Plant hormone regulation of abiotic stress responses

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Abstract | Plant hormones are signalling compounds that regulate crucial aspects of growth, development and environmental stress responses. Abiotic stresses, such as drought, salinity, heat, cold and flooding, have profound effects on plant growth and survival. Adaptation and tolerance to such stresses require sophisticated sensing, signalling and stress response mechanisms. In this Review, we discuss recent advances in understanding how diverse plant hormones control abiotic stress responses in plants and highlight points of hormonal crosstalk during abiotic stress signalling. Control mechanisms and stress responses mediated by plant hormones including abscisic acid, auxin, brassinosteroids, cytokinins, ethylene and gibberellins are discussed. We discuss new insights into osmotic stress sensing and signalling mechanisms, hormonal control of gene regulation and plant development during stress, hormone-regulated submergence tolerance and stomatal movements. We further explore how innovative imaging approaches are providing insights into single-cell and tissue hormone dynamics. Understanding stress tolerance mechanisms opens new opportunities for agricultural applications.

Abiotic stresses
Environmental stresses that are associated with the non-living environment, such as weather conditions or the quality of the soil in which plants grow.

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Plants are a major source for food, fuel and fibre, and are important contributors to the ecological diversity and sustainability of our planet. To optimize growth and productivity under changing environmental conditions, which are intensified as a result of climate change, plants have developed sophisticated mechanisms to sense and respond to external stresses[1,2]. Among them, abiotic stresses appear in various forms, associated either with changes in weather conditions such as rainfall, temperature and irradiation from the Sun or with the quality of the soil in which plants grow (for example, the content of water, nutrients and soil contaminants)[1]. In particular, changes in water availability and temperature leading to drought and heat stress have been associated with climate change[2].

To develop concepts and approaches for protecting plants from the negative effects of abiotic stresses, and to secure the future demands for plant products, we need to understand the mechanisms of plant stress responses at the molecular level. Plant hormones — that is, abscisic acid (ABA), auxin, brassinosteroid, cytokinin, ethylene, gibberellin, jasmonate, salicylic acid and strigolactone — are well-known plant growth regulators that mediate adaptations to environmental conditions. For an overview of their functions and respective signalling mechanisms, see a recent review[3]. The most notable roles of phytohormones in abiotic stress responses are listed in TABLE 1.

In this Review, we describe the current understanding of how plant hormones and other signalling compounds mediate plant responses to abiotic stresses, including drought, osmotic stress and flooding. We discuss the current view on how osmotic stress is sensed by plants, and how this leads to the activation of SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2 (SnRK2)-type protein kinases and interactions with plant hormone signalling modules. Then, we elaborate on phytohormone-dependent gene regulatory mechanisms that mediate abiotic stress responses in plants. We also highlight the effects of stress-dependent hormone responses on seed germination and flowering time, how ABA and auxin coordinate root growth under stress, the ethylene-mediated and gibberellin-mediated regulation of plant responses to flooding, and the ABA and abiotic stress sensing mechanisms regulating the aperture of stomata. Abiotic stresses also cause bud dormancy, leaf senescence and organ abscission, which are reviewed elsewhere[4]. Finally, we review how biosensor-based hormone imaging techniques are contributing to the elucidation of hormone dynamics under abiotic stress, and discuss how understanding stress tolerance mechanisms might open new avenues for agricultural applications.

Osmotic stress sensing and signalling

Water uptake from the soil and water movements within plants are driven by water potential gradients. Hypo-osmotic stress, such as flooding, leads to cell swelling, whereas hyperosmotic stress, such as drought

Phytohormones
Plant-derived compounds that function as plant growth regulators either locally or over long distances and at low (submicromolar) concentrations.

Osmotic stress
A sudden change in the ambient solute concentration resulting in the water potential difference between cells and environments effects the tendency of water movement across cell membranes. Hypo-osmotic stress leads to water influx into cells, whereas hyper-osmotic stress leads to water efflux from cells.

Stomata
Small pores in the leaf epidermis that are formed by guard cells to allow the uptake of CO_2 for photosynthesis in exchange for water loss.

Table 1 | **Examples of roles of phytohormones in abiotic stress responses**

Stress type	Adaptive responses	Refs
ABA		
Drought and osmotic stress in roots	Induction of ABA biosynthesis in shoots via hydraulic signals and CLE25 peptide-mediated induction of *NCED3* expression	26,27,32
Osmotic stress and salt stress	Activation of SnRK2-type protein kinases, which is mediated by subgroup B Raf-like kinases	34,36,39,42,44
Drought stress and osmotic stress in roots (uneven distribution of water in the soil)	Root hydrotropism, which requires ABA signalling in the cortex of the elongation zone	101
Salt stress	Inhibition of lateral root development, which depends on ABA synthesis and endodermal ABA signalling; ABA interferes with auxin signalling	104,116
Salt stress, K^+ and SO_4^{2-} deficiency	Endodermal suberization	207
Cold stress	Phosphorylation of the transcription factor ICE1 by SnRK2.6 (also known as OST1)	208
Heat stress	Promotion of seedling survival	209
Auxin		
Salt stress	Root bending to promote halotropism, the preferential growth away from areas of high salinity	105,106
Drought stress in roots (uneven distribution of water in the soil)	Hydropatterning, the preferential formation of lateral roots near water, which is mediated by the auxin response factor ARF7	114,115
Drought stress	Expression of *IAA5* and *IAA19*, two transcriptional repressors of auxin responses	76
Heat stress	Hypocotyl elongation via PIF4-mediated induction of auxin biosynthesis, the stabilization of auxin co-receptors and the regulation of gene expression by auxin response factors	210–212
Brassinosteroids		
Drought stress	Crosstalk with ABA signalling at the level of BES1-mediated and RD26-mediated transcriptional regulation	71
Cold and freezing stress	Modulation of *COR* and *CBF* expression	213,214
Increased temperature	Thermomorphogenesis via PIF4-mediated induction of BR biosynthesis; the BR-activated transcription factor BZR1 functions in a feedforward loop downstream of auxin and PIF4 to further induce *PIF4* expression	215,216
Cytokinins		
Drought stress and salt stress	Reduction of CK content and signalling, leading to increased ABA sensitivity; crosstalk with ABA signalling via interaction of SnRK2s with Type-A and Type-B ARR proteins	74,181,217
Osmotic stress (uneven distribution of water in the soil)	Root hydrotropism, which depends on the asymmetric distribution of CK signalling in the root tip; CK signalling is enhanced at the lower water potential side	202
Ethylene		
Salt stress	Induction of ET and ET signalling	121
Flooding or submergence	ET production; group VII *ERF* genes in *Arabidopsis* and other related *ERF* genes in rice (*SUBMERGENCE TOLERANCE 1*, *SNORKEL1* and *SNORKEL2*) likely induce gibberellin biosynthesis	128,129,135
Metal deficiency	Reduction of endodermal suberization	207
Gibberellin		
Drought stress	Interference with ABA signalling via DELLA protein interactions with the ABA-regulated transcription factor ABF2	218
Salt stress	Reduction of bioactive gibberellin levels likely via ABA signalling; *della* quadruple mutants are hypersensitive to salt stress	121
Cold stress	Accumulation of DELLA proteins, which interact with GRF-type transcription factors, leading to altered gene expression	219
Heat stress	Hypocotyl elongation via induction of gibberellin biosynthesis and degradation of DELLA proteins in a COP1-dependent manner	220
Water submergence	Internode elongation in rice, which is mediated by the induction of gibberellin production	135–137

Table 1 (cont.) | **Examples of roles of phytohormones in abiotic stress responses**

Stress type	Adaptive responses	Refs
Jasmonic acid		
Cold stress	Induction of JA production; JAZ degradation releases ICE1 and ICE2 from JAZ-mediated repression	221
Heat stress	Accumulation of the JA receptor COI1 to enhance downstream JA responses	185
Strigolactones		
Drought stress and salt stress	Modulation of stomatal development and function via ABA-dependent and ABA-independent pathways	179,222

ABA, abscisic acid; ABF2, ABSCISIC ACID BINDING ELEMENT-BINDING FACTOR 2; ARF7, AUXIN RESPONSE FACTOR 7; ARR, ARABIDOPSIS RESPONSE REGULATOR; BES1, BRI1-EMS-SUPPRESSOR 1; BR, brassinosteroid; CK, cytokinin; CLE25, CLAVATA3/ESR-RELATED 25; COI1, CORONATINE-INSENSITIVE 1; COP1, CONSTITUTIVE PHOTOMORPHOGENETIC 1; DELLA, plant-specific GRAS family proteins functioning as repressors of the gibberellin signalling pathway; ET, ethylene; GRF, GROWTH REGULATORY FACTOR; ICE1, INDUCER OF CBF EXPRESSION 1; ICE2, INDUCER OF CBF EXPRESSION 2; JA, jasmonic acid; JAZ, JASMONATE ZIM DOMAIN PROTEIN; PIF4, PHYTOCHROME-INTERACTING FACTOR 4; RD26, RESPONSIVE TO DESICCATION 26; SnRK2, SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2.

and salinity, leads to plant wilting. Plants have evolved osmotic stress adaptive mechanisms, including the regulation of cellular osmoticum concentrations, stomatal movements and plant development through ABA-dependent and ABA-independent pathways[1,5]. Here, we summarize the current understanding of osmotic sensory and signalling mechanisms in plants that lead to stress adaptation.

