2009) (Fig. 3). In addition, transcriptome analyses show that a number of *RLK* genes are also up-regulated by biotic stresses, suggesting their important roles in regulation of biotic responses (Chae *et al.*, 2009; Lehti-Shiu *et al.*, 2009). There is also a significant overlap among *RLK* genes induced by biotic and abiotic stresses (Chae *et al.*, 2009; Lehti-Shiu *et al.*, 2009), indicating that the RLKs might mediate the cross-talk between biotic and abiotic stress responses.

Likewise, the RLKs involved in sensing environmental signals and in abiotic stress signalling pathways have been identified in various plant species, such as *Arabidopsis* (Yang *et al.*, 2004; Osakabe *et al.*, 2005; Bai *et al.*, 2009; Y. Osakabe *et al.*, 2010; Lee *et al.*, 2011; Tanaka *et al.*, 2012), rice (*Oryza sativa*) (Ouyang *et al.*, 2010), *Medicago truncatula* (de Lorenzo *et al.*, 2009), and *Glycine soja* (L.A. Yang *et al.*, 2010) (Fig. 2, Table 1; Supplementary Table S1 at *JXB* online). These RLKs have a variety of extracellular domains, such as an LRR, an extensin-like, or a cysteine-rich domain, suggesting that various environmental stimuli may activate the signalling pathways mediated by RLKs. Increasing evidence suggests that RLKs can have either a positive or a negative regulatory role in abiotic stress response.

LRR-RLKs involved in water stress responses

RECEPTOR-LIKE PROTEIN KINASE1 (RPK1) is an LRR-RLK whose expression is induced by ABA, dehydration, high salt, and low temperature. Loss of function of *RPK1* resulted in ABA insensitivity and reduced expression levels of various water stress-responsive genes, indicating that RPK1 positively regulates ABA/stress signalling. Microarray analysis of the *Arabidopsis RPK1* loss-of-function mutant identified a number of down-regulated stress-related genes, including reactive oxygen species (ROS)-related genes (Osakabe *et al.*, 2005). ROS production is activated during both biotic and abiotic stresses, including pathogen attack, high light, osmotic stress, heavy metal stress, and herbicides. ROS are important second messengers for stress signal transduction pathways (Gill and Tuteja, 2010; Le *et al.*, 2010; Takahashi *et al.*, 2011; Choudhary *et al.*, 2012c). Many water stress-responsive genes were consistently up-regulated in *Arabidopsis RPK1*-overexpressing plants, including ROS-related genes (Y. Osakabe *et al.*, 2010). *RPK1* transgenic plants exhibited increased tolerance to both drought and oxidative stress, suggesting that RPK1 controls ROS homeostasis and mechanisms regulating both the water and oxidative stress response in *Arabidopsis*. In an independent study, Lee *et al.* (2011) reported that RPK1 functions in ABA-dependent leaf senescence as well. RPK1 has been primarily identified in the genomes of *Brassica* species, suggesting that RPK1 might play a specific regulatory role in *Brassica* species, whereas the *Arabidopsis* RPK1 homologous protein, RPK2/TOAD2, with high similarity in the kinase domain of RPK1, was identified in diverse plant species (Sawa and Tabata, 2011) (Fig. 2). RPK2 controls cell fate in anthers (Mizuno *et al.*, 2007), embryo development (Nodine *et al.*, 2007), and stem cell homeostasis in the shoot apical meristem through the mediation of CLV3 (Kinoshita *et al.*, 2010; Betsuyaku *et al.*, 2011). These studies indicate that RPK2 is one of the important RLKs governing plant development in various species. RLK7, an LRR-RLK member of the LRR XI subfamily, was identified as an important regulator in oxidative stress responses and shown to be involved in maintaining seed longevity (Pitorre *et al.*, 2010). Recently, an LRR-RLK that controls ABA- and H_2O_2-regulated activation of S-type anion channels in guard cells has been identified (Hua *et al.*, 2012). This RLK, named GUARD CELL HYDROGEN PEROXIDE-RESISTANT1 (GHR1), physically interacted with and activated the S-type anion channel SLOW ANION CHANNEL-ASSOCIATED1 (SLAC1) by phosphorylation, resulting in stomatal closing in response to drought stress. The ligands of these RLKs have not been resolved yet, and

further studies would elucidate the whole picture of the signalling mechanisms.

