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Chapter 9

Advances in Plant Tolerance to Abiotic Stresses

Geoffrey Onaga and Kerstin Wydra

Additional information is available at the end of the chapter

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Abstract

During the last 50 years, it has been shown that abiotic stresses influence plant growth and crop production greatly, and crop yields have evidently stagnated or decreased in economically important crops, where only high inputs assure high yields. The recent manifesting effects of climate change are considered to have aggravated the negative effects of abiotic stresses on plant productivity. On the other hand, the complexity of plant mechanisms controlling important traits and the limited availability of germplasm for tolerance to certain stresses have restricted genetic advances in major crops for increased yields or for improved other traits. However, some level of success has been achieved in understanding crop tolerance to abiotic stresses; for instance, identification of abscisic acid (ABA) receptors (e.g., ABA-responsive element (ABRE) binding protein/ABRE binding factor (AREB/ABF) transcription factors), and other regulons (e.g., *WRKYs*, *MYB*/*MYCs*, *NACs*, *HSFs*, *bZIPs* and nuclear factor-Y (NF-Y)), has shown potential promise to improve plant tolerance to abiotic stresses. Apart from these major regulons, studies on the post-transcriptional regulation of stress-responsive genes have provided additional opportunities for addressing the molecular basis of cellular stress responses in plants. This chapter focuses on the progress in the study of plant tolerance to abiotic stresses, and describes the major tolerance pathways and implicated signaling factors that have been identified, so far. To link basic and applied research, genes and proteins that play functional roles in mitigating abiotic stress damage are summarized and discussed.

Keywords: abiotic stress, climate change, crop improvement, transcription, regulatory proteins

1. Introduction

Abiotic stress is defined as the negative impact of non-living factors on living organisms in a specific environment. Abiotic stresses, such as drought, salinity, low or high temperatures and other environmental extremes are the major cause of poor plant growth and reduced crop yields in the world [1]. Drought alone affects 45% of the world's agricultural land, whereas

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19.5% of irrigated agricultural lands are considered saline [2, 3]. Moreover, 16% of the agricultural rice land of the world suffers from flash flooding [4]. A combination of two or more abiotic stresses, e.g., drought and heat stress also occurs in field situations and causes more severe crop yield reductions than a single stress [5]. With increasing challenges posed by climate change, it is predicted that warming, drought, floods and storm events will become even more frequent and severe, and will further reduce crop yields, especially in the tropics and subtropics.

Abiotic stresses commonly induce overproduction of reactive oxygen species (ROS) causing extensive cellular damage and inhibition of physiological processes in plants. Although antioxidative mechanisms would be an immediate endogenic choice of the plants to counter ROS production, this mechanism can be impaired by abiotic stresses causing a rise in ROS intracellular concentration and an increase in the damage. To survive under such conditions, plants have evolved intricate mechanisms, allowing optimal responses that enable adaptation or avoidance of the stress. These plant responses are regulated at all levels of organization. At the cellular level, responses include adjustments of the membrane system, modifications of cell wall architecture, changes in cell cycle and cell division, and synthesis of specific endogenous and low-molecular-weight molecules, such as salicylic acid, jasmonic acid, ethylene and abscisic acid [6]. An overview of changes that may occur under different abiotic stress conditions is presented in Figure 1.

At the genomic level, plant responses include the expression of stress-inducible genes involved in direct plant protection against stresses [3, 7, 8]. A broad range of abiotic stress induced genes are divided into two functional categories: and regulatory proteins. The first group consists of genes encoding for membrane proteins, enzymes for osmolyte biosynthesis, detoxification (glutathione S-transferases, superoxide dismutases, dehydrins, dehydroascorbate reductases, quinine reductases and ascorbate peroxidases) and proteins for macromolecular protection (such as LEA protein, anti-freezing proteins, chaperons and mRNA binding protein) [2]. The second group comprises genes encoding for transcription factors (e.g., *DREBPs*), protein kinases (e.g., *SRK2E*), receptor protein kinases, ribosomal-protein kinases and signal transduction proteinases (such as phosphoesterases and phospholipase C). Alterations in the phenylpropanoid pathway in which lignin biosynthesis intermediates are produced also occur under abiotic stress conditions. Moreover, increased accumulation of wall-linked phenolic compounds, for instance, in maize root elongation zone and the polyphenol content in cotton have been linked to stress response [9]. The same authors have shown the role of flavonoids, isoflavonoids, terpenoid and nitrogen-containing secondary metabolites such as glucosinolates alkaloids in abiotic stress response.

Thus, abiotic stress tolerance in plants is a complex trait, involving many different metabolic pathways and cellular and molecular components.

In the past 100 years, conventional breeding (Figure 2; based on observed variation and controlled mating) approaches have randomly exploited these plant tolerance mechanisms with limited success. Moreover, *in vitro* induced variations have also shown little progress in the improvement of plants against abiotic stresses. These conventional breeding approaches are limited by the complexity of stress tolerance traits coupled with less genetic

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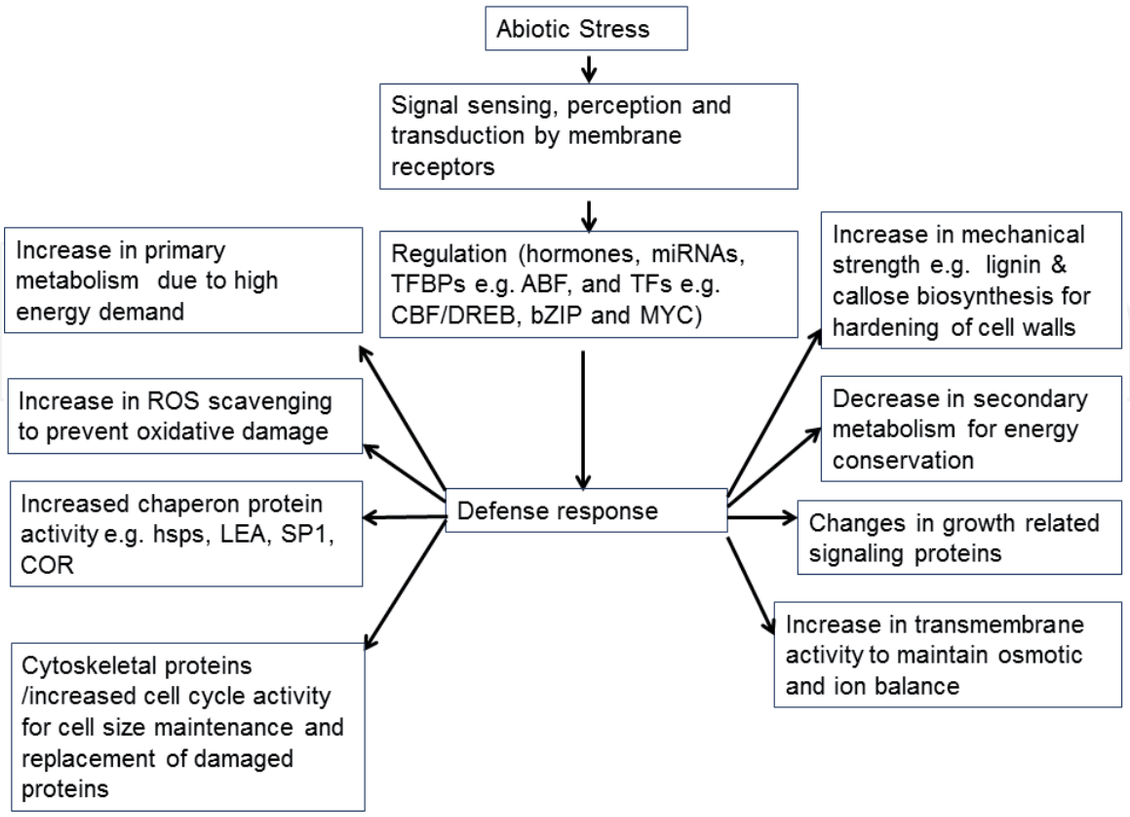


Figure 1. Abiotic stress response in plants. Primary stresses, including drought, salinity, cold, heat, and submergence, are often interconnected and cause cellular damage and secondary stresses, such as osmotic and oxidative stresses. The initial stress signals (e.g., osmotic and ionic effects or membrane fluidity changes) are perceived by membrane receptors that transmit the signals downstream to trigger transcription, which is regulated by hormones, transcription factor binding proteins (TFBPs), miRNAs, and transcription factors (TFs) to precisely activate stress responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes. Inadequate response at one or several steps in the signaling and gene activation levels may ultimately result in irreversible changes of cellular homeostasis and in the destruction of functional and structural proteins and membranes, leading to cell death.

variation exhibited by most crops due to domestication bottlenecks. The recent reports that the cultivated gene pool of major cereal crops, e.g., rice, maize and wheat, has reduced in genetic variation compared to wild relatives [10–12], raises concern, and could probably undermine the current efforts to identify genetic sources of resistance within the cultivated genepools. It is important, therefore, to consider exploring alternative sources of resistance by incorporating modern techniques into traditional breeding strategies to develop stress-tolerant crops (Figure 2).

Recently, with the support of genomics, targeted genetic studies involving QTL mapping and validation, identification of key regulatory genes, e.g., genes encoding for ABA receptors, developments in transcriptional and post-transcriptional regulation of stress-responsive genes and studies on hormonal interactions during plant response to stress, have provided opportunities for understanding cellular stress responses in plants. Moreover, the emergence of deep sequencing technologies, proteomics, metabolomics and epigenetics, has remarkably provided novel possibilities to understand the biology of plants and consequently to precisely develop stress-tolerant crop varieties. Amongst the techniques that are currently being

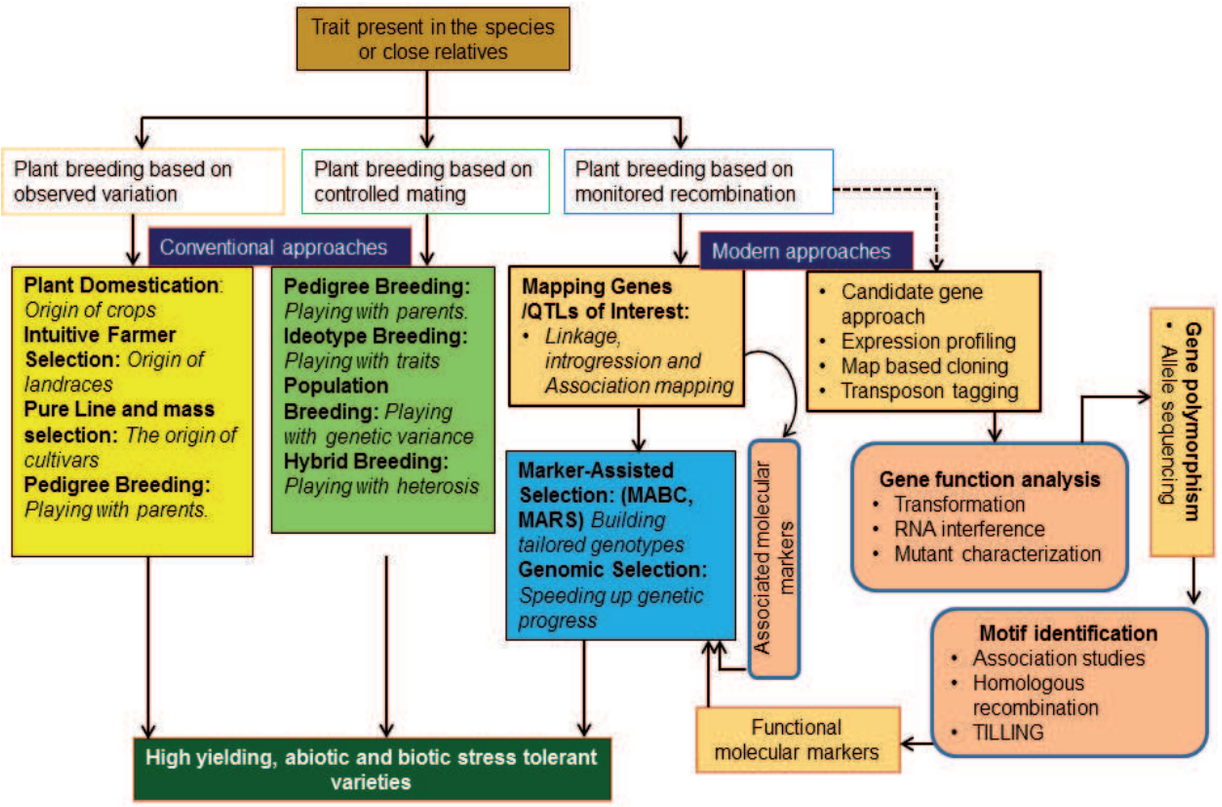


Figure 2. Overview of the traditional and modern approaches in plant breeding. In conjunction with the technological advancements, marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) schemes, which target an individual marker or set of markers showing significant association with QTLs, are progressively evolving into a modification of MAS, permitting the selection of the desirable genotypes on the basis of genome-wide marker information or genomic selection (GS).

exploited to develop stress-tolerant plants, alongside basic molecular biology, there are molecular breeding methods, including development of functional molecular markers to aid in marker-assisted selection, horizontal gene transfer and genome editing tools such as CRISPR/Cas9, to develop genetically modified plants with new or improved characteristics.

In this chapter, we reviewed the plant responses to various abiotic stresses, and focus on genetic and molecular components that function to confer stress tolerance in plants.

2. Advances in plant tolerance to drought

Drought tolerance in plants is the ability to survive and produce stable yields under water scarcity during various stages of crop growth. Principally, drought stress occurs when the soil water potential falls between −0.5 and −1.5 MPa. This affects plants by decreasing the photosynthetic rate through photo-oxidation and enzyme damage, thereby decreasing the amount of assimilates available for export to the sink organs [13]. Besides this, carbohydrate metabolism in plants is severely altered, ultimately affecting both biological and economical yield [14].