Osmotic and salt stress sensing. Plants can sense the alteration of turgor pressure, the mild change of solute concentrations in cells, and the mechanical effects on cellular structures caused by osmotic stress. Calcium signalling is suggested to play a key role in osmosensing because cytosolic free calcium concentrations ($[Ca^{2+}]_{cyt}$) in plants rapidly and transiently increase within seconds of exposure to osmotic shock[6,7]. The roles of mechanosensitive ion channels in osmotic stress sensing have been investigated (FIG. 1a). MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE-LIKE (MSL) proteins are non-selective ion channels activated by membrane tension for osmoregulation during hypo-osmolality in organelles, hydration and germination in pollen, touch responses in roots and cell swelling[8–10]. MID1-COMPLEMENTING ACTIVITY (MCA)-type Ca^{2+}-permeable channels are activated by membrane tension and are suggested to mediate hypo-osmotic shock and touch sensing in roots[11]. They also function in mediating cold tolerance and cold-induced $[Ca^{2+}]_{cyt}$ increases[12]. Another mechanosensitive ion channel, PIEZO1 (PZO1), is required for mechanotransduction at root tips[13]. Interestingly, plant PZO proteins are localized in the vacuolar membrane to regulate $[Ca^{2+}]_{cyt}$ oscillations and tip growth in *Physcomitrium patens* caulonemal cells and to mediate vacuole tubulation in the tips of *Arabidopsis thaliana* (referred to hereafter as '*Arabidopsis*') pollen tubes[14], suggesting a potential function under hypo-osmotic stress.

REDUCED HYPEROSMOLALITY-INDUCED $[Ca^{2+}]_{cyt}$ INCREASE 1 (OSCA1), a potential osmosensor, is involved in hyperosmotic stress-induced $[Ca^{2+}]_{cyt}$ increases for osmotic stress tolerance[15] (FIG. 1b). OSCA1 was initially characterized as a hyperosmolality-activated Ca^{2+}-permeable cation channel[15]. Further studies reported that OSCA family proteins function as stretch-activated channels[16,17]. Ca^{2+}-responsive phospholipid-binding BONZAI (BON) proteins were recently reported to mediate hyperosmotic stress tolerance by positively regulating osmotic stress-induced $[Ca^{2+}]_{cyt}$ increases, ABA accumulation and gene expression[18]. These membrane-associated Ca^{2+}-responsive BON proteins may be involved in osmotic sensing and signalling by regulating the initial $[Ca^{2+}]_{cyt}$ elevation together with plasma membrane Ca^{2+} transporters[19] (FIG. 1b). Defects in plant growth and ABA accumulation under osmotic stress in *bon* mutants can be restored by crossing *bon* mutants with *snc1-11* and *pad4* mutant alleles that are impaired in nucleotide-binding domain and leucine-rich repeat (NLR) immune signalling[18]. Therefore, BON proteins may confer osmotic stress responses by suppressing NLR immune signalling. Although several osmotic stress-linked mechanisms have been characterized, further research is needed to dissect their differential functions. Notably, the activation of SnRK2-type protein kinases in response to hyperosmotic shock is not impaired in *osca* septuple and *bon1 bon2 bon3* triple mutants[18,20], indicating the need to identify additional osmotic stress sensing and signalling mechanisms.

Plants sense salinity and induce rapid and transient $[Ca^{2+}]_{cyt}$ elevations[6,21] to trigger salt tolerance responses through the salt overly sensitive pathway[1] (FIG. 1c). Under salt stress, the receptor-like kinase FERONIA may sense cell wall defects caused by salinity and elicits cell-specific $[Ca^{2+}]_{cyt}$ signals for maintaining cell wall integrity[22]. FERONIA potentially also interferes with ABA signalling via interaction with the PROTEIN PHOSPHATASE 2C (PP2C) ABA-INSENSITIVE 2 (ABI2)[23]. In *Arabidopsis*, plasma membrane glycosyl inositol phosphorylceramide (GIPC) sphingolipids function in salt sensing[24]. In this model, GIPC sphingolipid formation is catalysed by the protein MONOCATION-INDUCED $[Ca^{2+}]_i$ INCREASES 1 (MOCA1; also known as IPUT1), and binding of Na^+ ions to GIPC sphingolipids activates Ca^{2+} influx channels[24]. Disruption of the annexin gene *ANN4*, which codes for a putative Ca^{2+}-permeable channel component, impairs salt stress-induced $[Ca^{2+}]_{cyt}$ elevations by ~40%, while Ca^{2+}-activated

Mechanosensitive ion channels
Ion channels that respond to mechanical forces, for example, induced by membrane tension.

SCaBP8(also known as CBL10) in complex with SOS2 negatively regulate ANN4 by phosphorylation to fine-tune salt tolerance responses[25].

Osmotic stress-induced ABA biosynthesis. Endogenous ABA concentrations increase approximately 2.5–6 h after exposure to water deficiency[26–29]. Stress-induced de novo ABA synthesis depends on the induction of the *NCED3* gene, which encodes a 9-*cis*-epoxycatoteinoid dioxygenase catalysing the rate-limiting step for ABA biosynthesis[30]. Post-translational processing of NCED3 in the chloroplast has also been reported to regulate ABA accumulation[31]. In response to water deficiency in roots, a hydraulic signal contributes to a rapid root-to-shoot water deficiency signal to trigger ABA biosynthesis in *Arabidopsis* leaves and stomatal closure[26]. In addition, a small peptide, CLAVATA3/ESR-RELATED 25 (CLE25), is induced in the root vasculature during drought stress and moves to aerial tissues to induce *NCED3* expression likely through BARELY ANY MERISTEM 1 (BAM1) and BAM3 receptor-like kinases[32] (FIG. 1b). At the transcriptional level, an *Arabidopsis* NAC transcription factor, ATAF1, was suggested to regulate *NCED3* expression to enhance ABA accumulation[33]. Moreover, the NGATHA (NGA) protein family, including four members in *Arabidopsis*, were identified as transcriptional activators regulating *NCED3* expression through direct binding to a *cis*-acting element (CACTTG) in the 5′ untranslated region of the *NCED3* promoter[30].