A number of reports have demonstrated that RLKs from legume and crop plants regulate the abiotic stress response (de Lorenzo *et al.*, 2009; Ouyang *et al.*, 2010; L.A. Yang *et al.*, 2010; Giri *et al.*, 2011; Gamuyao *et al.*, 2012) (Table 1). A salt stress-inducible *LRR-RLK* gene, *SRLK*, identified from the legume *M. truncatula* was reported to be involved in adaptation to salt stress (de Lorenzo *et al.*, 2009). Root growth inhibition by high salinity stress was alleviated in *SRLK* RNA interference (RNAi) transgenic *Medicago* roots. Epidermal cell-specific expression of the *SRLK* gene was observed in roots under salt stress, and SRLK was shown to control the expression level of several salt-responsive genes. These findings suggest that SRLK activates the signalling pathway involved in the adaptive response of *Medicago* roots to salt stress.

Over 1000 *RLK* genes have been identified in the rice genome. Among 267 abiotic stress-responsive *RLK* genes in rice, *Oryza sativa stress-induced protein kinase gene 1* (*OsSIK1*), which is induced by salt, drought, and oxidative stresses, was selected for functional analysis (Ouyang *et al.*, 2010). *OsSIK1*-overexpressing transgenic rice plants exhibited increased salt and drought tolerance, whereas the loss of function of *OsSIK1* resulted in sensitivity to these stresses. Increased activity of peroxidases, superoxide dismutases, and catalases was found in the *OsSIK1*-overexpressing transgenic plants, and ROS accumulation was decreased in their leaves. The OsSIK1 kinase domain has high similarity to the kinase domains of the *Arabidopsis* ER family, and OsSIK1 affects stomatal density. These findings indicate that OsSIK1 plays an important role in water stress tolerance in rice.

Collectively, the reports on the functions of LRR-RLKs in water stress responses demonstrate the diversity of the receptor domains. Further studies to identify their ligands and kinase functions are required to elucidate how the RLKs integrate their individual roles in controlling water stress responses.

PERK family

A member of the proline-rich extensin-like receptor kinase family, *PERK4*, was identified as a positive regulator in ABA responses (Bai *et al.*, 2009). Decreased sensitivity to ABA with respect to seed germination, seedling growth, and root tip growth was observed in *perk4* mutant plants. ABA-induced elevation of the concentration of cytosolic free calcium and Ca^{2+} channel activity were impaired in *perk4* root cells. PERK4 was shown to be an ABA- and Ca^{2+}-activated protein kinase that functions at an early stage of ABA signalling involved in the inhibition of root cell elongation (Bai *et al.*, 2009), suggesting that PERK4 may perceive changes in the levels of ABA. The PERK family is predicted to have similar functions to cell wall-associated kinases (WAKs) which serve as cell wall receptors and also bind to pectin (Nakhamchik *et al.*, 2004). Furthermore, Bai *et al.* (2009) demonstrated that PERK4 may also perceive changes in the cell wall.

CRR-RLK (CRK) family

A cysteine-rich RLK (CRK), CRK36, has recently been identified in *Arabidopsis* that acts as a negative regulator in osmotic stress and ABA signalling (Tanaka *et al.*, 2012). *CRK36*, an abiotic stress-inducible CRK (Wrzaczek *et al.*, 2010), was first identified by co-expression analyses and yeast two-hybrid screening as a potential interacting factor with *ARCK1*, which encodes a receptor-like cytoplasmic kinase (RLCK). CRK36 can physically interact with and phosphorylate ARCK1. The knock-down of *CRK36* in transgenic *Arabidopsis* resulted in increased sensitivity to ABA and osmotic stress during post-germinative growth, and the up-regulation of ABA-responsive genes, such as *LEA* genes, oleosin, *ABA-INSENSITIVE 4* (*ABI4*), and *ABI5*. Collectively, these results suggest that CRK36 and ARCK1 form a complex and function in a negative feedback mechanism regulating ABA and osmotic stress responses (Tanaka *et al.*, 2012).