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Evidence from several studies has shown that plants respond to drought, like many other abiotic stresses, by inducing cellular damage and secondary stresses, such as osmotic and oxidative stresses. These secondary stresses induce initial stress signals (e.g., osmotic and ionic effects and membrane fluidity changes) that are perceived by membrane receptors (sensors). The perceived signals are transmitted downstream to trigger transcription, which is regulated by phytohormones, transcription factor binding proteins (TFBPs), *cis*-acting elements and miRNAs. Based on the biological functions, the role of these transcriptional regulators and the regulated genes that encode functional proteins or other products to protect plant cells directly from damage is well described [15].

The phytohormone—abscisic acid—acts as a central regulator in the response and adaptation of plants to drought conditions. The various physiological reactions regulated by ABA, including stomatal closure, accumulation of osmoprotectants, changes in gene expression, and other phytohormones have been characterized at the molecular level [16]. The molecular mechanisms of ABA synthesis, transport and signaling in relation to the plant's response to stress are also reasonably well described [17]. ABA signals are perceived by different cellular receptors. The nucleocytoplasmic receptors *PYR/PYL/RCARs* (pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptors) have been suggested to be the primary sensors that bind ABA and inhibit type 2C protein phosphatases (*PP2Cs*) [18]. Inactivation of *PP2Cs* leads to accumulation of active *sucrose non-fermenting-1* (*SNF1*)-related protein kinases (*SnRK2s*), which interacts with ABA-responsive TFs, *ABA-responsive promoter elements* (*ABREs*) and *ABRE*-binding protein/*ABRE*-binding factors (*AREB*/*ABF*) to regulate transcription of downstream target genes and related physiological processes [19]. Drought also induces changes in calcium ion levels, which activates calcium-dependent protein kinases (*CDPKs*) via calmodulin-like domain. The activated *CDPKs* regulate downstream components of calcium signaling. For instance, *OsCPK4* overexpressing rice plants exhibit increased water-holding capacity under drought or salt stress [20]. Genetic manipulation of *RLK* genes, including *OsSIK1* that acts as a positive regulator of drought stress responses, is also well reported [21]. Other secondary signaling molecules, including phosphatases (serine/threonine phosphatases) and phospholipids such as phosphoinositides, nitric oxide, cAMP and sugars, play an important role in signal transduction [22]. Examples of phosphatases include the wheat phosphatase *TaPP2Ac-1* that exhibited less wilting under water-deficit conditions than non-transformed controls [23].

Numerous TF families such as myeloblastosis oncogene (*MYB*), dehydration-responsive element binding proteins (*DREB*), basic leucine zipper domain (*bZIP*), *WRKYs* and the *NAC* (*NAM, ATAF* and *CUC*) are directly or indirectly regulated by endogenous ABA signaling during drought stress [24]. Many *MYB* genes involved in plant response to drought stress are functionally characterized, including *AtMYB15,* which was shown to enhance drought tolerance, and sensitivity to ABA [25]. *WRKY* proteins, including ABA-inducible *OsWRKY45*, *OsWRKY11* and *OsWRKY08,* are upregulated by drought stress [26]. *AP2/ERF* family is another large group of plant-specific TFs that have been demonstrated to be effective in enhancing drought tolerance in plants. For instance, overexpression of *AP2/ERF* genes, e.g., *GmERF3* in soybeans, has been reported to enhance tolerance to drought [27]. In addition, *DREB2s*, e.g.,

ZmDREB2.7, are candidates for drought stress tolerance in maize [28]. The *bZIP* TFs have also been reported to enhance plant tolerance to stress and hormone signal transduction, e.g., *OsbZIP23* in rice [29] and *ZmbZIP72* in maize [30]. Within the *NAC* family, *RD26* (*responsive to dehydration 26*) was the first *NAC* gene identified as a regulator in mediating crosstalk between abscisic acid and jasmonic acid (JA) signaling during drought stress responses in *Arabidopsis* [31]. Overexpression of other *NAC* genes, including *ANAC019*, *ANAC055* and *ANAC072*, has been shown to confer drought tolerance in transgenic *Arabidopsis* [32]. Similarly, overexpression of *SNAC1*, *OsNAC10* and *OsNAC5* driven by a root-specific promoter *RCc3* confers increased drought resistance under field conditions [33, 34]. The nuclear factor Y (*NF-Y*) TFs are emerging as important regulators of drought-stress response, particularly with respect to ABA biosynthesis. For instance, ectopic expression of *Amaranthus hypochondriacus NF-YC* gene (*AhNF-YC*) in *Arabidopsis* and overexpression of Bermuda grass *Cdt-NF-YC1* in rice has shown that these genes confer drought tolerance [35, 36]. *Cdt-NF-YC1* induces expression of both ABA-responsive genes (e.g., *OsRAB16A*, *OsLEA3*, *OsP5CS1* and *OsLIP9*) and signaling genes (e.g., *OsABI2* and *OsNCED3*), as well as, ABA independent genes (e.g., *OsDREB1A*, *OsDREB2B* and *OsDREB1B*). In fact there is an increasing evidence that some NAC genes, e.g., *SNAC3*, contribute to drought resistance and osmotic adjustment independent of ABA [37]. *SNAC3* interacts with *phosphoglycerate mutase*, *cytochrome P450 72A1*, *PP2C*, WD domain-containing protein and *oxidoreductase* to modulate ROS in rice. These findings suggest a complex regulatory mechanisns of drought response and tolerance in plants, involving both ABA and other signaling pathways.

Recent work on inhibitors of phosphoinositide-dependent phospholipases C (*PI-PLCs*) in *Arabidopsis* has also provided considerable insight into the drought-stress-related lipid signaling by identifying links of phosphoinositides to the *DREB2* pathway [38]. Moreover, overexpression of phosphatidylinositol synthase gene *(ZmPIS)* in tobacco plants changed membrane lipids' composition and improved drought stress tolerance [39]. The best characterized lipid derivative, so far, is inositol 1,4,5-trisphosphate (IP_3). IP_3 levels have been shown to increase in response to exogenous ABA in *Vicia faba* guard cell protoplasts and in *Arabidopsis* seedlings, for review see [40]. IP_3 acts as a second messenger involved in releasing Ca^{2+} from internal stores such as vacuoles. This pathway has been reported to induce osmotic-stress-responsive genes, as well as ABA stress-responsive genes [40]. Another lipid derivative, phospholipase D (PLD), has been reported by the same authors to be functionally associated with ABA; and the application of phosphatidic acid (PA), a PLD derivative, has been shown to mimic the effect of ABA in inducing stomatal closure [41]. This could probably suggest that lipid signaling is linked to ABA in drought stress response, and it is worthwhile to study how the different lipid derivatives enter in action, either simultaneously or timely synchronized with ABA.

Downstream of the TFs are numerous responsive genes that function either in a constitutive manner (i.e., also expressed under well-watered conditions) or a drought-responsive manner (i.e., expressed only under pronounced water shortage). Amongst them, genes encoding for receptor-like kinases (RLKs) with Ser/Thr kinase domain could play an important role in optimizing plant responses to drought stress [18]. Other genes that have been shown to be up-

or downregulated by drought stress to enable dehydration avoidance or tolerance in various plant species are documented in several studies [18, 42]. Another process, downstream of transcriptional regulatory networks, is the induction of a large range of genes encoding for enzymes involved in osmotic adjustments, osmoprotection, wax biosynthesis and changes in fatty acid composition (Figure 3). Adjustment of osmotic pressure allows the plant to take up more soil water and maintain turgor and cell function for a longer time under drought.

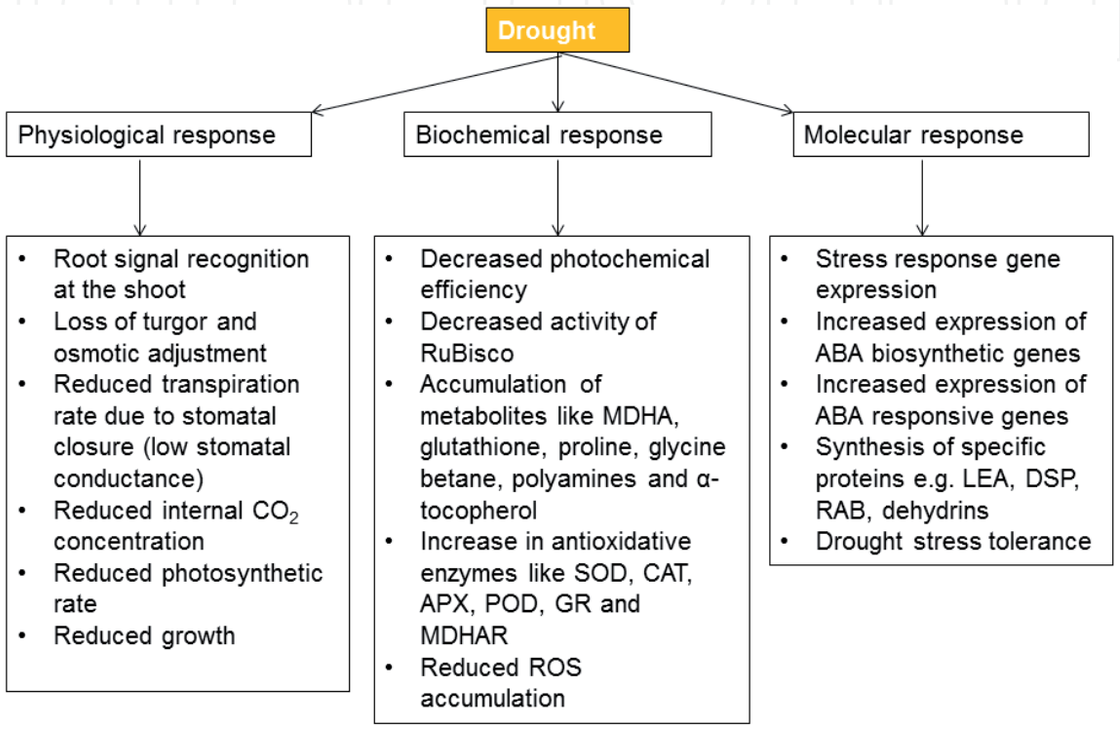


Figure 3. Physiological, biochemical, and molecular basis of drought stress tolerance in plants. Both major and minor changes that occur downstream of the transcriptional regulatory network are shown, although some of them, e.g. proline, glycine betaine and other amino acids, were previously shown not to be important in plant resistance to drought stress.

Water-channel proteins, e.g. aquaporins (*AQPs*), and sugar transporters are believed to facilitate the adjustment of osmotic pressure under stress by transporting water and sugars to the cytosol [42]. More recently, *AQPs* encoding genes (e.g., *MaPIP1;1*) were shown to be strongly induced in banana plants exposed to drought [43]. The same authors indicate that overexpression of *MaPIP1;1* in *Arabidopsis* exhibited better growth, reduced water loss and higher survival rates. Li et al. [44] also showed that *AQPs* were elevated under drought stress in Tibetan *Sophora moorcroftiana*, which is consistent with the previous reports [45]. However, the same authors indicate conflicting functions of plasma membrane intrinsic proteins (*PIP*s). For instance, overexpression of *GoPIP1*, cloned from *Galega orientalis*, showed increased sensitivity to drought in transgenic *Arabidopsis* plants. This indicates that AQPs are able to facilitate both tolerance and sensitivity, which warrants further research to delineate *AQP*s that are potentially helpful in improving drought tolerance in plants.

Studies have also shown that the *K⁺ uptake transporter 6* (*KUP6*) subfamily of transporters act as key factors in osmotic adjustment by balancing potassium homeostasis in cell growth and drought stress responses [46]. *KUP6* is apparently under the control of abscisic acid and interacts with ABA-activated *SnRK2*-type protein kinase, *SnRK2E*, resulting in phosphorylation of the *KUP6* C-terminal domain. This indicates that *KUP6* is a downstream target for *SnRK2E* in the control of water stress responses. However, other interacting proteins, and probably hormones, e.g. auxins, could regulate the activity of *KUP6* in the maintenance of water status during drought stress. Indeed, it was reported previously that a variant of *KUP6*, *KUP4*/*TRH1*, facilitates root-specific auxin distribution [47]. This was substantiated by the findings that triple mutants of the *KUP* genes (i.e., *kup2 kup6 kup8*and*kup6 kup8 gork*) showed enhanced cell expansion and auxin responses in lateral root formation [54]. Moreover, auxin-responsive TFs, *LBD18* and *LBD29*, were highly expressed in the triple mutants in the presence of IAA, indicating that auxin could be modulating K^+ and proton fluxes during drought stress.

The biosynthesis of osmoprotectants such as amino acid, amines and carbohydrates is another indispensable strategy for plant resistance to drought stress. The most common osmoprotectants are proline (Pro), γ-aminobutyric acid (GABA), glycine betaine (GB), fructans, starch, mono- and disaccharides, trehalose (Tre) and raffinose family oligosaccharides (RFO). The biosynthesis and transport of trehalose and raffinose is particularly relevant in drought stress response. More recently, genes encoding for trehalose and raffinose biosynthesis were significantly upregulated in the roots and leaves of *Jatropha curcas* under drought [48], suggesting that these compounds may have major impacts on osmotic adjustment and ROS scavenging during drought stress. The same authors indicated that dozens of genes involved in wax biosynthesis, including *KCS* and *WSD*, and their regulators (e.g., *MYB96, CER*) were upregulated more than four-fold in leaves under drought conditions. Overexpression of genes encoding for *MYB96, CER KCS* and *WSD* could probably strengthen the hydrophobic barrier that prevents non-stomatal water loss and increase plant tolerance to drought.