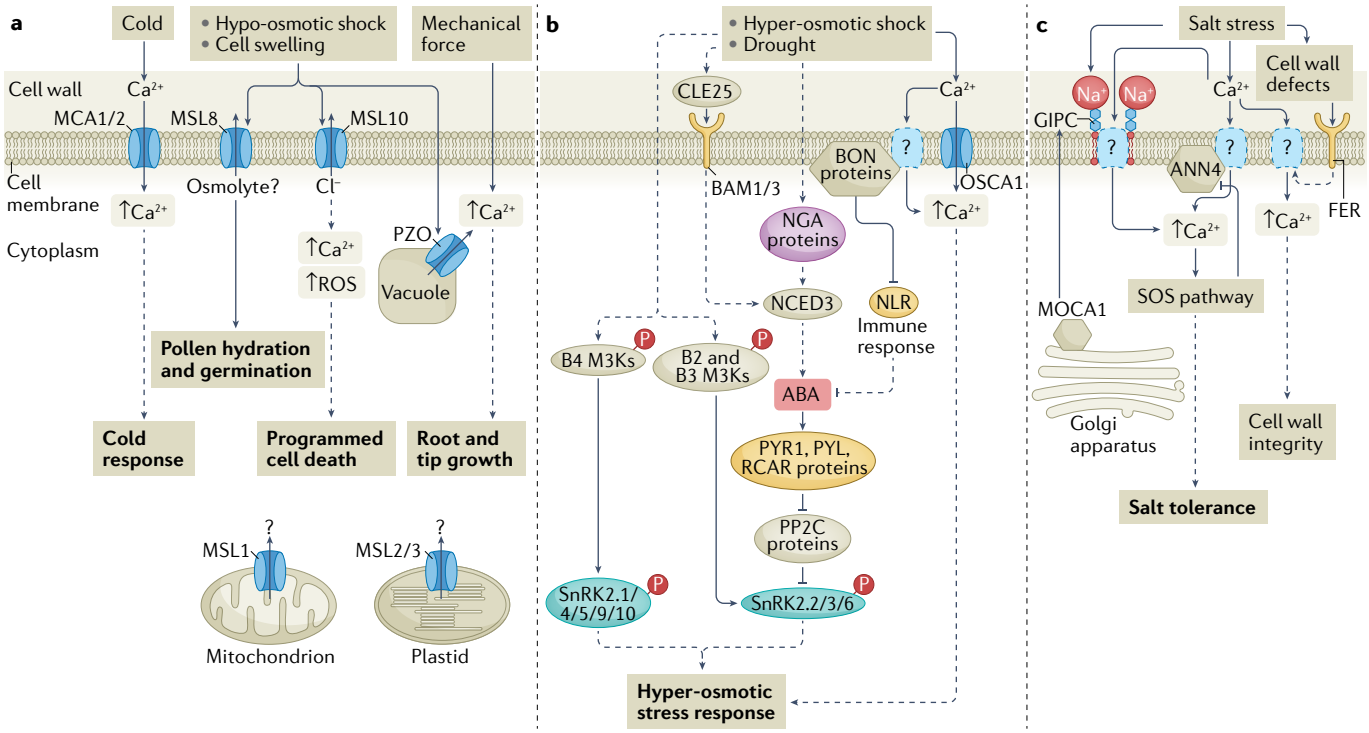

Fig. 1 | **Osmotic stress and salinity sensing and signalling in plants.** **a** | Mechanosensitive channels have been proposed to be involved in sensing the alterations of membrane tension caused by hypo-osmotic stress and other abiotic stresses. MID1-COMPLEMENTING ACTIVITY 1 (MCA1) and MCA2 mediate hypo-osmotic and cold-induced $[Ca^{2+}]_{cyt}$ increases and promote cold tolerance[12]. MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE-LIKE 8 (MSL8) prevents bursting of pollen during hydration and germination[8]. MSL10 potentiates hypo-osmotic stress-induced and cell swelling-induced transient cytosolic free calcium concentration ($[Ca^{2+}]_{cyt}$) increases, reactive oxygen species (ROS) production and programmed cell death[10]. MSL1, MSL2 and MSL3 control mitochondrial and plastidial osmotic pressure[223]. PIEZO (PZO) proteins are required for $[Ca^{2+}]_{cyt}$ oscillations in tip-growing cells[14] and mechanical stress-induced $[Ca^{2+}]_{cyt}$ increases in the root tip to regulate root penetration into denser barriers[13]. **b** | Hyperosmotic stress-induced $[Ca^{2+}]_{cyt}$ increases have been reported to function in early hyperosmotic stress signalling. REDUCED HYPEROSMOLALITY-INDUCED $[Ca^{2+}]_{cyt}$ INCREASE 1 (OSCA1) is an osmotic stress-sensitive and mechanical stress-sensitive channel required for hyperosmotic stress-induced $[Ca^{2+}]_{cyt}$ increases[15]. Ca^{2+}-responsive phospholipid-binding BONZAI (BON) proteins regulate hyperosmotic stress-induced $[Ca^{2+}]_{cyt}$ increases and suppress nucleotide-binding domain and leucine-rich repeat (NLR) immune signalling to trigger a hyperosmotic stress response[18]. Drought induces abscisic acid (ABA) biosynthesis via induction of *NCED3* expression. Root-derived CLAVATA3/ESR-RELATED 25 (CLE25) peptides activate *NCED3* expression in the shoot in response to dehydration likely through the receptor-like kinases BARELY ANY MERISTEM 1 (BAM1) and BAM3 (REF. [32]). NGATHA (NGA) transcription factors are responsible for the drought-induced transcriptional activation of *NCED3* (REF. [30]). Hyperosmotic stress activates Raf-like mitogen-activated protein kinase kinase kinases (M3Ks) via phosphorylation through an unknown osmotic stress sensor-mediated signal transduction mechanism. Members of the B2 and B3 subgroups of Raf-like M3Ks mediate both the rapid osmotic stress-induced and the slower, post-ABA-synthesis, activation of SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2.2 (SnRK2.2), SnRK2.3 and SnRK2.6, whereas the B4 subgroup of Raf-like M3Ks activate only osmotic stress-responsive SnRK2.1, SnRK2.4, SnRK2.5, SnRK2.9 and SnRK2.10 (REFS[20,42–44]). **c** | A salt-induced $[Ca^{2+}]_{cyt}$ increase triggers tolerance responses through the salt overly sensitive (SOS) pathway. Glycosyl inositol phosphorylceramide (GIPC) sphingolipids synthesized by MONOCATION-INDUCED $[Ca^{2+}]_i$ INCREASES 1 (MOCA1; also known as IPUT1) are involved in Na^+ sensing[24]. The ANNEXIN 4 (ANN4)-mediated $[Ca^{2+}]_{cyt}$ increase is feedback inhibited by the SOS pathway for fine-tuning salt tolerance[25]. FERONIA (FER) is required for maintenance of cell wall integrity under salt stress[22]. PP2C, PROTEIN PHOSPHATASE 2C; PYL, PYR1-LIKE; PYR1, PYRABACTIN RESISTANCE 1; RCAR, REGULATORY COMPONENT OF ABA RECEPTOR.

An emerging role of Raf-like M3Ks. An ABA-independent rapid osmotic stress signal transduction pathway and an ABA-dependent pathway converge at the level of SnRK2-type protein kinase activation. The *Arabidopsis* genome encodes ten SnRK2 genes. Except for SnRK2.9, the other SnRK2 proteins are activated by osmotic stress, whereas SnRK2.2, SnRK2.3 and SnRK2.6 (also known as OST1) are clearly activated by ABA[34–37]. ABA-dependent SnRK2 activation through the PYRABACTIN RESISTANCE 1 (PYR1)/PYR1-LIKE (PYL)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) and PP2C ABA sensing module has been well described[38]. Since SnRK2 activation by osmotic stress is not impaired in ABA-insensitive dominant negative PP2C *Arabidopsis* mutants[34,39], osmotic stress uses other signalling mechanisms to activate SnRK2 kinases. This ABA-independent pathway is still largely unknown, including the identity of the contributing osmotic stress sensors.

SnRK2 proteins have an autophosphorylation activity that enhances the kinase activity itself[40]. In vitro studies identified a key phosphorylation site at Ser175 within the activation loop of the SnRK2.6/OST1 kinase domain[40]. In vivo analyses revealed that both ABA and osmotic stress induce phosphorylation at residues Ser171 and Ser175 (REF.[41]).

Recent studies identified Raf-like mitogen-activated protein kinase kinase kinases (M3Ks) to be required for phosphorylation-dependent SnRK2 activation via osmotic stress and ABA signalling[20,42–44] (FIG. 1b). SnRK2.6/OST1 was found to be impaired in autoactivation after dephosphorylation by PP2C proteins[42]. The reactivation of SnRK2.6/OST1 requires the initial transphosphorylation at Ser171 or Ser175 by members of the *Arabidopsis* B2 and B3 subgroups of Raf-like M3Ks[42,44]. Moreover, the *raf-like m3kδ1 m3kδ6 m3kδ7* triple-knockout mutant exhibited not only a reduced SnRK2 kinase activation by ABA but also impairment in SnRK2 activation by osmotic stress[42]. Important functions of B4 subgroup Raf-like M3Ks in osmotic stress-induced but not in ABA-induced rapid SnRK2 activation were identified[20,43] (FIG. 1b).

Roles for Raf-like M3Ks in ABA responses were initially identified in genetic ABA response and stress response mutant screens in *Arabidopsis* and in moss[45,46]. In the moss *Physcomitrium patens*, the ABA and abiotic stress-responsive Raf-like kinase ARK (also known as ANR), which is encoded by a single ancestral gene similar to the *Arabidopsis* B3 subgroup, has a role in osmotic stress signalling and ABA signalling[46–48]. A recent study reported that ARK is activated by ABA[47]. The *Arabidopsis* B3 Raf-like M3K subgroup contains another well-studied gene, *CONSTITUTIVE TRIPLE RESPONSE 1* (*CTR1*), functioning as a negative regulator of ethylene signalling. Ethylene deactivates CTR1 through ethylene receptors, a family of histidine kinases including ETHYLENE RESPONSE 1 (ETR1), which directly binds to CTR1 (REF.[49]). Interestingly, *Physcomitrium patens* ARK/ANR (also known as CTR1L) mediates not only ABA signalling in moss but also ethylene responses[50], which might suggest a role of Raf-like M3Ks as a signalling 'hub'. How osmotic stress sensors are linked to Raf-like M3Ks, SnRK2 activation and downstream components remains to be determined.

Gene regulation under abiotic stress

Changes in gene expression mediate many of the effects of phytohormones. Early genomic technologies revealed that abiotic stress-linked ABA level increases change the mRNA levels of thousands of genes[51]. This, together with the discovery that many classic ABA-insensitive mutations were mapped to transcriptional regulators, suggested a prominent role for gene regulation in abiotic stress resistance[52].

ABA-mediated transcriptional regulation and hormone crosstalk. Early studies discovered a conserved *cis*-acting regulatory element known as the ABA-responsive element (ABRE) in the promoters of drought-induced genes[53]. ABREs are recognized by basic leucine zipper-type transcription factors, including a family of four ABRE-binding proteins/ABRE-binding factors (AREBs/ABFs)[54], and the closely related ABI5 (REFS[52,55,56]) (FIG. 2). During ABA signalling, ABA-dependent SnRK2-type protein kinases directly phosphorylate and activate AREBs/ABFs and ABI5 (REFS[57–59]). In *Arabidopsis*, the four partially redundant AREBs/ABFs are responsible for most of the transcriptional responses to ABA during vegetative growth[60], whereas ABI5 is more important during seed germination[61] (see later). Many of the AREB/ABF targets are other transcription factor genes, implying that a multilevel transcription factor hierarchy controls ABA-dependent transcriptome remodelling[62–65]. A seminal study using chromatin immunoprecipitation followed by sequencing to profile the genome-wide binding sites of 21 transcription factors during the ABA response revealed that many binding events are dynamic, and that multiple transcription factors can target the same gene[64]. Crucially, the ABA-induced binding of some transcription factors was positively correlated with the presence of adjacent ABRE sites, suggesting that some transcription factors may act cooperatively with AREBs/ABFs. Indeed, several NAC family transcription factors are required for ABA-dependent transcription events, including ANAC096, which interacts with ABF2 to activate *RD29A* transcription[62,66,67].