RLCK family

Calcium-mediated signalling plays a pivotal role in plant responses to abiotic stresses, including cold and drought stress. Calcium/calmodulin-regulated RLCKs have been shown to be involved in abiotic stress signalling. For instance, the cold-responsive *CRLK1*, which encodes an RLCK with two sites for calcium/calmodulin binding to stimulate kinase activity, was reported to regulate cold stress signalling (T. Yang *et al.*, 2010a). The *crlk1* knock-out mutant plants exhibited an increased sensitivity to chilling and freezing stress, and cold-responsive genes were down-regulated in *crlk1* mutants (T. Yang *et al.*, 2010a). Further analysis revealed that CRLK1 interacts with MEKK1, a member of the MAPKKK family, both *in vitro* and *in planta* (T. Yang *et al.*, 2010b). MAPK activation by cold treatment was abolished in the *crlk1* mutant, and the expression levels of cold-inducible genes involved in MAPK signalling were altered. Calcium-dependent calmodulin-binding RLCKs have also been isolated from *Glycine max* (GmCaMK1) (DeFalco *et al.*, 2010) and *Glycine soja* (GsCBRLK) (L.A. Yang *et al.*, 2010). Although GmCaMK1 was identified as a homologue of *Arabidopsis* CRLK1, the function of GmCaMK1 in the cold stress response is still unclear. GsCBRLK activity is regulated by its calcium/calmodulin binding, and the overexpression of *GsCBRLK* in transgenic *Arabidopsis* enhanced plant tolerance to high salinity and ABA, as well as increasing the expression level of stress marker genes.

Perception and cross-talk of osmotic stress signalling by non-ethylene histidine kinases

The His–Asp phosphorelay is involved in the regulation of responses to various abiotic stresses, including drought, high salinity, and cold (Tran *et al.*, 2007b, 2010b; Wohlbach *et al.*, 2008; Jeon *et al.*, 2010; Ha *et al.*, 2012; Pham *et al.*, 2012). The *Arabidopsis* genome contains eight HK members. Five

HK members (ETR1, ERS1, AHK2, AHK3, and AHK4) are implicated in the perception of two plant hormones, ethylene and CKs (Schaller *et al.*, 2008). The CK receptor AHKs, AHK2, AHK3, and AHK4, are involved in phosphorelay along with five AHPs and numerous type-B ARRs which control the transcription of certain type-A *ARR* genes (Tsai *et al.*, 2012) (Fig. 4). On the other hand, type-A *ARR* genes, whose expression is rapidly induced by CKs for negative feedback regulation, were reported to regulate the activity of type-B ARRs indirectly via a negative feedback loop (Muller and Sheen, 2007; Ha *et al.*, 2012) (Fig. 4).

Among the non-hormonal receptor HKs (AHK1, AHK5, and CKI1), AHK1 was identified as a unique osmosensor with *in planta* positive regulatory function in the osmotic stress response (Tran *et al.*, 2007b; Wohlbach *et al.*, 2008). AHK1 complements a deletion of SLN1, an osmosensor HK in yeast, and acts as an osmosensor by activating downstream cascades in yeast cells (Urao *et al.*, 1999). Overexpression of *AHK1* in *Arabidopsis* resulted in increased tolerance to osmotic stress, whereas the *ahk1* knock-out mutant plants exhibited enhanced sensitivity to osmotic stress (Tran *et al.*,

Fig. 4. Schematic model of cytokinin and osmotic stress signal transduction pathways via a His–Asp phosphorelay in *Arabidopsis*. The activated HKs transfer the phosphoryl group to AHPs which are subsequently activated. Activated AHPs then transport the signals to type-B ARRs, which act as transcriptional factors, in the nucleus. The target genes of the type-B ARRs contain type-A *ARR* genes that function as negative regulators in cytokinin signalling in a negative feedback loop. The osmotic stress signalling that is controlled by AHK1 and the cytokinin signalling mediated via the His–Asp phosphorelay have antagonistic functions in the control of the osmotic stress responses.