Genes encoding for proteins involved in cellular structure stabilization have also been reported to be induced in plant tolerance to drought. For instance, dehydrins (DHNs) function to protect cells from damage caused by drought stress-induced dehydration [49]. Proteins related to lignin biosynthesis, such as caffeoyl-CoA 3-O-methyl-transferases and class III plant peroxidases, were also found to be induced by drought in wild watermelon [50] and in maize roots [51]. In winter triticale, water-deficit-induced leaf rolling was correlated with a higher level of cell wall-bound phenolics in the leaves [52]. These adaptive mechanism could probably limit water loss by restricting the leaf transpiration surface. In addition, carbon/nitrogen-metabolism-related proteins have been reported to be more abundant in roots of soybean [53], wild watermelon [50] and rapeseed [54] after drought treatment, suggesting an increased energy demand as well as enhanced cellular activities in the root tissues during drought stress. The same authors reported a relative increase in the root growth rate and abundance of root-growth-related small G-protein family members such as *Ran GTPases*, which suggests increased membrane trafficking activity in an effort by the plant roots to absorb water from deep soil layers.

Taken together, the vast amount of data from 'omic' tools provide a basis for identification of more functional genes, which could contribute directly to cellular drought stress tolerance. In addition, understanding expression networks of genes encoding for the aforementioned proteins, especially genes involved in cellular structure stabilization, molecular chaperones, enzymes for detoxification of reactive oxygen species, and those for the biosynthesis of sugars, wax and dehydrins, which are important as protectants [55], may allow for the realization of significant genetic gains in breeding for plant tolerance to drought. Further genomic scale investigations will enable understanding of transcriptional regulators behind co-expressed genes and their association with particular genomic regions (QTLs). Although QTL identification for tracing drought tolerance remains a challenge due to the large number of genes influencing drought tolerance traits, continued investigation into the basis of tolerance in crops like *Jatropha curcas* will probably provide a clearer understanding of drought tolerance. Besides this, the mechanism by which drought tolerance associated protein networks effectively protect PSII and granal stability, as well as maintain photosynthetic competence will need further elucidation.

3. Advances in plant tolerance to heat stress

Temperatures above the normal optimum cause heat stress (HS) at different levels in all living organisms. Heat stress disturbs cellular homeostasis, and causes denaturation and dysfunction in many proteins, leading to severe retardation in growth, development and even death. In plants, the major sites of heat stress injury are the oxygen-evolving complex (OEC) along with associated biochemical reactions in photosystem II (PSII). Ultimately, efficiency of electron transport is reduced or altered affecting electron flow from OEC towards the acceptor side of PSII. These alterations affect the generation of ATP and the regeneration of Rubisco for carbon fixation [56]. Starch synthesis is also negatively affected by heat stress because of the reduced activity of enzymes such as invertase, sucrose phosphate synthase and ADP glucose pyrophosphorylase. Usually, ROS induction and accumulation in the chloroplasts precedes these changes. Accumulated ROS can severely damage DNA and cause autocatalytic peroxidation of membrane lipids and pigments, altering membrane functions and cell semi-permeability. Physiological changes associated with biochemical damage may include a decrease in chlorophyll a:b ratio, inhibitions of stomatal conductance and net photosynthesis, and low plant water potential. These changes ultimately reduce the partitioning of photosynthates, which morphologically manifest as retarded growth, reduced economic yield and harvest index. Scorching and sunburns of leaves and twigs, branches and stems, leaf senescence and abscission, and fruit discoloration and damage are other morphological damages associated with heat stress [57].

Perception of heat stress by plants usually triggers sensors at the plasma membrane and causes a transient opening of Ca^{2+} channels, possibly via modulation of membrane fluidity (Figure 4) [58]. Upon entry of Ca^{2+}, putatively through channels possessing cytosolic C-terminus with a calmodulin-binding domain, multiple kinases are activated.

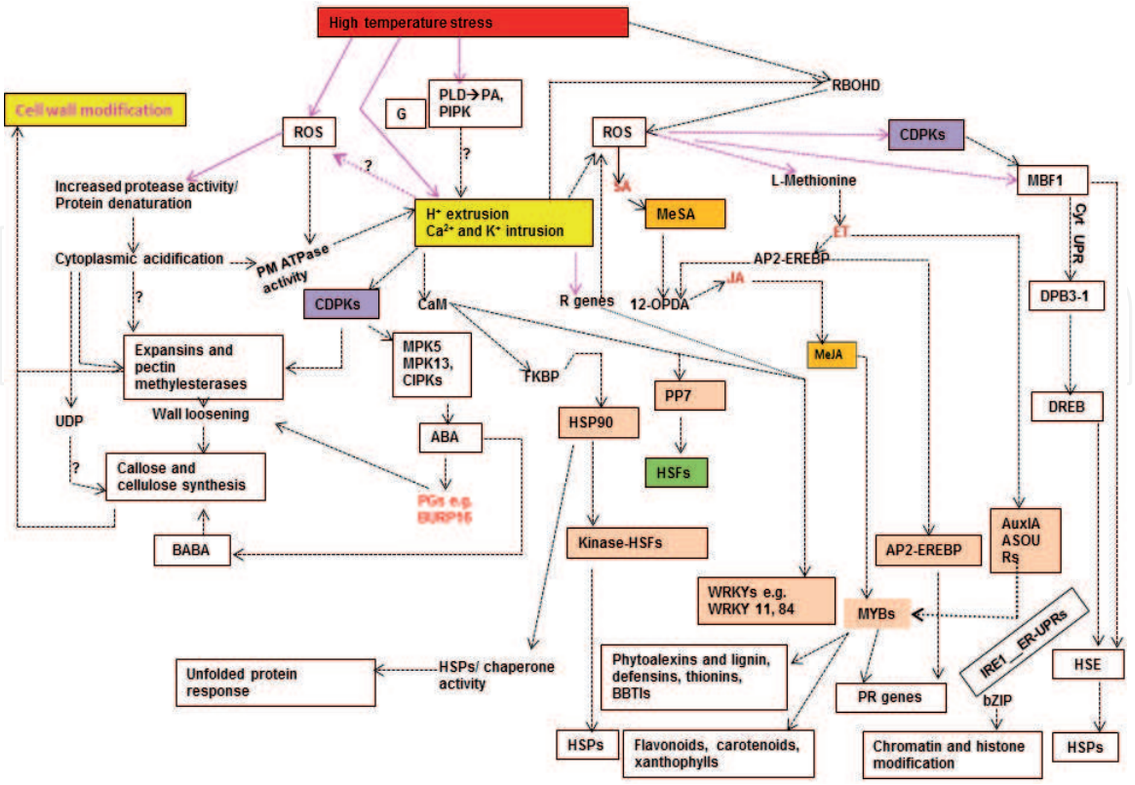


Figure 4. Hypothetical model for high-temperature signal sensing and induction of molecular pathways leading to plant defence response. Prolonged high-temperature stress causes membrane depolarization leading to Ca^{2+} influx or directly activates apoplastic enzymes including GLPs. Increased levels of cytosolic calcium activate the ROS-producing enzyme, RBOHD, which catalyses ROS production. Effect of temperature on R genes through an unknown pathway is likely to further enhance ROS production. ROS/ Ca^{2+} signaling causes activation of plasma membrane ATPase, which extrude H^+. Alternatively, heat-stress-induced protein damage and protease activity decreases cytosolic pH. Low cytosolic pH and H_2O_2 accumulation reduces CO_2 assimilation, thereby increasing endogenous carbohydrate metabolism. Cytosolic acidification and ATPase activity may also increase accumulation of expansins and methylesterases that eventually affect the cell wall integrity. Activating plasma membrane ATPase is probably reverse phosphorylated by FKBP65 leading to H^+ extrusion and K^+ intrusion. A part from its targeted role in the nucleus, FKBP65 could be targeted to the chloroplast through the tat pathway to activate photosystem II 10 kDa polypeptides or for directing chaperone functions. Activated HSPs probably cause chromatin remodelling and histone displacement. In addition to activating PM ion channels, heat-induced changes in membrane fluidity triggers lipid signaling. Plants deploy phospholipids, including phospholipase D (PLD), PIPK (phosphatidylinositol 4,5-bisphosphate kinase), phosphatidic acid (PA), PIP2 (phosphatidylinositol phosphate kinase) and IP3 (D-myo-inositol-1,4,5-trisphosphate) to specific intracellular locations. The accumulation of lipid signaling molecules also triggers Ca^{2+} influx, which initiates downstream signaling, including activation of CDPKs, hormonal changes, transcription factor activation and secondary metabolism. Question marks indicate the unknown players.

The *MPK6* activity has been particularly shown to increase under heat stress. *MPK6* activates a vacuolar processing enzyme (*VPE*), which has been suggested to play a role in HS-induced programmed cell death [59]. Transcriptional regulators, such as *HSFs*, *WRKY*, *Zat* and *MBF1c*, a transcriptional regulator of *DREB* genes [60], are activated to regulate expression of HSPs and other heat stress response genes.

Heat-induced accumulation of Ca^{2+} in the cytoplasm also activates the ROS-producing enzymes *RBOHD* and *NADPH oxidase*, by direct interaction or through activation of calcium-dependent protein kinases (*CDPK*) that phosphorylate *RBOHD* [61]. When activated, *RBOHD*

catalyzes the production of ROS, causing membrane depolarization and/or initiation of ROS/redox signaling network, which interacts with the above-mentioned *MBF1c*, *HSFs*, *MAPKs* and *SnRK*s to trigger downstream signaling networks [61].

Calcium/calmodulin-binding protein kinases (*CBK*), which also regulate the expression of *HSPs*, are activated via *CaM3*. A well-known example is the activation of *CBK3*, which enhances thermotolerance in *A. thaliana* seedlings by phosphorylating *HsfA1a* and a *CaM* protein phosphatase (*PP7*) [62]. *PP7* interacts with both *AtCaM3* and *AtHsfA1a*. *AtCaM3* increases thermotolerance by activating *WRKY39* and *HSFs*, indicating that *CBK3* plays a key role in heat stress signaling. The TF *Zat* is necessary for the activation of *WRKYs* and ascorbate peroxidase [63]. *MBF1c* modulates the induction of SA and trehalose, which are regulators of plant stress response [64]. SA has been shown to alleviate heat stress by increasing proline production and restricting the formation of ethylene in heat-stressed plants [65].

Another HS-response-associated signaling pathway was shown in the *Hsp90–ROF1* interaction in the cytoplasm and their subsequent translocation to the nucleus. The *Hsp90–ROF1* complex localizes in the nucleus only in the presence of *HsfA2* [66]. The interaction of these three proteins modulates *HSP* gene expression under HS. Although, *ROF1* has been reported to induce expression of small *HSPs*, which increases plant survival rate under HS, to date the upstream signal that regulates the subcellular localization of *Hsp90–ROF1* remains elusive. Interestingly, just like *MBF1c, ROF1* is involved in calcium-dependent phosphorylation of *HSFs*, which suggests that Ca^{2+}-dependent activation of *RBOHD* or *CDPK*s could be the upstream signal for *ROF1*.

Heat stress also triggers lipid signaling. Activation of *phospholipase D* (*PLD*) and a *phosphatidylinositol 4, 5-bisphosphate kinase* (*PIPK*) increases the accumulation of phosphatidic acid (*PA*), phosphatidylinositol phosphate kinase and D-myo-inositol-1,4,5-trisphosphate (*IP3*); and an active cycling of a G protein appears necessary in this process. The accumulation of lipid signaling molecules could in turn cause the opening of channels and the triggering of Ca^{2+} influx [67].

Downstream effects of heat stress signals have been reported to activate a signaling pathway called unfolded protein response (UPR) in the endoplasmic reticulum, which requires specific calcium signals from the plasma membrane [58]. Within the endoplasmic reticulum, the activity of UPRs involves two signaling pathways: one involving proteolytic processing of membrane-associated *bZIP* TFs and the other involving RNA splicing factor, inositol requiring enzyme-1 (*IRE1*) and its mRNA target [68]. *IRE1* is a dual functional enzyme possessing both serine/threonine protein kinase and endoribonuclease activity. In *Arabidopsis*, heat signals activate *IRE1* to splice *bZIP60* mRNA in the cytosol, causing a frameshift, which triggers the synthesis of a tissue factor without a transmembrane domain, but having a nuclear targeting signal [69]. The *bZIP60* (*bZIP60*(s)) spliced forms activate UPR target genes in the nucleus. A cytosolic form of UPR, which is triggered by the presence of unfolded proteins in the cytosol, was also previously reported [70]. Together, these UPRs are associated with the heat shock promoter elements and the involvement of specific *HSFs*, notably *HSFA2*, regulated by alternative splicing and non-sense-mediated decay. Under severe HS (42–45ºC), a novel post-transcriptional regulatory mechanism governing *HSFA2* expression has also been shown to

occur. Moreover, a new splice variant of *HSFA2-III* is reported to be generated through the use of acryptic 5′ splice site in the intron. *HSFA2-III* can be translated into the small *HSFA2* (*S-HsfA2*), which binds to the TATA box proximal clusters of HS elements (*HSE*) in the *HSFA2* promoter to activate its own gene expression, thus constituting a positive auto-regulatory loop [71]. Although the TFs interacting with *S-HsfA2* are yet to be validated, this finding suggests that severe HS may alter the splicing pattern of *Hsf* genes, generating isoforms that may auto-activate self-expression and consequently rapidly induce the expression of HSPs required for enhanced response to HS.