In the absence of abiotic stress, repression of ABA signalling promotes optimal growth. For instance, under non-stress conditions, mRNA levels of *ABI5* are low, and *ABI5* transcription is increased upon exposure to ABA or osmotic stress[55]. This repression of *ABI5* requires the SWI2/SNF2 chromatin remodelling ATPase BRAHMA[68]. In the absence of ABA, BRAHMA inhibits the transcription of *ABI5* by promoting nucleosome occupancy at the transcription start site of the *ABI5* gene. Interestingly, phosphorylation of BRAHMA by SnRK2.2, SnRK2.3 and SnRK2.6 appears to inhibit its action and, in turn, this inhibitory phosphate is removed by group A PP2C proteins[69] (FIG. 2).

Different hormone pathways interact to control numerous aspects of plant life in the absence of abiotic stress, and we direct readers to a review[70] for an overview of this topic. Here, we focus on how transcriptional

mRNA decapping
The removal of the 5′ methylguanosine cap, a key step in the regulated degradation of mRNAs.

regulation enables hormone crosstalk during drought stress responses. For instance, the ABA-induced transcription factor RESPONSIVE TO DESICCATION 26 (RD26) represses a subset of brassinosteroid-induced genes[62,71]. Furthermore, brassinosteroid-activated transcription factors repress the expression of *RD26*, suggesting that antagonistic crosstalk between ABA signalling and brassinosteroid signalling contributes to drought stress responses[72] (FIG. 2). Intriguingly, overexpression of the vascular-enriched brassinosteroid receptor BRL3 causes constitutive expression of some drought-induced genes, including *RD26*, and promotes drought resistance[73]. The cytokinin and ABA pathways also converge on the level of transcriptional control. SnRK2-mediated phosphorylation of the Type-A ARABIDOPSIS RESPONSE REGULATOR 5 (ARR5), a negative regulator of cytokinin signalling, promotes its protein stability, thereby downregulating cytokinin responses during drought stress[74]. Oppositely, cytokinin can trigger the degradation of ABI5 to promote seed germination[75] (FIG. 2). Furthermore, dehydration induces the expression of IAA5 and IAA19, two transcriptional repressors of the auxin signalling pathway, indicating that auxin responses are repressed during drought stress[76].

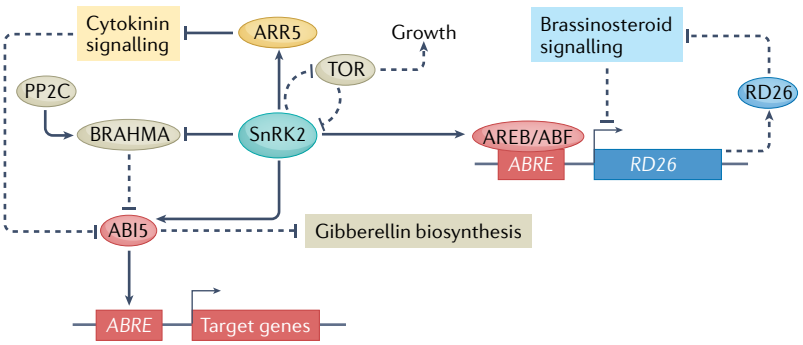

Fig. 2 | **Hormonal crosstalk through transcriptional regulation.** Plant hormones control abiotic stress responses by altering transcriptional programmes. The abscisic acid (ABA) signalling system intersects with many other hormone pathways during transcription. This figure summarizes the relationships between ABA signalling and key transcriptional regulators during hormonal signalling. During ABA responses, SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2 (SnRK2)-type protein kinases phosphorylate *ABA-RESPONSIVE ELEMENT* (*ABRE*)-binding proteins/*ABRE*-binding factors (AREBs/ABFs) and the transcription factor ABA-INSENSITIVE 5 (ABI5). AREBs/ABFs and ABI5 activate target genes with *ABRE*s in their promotors to drive ABA responses. For instance, during drought stress AREBs/ABFs activate transcription of *RESPONSIVE TO DESICCATION 26* (*RD26*), which encodes a transcription factor that can repress brassinosteroid signalling. SnRK2-type protein kinases further promote *ABI5* expression by phosphorylating and inhibiting the SWI2/SNF2-type chromatin remodelling ATPase BRAHMA, which represses *ABI5* transcription. Oppositely, PROTEIN PHOSPHATASE 2C (PP2C)-type phosphatases activate BRAHMA through dephosphorylation. Additionally, in dormant seeds, ABI5 target genes repress gibberellin biosynthesis and thereby block germination. Cytokinin signalling can repress ABA responses possibly by triggering the degradation of ABI5. ABA-activated SnRK2-type protein kinases promote transcriptional ABA responses by phosphorylating Type-A ARABIDOPSIS RESPONSE REGULATOR 5 (ARR5), a negative regulator of the cytokinin pathway. Finally, in unstressed conditions the protein kinase TARGET OF RAPAMYCIN (TOR) promotes plant growth by inhibiting SnRK2-type protein kinase-mediated ABA responses through phosphorylation of ABA receptors. Conversely, during stress, SnRK2-type protein kinases phosphorylate and inhibit the TOR regulatory protein REGULATORY-ASSOCIATED PROTEIN OF TOR 1B (RAPTOR1B), leading to growth repression. Not all mechanisms shown here are necessarily present at the same time or in the same cell or tissue. Dashed lines indicate indirect mechanisms.

Post-transcriptional abiotic stress responses. Post-transcriptional processes expand gene regulatory possibilities beyond transcriptional control, and recent research has uncovered the contributions of such mechanisms in shaping ABA responses. The discovery of an ABA hypersensitive mutant of a gene encoding an mRNA cap-binding protein known as ABA HYPERSENSITIVE 1 (ABH1) established an early link between mRNA processing and ABA signalling[77]. Alternative mRNA splicing is modulated by abiotic stress and regulates ABA responses[78–82]. *HAB1*, for instance, a group A PP2C gene, encodes multiple splice isoforms, of which *HAB1.2* retains an intron leading to a non-functional protein and ABA hypersensitivity[79,80].

Recently mRNA decay has emerged as an additional mechanism contributing to abiotic stress responses. The degradation of mRNA molecules is mediated by mRNA decapping[83]. During osmotic stress, ABA-independent subclass I SnRK2-type protein kinases phosphorylate the decapping activator VARICOSE (VCS), leading to the destabilization of some transcripts[84]. The 5′ end of mRNAs can be alternatively modified by the addition of nicotinamide adenine dinucleotide (NAD^+). In plants, the NAD^+ cap occurs on many transcripts and is thought to downregulate gene expression by promoting the degradation of marked mRNAs[85,86]. A recent study demonstrated that the NAD^+-capped transcriptome undergoes extensive changes in response to ABA and that many ABA-induced transcripts lose their NAD^+ caps following ABA treatment, possibly promoting their stability[87].

Growth regulation under abiotic stress

Phytohormones regulate many aspects of plant growth and development. Recent discoveries have begun to illuminate how they control different strategies of plant growth and development in response to abiotic stress.

TOR interaction with abiotic stress responses. The protein kinase TARGET OF RAPAMYCIN (TOR) is a central developmental and metabolic regulator in plants[88]. A reciprocal regulation between the ABA pathway and the TOR pathway has been proposed to coordinate plant growth and abiotic stress responses[89]. TOR phosphorylates PYL/RCAR ABA receptors to inhibit ABA signalling and promote growth under non-stress conditions, whereas ABA-activated SnRK2-type protein kinases phosphorylate REGULATORY-ASSOCIATED PROTEIN OF TOR 1B (RAPTOR1B) to inhibit TOR kinase activity and repress growth in response to abiotic stress conditions (FIG. 2).

Gibberellin, ABA and the decision to germinate. The regulation of seed germination promotes seedling survival by coordinating embryo development and emergence with environmental conditions. The balance of two competing hormone signalling pathways, gibberellin and ABA, dominates the decision to germinate[52]. During seed maturation, a network of transcription factors, including the ABA-regulated transcription factors ABI3, ABI4 and ABI5, induce genes required for seed desiccation and ABA biosynthesis and repress gibberellin