2007b; Wohlbach *et al.*, 2008). Additionally, *ahk1* mutants exhibited decreased sensitivity to ABA, and expression levels of ABA and/or stress-responsive genes were down-regulated. These studies indicate that the osmosensor AHK1 acts as a positive regulator in osmotic stress signalling and regulates the expression of downstream genes in both an ABA-dependent and ABA-independent manner (Tran *et al.*, 2007b; Wohlbach *et al.*, 2008). While downstream cascades are potentially controlled by AHPs and ARRs in the multiple His–Asp phosphorelay, the factors that receive the signals from AHK1 and the identification of the precise signalling cascades remain to be determined. AHK5, which is localized to the plasma membrane, was identified as a regulator of stomatal closure in response to ROS (H_2O_2) (Desikan *et al.*, 2008). Guard cells of *ahk5* mutant plants exhibited reduced sensitivity to the ROS-induced stomatal closure response but were not significantly altered in their response to ABA, suggesting that AHK5 regulates the H_2O_2 response in an ABA-independent manner (Desikan *et al.*, 2008). Recently, the same group provided evidence showing that AHK5 acts as a negative regulator of the salt stress response during seed germination and growth of both young seedlings and mature plants in *Arabidopsis* (Pham *et al.*, 2012).

Among the hormonal receptor HKs, AHK2, AHK3, and AHK4 were shown to negatively regulate ABA and stress signalling (Tran *et al.*, 2007b, 2010b; Jeon *et al.*, 2010) (Fig. 4). Mutations of *ahk2*, *ahk3*, and *ahk4* in various combinations resulted in increased sensitivity to ABA and tolerance to abiotic stresses including cold, salt, and drought (Tran *et al.*, 2007b; Jeon *et al.*, 2010). The expression levels of ABA and/or stress-responsive genes were up-regulated in *ahk2ahk3* mutant plants. These studies suggest that cross-talk exists among ABA, CK, and stress signalling pathways. The ability of CKs to have an impact on the negative regulatory function of AHK4 suggests the involvement of CKs as a negative regulator of the stress response (Tran *et al.*, 2007b). This has been recently verified by a series of molecular analyses of CK-deficient mutants subjected to drought and salt stress (Nishiyama *et al.*, 2011, 2012). Reduced CK content found under drought and salt stresses may help plants adapt to these stresses through a leaf senescence strategy (Guo and Gan, 2007; Nishiyama *et al.*, 2011; Ha *et al.*, 2012). However, the conditional and stage-specific stimulation of CK biosynthesis by stress-inducible promoter(s) may delay the stress-induced acceleration of leaf senescence and enable the maintenance of photosynthetic activity under stress conditions, providing an effective biotechnological strategy for agricultural applications (Rivero *et al.*, 2007; Peleg *et al.*, 2011). For instance, the overexpression of a CK biosynthetic gene, an *isopentenyltransferase* (*IPT*) gene derived from *Agrobacterium tumefaciens*, under the control of a drought- and senescence-inducible promoter was reported to enhance both plant longevity and stress tolerance in tobacco (*Nicotiana tabacum*) and rice. The transgenic plants exhibited increased ROS scavenging and photosynthetic capacity as well as the ability to maintain homeostasis under stress conditions (Rivero *et al.*, 2007; Peleg *et al.*, 2011).

Several downstream components of CK signalling have also been shown to be involved in the stress response. For instance,
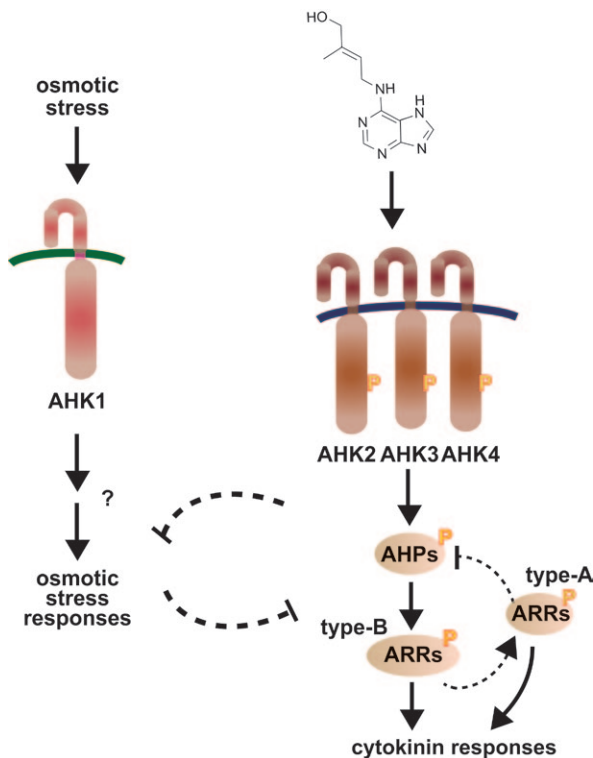
loss of function of *ARR5*, *ARR6*, or *ARR7* improved cold tolerance (Jeon *et al.*, 2010), while that of *ARR1* and *ARR12* enhanced salt tolerance (Mason *et al.*, 2010). Additionally, altered expression of phosphorelay components by abiotic stresses has been reported in various plant species, including *Arabidopsis*, rice, and soybean (Osakabe *et al.*, 2002; Tran *et al.*, 2007b; Karan *et al.*, 2009; Jeon *et al.*, 2010; Le *et al.*, 2011). Overall, the collective reports support the premise that cross-talk between hormone and stress signalling networks plays an important role in plant stress response.