Apart from *HSFs*, overexpression of *DPB3-1,* which regulates expression of *DREB2A* and *DREB2B*, increases thermotolerance [72]. Other studies have also shown the role of *bZIP28* [73] and *WRKY* proteins in plants thermotolerance [74, 75]. Furthermore, the basic helix-loop-helix (*bHLH*) TF, *phytochrome interacting factor 4* (*PIF4*), was reported to control acclimation to changes in ambient temperature by regulating important hormonal and developmental pathways modulating the acclimation mechanisms [76]. *PIF4* alleles control floral timing by modulating *FLOWERING LOCUS T (FT). PIF4* also controls early inflorescence internode elongation and high-temperature-induced hypocotyl elongation by modulating levels of free indole-3-acetic acid (*IAA*) through the triggering of *YUC8 (YUCCA8)* or *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS* (*TAA1*) gene expression [57, 77]. Thus, *PIF4* is a potential regulator of plant responses to high temperature. However, its physical interaction with *cryptochrome 1* (*CRY1*) on nuclear DNA suggests that these two proteins co-regulate temperature responses in plants. Another regulator, *E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1),* was shown to be essential for plant responses to HS [77]. However, it is not known whether *COP1* signaling is independent of *PIF4*. Orthologs of *PIF4* have been identified in several crop species. Thus, if the interaction with other associated proteins is resolved, *PIF4* has a potential promise to improve plant tolerance to HS in several crops through genetic engineering.

Other components of heat sensing that could be linked to these signaling pathways include the transcriptional modulator, the *nuclear actin-related protein 6* (*ARP6*), which is part of the Snf-2-related CREB-binding activator protein (*SRCAP*) encoding a subunit of the *SWR1* chromatin remodelling complex is necessary for inserting the alternative histone, *H2A.Z*, into nucleosomes, while replacing the core histone H2A [78]. Heat stress induces a decrease in *H2A.Z* occupancy in nucleosomes located at the transcription start site of heat response genes, a process that probably allows nucleosome opening and enhanced transcription of these genes.

Plant adaptation to thermotolerance also involves the activity of superoxide reductase (*SOR*), *S*-nitrosoglutathione reductase (*GSNOR*) and rubisco activase (*RCA*). The functions of these proteins are reasonably well described in a review by [67]. Other commonly reported anti-oxidant enzymes produced by plants under HS include superoxide dismutase (*SOD*), catalase (*CAT*), guaiacol peroxidase (*GPX*), ascorbate peroxidase (*APX*), dehydroascorbate reductase (*DHAR*), glutathione reductase (*GR*), glutathione S-transferase (*GST*) and non-enzymatic anti-oxidants such as flavanoids, anthocyanin, carotenoids and ascorbic acid (*AA*) [60]. The accumulation of other osmolytes such as glycine betaine and trehalose is another well-known adaptive mechanism in plants against HS. Generally, most of these compounds are involved

in ROS removal (anti-oxidants), osmotic adjustment, saturation of membrane-associated lipids, protection of photosynthetic reactions, production of polyamines and protein biosynthesis [94], which enable plants to exhibit basal or acquired thermotolerance. Proline and glycine betaine application considerably reduce the H_2O_2 production, improve the accumulation of soluble sugars and protect the developing tissues from HS [79]. Tocopherol is another important lipid-soluble redox buffer and an important scavenger of singlet oxygen species and other ROS. Moreover α-tocopherol has the highest anti-oxidant activity of all the tocopherol types reported in plants [80].

A number of studies have demonstrated the presence of QTLs associated with most HS-related traits and promise to the use of molecular markers in breeding for heat stress tolerance. More than 50 QTLs have been identified in various crops so far, including maize, wheat, rice, cowpea, lettuce, *Medicago truncatula* and *Brassica napus*. Recent studies in transcriptomics [81, 82], proteomics [83, 84], metabolomics [85, 86] and microRNAs [87] have also provided additional information on the mechanisms controlling plant responses to HS. Understanding the relationship between these mechanisms and the genomic regions mapped and delineated as QTLs would validate the genes controlling plant responses to HS, and subsequently improve genetic gains in plant improvement programmes. Besides, the possibility of developing transgenic plants with enhanced tolerance to HS would also gain significance. This approach has already been demonstrated in cotton [88], *Arabidopsis* [89], tobacco [90] and rice [91], but needs further validation, especially in economically important crops where it has not been applied before. Taken together, heat stress responses discussed here demonstrate that heat stress is a quantitative trait, which requires a combination of several disciplines to improve plant tolerance.

4. Advances in plant tolerance to cold stress

Cold stress occurs at temperatures less than 20ºC and varies with the degree of temperature duration and plant type. Chilling (<20ºC) or freezing (<0ºC) temperatures can trigger the formation of ice in plant tissues, which causes cellular dehydration [92]. Ultimately, cold stress reduces plasma membrane (PM) integrity, causing leakage of intracellular solutes. Cold stress severely affects plant growth and survival, and leads to substantial crop losses in temperate climatic regions and hilly areas of the tropics and subtropics [93]. In rice, for instance, losses due to cold stress can range from 0.5 to 2.5 t/ha and grain yields can drop by up to 26%, especially when low temperatures occur during the reproductive stage [94].

To cope with this adverse condition, plants adapt several strategies such as producing more energy by activation of primary metabolisms, raising the level of anti-oxidants and chaperones, and maintaining osmotic balance by altering cell membrane structure [95]. These mechanisms of plant response to cold stress are closely similar to that of heat stress. However, the difference lies in the fact that membrane rigidification occurs in cold stress as opposed to heat stress. Thus, membrane rigidification is the upstream trigger for the induction cytosolic Ca^{2+} signatures leading to a transient increase in cytosolic Ca^{2+}levels [96]. It is assumed that dimethyl

sulfoxide (DMSO) mediates the perception of membrane rigidification by mechanosensitive Ca^{2+}channels [97]. Other upstream factors such as changes in the metabolic reactions and metabolite concentrations, protein and nucleic acid conformation could contribute to enhance perception of cold stress. These factors as well, either directly or indirectly, induce an increase in cytosolic Ca^{2+}, which is a well-known upstream second messenger, regulating cold regulated (*COR*) gene expression.

Cold-stress-induced cytosolic Ca^{2+} signals can be decoded by different pathways. More recently, Ca^{2+} signal was reported to be transduced directly into the nucleus. The concentration of nuclear Ca^{2+} is monitored by a chimera protein formed by the fusion of aequorin to nucleoplasmin, which is also transiently increased after cold shock [95]. Aequorin possesses several EF-hand-type binding sites for Ca^{2+} ions. The binding of Ca^{2+} to these sites causes a conformational change in aequorin which enables the monitoring of Ca^{2+} concentration. It has been reported that nuclear Ca^{2+} concentration peaks at about 5–10s later than the cytosolic Ca^{2+} [95]. The same authors have reported that nuclear Ca^{2+} signal may be initiated from the nuclear envelope and is assumed to be propagated by cytosolic Ca^{2+} transients in plants.

In the cytoplasm, a range of Ca^{2+} sensors have been reported, including calmodulin (*CaM*), CaM-like (*CMLs*), Ca^{2+}-dependent protein kinases (*CDPKs*), Ca^{2+}-and Ca^{2+}/CaM-dependent protein kinase (*CCaMK*), CaM-binding transcription activator (*CAMTA*), calcineurin B-like proteins (*CBLs*) and *CBL*-interacting protein kinases (*CIPKs*) [98]. Some of the sensors work as negative regulators of cold tolerance in plants, e.g., calmodulin3, a SOS3-like or a CBL calcium-binding protein and a protein phosphatase 2C (*AtPP2CA*). The positive regulators, e.g., CDPKs and probably some *CBLs*, relay the Ca^{2+} signal by interacting with and regulating the family of *CIPKs*. For instance, *CBL1* has been shown to regulate cold response by interacting with *CIPK7* [99], whereas *CAMTA3* has been identified as a positive regulator of *CBF2*/*DREB1C* through binding to a regulatory element (*CG-1*, *vCGCGb*) in its promoter [100]. Although *CBF2*/*DREB1C* was previously reported to negatively regulate *CBF1*/*DREB1B* and *CBF3*/*DREB1A*, its expression appears to be necessary for integrating cold-inducible calcium signaling with gene expression, but under transient and tight control to avoid repression of freezing tolerance. Both *CBF1*/*DREB1B* and *CBF3*/*DREB1A* are required for constitutive expression of cold-inducible genes in *Arabidopsis,* and play an important role in cold acclimation (see discussion below).

Ca^{2+} influx into the cytoplasm also apparently activates phospholipase C (PLC) and D (PLD), which are precursors for IP_3 and PA, respectively. IP_3 activates IP_3-gated Ca^{2+} channels that can amplify Ca^{2+} signatures in the cytoplasm, leading to higher induction of *COR* genes and CBFs, for review see [101].

There are some reports that the chloroplast may also play a role in sensing low temperature [98]. Cold stress is considered to cause excess photosystem II (PSII) excitation pressure, as a result of the imbalance between the capacity for harvesting light energy and the capacity to consume this energy on metabolic activity in the leaves, which probably leads to ROS generation. The damaging effect of ROS on the photosynthetic apparatus presumably leads to photoinhibition, which occurs even under relatively low irradiance [102] and is apparently a mechanism of cold acclimation or freezing tolerance. ROS also acts as the second messenger

and may reprogramme transcriptome changes through induction of Ca^{2+} signatures and activation of MAPKs and redox-responsive TFs. The MAPK cascades in *Arabidopsis* , including AtMEKK1/ANP1 (MAPKKK)–AtMKK2 (MAPKK)–AtMPK4/6 (MAPK), positively regulate cold acclimation in plants [103].

The downstream signals that promote the production of *COR* proteins and cold response to metabolites are reasonably discussed in references [95, 104]. Specific examples include the upregulation of the TFs, *CBF*/*DREB1s* (CRT (C-repeat)/DRE binding proteins) [103], which initiate the transcription process. The *CBF*/*DREB1* (mainly *CBF3*/*DREB1A*) pathway is controlled by a myelocytomatosis oncogene (*MYC*)-type TF, inducer of CBF expression1 (*ICE1*), which binds to the *MYC* recognition cis-elements (*CANNTG*) in the promoter of *CBF3*/*DREB1A,* and induces the expression of *CBF3*/*DREB1A* and its regulons during cold acclimation [105]. The function of *ICE1* in cold response is conserved; and overexpression of *Arabidopsis ICE1* improves chilling tolerance and enhances the accumulation of soluble sugars and proline concentration in cucumber [106]. In rice, *OsICE1* and *OsICE2* are induced by cold stress and sequentially upregulate *OsDREB1B*, rice heat shock factor A3 (*OsHsfA3*) and rice trehalose 6-phosphate phosphatase (*OsTPP1*). The *CBF*/*DREB1s* can bind to *CRT*/*DRE* cis-elements, A/GCCGAC, in the promoter of *COR* genes to regulate expression of *COR* genes [107]. Moreover, *CBF*/*DREB1* genes are organized in tandem (*CBF1*/*DREB1B*-*CBF3*/*DREB1A*-*CBF2*/*DREB1C*) on *Arabidopsis* chromosome IV and have been reported to be induced at the same time, suggesting that combining these TFs in one genotype could probably improve cold tolerance. However, the inconsistent target specificity amongst the three *CBF* factors in *CBF*/*DREB1*-overexpressing transgenic plants reveals variability in their roles [108]. Indeed, *CBF2*/*DREB1C* has been shown to be a negative regulator of both *CBF1*/*DREB1B* and *CBF3*/*DREB1A* [109], while *CBF1*/*DREB1B* and *CBF3*/*DREB1A* act as positive regulators of cold acclimation by activating the same subset of *CBF*/*DREB1*-target genes [110]. *CBF1*/*DREB1B* and *CBF3*/*DREB1A* are therefore concertedly required to induce the whole *CBF*/*DREB1*-regulon to complete the development of cold acclimation, while the expression of *CBF2*/*DREB1C* is tightly controlled to avoid its negative modulation of *CBF1*/*DREB1B* and *CBF3*/*DREB1A*. The exact mechanism by which this happens is unknown.

Downstream of these TFs are *COR* genes, which are mainly linked to the onset of tolerance mechanisms and ultimately lead to acclimation. Genes encoding for annexin; hyper-sensitive-induced response (HIR) protein families (e.g., prohibitins and stomatins); dehydrins (e.g., 25 kDa dehydrin-like protein, *ERD14*, and *cold acclimation-specific protein 15* (*CAS15*)); antioxidants (e.g., superoxide dismutase, catalase and ascorbate peroxidase); *HSPs* (e.g., *HSP70* family being the most abundant); defence-related proteins such as protein disulfide isomerase; disease resistance response proteins, peptidylprolyl isomerase *Cyp2* and cysteine proteinase; amino acids, polyamines and polyols; and cellulose synthesis, such as UDP-glucose pyrophosphorylase, are commonly reported in expression studies [111]. Several metabolism-associated proteins, including carbohydrate metabolism enzymes, such as phosphogluconate dehydrogenase, NADP-specific isocitrate dehydrogenase, fructokinase, cytoplasmic malate dehydrogenase, pyruvate orthophosphate dikinase precursors (PPDK), aconitate hydratase, glycine dehydrogenase and enolase, have also been reported to be activated during cold stress

[112]. Thus, several genes and the corresponding proteins are associated with the regulation of the metabolic pathways operating under cold stress.

However, identification of functional polymorphism in these genes remains a daunting task. A similar challenge is observed in the QTLs identified, so far, in various crops, including maize, barley, rice, wheat, sorghum and many other economically important crops. Identification of effective cold sensors also remains elusive, as multiple primary sensors are thought to be involved in sensing low temperatures. Thus, a comprehensive understanding of the defence mechanism from sensors, cold signaling, to the defence response will require further research on both upstream and downstream regulations of *ICE1-CBF/DREB1*-dependent pathway, as well as proteins that may be functioning independent of this pathway.

5. Advances in plant tolerance to salinity

Salinity is increasingly becoming a major threat to crop production, particularly due to inappropriate irrigation regimes and increasing use of brackish water for irrigation. As much as 6% of the total world land is subjected to salinity [113], and more than 20% of irrigated land is affected by salinity [114]. Moreover, major reductions in cultivated land area, crop productivity and quality that have been reported in the recent past are due to salt-induced stress [115]. Climate-change-associated rise in sea levels and coastal floods are expected to further contribute to this phenomenon in the future.