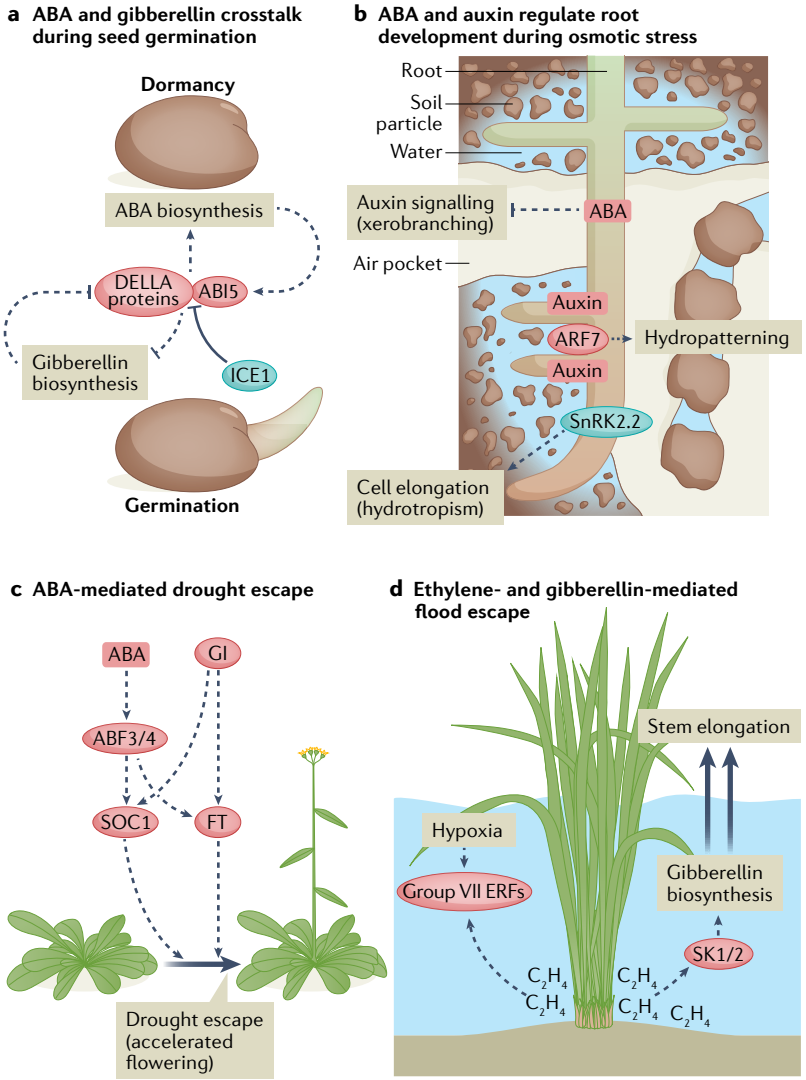

Fig. 3 | **Hormonal control of growth and development during abiotic stress. a** | Abscisic acid (ABA) and gibberellin signalling pathways antagonistically control germination. In dormant seeds, DELLA proteins and ABA-INSENSITIVE 5 (ABI5) promote ABA signalling by stimulating expression of ABA biosynthesis genes and the *ABI5* gene and inhibit gibberellin responses by repressing gibberellin biosynthesis. INDUCER OF CBF EXPRESSION 1 (ICE1) antagonizes DELLA and ABI5 activity to promote germination. During germination, gibberellin levels increase, and gibberellin triggers the degradation of DELLA proteins, leading to decreased ABA signalling. **b** | Water is unevenly distributed in soil, and large air pockets form between soil particles. Primary roots display hydrotropism or biased growth towards areas of higher water content. This process depends on SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2.2 (SnRK2.2) activity in cortex cells of the elongation zone. When roots enter air spaces, lateral root formation is repressed (xerobranching), a process that depends on the ABA inhibition of auxin signalling. Roots growing in areas where water is asymmetrically distributed display a growth programme known as hydropatterning, where lateral roots preferentially form on the water-contacting side. In hydropatterning, the auxin response factor ARF7 stimulates preferential lateral root initiation. **c** | During prolonged drought, plants accelerate flowering to reproduce in a process called drought escape. Under drought stress, the ABA-activated transcription factors ABA-RESPONSIVE ELEMENT-BINDING FACTOR 3 (ABF3) and ABF4 and the floral regulator GIGANTEA (GI) stimulate expression of the flowering inducers *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*) to promote flowering. **d** | Submerged plant tissues experience hypoxia and elevated levels of ethylene gas. These cues activate transcription factors known as group VII ETHYLENE RESPONSE FACTORS (ERFs). Group VII ERFs initiate a conserved hypoxia-induced transcriptional programme. In deepwater rice varieties, elevated ethylene concentration activates the ERFs SNORKEL1 and SNORKEL2 (SK1/2), which induce gibberellin biosynthesis. Gibberellin signalling promotes a flood escape strategy where stems elongate to emerge into the air. Dashed lines indicate indirect mechanisms.

biosynthesis genes. Environmental signals, such as cold and light, that trigger seeds to break seed dormancy do so by flipping the balance towards gibberellin[52]. This antagonistic relationship between ABA and gibberellin arises at multiple points in their respective pathways (FIG. 3a).

DELLA proteins are members of the plant-specific GRAS (GIBBERELLIN-INSENSITIVE, REPRESSOR of *ga1-3*, SCARECROW) family of transcriptional regulators, which lack DNA-binding activity and function by interacting with other transcription factors[90]. DELLA proteins inhibit gibberellin responses, and gibberellin signalling inactivates DELLA proteins in part by triggering their proteasomal degradation[90,91]. DELLA proteins interact with ABI3 and ABI5, and together these protein complexes stimulate the transcription of *SOMNUS*, a key dormancy-promoting factor that activates ABA biosynthesis genes and represses gibberellin biosynthesis genes[92]. Interestingly, the action of the DELLA–ABI5 complex is inhibited by the basic helix–loop–helix transcription factor INDUCER OF CBF EXPRESSION 1 (ICE1)[93]. Binding of ICE1 blocks the DNA-binding activity of ABI5, and this interaction is stimulated by gibberellin treatment, possibly due to the degradation of DELLA proteins, providing a possible mechanism through which prior exposure to low temperatures may promote germination.

The control of *ABI5* expression appears to be a major regulatory point for multiple environmental signals during germination. The light signalling component ELONGATED HYPCOTYL 5 (HY5) directly activates *ABI5* transcription in response to light[94]. The DELLA protein RGL2 further promotes ABA signalling by enhancing the transcription of *ABI5*. Gibberellin production during germination initiation could then reduce *ABI5* expression through RGL2 degradation[95]. High salinity inhibits seed germination, and two different transcription factors, AGL21 and RSM1, were reported recently to enhance *ABI5* expression during exposure to NaCl (REFS[96,97]).

Auxin, ABA and root growth under stress. Root development is shaped by environmental conditions, and this topic has been the subject of multiple excellent reviews[98]. Here, we focus on the mechanisms by which auxin and ABA control the architecture of the root system in response to water and salinity stresses (FIG. 3b).

While high concentrations of exogenous ABA inhibit root growth, lower concentrations (nanomolar range) stimulate primary root growth[99]. Water distribution in soil is uneven, and plants partly address this situation through hydrotropism, the directional growth of roots towards water. Hydrotropism is impaired in ABA-deficient mutants, and ABA accumulates in root tissues during water stress, suggesting an important role for ABA signalling[100]. For hydrotropism the protein kinase SnRK2.2 is required specifically in cortical cells of the root elongation zone, where it promotes the cell elongation necessary for differential growth[101] (FIG. 3b). Low concentrations of ABA (100 nM) stimulate primary root growth by abating PP2C-mediated inhibition of apoplastic H^+ efflux through AUTOINHIBITED H^+-ATPASE 2 (AHA2)[102]. This mechanism provides a

Seed dormancy
A state in which seed germination is inhibited. ABA signalling promotes seed dormancy, while gibberellin signalling can repress it.

Hydrotropism
The directional growth of roots towards regions of the soil environment with higher water content.

Halotropism
The directional growth of roots away from regions of high salinity.

Xylem
A vascular tissue that conveys water and nutrients from roots to stems and leaves.

Hydropatterning
A water-responsive root developmental programme active when water is asymmetrically available around the circumference of the root. Lateral roots preferentially form on the water-contacting side.

Xerobranching
A water-responsive root developmental programme where the formation of lateral roots is repressed in regions of the soil environment that lack water.

Pericycle cells
A layer of cells that encircle the vascular tissue.

Drought escape
An adaptive response to prolonged drought stress where plants accelerate the transition to flowering in order to reproduce.

contribution to the hydrotropic response[102]. Two recent studies have implicated brassinosteroid signalling in the hydrotropic response as well, although the mechanism is currently unclear[73,103]. By contrast, in high-salinity environments, lateral roots enter a prolonged growth arrest which requires endodermal ABA signalling[104]. Roots also exhibit preferential growth away from areas of high salinity — a phenomenon called halotropism[105]. Salt treatment induces internalization of the auxin transporter PIN-FORMED 2 (PIN2), and when roots encounter a longitudinal salinity gradient, auxin accumulates on the side of the root furthest from the salt source, which then leads to root bending[105,106]. Interestingly, hydrotropism does not appear to act through auxin redistribution, suggesting that halotropism is a distinct process[107,108].

ABA also regulates root tissue patterning during water stress. Endodermal ABA signalling stimulates xylem differentiation by inducing the expression of the microRNAs miR165 and miR166, two key regulators of vascular development[109,110]. ABA functions within xylem cells as well, where it activates expression of several *VASCULAR-RELATED NAC DOMAIN* (*VND*) transcription factors which promote xylem differentiation[111].

Research on two related water-dependent root-branching strategies, hydropatterning and xerobranching, has uncovered requirements for auxin and ABA signalling[98]. Lateral roots initiate from pericycle cells within the primary root, and this is timed by an auxin-regulated transcriptional network[112,113]. The position of these initiation events was shown to respond to water availability in a process termed 'hydropatterning'[114]. In hydropatterning, differences in water content around the circumference of primary roots lead to preferential lateral root initiation where water is available. Hydropatterning was correlated with auxin biosynthesis and signalling on the side of the root in contact with water[114]. A recent study demonstrated that hydropatterning requires the auxin response factor ARF7 (REF. [115]) (FIG. 3b). Removal of seedlings from agar plates and their exposure to air triggered the post-translational modification of ARF7 with a SUMO protein, and sumoylated ARF7 had reduced DNA-binding activity. Roots can encounter large air spaces in soil, and in these regions lateral root formation is repressed. This repression of branching along the entire root circumference has been termed 'xerobranching'. A recent study implicated ABA signalling in the xerobranching response[116]. The roots of barley plants were found to accumulate ABA following a transient water deficit. Short-term ABA treatment of aeroponically grown maize and barley roots led to a zone of lateral root repression, showing that ABA can mimic a xerobranching response. Furthermore, ABA treatment disrupted auxin signalling in roots, suggesting a possible mechanism for lateral root repression[116] (FIG. 3b).