Conclusions and future perspectives

Plants have evolved the ability to adapt to and tolerate natural environmental stresses. Tolerance mechanisms are constructed of complex processes involving various signalling pathways. Changes in surrounding environmental conditions, sensed by molecular sensors, can activate cellular signalling events that control plant homeostasis in an effort to maintain 'normal' growth and development (Fig. 3). HKs involved in the His–Asp signalling systems have been identified in many plant species, indicating that the fundamental roles of HKs in stress responses are conserved. *In planta* elucidation of each member of the phosphorelay and their downstream genes will provide a greater insight into the functions of the phosphorelay and the cross-talk that exists among various hormones involved in stress responses.

The large RLK family, which has evolved during plant evolutionary history, may enable RLKs to recognize a wide range of environmental signals. This premise implies that the diverse functions of RLKs are critical to maintaining 'normal' plant growth and development under various conditions. Due to the multiple functions of RLKs in the abiotic stress response, signalling networks governing plant responses to stress are complex. The reports discussed in this review suggest that various abiotic stresses serve as signals to trigger the activity of RLKs. These stress-specific 'ligands' and RLKs coordinately control various molecular events and downstream cascades, such as phosphorylation cascades, activation of secondary messengers, gene expression, and protein dynamics. Further analyses of tissue- and/or cellular-specific responses, as well as developmentally regulated events, will provide new insights into the integrated molecular relationship of these signalling pathways. The study of cross-talk between regulatory pathways involving different plant hormones and/or biotic stresses will also help to identify the specific molecular cascades that control plant homeostasis. The precise ligands and the downstream signalling molecules sensed by RLKs remain to be identified. Extracellular signals, such as hormones, small peptides, small chemical molecules, and physical stimuli, are all potential candidates. The intracellular events downstream of RLKs may include kinase cascades (e.g. MAPK), Ca^{2+} ions, ROS signalling, metabolic adjustments, and membrane dynamics. It will be important to analyse the cellular or tissue-specific events in stress responses as well as growth, developmental, and ageing stage-specific events to understand precisely the signalling pathways controlled by RLKs, especially those involved in cell–cell communication. These efforts will provide new insights that will enable us to better understand the complex and well-coordinated strategies that plants have evolved to adapt to adverse environmental conditions.

Environmental stress tolerance is an important agronomic trait and is essential for maintaining high yield under adverse conditions. It is important to understand how plants sense the environment and regulate signalling networks. This knowledge will provide important information on how plants attempt to maintain 'normal' growth and development under suboptimal environmental conditions. Genetic engineering of plant membrane signalling factors, including RLKs and HKs, can have a positive impact on stress tolerance by influencing many downstream signalling factors. Furthermore, emerging technologies utilizing synthetic restriction endonucleases (Shukla *et al.*, 2009; K. Osakabe *et al.*, 2010; Zhang *et al.*, 2010) will enable genome editing of RLKs and HKs, which will help to elucidate how these kinases have evolved to control downstream signalling pathways in various plant species. A more precise knowledge of how signalling networks function and are regulated, their impact on the physiology and biochemistry of a plant, and how different signalling networks are integrated to maintain plant homeostasis (i.e. normal growth and development) will increase our ability to produce superior plant genotypes that exhibit high levels of productivity in a rapidly changing and ever more stressful environment.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Plant receptor-like kinases that control plant growth and development and plant responses to stresses.

Acknowledgements

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