Salt stress in plants occurs when electrical conductivity of saturated soil paste extract (ECe) reaches 4.0 deci-Siemens per meter (dS/m; approximately 40 mM NaCl). The minimum level may, however, vary from crop to crop. For instance, the salinity threshold for rice is 3.0 dS/m [163]. Beyond this threshold, a yield reduction of 12% per dS/m has been reported to occur. When plants gradually accumulate salts, osmotic stress, nutrient imbalance and oxidative stress occur [116]. These salt effects disrupt intracellular ion homeostasis, membrane function and metabolic activity [117]. As secondary effects, salt-induced osmotic stress decreases root epidermal cell division and elongation rates, reducing primary root growth, eventually resulting in inhibition of growth and reduction of crop yields [118].

Alkalinity stress is a heightened version of salinity stress which has been reported to be much harsher than equimolar salinity, especially at neutral pH [119]. Although it is fairly understood that alkalinity causes osmotic challenge and ionic stress, and precipitates nutrients such as metallic micronutrients and phosphates, and disrupts the integrity of root cellular structure, the molecular signals and adaptive mechanisms are not well understood. Because many saline soils are also alkaline due to the presence of sodium (Na) carbonates, in this section we will exclusively focus on salinity, which is wide spread, and has been extensively researched and discussed in several studies.

To cope with saline soils, plants deploy a range of mechanisms that range from exclusion of Na^+ from the cells to tolerance within the cells. When plants are subjected to salinity, a series of responses ranging from genetic molecular expression through biochemical metabolism to physiological processes occur (Figure 5).

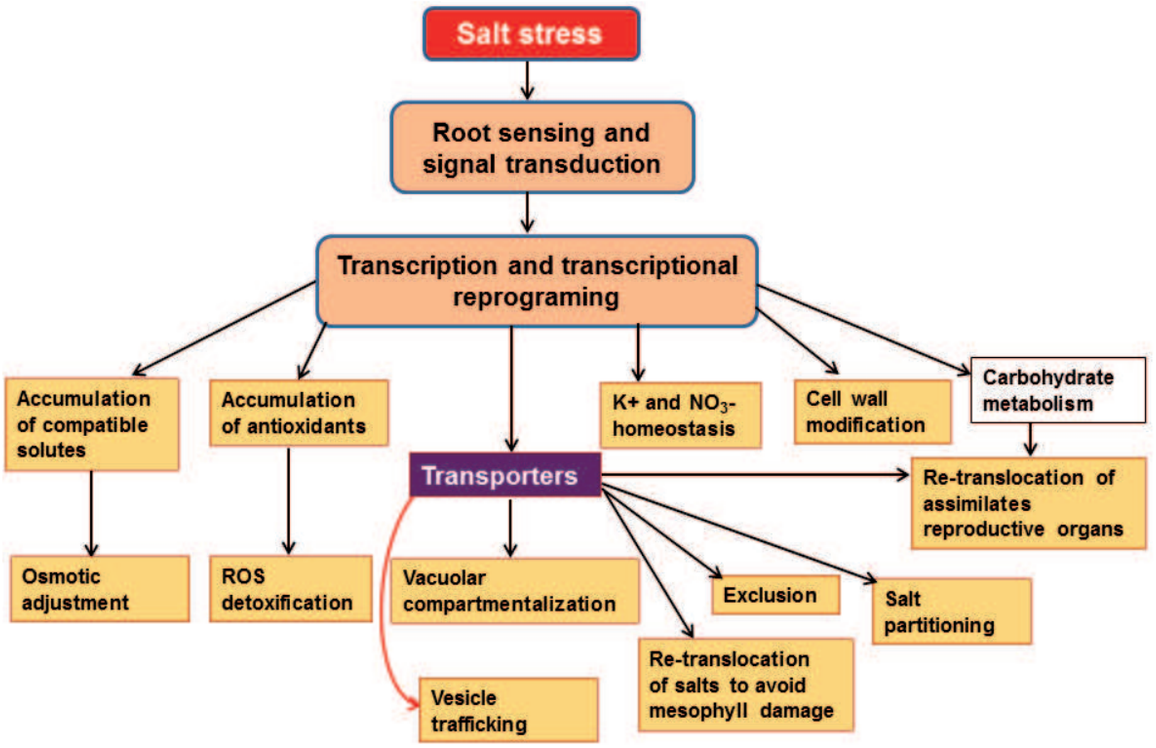


Figure 5. Adaptive mechanisms of salt tolerance. Cellular functions that would apply to all cells within the plant are the first adaptation mechanisms, followed by the functions of specific tissues or organs. Most of these functions are explained in the text (modified from [140].

Amongst the receptor proteins identified as the first detectors of salt stress are G-protein-coupled receptors, ion channel, receptor-like kinase or histidine kinase. These receptors transduce signals that generate secondary signals such as Ca^{2+}, inositol phosphates, ROS, nitric oxide (NO) and ABA. The signaling pathway associated with increased concentration of cytosolic Ca^{2+} is the most reported.

Cytosolic Ca^{2+} activates calcium-dependent protein kinases (*CDPKs*), calcineurin B-like proteins (*CBLs*) and CBL-interacting protein kinases (*CIPKs*) to transduce signals to downstream protein activity and gene transcription [120]. Transcription factors such as calmodulin-binding transcription activators (*CAMTAs*), GT element-binding-like proteins (*GTLs*) and MYBs have been reported to be activated by Ca^{2+}/calmodulin directly [121–123]. Other commonly expressed TFs in response to salt stress include the basic leucine zipper (*bZIP*), e.g., *OsbZIP71* in rice [124], *WRKY* [125], *APETALA2/ETHYLENE RESPONSE FACTOR* (*AP2/ERF*) [126], *MYB* [127], basic helix–loop–helix [128] and *NAC* [42] families. These TFs regulate the expression of genes related to water potential decrease, which results from osmotic stress caused by salinity.

Downstream of these TFs, there are several genes associated with salinity tolerance. The most reported are genes encoding for salt exclusion proteins, e.g., *SOS1*, cation:proton **antiporter family**1 of Na^+/H^+ anti-porters, salt compartmentalization genes, e.g., *vacuolar H⁺-pyrophosphatase* [129], and osmotic adjustment, e.g., *pyrroline-5-carboxylate synthetase* [130].

The salt overly sensitive (*SOS*) Ca^{2+} sensor regulatory mechanism is believed to be conserved in higher plants including monocots and dicots [131]. *SOS* consists of three functionally interlinked proteins, *SOS3*/*SCaBP8*–*SOS2*–*SOS1*. *SOS3* mainly functions in the roots, while *CBL10*/*SCaBP8*, an alternative regulator of *SOS2* that has been described as *SOS3*-like, primarily functions in the shoots. At high Na^+ concentrations, increased influx of Ca^{2+} is perceived by *SOS3* that encodes a myristoylated EF hand (a domain of five serially repeated helix–loop–helix calcium-binding motifs). Upon Ca^{2+} binding, a conformational change occurs and *SOS3* activates the downstream serine/threonine protein kinase, *SOS2*, and recruits it to the plasma membrane. Subsequently, the *SOS3*–*SOS2* complex stimulates the plasma membrane-localized Na^+/H^+ anti-porter (*SOS1*), leading to the extrusion of the excess Na^+ out of the cells [132]. Different from *SOS3, SOS3*-like proteins (*CBL10*/*SCaBP8*) are phosphorylated by their interacting protein kinases apparently regulating *CBL*/*SCaBP*–*CIPK*/*PKS* modules [133].

Besides extruding Na^+, the adaptive *SOS* module also links cytosolic Na^+ with Ca^{2+} binding proteins. The PM-localized *NHX7*/*SOS1* and the intracellular localized cation:proton **antiporter family**1 (*CPA1*) of Na^+/H^+ anti-porters (*NHX1-NHX4*; tonoplast-localized) are a ubiquitous family of transporters that mediate the exchange of K^+ or Na^+ for H^+ while regulating cytoplasmic salt overloads [134]. In the cytosol, increased influx of Ca^{2+} associated with excess Na^+ levels is perceived by Ca^{2+}-binding calmodulins/calmodulin-like proteins, which interact with *NHX1* transporters to sequester excess Na^+ in the vacuole. In *Arabidopsis*, a calmodulin-like protein, *AtCaM15,* regulates the tonoplast localized *AtNHX1* [135]. The interaction of *AtCaM15* with *AtNHX1* occurs in the vacuolar lumen and is dependent on Ca^{2+} and pH. The C-terminus of *AtNHX1* has been shown to localize in the cytosol, which might suggest that this strategic placement is targeted for phosphorylation by protein kinases or for sensing changes in cytosolic pH. However, the protein kinase targeting *AtNHX1* is unknown, and further studies on the interaction of this transporter with other proteins, especially protein kinases, will be necessary.

Interestingly, at moderate salt levels, the role of these transporters is less clear. Indeed, the *nhx1*/*nhx2* **double mutants** are not sensitive to moderate external Na^+ concentrations, yet they are sensitive to moderate external K^+ concentrations, for review see [134]. Conversely, the trans-Golgi network-localized *NHX* double knockouts, *nhx5*/*nhx6*, highly respond to moderate salinity and interfere with vesicle trafficking to the vacuole. This suggests that the endosomal *NHXs* are more sensitive to Na^+ accumulation than vacuolar *NHXs*. This difference has implications on Na^+ tolerance in plants. Recently, another *CPA* family member, a cation/H^+ exchanger (*CHX*), *GmSALT3*, was shown to improve shoot Na^+ exclusion and salt tolerance in soybean [136]. Fluorescent protein fusions suggested that *GmSALT3* and other *CHX* proteins are localized to the endoplasmic reticulum, further indicating that endosomal *NHXs* could be more reliable in sensing abnormal Na^+ levels in the cell and has a positive implication on salt tolerance in plants.

Other genes encoding for *Mannose-1-phosphate guanyl transferase* **(*OsMPG1*)** and **the rice** homologue of Shaker family K+ channel *KAT1* (*OsKAT1*) have also been reported to confer salinity tolerance [137, 138]. *OsMPG1* is an important enzyme for the biosynthesis of ascorbic acid in plants, whereas *OsKAT1* reduces the cellular Na^+ to K^+ ratio by increasing the cellular K^+ content. Another rice potassium transporter (*OsHAK5*) was shown to accumulate more K^+

and less Na^+ when constitutively expressed in *Nicotiana tabacum* cv. Bright Yellow 2 under salinity stress [198]. Several other genes were recently identified by Chen et al. [139] while studying the halophyte seashore Paspalum (*Paspalum vaginatum*).

Another process, downstream of transcriptional regulatory networks, involves accumulation of sufficient solutes (e.g., proline and glycine betaine) to balance extra osmotic pressure in the soil solution to maintain turgor [140]. Moreover, plants can also accumulate sufficient Na^+ and Cl^- to balance those in the soil solution, but this is tightly controlled through strict ionic regulation in various cell compartments ('tissue tolerance'). These tolerance strategies are achieved through a series of ion transporters and their localization in key cell types. Na^+/H^+ anti-porter proteins are the key regulators of these tolerance strategies. Examples include *TaHKT1;5-D* protein, which maintains high cytosolic K^+/Na^+ ratios in bread wheat shoots by restricting Na^+ loads in the root xylem before entering the shoot [141]. Recently, the introgression of the *Triticum monococcum HKT1;5-A* into durum wheat improved shoot Na^+ exclusion and improved grain yield in the field by 25% [142], indicating the significance and functional stability of these transporters even in interspecific hybrids. Additionally, Eswaran et al. [143] used the yeast Full-length cDNA Over-eXpressor (*FOX*) gene hunting to identify several salt-responsive genes in *Jatropha curcas*. The late embryogenesis-abundant protein **(*LEA-5*),** aquaporins and a cytosolic ascorbate peroxidase-1 (*Apx1*) were amongst the identified genes involved in salinity tolerance. *LEA5* are group 5 LEA genes that have been shown to play roles in the combining of concentrated ions and dehydration [143]. This group of LEA proteins have attracted fewer investigations and will require further studies at salt stress conditions. Aquaporin proteins are members of a large multigenic family that regulates a large proportion of water transport across membranes. Aquaporins are rapidly influenced both transcriptionally and post-translationally, and enhance salt stress tolerance in plants. For instance, a plasma membrane intrinsic protein (*GmPIP1;6*, which belongs to a subfamily of aquaporin specifically located in the PM) in soybean increases shoot Na^+ exclusion and improves the seed yield from a saline field [144]. Orthologous *PIP* proteins are found in *Arabidopsis* , tobacco, barley, rice and wheat. For instance, *GmPIP1;6* is the ortholog of *AtPIP1;2, NtAQP1, HvPIP1;6/1;1* and *TaAQP8.* Overexpression of *NtAQP1* in tobacco increases photosynthetic rate, water use efficiency and yield under salt stress [145]. Overexpression of *TaAQP8, TaNIP* and *TaAQP7* genes in Arabidopsis or tobacco also increases salt tolerance of transgenic plants [146–148]. Root stellar cells also confer control over shoot Cl^- accumulation [149]. The expression of *GmPIP1;6* in roots was recently shown to be correlated with rapid and longer term changes in root hydraulic conductance (L_o) in response to shoot treatments and appeared to be more concentrated in stellar tissue [150]. These results indicated that *GmPIP1;6* could be the protein responsible for the control of root water transport, particularly in response to shoot signals. More recently, overexpression of *GmPIP1;6* was shown to significantly increase salt tolerance of soybean by improving root L_o and Na^+ exclusion, which provided additional evidence that *GmPIP1;6*'s activity is in the stellar tissue. However, as there is no conclusive interactive or independent role of *AQPs* in salt tolerance, *AQPs* could instead be playing an indirect role through their impact on osmotically driven water and solute flow in roots and leaves. Further research will probably provide clear insight as to whether *GmPIP1;6* is responsible for salt regulation in the stellar cells, and whether there are other co-factors involved.