Gibberellins, ABA and ethylene regulate flowering during abiotic stress. A core genetic network regulates flowering time in plants, and this network receives inputs from endogenous, environmental and seasonal cues[117]. Here, we explore how hormone signalling intersects with core flowering regulators to mediate the effects of abiotic stress on flowering time.

During periods of prolonged drought many species will accelerate the flowering transition to reproduce before death, and this response is known as drought escape[118]. The exact role of ABA during the flowering transition is currently unclear, and puzzlingly *snrk2.2 snrk2.3 snrk2.6* triple mutants are early flowering[119], while ABA-deficient mutants and *areb1 areb2 abf3 abf1* quadruple mutants are late flowering[60,118]. Here, we focus on the case of drought-accelerated flowering, where emerging evidence suggests a positive role for ABA signalling. Under long-day conditions, flowering time is delayed in ABA biosynthesis mutants and advanced in an ABA-hypersensitive *pp2c* triple mutant[118]. Crucially, drought stress magnifies this delay, suggesting that ABA is required to promote drought escape[118]. This positive role of ABA in drought-induced flowering requires the core photoperiod-dependent flowering regulatory protein GIGANTEA (GI)[118,120]. Additionally, the drought escape response is abolished in *abf3 abf4* double mutants, and ABF3 and ABF4 can directly induce the transcription of *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), another key flowering gene[65] (FIG. 3c).

In contrast to drought, salt stress causes an ethylene-dependent delay in flowering time in *Arabidopsis*[121,122]. Salt stress leads to ethylene accumulation by inducing the expression of ethylene biosynthesis genes[121]. Although the underlying mechanism is unclear, ethylene interferes with gibberellin signalling, leading to the accumulation of DELLA proteins. DELLA proteins can then delay flowering by inhibiting the flowering-stimulating transcription factor CONSTANS[123]. In addition, salt stress also represses flowering by inducing the degradation of GIGANTEA[124].

Ethylene and gibberellins control flooding responses. Floods are a major cause of crop loss in agriculture and a clear environmental challenge for some plants in natural ecosystems[125]. Plants possess an array of developmental and physiological strategies to adapt to flooding, with different strategies exhibited in different species. Here, we discuss advances related to hormone signalling during submergence and provide focused coverage of recent work on the hormonal control of a flood-escape strategy in rice. We further direct readers to recent reviews on the broader topic of flooding responses[126,127].

The submergence of plant tissues impedes cellular access to O_2 and CO_2, which can severely disrupt metabolism. Additionally, restricted gas diffusion underwater leads to an accumulation of ethylene within flooded plant tissues[125]. Prolonged flooding can cause hypoxia, which activates a conserved gene expression programme that supports plant survival in limiting O_2. In *Arabidopsis*, the transcription of these hypoxia-responsive genes requires five transcription factors known as group VII ETHYLENE RESPONSE FACTORS (ERFs)[128]. Additionally, *SUBMERGENCE TOLERANCE 1*, a major quantitative trait locus associated with increased flood tolerance in rice, contains a cluster of three related *ERF* genes[129]. Both hypoxia and high concentrations of ethylene enhance the protein stability of group VII ERFs, leading to target gene transcription[130–133] (FIG. 3d). Group VII ERFs bind to

Deepwater rice
Varieties of rice (*Oryza sativa*) that avoid submergence stress by activating stem and leaf elongation to rise above the water surface. This developmental programme depends on the hormones ethylene and gibberellin.

conserved *cis*-regulatory elements, and the chromatin accessibility at these regulatory elements increases in response to flooding[128,134]. Interestingly, accessible group VII ERF binding sites were more prevalent in the flood-adapted rice genome than in the dryland-adapted tomato *Solanum pennellii*[134].

Some flood-adapted species display an escape strategy where underwater shoots and leaves elongate to emerge into the air[125]. Research on a flooding-tolerant rice variety known as deepwater rice has begun to reveal how ethylene signalling and gibberellin signalling control this underwater growth response. Gibberellin stimulates stem elongation by promoting internode growth, and this relationship has been exploited during plant domestication. In rice, ethylene accumulates in submerged stem and leaf tissues, and this elevated ethylene concentration induces the expression of the ERF-encoding genes known as *SNORKEL1* and *SNORKEL2*, two major quantitative trait loci associated with deepwater rice internode elongation[135]. SNORKEL1 and SNORKEL2 may stimulate stem elongation by inducing gibberellin biosynthesis (FIG. 3d). More recently, an additional locus associated with internode elongation was mapped to *SEMIDWARF1* (*SD1*), a key gibberellin biosynthesis gene[136]. In contrast to the more common semidwarf rice variety, which carries a null allele of *SD1*, deepwater rice plants induce *SD1* expression in submerged tissues. The resulting increased gibberellin levels act together with an additional locus called *ACCELERATOR OF INTERNODE ELONGATION 1* (*ACE1*) to promote cell division in the intercalary meristem[137].

Interestingly, a recent study reported that compacted soil leads to ethylene accumulation in roots, which subsequently inhibits further growth, possibly allowing plants to avoid regions with poor soil aeration[138]. This suggests that elevated ethylene concentration may be a common and early cue for air deficiency stress that plants use to adapt their growth.

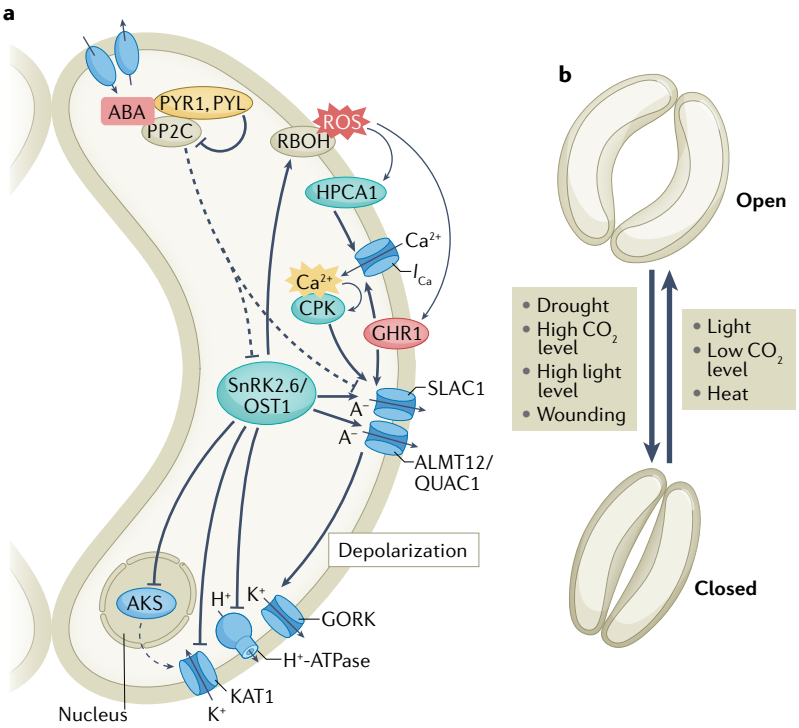

Fig. 4 | **Guard cell signal transduction and stomatal responses to environmental stimuli. a** | Schematic model of abscisic acid (ABA) signal transduction in guard cells. ABA transporters mediate ABA import to or export from guard cells. In the presence of ABA, the key regulator SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2.6 (SnRK2.6; also known as OST1) phosphorylates and activates SLOW ANION CHANNEL-ASSOCIATED 1 (SLAC1), ALUMINIUM-ACTIVATED MALATE TRANSPORTER 12 (ALMT12; also known as QUAC1) and RESPIRATORY BURST OXIDASE HOMOLOGUE (RBOH) proteins. Activation of the slow-type anion channel SLAC1 and the rapid-type anion channel ALMT12/QUAC1 leads to long-term plasma membrane depolarization, which causes K^+ efflux through the voltage-dependent K^+_{out} channel GUARD CELL OUTWARD RECTIFYING K^+ CHANNEL (GORK). Activated RBOH NADPH oxidases produce reactive oxygen species (ROS) that mediate HYDROGEN-PEROXIDE-INDUCED Ca^{2+} INCREASES 1 (HPCA1) sensor-dependent activation of Ca^{2+}-permeable I_{Ca} channels, resulting in an elevation of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). Elevated $[Ca^{2+}]_{cyt}$ activates Ca^{2+}-sensor proteins, including Ca^{2+}-dependent protein kinases (CPKs) that phosphorylate and activate SLAC1. The (pseudo-)kinase GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1 (GHR1) mediates the activation of I_{Ca} and SLAC1 channels through an unknown mechanism, possibly as a scaffolding protein. SnRK2.6/OST1 inhibits the K^+_{in} channel K^+ CHANNEL IN ARABIDOPSIS THALIANA 1 (KAT1), which mediates K^+ uptake, by direct phosphorylation. In addition, SnRK2.6/OST1 causes a long-term decrease of *KAT1* expression by inhibition of ABA-RESPONSIVE KINASE SUBSTRATE (AKS) transcription factors. ABA also inhibits H^+-ATPase activity through SnRK2.6/OST1, but the detailed mechanism is unknown. Dashed lines indicate steps that are inhibited in the presence of ABA. Only guard cell ABA signalling regulating ion transport across the plasma membrane is depicted in this figure. **b** | In addition to drought stress, several other environmental stimuli can be perceived by guard cells and affect stomatal aperture though sophisticated signalling crosstalk and integration. PP2C, PROTEIN PHOSPHATASE 2C; PYL, PYR1-LIKE; PYR1, PYRABACTIN RESISTANCE 1.

Regulation of stomatal movements

Stomatal pores formed by guard cells in the leaf epidermis allow the uptake of CO_2 for photosynthesis in exchange for water. To optimize plant water-use efficiency, guard cells sense and respond to several abiotic factors, including light, CO_2 and drought. ABA is a central regulator of guard cell physiology (FIG. 4a), and here we discuss the crosstalk with abiotic factors and other hormones.

Stomatal response to drought. Drought stress has been reported to trigger ABA synthesis in vascular tissues and guard cells[139,140]. ABA signalling in guard cells regulates plasma membrane ion channels to trigger long-term efflux of anions and K^+, resulting in the reduction of guard cell turgor and stomatal closure. Anion release from guard cells and subsequent plasma membrane depolarization is mediated by slow-type and rapid-type anion channels[141]. A major slow-type anion channel in *Arabidopsis* guard cells is encoded by *SLOW ANION CHANNEL-ASSOCIATED 1* (*SLAC1*)[142,143]. ALUMINIUM-ACTIVATED MALATE TRANSPORTER 12 (ALMT12; also known as QUAC1) contributes to 40% of rapid-type anion currents[144]. The anion channel-triggered depolarization in turn induces K^+ efflux through the voltage-dependent outward-rectifying K^+ (K^+_{out}) channel[145] GUARD CELL OUTWARD RECTIFYING K^+ CHANNEL (GORK)[146]. The protein kinase SnRK2.6 (also known as OST1) is a major positive regulator of ABA signalling in guard cells[35]. SnRK2.6/OST1 phosphorylates and activates both SLAC1 (REFS[147,148]) and ALMT12/QUAC1 (REF.[149]). Group A PP2C proteins, as negative ABA signalling

Depolarization
Changes in the cell membrane potential making it more positive.

Hyperpolarization
Changes in the cell membrane potential making it more negative.

Genetically encoded phytohormone indicators
Indicators that allow the in vivo monitoring of hormone levels and downstream hormone signalling responses.

regulators, directly dephosphorylate and inactivate not only SnRK2.6/OST1 (REFS[150,151]) but also SnRK2.6/OST1 substrates, such as SLAC1 (REF.[152]). Ion transport at the vacuolar membrane is also required for ABA-induced stomatal closure, and detailed information has been reviewed[153].

Cytosolic Ca^{2+} fine-tunes ABA-mediated stomatal closure by regulating Ca^{2+}-sensor proteins, such as Ca^{2+}-dependent protein kinases, which contribute to the activation of SLAC1 (REFS[152,154]). ABA can induce elevations in $[Ca^{2+}]_{cyt}$ in guard cells, and one of the underlying mechanisms is plasma membrane Ca^{2+} influx through hyperpolarization-activated Ca^{2+}-permeable cation (I_{Ca}) channels[155,156]. ABA-activation of I_{Ca} channels involves several steps. First, SnRK2.6/OST1 triggers extracellular reactive oxygen species production, which includes SnRK2 regulation of NADPH oxidases[157,158]. Reactive oxygen species mediate I_{Ca} channel activation in the plasma membrane via the hydrogen peroxide sensor kinase HYDROGEN PEROXIDE-INDUCED Ca^{2+} INCREASES 1 (HPCA1)[159] and the receptor-like (pseudo-)kinase GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1 (GHR1)[160]. GHR1 also contributes to SLAC1 activation[161]. In addition to Ca^{2+} and reactive oxygen species, other small molecules, such as hydrogen sulfide[162,163] and γ-aminobutyric acid[164], have recently been shown to modulate guard cell ABA signalling.

Abiotic signal integration in guard cells. Guard cells can perceive and integrate several environmental stimuli (FIG. 4b). Among them, light and CO_2 are major abiotic stimuli that regulate stomatal aperture. Blue light and red light induce stomatal opening mechanisms to maximize photosynthesis. Light-induced stomatal opening is mediated by H^+-ATPase activation and subsequent K^+ uptake through voltage-dependent inward-rectifying K^+ (K^+_{in}) channels at the guard cell plasma membrane[145,165,166]. ABA suppresses light-induced stomatal opening via inhibition of H^+-ATPases and K^+_{in} channels. Group D PP2C proteins and their negative regulators, the SMALL AUXIN-UP RNAs (SAURs) also contribute to the regulation of H^+-ATPases in *Arabidopsis* guard cells[167,168]. To what extent auxin is involved in this mechanism remains to be elucidated. Rapid downregulation of K^+_{in} channels is mediated by SnRK2.6/OST1-dependent phosphorylation of the K^+_{in} channel KAT1 (REF.[169]) and also by $[Ca^{2+}]_{cyt}$ elevation[170]. On a slower timescale, the expression of K^+_{in} channel-encoding genes, including *KAT1*, is inhibited via SnRK2-dependent inactivation of ABA-RESPONSIVE KINASE SUBSTRATE (AKS) transcription factors[171].

High CO_2 concentration induces stomatal closure, whereas low CO_2 concentration induces stomatal opening. In response to ABA, stomata close and do not easily reopen in the short term. By contrast, high CO_2 concentration-mediated stomatal closure is rapidly reversible[172]. The mechanisms by which high CO_2 concentration mediates stomatal closure and activates SLAC1 differ from those of ABA[172–174]. In contrast to ABA signalling, elevated CO_2 concentration does not rapidly activate SnRK2-type protein kinases[172,175]. Raf-like kinases, such as CONVERGENCE OF BLUE LIGHT AND CO_2 1 (CBC1), CBC2 (REF.[165]) and HIGH LEAF TEMPERATURE 1 (HT1)[176,177], inhibit slow-type anion channel activation via an unknown mechanism. CBC kinases function at a convergence point between blue light and low CO_2 concentration-induced stomatal opening signalling pathways[165].

Signalling crosstalk between ABA and other hormones contributes to guard cell abiotic stress responses. The F-box protein MORE AXILLARY GROWTH 2 (MAX2) is a central regulator of both strigolactone signalling and karrikin signalling[178]. MAX2-dependent signalling induces the upregulation of ABA-signalling genes, such as *SnRK2.6/OST1*, thereby enhancing ABA-induced stomatal closure and drought tolerance[179,180]. The Type-B ARRs ARR1, ARR10 and ARR12, acting as positive transcriptional regulators of cytokinin signalling, negatively regulate stomatal closure and drought tolerance[181]. ABA and water deficit suppress cytokinin signalling via downregulation of Type-B *ARR* genes, as a proposed adaptive mechanism to survive drought[181]. It was reported that excess high light stress triggers local and whole-plant systemic stomatal closure, which is likely mediated by the NADPH oxidase RBOHD in coordination with ABA, salicylic acid and jasmonate signalling[182]. Darkness and high CO_2 concentration, however, do not induce stomatal closure in systemic leaves of *Arabidopsis*[183]. Under heat stress, plants open stomata to cool the leaves by transpiration. Jasmonate has been suggested to fine-tune stomatal apertures during a combination of heat and other stresses, such as high light levels and wounding[184,185]. Brassinosteroids can positively mediate stomatal opening[186]. In the brassinosteroid-biosynthesis mutant *dwarf5* (*dwf5*), *KAT1* expression is downregulated via an AKS-independent pathway and the light-driven activation of H^+-ATPases remains intact, suggesting that brassinosteroid regulation of stomatal opening is independent of ABA signalling[186].

Monitoring hormone responses in plants

To understand phytohormone signalling processes during abiotic stress, it is important to determine under which stress conditions and in which cell types or tissues and on which time frames phytohormone responses appear. Furthermore, it is relevant to determine at which level (that is, biosynthesis, transport, perception, transduction and transcriptional response) abiotic stresses interfere with a certain hormone signalling pathway. Genetically encoded phytohormone indicators are biosensors that allow the in vivo monitoring of cellular hormone responses at high spatiotemporal resolution and at various levels. Their functional principles, their advantages over other methods and their limitations have been extensively discussed[3,187,188] and are summarized in BOX 1. Here, we review their contribution to abiotic stress response analyses in plants.

Genetically encoded phytohormone indicators that enable the direct detection of phytohormone concentration changes were initially developed for ABA[28,189], followed by reporters for gibberellin, strigolactones and auxin[190–192]. However, only the Förster resonance energy

Box 1 | **GEPHIs**

Genetically encoded phytohormone indicators (GEPHIs) enable the in vivo analysis of hormone concentration changes and subsequent downstream signalling processes at tissue resolution and cellular resolution. Several GEPHIs have been developed and used in plants, and more comprehensive information can be found elsewhere[3,187,188].