Wheat tonoplast intrinsic protein (*TIP2; 2*) is also reported to enhance salt tolerance [151]. However, the functional role of this protein is regulated by methylation following salt treatment as is *HKT1* in *Arabidopsis* [152]. This suggests that aquaporin methylation could also play a role in regulating salt tolerance in plants and is worth further exploration.

Accumulation of ROS scavenging enzymes has also been reported to lower cellular damage, maintain photosynthetic energy capture, and improve shoot and root growth under saline conditions. For instance, salt-stress-induced accumulation of SOD has been reported to play a protective role in *Canola*, *S. europaea*, *S. chilense* and *K. candel* [153–155]. Furthermore, expression levels of anti-oxidant enzymes *APX* (e.g.,***Apx1)*** , *Trx, Prx, GPX* and *GST* were observed to be enhanced in *Tangut nitraria* [156] under salinity conditions. Moreover, the same authors have reported that a photosynthetic enzyme, Ferredoxin—NADP (+) reductase (*FNR*), activity also increased in *T. nitraria*. Pea plants grown under saline stress also showed an enhancement of both APX activity and S-nitrosylated APX, which suggests that APX plays a significant role in plant tolerance to salt stress. However, apart from ascorbic acid biosynthesis, which has been shown to be modulated by *OsMPG1*, the molecular regulation of most anti-oxidants in response to salinity remains to be explored.

The recent discovery that salt-tolerant plant growth promoting rhizobacteria (PGPR) populations reduce Na^+ concentration in the plant shoots [157] provides further insights into plant tolerance to saline conditions. The PGPRs increase the expression of stress-responsive TFs, induce greater proline synthesis, enhance ROS scavenging and improve plant biomass under salinity stress. Therefore, treatment with rhizospheric organisms, and understanding the mechanisms associated with these PGPRs leading to salt tolerance, is an attractive option to improve crop yields under saline conditions.

Fundamental insights into genetic control of salt tolerance mechanisms have also led to identification of more than 100 QTLs in various crops including *Arabidopsis* , barley, rice and wheat, amongst others. The earlier mentioned **salt overly sensitive** (*SOS*) pathway genes and *AtCIPK16* are amongst the salt tolerance factors spanning several QTLs identified [158]. *CIPK16* is an SNF1-related kinase/CBL-interacting protein kinase, underlying a quantitative trait locus for Na^+ exclusion in the *Arabidopsis* Bay-0×Shahadara mapping population. *CIPK16* was also recently shown to be expressed in barley and improves Na^+ exclusion and biomass in a saline field.

Taken together, several genes and proteins have been shown to enhance salt tolerance in plants. However, the limited number of genes with functional polymorphism for salt tolerance makes it difficult to employ marker-assisted breeding for salt tolerance traits. In addition, the complex molecular mechanisms underlying the difference between seedling and reproductive stage salt tolerance in plants, e.g. rice [159], suggest the need for further research. The importance of the apoplastic bypass flow in delivering Na^+ to the xylem, thus reducing leaf Na^+ concentration and improving tolerance as suggested by [160], is also worth exploring further. Moreover, more insights into the molecular regulation of salt response will provide avenues for combining tolerance mechanisms to develop varieties that are widely adapted to salt stress.

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6. Advances in plant tolerance to submergence/flooding

Over the past 25 years, yield losses caused by flooding have been increasing in various parts of the world, including the United States, China, Europe, Pakistan and Australia [161, 162]. Flooding is expected to increase as a result of erratic weather patterns, including frequent and lengthy storms associated with climate change, and could severely affect food production if mitigation measures are not sought.

Generally, submergence/flooding stress results from reduced oxygen levels in the plant root zone due to the low diffusion rate of oxygen in water. Submergence inhibits electron flows that underpin photosynthesis and aerobic respiration from the air causing energy shortfalls that can prove injurious to the plant [162]. Flooding also leads to accumulation of gases such as ethylene and carbondioxide by preventing their diffusive escape and oxidative breakdown [163]. A high concentration of ethylene limits root extension, while carbon dioxide can severely damage plant roots. Trapped carbondioxide may also form bicarbonate ions that can accentuate the effect of high lime content, leading to iron unavailability and chlorosis. The hypoxic environment also leads to restricted production of ATP, forcing cells to rely on glycolysis and fermentation to generate ATP and regenerate NAD^+ to cope with the energy crisis [164]. Moreover, survival through prolonged inundation hypoxia involves the use of inorganic pyrophosphate (PPi) as an alternative energy source and induction of enzymes that reduce reactive oxygen species (ROS) or cytoplasmic acidosis, which are equally energy consuming processes. Because translation is a tremendously energy-intensive process, protein synthesis is affected in such oxygen-deprived conditions. Subsequently, essential metabolic processes slow down affecting the overall growth of the plant. In rice, soybean and wheat, various deleterious effects have been observed, such as suppression or reduction of hypocotyl and root elongation, and suppression of lateral root development [162, 164, 165].

Plant tolerance to submergence/flooding is generally a metabolic adaptation in response to anaerobiosis that enables cells to maintain their integrity so that the plant survives hypoxia without major damages. Several defence-related changes occur in submergence tolerant plants, including anatomical (e.g. formation of higher aerenchyma tissue in the nodal region in rice), physiological (more shoot elongation) and biochemical (inhibition of chlorophyll degradation, less utilization of storage carbohydrates and increased activity of anti-oxidative enzymes). At the molecular level, plants need to adapt these several changes in their gene expression profiles as well as cellular protein profiles. We will focus more on molecular adaptation, with a preference for adaptive QTLs, genes and proteins of significance to crop tolerance to flooding.

One of the early responses to submergence involves the differential regulation of a suite of TFs belonging to the ethylene response factor (*ERFs*) gene family. In rice, a major QTL locus belonging to ERF family, which is responsible for submergence tolerance, was mapped to chromosome 9, designated as Submergence1 (*Sub1*) [166]. This QTL was reported to account for about 70% of the phenotypic variation under submergence [167]. One of the genes adhered to *Sub1* locus is *Sub1A*, which limits shoot elongation during submergence by repressing gibberellic acid (GA) levels and modulating GA signaling. In the process, the consumption of

energy reserves is reduced, and upon de-submergence, genotypes with *SUB1A* are able to resume development when flood water subsides.

Two *ERFs*, *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) from Thai deep water accession C9285, also confer submergence adaptation in deep water rice by inducing rapid internode elongation [168]. SKs have also been found in the genomes of accessions of wild *O. rufipogon* from Asia and *O. glumaepatula* from South America but missing in most cultivated rice varieties, which suggests that an ancient genomic region of *Oryza* was lost during the establishment of rice grown in shallow paddies, but was safeguarded in deep water ecosystems. More recently, two QTLs on chromosome 3 and 12, including *O. sativa-GROWTH-REGULATING FACTOR7* (*OsGRF7*), were reported to be involved in GA-dependent stem elongation and meristem maintenance in deep water rice [169]. *OsGRF7* on chromosome 12 could probably be a regulator of GA responsiveness for internode elongation, whereas a QTL on chromosome 3 and other QTLs may regulate the *DELLA* function or act downstream of GA signaling. The *DELLA* proteins are the key regulators of GA signaling and suppress plant growth in the absence of GA.

In maize, a major QTL, *Subtol6*, was also recently shown to be associated with submergence tolerance [170]. Based on the expression differences between the parent inbreds, *subtol6* is associated with *HEMOGLOBIN2* (*HB2*), a gene which was previously reported to be associated with plant survival in low oxygen or low ATP conditions [171]. The same authors indicate that haemoglobin proteins in maize repress ROS levels and maintain the energy status of maize cells during hypoxia. Other notable candidate genes, including genes related to *ABA-INSENSITIVE3* (*ABI3*)/*VIVIPAROUS1* (*RAV1*), genes related to accumulation and metabolism of carbohydrates, e.g., alpha subunit of *PYROPHOSPHATE-DEPENDENT FRUCTOSE-6-PHOSPHATE 1-PHOSPHOTRANSFERASE* (*PFP*) and *ALCOHOL DEHYDROGENASE1* (*ADH1*), have been reported to be highly upregulated in response to submergence [170].

In association with these tolerance genes, a number of other QTLs have also been identified in various crops, including barley, wheat, *Brassica napus*, maize and *Lolium perenne*, amongst others.

In addition to these QTLs studies, several proteins have been reported to enhance submergence tolerance in plants. Enzymes involved in primary metabolism are differentially regulated in response to flooding. For instance, UDP-glucose dehydrogenase, UDP-glucose pyrophosphorylase, β-glucosidase G4 and rhamnose synthase, aspartate aminotransferase and lipoxygenase have been reported as early flood-responsive proteins in rice and soybeans [164, 172]. The same authors indicate that phenlypropanoid pathway and cell wall synthesis enzymes decrease in abundance during flooding, which could be an energy-conserving adaptive strategy towards enhanced flooding tolerance.

Together these findings suggest that during flooding several processes are inhibited to reduce energy consumption. It is crucial for the plant to preserve some carbohydrates for release of energy to support further growth when the water level recedes. The regulatory genes in this category may also serve some ABA-mediated water stress recovery and inhibition of GA-induced internodal elongation as quiescence strategies adopted by plants [173]. On the other

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hand, avoidance mechanisms employed under deep water conditions involve rapid internode elongation. In *R. palustris*, there are populations that show either the quiescence response or the avoidance response to submergence. This divergence shows that quiescence and avoidance are two strategies that can be employed by plants depending on the duration of flooding. Quiescence can be the optimal strategy for short-duration 'flash' floods, whereas avoidance via growth could be more reliable in prolonged deep flooding. Notwithstanding the above-mentioned tolerance genes and proteins, a deeper insight into the molecular regulation of quiescence and avoidance, and the associated regulatory networks, is still needed to provide sustainable avenues for improving plants specific to either flooding condition or able to grow in both.

7. Advances in plant tolerance to nutrient imbalances

7.1. Tolerance to nutrient deficiency

A total of 21 mineral nutrients are essential for crop growth and development. Most nutrients in the soil are primarily generated from the weathering of the parent material in the Earth's crust. Moreover, nutrient levels can vary widely across locations because of initial influence of the composition of the parent material. In most cases, inadequate replenishment from the parent material and from the adsorbed and complexed fractions causes nutrient deficiencies in the soil. In addition, natural factors, including acidity, alkalinity and human activities such as inadequate fertilization also cause nutrient deficiencies. In countries such as India and China, mineral deficiencies have significantly stagnated or limited crop yields. More than 30% of agricultural soils are boron deficient, not only in China and India, but in the whole world. Moreover, zinc deficiency is even more widespread, affecting approximately 50% of the soils. Significant zinc deficiencies occur in sub-Saharan Africa, Turkey, Iran and Pakistan [174].

Several studies have been conducted on understanding plant nutrition; the most noteworthy being the work of the German scientist Justus von Liebig, who stipulated that plant growth is controlled not only by the total resources (nutrients) available, but also by the scarcest resource (the limiting factor). This submission has stimulated a series of studies on nutrient management, including plant breeding for tolerance to nutrient deficiencies. Tolerance to nutrient deficiency is associated with the genotype's nutrient use efficiency. Genotypic variation in nutrient use efficiency is closely related to root nutrient acquisition capacity and utilization. In this section, we will focus on nitrogen and phosphorus, the two most limiting nutrients that are essential for several biological processes in plants.

7.1.1. Plant tolerance to nitrogen deficiency

Nitrogen is the most limiting nutrient to plant growth in most ecosystems despite its abundance in the atmosphere. This problem occurs because most plants can only take up nitrogen in two solid forms: ammonium ion (NH_4^+) and nitrate ion (NO_3^-). Ammonium is used less by plants because it is extremely toxic if taken up in large concentrations, so inorganic nitrate is the most usable form obtained by plants from the soil solution. Nitrogen-deficiency effect on

crop yields depends on the growth stage at which it occurs, as well as on its duration and extent [175]. However, reduced radiation interception, low radiation use efficiency, poor dry matter partitioning to reproductive organs, reduced leaf area index and decreased protein content of the plant and seed are the common effects of nitrogen deficiency.

Plants react in many different ways to changes in N provision; and physiological and molecular components governing N uptake, assimilation and remobilization during the plant life cycle have been studied extensively in the past decades, for review see [176, 177]. Three types of responses have been recently unraveled: (i) regulation of root N uptake systems, (ii) plasticity of root system architecture and (iii) fast modulation of shoot growth [178]. The first two responses generally improve efficiency of root N uptake under deficient conditions. The upregulation of specific high-affinity membrane transporters and enhanced foraging by the root system are implicated in these responses. When soil conditions for N uptake are seemingly unfavourable, e.g. limited water availability, plants will quickly slow down the overall N demand, as a nutrient conserving adaptive strategy, to prevent N starvation until conditions for N uptake become favourable.

In various plant species, nitrate transporters play a dominant role in N uptake. In *Arabidopsis*, three major families of nitrate transporters have been identified: Chlorate resistant 1 (*CHL1*/*NRT1*), *NRT2* and chloride channel (*CLC*) [177]. *NRT2* belongs to the high-affinity nitrate transporter group while most of the *NRT1* family members belong to low-affinity nitrate transporters. The only exception, so far, in the latter group is *NRT1.1* that is a dual affinity nitrate transporter. Thus, the high-affinity transporters that have been identified and primarily associated with nitrate uptake from the external environment include *NRT1.2*, *NRT2.1*, *NRT2.2* and the dual affinity transporter, *NRT1.1*.