FRET-based GEPHIs. To directly monitor hormone concentration changes, Förster resonance energy transfer (FRET)-based indicators have been developed for abscisic acid (ABA) (ABACUS and ABAleon)[28,189,224], auxin (AuxSen)[192] and gibberellins (GPS1)[190]. These indicators consist of a sensory domain that changes its structure in a hormone-bound configuration, thereby affecting the distance, orientation and the fluorescence ratio of a fluorescent protein FRET pair upon excitation of the FRET donor fluorescent protein. ABAleon-type ABA indicators have recently received two updates. Dual-reporting indicators, consisting of ABAleonSD1-3L21 fused via the self-cleaving P2A peptide linker to the red-fluorescing Ca^{2+} indicator R-GECO1 or the pH indicator PA-17, allow the simultaneous monitoring of ABA together with Ca^{2+} concentration or pH[224], whereas ABAleon2.1_Tao3s, which harbour a nanobody recognition domain and a secretion signal, can be recruited by a subcellular targeted nanobody to either side of the endoplasmic reticulum membrane[194]. For the analysis of signalling processes downstream of ABA perception, a FRET-based SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2 (SnRK2) activity sensor (SNACS) has been developed, providing an approach to investigate the activation of SnRK2-type protein kinases in response to abiotic stresses and SnRK2 interaction with other hormone signalling pathways[175].

Degradation-based hormone reporters. Several plant hormones induce downstream responses by regulating the ubiquitination and proteasomal degradation of transcriptional repressors[3]. Degradation-based GEPHIs monitor their protein levels using fluorescent protein fusions. More sophisticated reporters use only a hormone-dependent degradation domain (degron motif) fused to a fluorescent protein to report an increased hormone concentration and signalling strength via a decrease in fluorescent protein stability. To achieve a ratiometric readout, reporters for auxin, such as R2D2 (REF. [225]) and qDII[226], co-express a non-degradable fluorescent protein as a reference.

Synthetic hormone-activated Cas9-based repressors. Synthetic hormone-activated Cas9-based repressors (HACRs) consist of a deactivated Cas9 (dCas9) fused to a hormone-dependent degradation domain and a fragment of the repressor TOPLESS. The dCas9 component associates with a guide RNA and recruits the HACR to a target promoter. Hormone-dependent degradation of the HACR then leads to a derepression of the target (reporter) gene[227].

A reporter for ethylene-dependent translational regulation. The ethylene signalling pathway involves the EIN2 carboxy-terminal domain (CEND) association with 3′ untranslated regions of EIN3-binding F-box protein (EBF) mRNAs to repress their translation[228,229]. On the basis of this mechanism, a translational reporter has been designed consisting of a fluorescent protein coding sequence followed by three tandems of ethylene responsive RNA elements containing polyuridylates (EPUs). Upon induction of *EIN2*, the translation of this 6× EPU reporter is inhibited, and transgenic plants expressing this construct are insensitive to ethylene[228].

Hormone-activated transcriptional reporters. Several marker genes for plant hormone signalling have been identified, and their promoters have been used to drive the expression of reporter genes. In addition, the identification of *cis* elements that are targeted by hormone-specific transcriptional regulators led to the development of synthetic promoters. Synthetic promoters contain multiple repeats of *cis* element-containing promoter fragments upstream of a minimal 35S promoter to drive reporter gene expression. They have been developed for almost any plant hormone[3,187,188].

transfer (FRET)-based ABA indicators ABACUS1-2μ and ABAleon have thus far been used in research related to abiotic stress[28,189]. Analyses in *Arabidopsis* using ABAleon2.1 under non-stress conditions revealed the existence of an ABA gradient in roots and comparably higher basal ABA concentrations in guard cells, in the root–hypocotyl junction and in the root tip[28,193]. Experiments using ABAleon2.1_Tao3s, which were targeted to either side of the endoplasmic reticulum membrane, indicated that in tobacco protoplasts, ABA levels might be higher in the endoplasmic reticulum than in the cytosol[194]. How ABA gradients are maintained, and to what extent ABA biosynthesis and transport pathways contribute to distinct ABA concentration patterns, could be further analysed with use of ABA biosensors, similar to research conducted on gibberellin gradients in *Arabidopsis* roots[195]. There is increasing evidence that water deficit in *Arabidopsis* roots first induces the biosynthesis of ABA in leaves, via long-distance signals, before ABA accumulation is detected in roots[26,27,32]. Consistent with these findings, ABA indicator analyses could not detect rapid osmotic stress-induced or salt stress-induced ABA concentration elevations in roots under imposed experimental conditions. Instead, ABA concentration elevations were observed only several hours after exposure to stress[28,189]. Further analyses in *Arabidopsis* also revealed that sulfate and cysteine trigger ABA level increases in guard cells[196], whereas CO_2 concentration elevation did not cause a rapid ABA concentration increase[172,175]. More detailed analyses are required to determine the spatiotemporal parameters of ABA concentration elevation in response to water deficit and the intercellular transport routes of ABA. It will also be interesting to investigate whether recently developed indicators for auxin[192], gibberellin[190] and strigolactones[191] can detect respective hormone dynamics in response to abiotic stress.

Complementary to direct ABA indicators, the FRET-based SNACS reporter monitors downstream SnRK2-type protein kinase activity[175]. In *Arabidopsis* guard cells, SNACS responded to ABA, but not to elevated CO_2 concentrations or treatments with methyl jasmonate. These results were consistent with a lack of ABA accumulation under the same experimental conditions, providing evidence for the hypothesis that basal ABA signalling rather than further SnRK2 activation contributes to elevated CO_2 and methyl jasmonate responses in guard cells[172,175].

Early on, promoter fragments of marker genes, or synthetic hormone-responsive promoters, were used to drive reporter gene expression as a readout for the detection of phytohormone signalling patterns[3,187,188] (BOX 1). Although several transcriptional reporters were used for the analyses of abiotic stress responses, most of the research related to abiotic stress focused on ABA. In this context, ABA signalling reporters were used for the analyses of drought stress, osmotic stress, salt stress, cold stress and high CO_2 concentration responses[26,172,193,197], contributing to the hypothesis that in response to water shortage, ABA is largely synthesized in shoots rather than in roots of *Arabidopsis*[26]. Furthermore, the *proRD29A*-based ABA signalling reporter was used as a readout in genetic screens[197], contributing to the identification of ABA synthesis genes in *Arabidopsis*[198]. Also synthetic hormone-responsive promoter reporters were recently used for the reconstitution of ABA signalling in yeast[199] and for the analysis of ABA-mediated transcriptional regulation in *Arabidopsis*[200]. The latter *6xABRE* synthetic promoters reported basal ABA-independent activity in the root quiescent centre, and ABA-, salt- and osmotic stress-dependent increases in other root tissues[200], albeit with an apparent relatively

low dynamic range compared with the *proRAB18:GFP* reporter[193].

Reporters for other phytohormones also contributed to important observations on the roles of auxin, cytokinins and gibberellins in osmotic stress[201], the contribution of cytokinin signalling to the hydrotropic response[202] and the involvement of gibberellin signalling in the salt stress response[121]. The use of hormone reporters in species other than *Arabidopsis* is beginning to emerge[203] and will likely aid in determining differences and similarities in hormone signalling between different taxa.

Conclusions

Due to climate change, abiotic stresses, such as drought, salt, heat and flooding, are becoming increasingly challenging for plants[1,2]. Climate change and abiotic stresses can also intensify plant diseases[204]. Such alarming conditions demand innovative approaches. Recent advances in plant biology are providing crucial new insights into how plants sense and respond to abiotic stresses. While translating such findings into field applications remains challenging[205], the advanced understanding of individual hormone-regulated abiotic stress responses, reviewed here, has the potential to provide key insights for developing more resilient crops through both engineering and mining of traits from more resistant wild crop relatives[125]. The elucidation of the mechanisms, genes and pathways that control these traits can provide road maps for applications and translational research into enhancing or protecting yields in response to abiotic stressors. Many of the advances we have discussed in this Review were made in the model system *Arabidopsis thaliana*. Therefore, research will be needed to determine whether similar or divergent mechanisms are used in crops. Moreover, it has become clear that specific cell types have specific hormone signalling pathways and outputs, and therefore alteration of cell-targeted or tissue-targeted traits will require investigation of hormone signalling mechanisms in those cell types. New tools, including hormone reporters, protein complex identifications, single-cell sequencing and other approaches, will enable the dissection of abiotic stress-linked cell type-specific and species-specific signal transduction mechanisms. Genetic approaches, including genomics-accelerated breeding and CRISPR–Cas9 gene editing, provide new opportunities to accelerate the development of abiotic stress-resilient traits. Furthermore, the genomics revolution combined with automated phenotyping is enhancing our ability to understand or predict which of these genes and mechanisms could be primarily used by resilient wild relatives. This could lead to targeted breeding of improved traits into crops. Moreover, enhancing yields of climate change-resilient wild varieties through knowledge-guided de novo domestication of crops[206] provides an important new avenue for incorporating beneficial hormone signalling traits. Continued advances in understanding the interplay of plant hormones in diverse responses to abiotic stress will be important for developing abiotic stress-resilient crops.

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