NRT1.1 is functionally regulated by phosphorylation of a threonine residue, *Thr101*, which facilitates the switching of its activity from a low- to a high-affinity state. *AtNRT1.1*, which is also induced by auxin and is itself an auxin influx facilitator, is a dimer in the asymmetric unit cell despite being monomeric in solution. At low nitrate levels, *AtNRT1.1* is phosphorylated at the dimer interface, dissociates the *NRT1.1* dimer, changes into a high-affinity transporter and represses lateral root (LR) development by promoting basipetal auxin transport out of LR primordia (LRP) [179]. At high nitrate levels, *NRT1 1* is dephosphorylated, adopts a dimeric structure and adapts a low-affinity transporter configuration. In this state, trafficking of auxin out of the LR is blocked, and auxin accumulates in the LR initials promoting LR development. *NRT1.1* is also shown to act upstream of the *MADS box ARABIDOPSIS NITRATE REGULATED1* (*ANR1*) when modulating LR growth [179]. *ANR1* mediates localized N response and modulates the proliferation of LRs in N-dense patches. Moreover, *NRT1.1* has been shown to regulate genes encoding for other nitrate transporters, including *NRT2.1* and *NRT3.1* [180]. However, *NRT1.1* and *NRT2.1* are localized in different cell layers in the roots, and their adaptive/complementary strategy in nitrate uptake is not well elucidated. The *NRT1.1*-auxin modulation and nitrate signaling has also been a topic of interest and requires elucidation [181].

Amongst the *CLC* family members, *CLCa* and *CLCb* function as proton-nitrate exchanges, and have high selectivity for nitrates over chlorides [182]. Both transporters are known to mediate

nitrate accumulation in the plant vacuoles. Besides the above-mentioned transporters, the acquisition of nitrate is also regulated by slow anion channel (*SLAC1*) and *SLAC1* homologue (*SLAH*) and nitrate excretion transporter (*NAXT-1)*. Five *SLAC* genes were previously reported in *Arabidopsis* . Amongst these genes, *SLAC1* and *SLAH3* show nitrate transport activity, but their channel activity is co-regulated by kinases (e.g., *CPK21*) [183]. An efflux component operated by *NAXT-1*, associated with the nitrate transporter 1/peptide transporter (*NRT1/PTR*) family of proteins, mediates nitrate efflux under acid load in the cytosol [184]. Similarly, *NRT1.5*, which loads nitrates into the xylem for root-to-shoot translocation, also mediates nitrate efflux. However, the proton-coupling mechanism of *NAXT1* remains to be elucidated. Two other transporters, *NRT1.8* and *NRT1.9*, have been reported to regulate root-to-shoot nitrate translocation [185, 186]. Both transporters are apparently negative regulators of root-to-shoot nitrate transport. The subsequent nitrate allocation into the vegetative tissues, reproductive tissues and osmotic regulation of guard cells is reasonably described elsewhere [187].

Further studies on signaling, transcriptional and post-translational regulation have revealed evidence that a CBL-interacting protein kinase, *CIPK8*, regulates the activity of nitrate transporters and the expression of nitrate assimilation genes [188]. Like *CIPK8*, *CIPK23* is also suggested to be activated by a CBL protein, *CBL9*, but the exact mechanism is elusive. *CIPK23* directly interacts with *NRT1.1* in the plasma membrane and phosphorylates *NRT1.1* at *Thr101* to adopt a monomeric structure when the nitrate concentration is low. This process helps plants to adapt to low nitrogen levels.

Several TFs have been implicated in regulating *NRT1.1* activity. For instance, the activity of two *bZIP* TFs in *Arabidopsis*, *ELONGATED HYPOCOTYL5* (*HY5*) and *HY5-HOMOLOG* (*HYH*), was suggested to positively modulate *NITRATE REDUCTASE2* (*NIA2*) and negatively modulate *NRT1.1* [189]. The *NODULE INCEPTION* (*NIN*)-like TFs have also been shown to play a central role in the regulation of nitrate-inducible genes [190]. Nitrate signaling activates *NIN*-like transcription factors through their N-terminal regions. The activated factors promote the expression of nitrogen assimilation-related genes and genes encoding regulatory proteins. *NLP7* is the most reported in this family of TFs. *NLP7* is strongly induced in vascular tissues and root hairs, and is required for the induction of several nitrate uptake and assimilatory genes. Thus, *NLP7* is is probably a key regulator of nitrogen utilization mechanisms. More recently, the presence of nitrate in the external solution induced the expression of *NRT* accessory proteins (*NAR*), nitrate reductase, nitrite reductase and genes involved in the GS-GOGAT cycle, in *Arabidopsis*, as well as in maize and other plants [191]. These proteins likely play a role in nitrate sensing.

Strigolactones (SLs), a new class of plant hormones and rhizosphere signaling molecules, also appear to be upregulated in plants under low N conditions [192]; however, the impact of SL levels on root growth is yet to be determined. Changes in root system architecture (RSA) may also be induced depending on the prevailing available organic form of nitrogen, for review see [118]. The most commonly reported organic forms are l-glutamate or carnitine. In *Arabidopsis* seedlings, l-glutamate inhibits cell division in the root apical meristem (PRM) of the primary root (PR) tip and promotes LR formation and outgrowth. However, several

Arabidopsis auxin-signaling mutants display different levels of sensitivity to l-glutamate, suggesting that l-glutamate is rather a signaling molecule as opposed to a nitrogen source [193]. In addition, the rice glutamate receptor mutants display a host of RSA changes, including short PR and LR, reduced cell division and the cell death of root apical meristem [194], further suggesting that l-glutamate is a signaling molecule. l-Glutamate could be a major anchor in the signaling process leading to nitrate uptake and assimilation. This is supported by previous studies that have shown that glutamine synthetase (*GS1*) from alfalfa causes an increase in photosynthesis and growth under low N fertilization regime [195]. Glutamine synthetase also mediates ammonium assimilation into glutamine. Ammonium form of nitrogen is rapidly assimilated into organic nitrogen forms to avoid tissue toxicity, for review see [196]. Several other reviews have documented the genes and proteins regulating nitrogen use efficiency (NUE) in plants. The reader is referred to excellent reviews by [177, 196]. In addition, more than 50 QTLs for nitrogen use efficiency have been reported in plants, though few of them have been validated. Amongst the identified QTLs are nitrogen deficiency response QTLs in rice, nitrogen supply responses and yield in wheat and nitrogen use efficiency in barley.

Collectively, nitrogen use efficiency in plants is controlled by a complex array of physiological, developmental and environmental interactions that are specific to the genotype of a given species. Notwithstanding the aforementioned N uptake and utilization genes and QTLs, an extensive molecular survey of a wide range of genotypes covering the genetic diversity of a crop could provide further evidence on the genetic control of these trait. This can be achieved using the various available 'omics' techniques, combined with agronomic and physiological approaches in order to identify more elements controlling NUE in plants, both universal and specific, for use in crop improvement.

7.1.2. Plant tolerance to phosphorus deficiency

Phosphorus (P) is the second most limiting mineral nutrient in almost all soils, and >30% of the world's arable land has low P [197]. Phosphorus availability is particularly limiting on highly weathered acid soils of the tropics and subtropics due to its fixation by Al and Fe oxides on the surface of clay minerals. Plants take up phosphorus as phosphate (Pi), either directly by the root system or transferred through the fungal symbiont in arbuscular mycorrhizae host plants. Plants have elaborate sensing and signaling mechanisms in response to Pi deficiency, and both local and systemic signaling in response to Pi deficiency have been reported [197]. Key responses in the plant include changes in the root system architecture (RSA), a reduction in photosynthetic rate; increased activity of high-affinity Pi transporter activities; secretion of APases, ribonucleases and organic acids; membrane phospholipid replacement with glycolipids and sulfolipids; and increased availability of anthocyanin and starch [198]. Putative signaling molecules in response to Pi deficiency include sugars, hormones and microRNAs.

Under limiting Pi conditions, plants can monitor Pi deficiency both locally and systemically, and root foraging strategy to explore top soil layers for Pi is employed. The Pi foraging strategy is accomplished through one of the several different RSA and physiological changes [118]. The local external Pi rather than the systemic Pi status of the whole plant regulates the remodelling of RSA [199]. In maize and some species in the *Proteaceae* and *Casuarinaceae* families, the

remodelling of RSA involves production of adventitious roots and cluster roots [200, 201], which increases root surface area for Pi absorption. While a plant Pi receptor is yet to be identified, recent reports have suggested that ethylene biosynthesis and signaling are involved in the Pi-deficiency-triggered remodelling of RSA, for review see [118, 195]. The evidence is supported by previous finding that inhibition of ethylene biosynthesis with 2-*aminoethoxyvinyl glycine* (*AVG*) or ethylene perception with Ag^+ restricted the low Pi-induced meristem exhaustion of the primary root [202]. Correspondingly, application of Ag^+ was found to reduce the inhibition of primary root growth triggered by Pi deficiency. Moreover, Pi deficiency induced the formation of aerenchyma in adventitious roots, which is similarly induced by ethylene perception.

At the transcriptional level, Lei et al. [203], using an *Arabidopsis* transgenic line that carries a *LUC* gene fused to the promoter of the high-affinity Pi transporter, *AtPT2*, showed that the transcription of *AtPT2* is induced by Pi starvation. Using this marker line, the authors identified the *Arabidopsis* mutant *etr1/hps2* (*constitutive triple response 1/hyper-sensitive to Pi starvation2*), which showed hyper-induction of the *AtPT2::LUC* gene by Pi deficiency. Interestingly, the expression of *AtPT2* was partially blocked in *ethylene insensitive* 2 (*ein2)* mutants, but was enhanced in *ethylene over producer1* (*eto1*) mutants. A similar expression pattern was observed for several other Pi starvation-induced (*PSI*) genes in the *hps2* (negative regulator of ethylene response) and *ein2* mutants, including high-affinity phosphate transporter, *AtPT1* (*Pht1;1)*; a non-coding transcript, *At4*; an APase, *ACP5*; a ribonuclease, *Rxlink*; and *miR399d* [204]. Enhanced transcription of *PSI* genes was also observed in the mutant *hps3* and *hps4,* which are *ETO1* alleles [205, 206]. *ETO1* protein is a member of the broad complex/tramtrack/bric-a-brac (BTB) protein superfamily that participates in substrate recognition during ubiquitin-mediated protein degradation [204, 207]. *ETO1* directly binds to the C-terminal of *ACS5* and mediates its degradation. When *ETO1* is mutated, it causes an overproduction of ethylene in young seedlings [208]. Application of 25 μM ACC to young *Arabidopsis* seedlings under high Pi conditions barely induces the expression of *AtPT2*. Under Pi deficiency, however, 0.5 μM ACC dramatically increases *AtPT2* expression and induces ectopic root-hair development [203]. Thus, these results provide evidence that ethylene production and signaling is involved in the transcriptional responses of plants to Pi deficiency and primarily integrates with other Pi-deficiency-induced signaling pathways.

The other signaling component involving increased transcription of purple acid phosphatase 10 (*AtPAP10*) by Pi starvation in the whole seedlings of *hps3* and *hps4* has been reported [205, 206]. *AtPAP10* is a Pi starvation-induced *APase* (enzymes that scavenge Pi from organophosphate compounds) associated with the root surface. Functional analyses of *atpap10* mutants suggest that *AtPAP10* is important for plant tolerance to Pi starvation. However, the transcription of *AtPAP10* does not significantly increase in *ACC*-treated seedlings or the *constitutive triple response I* (*ctr1*) mutant under Pi deficiency, nor does the accumulation of *AtPAP10* proteins, which could suggest that ethylene has no effect on *AtPAP10* transcription. More recently, Zhang *et al.* [209] have shown that positive regulation of *AtPAP10* depends on sucrose and not ethylene. Moreover, they have also shown that ethylene does not affect *AtPAP10* activity without sucrose, but the opposite is true. This suggests that ethylene could be a local

but indirect signal for *AtPAP10* activity. However, as discussed before, ethylene could be regulating other components of Pi starvation response at the transcriptional level. Song and Liu [204] have demonstrated that accumulation of anthocyanin is lower in *hps2*, *hps3* and *hps4* mutants under low Pi but increases in Pi-starved *ein2* mutants. As mentioned before, accumulation of anthocyanins is an indicator of Pi-deficiency response in plants, thus ethylene could be a negative regulator of Pi-deficiency-induced anthocyanin accumulation probably through the regulation of genes involved in anthocyanin synthesis. Thus, ethylene likely participates at both the transcriptional and post-transcriptional levels, and this has implications on Pi starvation response in plants.

The systemic response to P starvation is also carried out through a complex signaling network that involves other plant hormones [210, 211], sugars [212] and nitric oxide [213], collectively resulting in the alteration of carbohydrate distribution between roots and shoots. Amongst the plant hormones, other than ethylene, auxin likely plays a role in response to Pi starvation. However, ethylene likely exerts its influence through regulating auxin activity, as it has been associated with RSA remodelling [198]. Indeed, ethylene has been reported to interact with auxin and sugars, and changes in auxin transport and localization appear to be at least partially responsible for Pi stress-induced LR development [214]. Decreased sensitivity to CK and GA also appears to be at least partially responsible for Pi-stress-induced LR development [215]. Under low Pi, GA has been shown to repress Pi-induced root architecture changes [216]. Moreover, Pi-deficient plants were shown to accumulate *DELLA* proteins, the negative regulators of GA-induced root growth, which are modulated by auxin.

As discussed before, amongst sugars, sucrose is key to Pi-deficiency response and appears to regulate ethylene activity. Amongst the TFs, phosphate starvation response proteins (e.g., *OsPHR1*, *OsPHR2*, *PvPHR1*, *ZmPHR1* and *TaPHR1*), which bind the promoter sequences of low Pi-induced genes, and their regulator *SMALL UBIQUITIN-LIKE MODIFIER1* [*AtSIZ1*; 217], a small ubiquitin-modified E3 ligase, and the downstream *PHOSPHATE2* (*PHO2*), an E2 conjugase, are involved in Pi-deficiency-related transcriptional changes. Other TFs, including the *bHLH*, *PTF1* (e.g., *OsPTF1* and *ZmPTF1*) and *MYB2P-1* (e.g., *OsMYB2P1*), *MYB62*, *WRKY* (e.g., *WRKY75*, *WRKY6*), *bHLH32* and *ZAT6* are also involved in the signaling network to regulate plant adaptation to P stress, for review see [218].

Based on genetic analysis, two proteins, the P_5 type ATPase encoded by *PHOSPHATE DEFICIENCY RESPONSE2* (*PDR2*), and multicopper oxidase *LOW PHOSPHATE ROOT1* (*LPR1*), were also previously shown to modulate Pi signaling in an endoplasmic-reticulum-localized pathway [219]. *PDR2* is required for maintaining the levels of the root patterning gene, *SCARECROW* (*SCR*), and *SHORT-ROOT* protein (*SHR*) trafficking from stele into endodermis. *PDR2* was proposed to act upstream of *LPR1*/*LPR2* to adjust meristem activity. A recent study has shown that *LPR1* is a ferroxidase [220]. Mutation of *LPR1* reduces Fe^{3+} levels in the meristemic tissues of Pi-deficient plants. In contrast, increased levels of Fe^{3+} have been reported in *pdr2* mutants leading to high production levels of reactive oxygen species (ROS). ROS signaling increases deposition of callose, which has been suggested to impair the trafficking of *SHR*, thus restricting root tip growth. Thus, PDR2 appears to modulate Pi-deficiency response by limiting Fe^{3+} accumulation in root tips.

More recently, molecular mechanisms defining the phosphate signaling pathway showed that *phosphate uptake 1* (*Pup1*)-specific protein kinase gene, named *phosphorus-starvation tolerance 1* (*PSTOL1*), was confirmed to be involved in regulating root growth and architecture during early stages of rice growth [221]. Allele-specific markers for this gene have been reported recently [222]. Interestingly, *OsPSTOL1* is located within the Kasalath-specific INDEL region and is absent from the rice variety Nipponbare reference genome. Thus, the configuration of the functional mechanism of *PSTOL1* is still elusive. We speculate that *PSTOL1* could be a local sensor of Pi starvation which transduces signals for sucrose or ethylene biosynthesis or both. The interplay of sucrose accumulation and ethylene biosynthesis is apparently the hallmark of Pi starvation response in plants.

The post-transcriptional regulation as well as long-distance signaling is carried out by microRNAs. As mentioned before, *miR399*, which is regulated by *PHR1*, a conserved *MYB* TF, maintains P homeostasis by regulating P transporter *PHO2* [223]. In tomato, overexpression of *Arabidopsis miR399* increases both the Pi accumulation and secretion of acid phosphatase and protons in the roots [223]. Thus, *miR399* is important for Pi acquisition, and could be acting downstream of sucrose and probably ethylene. Overexpression of *miR399* in *Arabidopsis* also increases P uptake and allocation to the shoot. Moreover, P remobilization from older leaves to young leaves is defective in *Arabidopsis miR399* transgenic lines [224]. This suggests that *miR399* is important for allocation and remobilization of P. The targets of *miR399* include a ubiquitin-conjugating E2 enzyme (*UBC24*) encoded by *PHO2*, which is upregulated under P-sufficient conditions and downregulated in P-starved plant roots. Homologues of *PHO2*/*UBC24* have a conserved structure in many species, and their 5′ UTR regions possess multiple *miR399*-complementary sequences. Thus, the regulatory mechanism of *miR399-PHO2* complex is evolutionarily conserved in angiosperms, making it a potential target for improving P nutrition efficiency in plants.

Strigolactones (SL) have also been shown to be induced by low Pi in many species, including tomato, *Arabidopsis*, pea and rice [225–229]. Strigolactones are terpenoid lactones that function as either endogenous hormones that control plant development or as components of root exudates that promote symbiotic interactions between plants and soil microbes. The production and exudation of SLs may depend on whether the plant is arbuscular mycorrhizal fungi (AMF)-compatible host or an arbuscular mycorrhizal symbiosis (AMS) for Pi and N uptake. A well-known synthetic SL, *GR24*, apparently increases LR formation under low Pi or decreases LR formation under sufficient Pi. In addition, SL biosynthesis (*more axillary growth; max4-1*) and signaling (*max2-1*) mutants have reduced number of root hairs under low Pi condition at the early stages of seedling development. This suggests that SLs mediate plant responses to low Pi; however, the mechanism by which SL exudation affects root growth is not fully understood.

In conclusion, although the molecular components of P stress signaling in plants have been fairly documented, the overall pathway is still less understood and requires further investigation. Nonetheless, the recent developments in whole genome sequencing technologies provide hope for more studies on plants with better P acquisition and utilization. Successes in QTL analysis have also set a stage for subsequent studies. Besides the success story of *PSTOL1*

in rice, QTL analysis in common bean has shown the importance of basal roots and adventitious roots for P acquisition [230–232]. Another study by Yan et al. [233] identified a large number of QTLs for Hþ exudation, root-hair density and length, associated with P efficiency. Additionally, QTLs for root traits related to P efficiency have also been identified in soybean [234, 235]. Moreover, QTLs controlling P deficiency tolerance were mapped by Zhang et al. [344] using 152 RILs derived from a cross between P-stress-tolerant and P-stress-sensitive parents. Thus, future studies will build on these present discoveries to facilitate genetic improvement for Pi-deficiency tolerance.

7.2. Advances in plant tolerance to nutrient toxicities

Metal toxicity is an important factor limiting the growth of plants in many environments. Some metals, such as copper and zinc, are micronutrients at low concentrations and become toxic at higher levels, whereas others (e.g., aluminium, iron, cadmium, chromium and lead) are well known for their toxicity [236]. These elements can be highly phytotoxic and seriously impair plant root growth. However, some crops are able to tolerate toxic environments, without significant display of toxicity symptoms. Three main strategies are employed by such plants to manage toxic soil compounds: (1) Producing root exudates that bind and neutralize the toxin in the rhizosphere, (2) actively transport the compound into the root, but neutralizing and sequestering it in vacuoles for safe accumulation or eliminating it through exudation and (3) excluding the toxic elements by preventing entry into the plant tissues. For the purpose of this chapter, we will focus on aluminium and iron toxicities as these elements have been frequently reported as major constraints in the production of economically important crops.

7.2.1. Plant tolerance to aluminium toxicity

Aluminium (Al) is a light metal that makes up 7% of the Earth's crust and is the third most abundant element after oxygen and silicon. Aluminium toxicity is one of the major constraints to crop productivity worldwide, especially in the acid soils of the tropics and subtropics that comprise almost 50% of all non-irrigated arable land in those regions [118, 237]. The soil pH has a crucial role for Al toxicity to occur, by affecting both solubility and the ability of plant roots to absorb Al. Al solubilizes into its toxic form (Al^{3+}) when the soil pH drops to 5.5 or less, and is most severe in solutions of low ionic strength and low cation concentrations. Al^{3+} is taken up by plants through diffusion [238], and toxic concentrations of >12 μM are detrimental to root growth. Possible exceptions of $Al(OH)_3^{4-}$ toxicity at higher pH values have also been reported [239].

The initial effects of Al^{3+} toxicity on the roots include rapid inhibition of cell division and a reduction in root apical cell expansion and elongation. Consequently, plants develop stubby and brittle roots with swollen malformed root tips. Moreover, lateral root initiation and outgrowth are also inhibited. Root-hair malformation is often reported, and nutrient (mainly P, K, Ca and Mg) and water uptake capacity is impaired [238]. Plant responses in the shoots include reduced stomatal opening, chlorosis, foliar necrosis and reduced photosynthetic activity.

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Plant tolerance to aluminium toxicity occurs through (1) external avoidance, which involves root secretion of organic acids to chelate Al^{3+} in the rhizosphere, limiting its diffusion into the roots [240], and (2) true or internal tolerance, which involves regulation of Al^{3+} uptake, and organic acid chelation and sequestration of aluminium bound substrates [241]. In rice, the latter is the main tolerance mechanism, and is apparently associated with the differential expression and transport properties of membrane transporters, e.g., *NRAMP Al $^{3+}$ transporter 1* (*NRAT1*) [242]. Most other plant species also vary significantly in these mechanisms; however, there are some tolerance mechanisms that are largely shared. Cereal crops, such as wheat, barley, sorghum (*Sorghumbicolor* L.) and oat were reported to have simple genetic mechanisms of Al tolerance, whereas rice and maize (*Zea mays* L.) have over time developed complicated inheritance controlled by numerous genes/loci involved [118, 243].

Genetic control of organic acid exudation either rests on the Multidrug and Toxin Efflux (MATE) family encoding a citrate transporter or on the membrane localized Al^{3+}-activated malate transporters (*ALMT*). Several transporters in these families, including *HvAACT1* in barley [244], *TaALMT1* and *TaMATE1* in wheat [245] and *ZmMATE1* and *ZmMATE2* in maize [246] are responsible for organic acid exudation and Al tolerance. Specific markers for *HvAACT1* and the MATE gene, *HvMATE-21*, have been developed and can be used to differentiate tolerant and sensitive barley cultivars. Differences amongst these transporters however exist. For instance, *TaALMT1* encodes a malate transporter on chromosome 4D and is constitutively expressed on root apices, whereas *TaMATE1* reportedly responds to Al stress based on citrate efflux. ZmMATE1 and *ZmMATE2* co-segregate with two major Al-tolerance QTLs [247]. *ZmMATE1* was shown to be induced by Al and enhances Al tolerance, whereas *ZmMATE2* did not respond to Al [246], suggesting variability in their roles. In sorghum, Al tolerance is controlled by *SbMATE*, encoded by a major Al-tolerant locus Alt_{SB} on chromosome 3 [248]. In *Arabidopsis* , two genes were reportedly responsible for Al tolerance: *AtALMT1* that also encodes a malate transporter responsible for malate efflux on chromosome 1 [249] and *AtMATE* that encodes an Al-activated citrate transporter [389]. These two genes function independently, but both are regulated by the C2H2-type zinc finger transcription factor *STOP1* [250], which is also reportedly induced by with low pH tolerance [366]. In rye, *ScALMT1*, which is mainly expressed in the root apex and upregulated by Al, co-segregates with the *Alt4* locus on chromosome 7RS [367]. Another candidate gene *ScAACT1* on chromosome 7RS was mapped to 25 cM from *ScALMT1* [251].

At the transcriptome level, two genes, *SENSITIVE TO ALUMINUM RHIZOTOXICITY1* and *2* (*STAR1* and *2*), which encode the nuclear binding domain and the transmembrane domain, respectively, of an ABC transporter, with specificity for uridine diphosphate (UDP) glucose, are upregulated following root exposure to Al^{3+} [252]. Both *STAR* genes were previously reported to be upregulated by the constitutively expressed rice root *ALUMINUM RESISTANT TRANSCRIPTION FACTOR1* (*ART1*), which also upregulates several other genes implicated in different aluminium tolerance mechanisms [253]. More recently, *ASR5* was reported to act as a key TF that is essential for Al-responsive *STAR1* and other Al response genes [254]. Rice homologues, which encode α-expansin (e.g., *EXPA10*), belong to this family of TFs, and have been implicated in the regulation of root elongation and cell wall elasticity. The members of

EXPA10 decrease cell wall extension potential when exposed to Al^{3+} [255] and are downregulated during Al^{3+} stress. The functions of *STAR1*, *STAR2*/*ALS3* and *ALS1* in Al tolerance are fairly conserved and ubiquitous in monocot and dicot species. However, these genes are differentially expressed between species. For instance, the expression and induction levels of these genes in response to Al^{3+} stress are higher in the Al-tolerant species of rice than in the Al-sensitive species of *Arabidopsis* , suggesting that Al-tolerant species may require increased expression of these conserved Al-tolerance genes to overcome Al^{3+} stress [256]. The same authors show that Tartary buckwheat shows high expression of the Al-tolerance gene homologues under Al^{3+} stress. Al-tolerance in buckwheat is evolutionarily closer to *Arabidopsis* than rice, suggesting that buckwheat could have rapidly evolved higher expression of Al-tolerance genes to detoxify Al^{3+} than *Arabidopsis* . In addition, the gene duplication of *ART1*/*STOP1*, *STAR1* and *ALS1* has been suggested to play a significant role in Al tolerance. This is consistent with the previous findings that duplication of key genes responsible for metal translocation and detoxification in *Arabidopsis halleri* facilitates hyper-accumulation of zinc/cadmium [257]. However, further functional analysis by creating knockdown or knockout mutants will be necessary to provide additional insights into the role of each homologous gene in Al detoxification and accumulation in buckwheat.

An *Arabidopsis* cell-wall-associated putative endochitinase, CHITINASE A (*AtCHIA*), likely involved in modulating cell wall extension by regulating chitin levels, has also been suggested to play a role in Al tolerance [258]. Another signal of Al^{3+}-induced cellular response is the induction of *1,3-β-d-glucan synthase*, which leads to the accumulation of callose in root apices, especially in endodermal and cortical cell walls [259, 260]. This callose deposition is suggested to be an inhibitory process that may block symplastic and apoplastic flows. Whether callose deposition represents Al^{3+}-induced injury or a defence response to block further Al^{3+} binding and movement remains to be confirmed.

In *Arabidopsis*, the ethylene receptor gene *ETHYLENE RECEPTOR1* (*ETR1*) and the ethylene signal transducer *ETHYLENE INSENSITIVE2* (*EIN2*) were found to be important for Al^{3+}-induced inhibition of root elongation [261]. These genes apparently regulate Al^{3+}-induced upregulation of the *Arabidopsis* ethylene synthesis genes *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE2*, *6*, and *8* and *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE1* and *2*. Moreover, ET was recently shown to negatively regulate aluminium-induced malate efflux from wheat roots and tobacco cells transformed with *TaALMT1* [262], which suggests that ethylene could be a negative regulator of root secretion of organic acids. The upregulation of auxin transporters *PIN FORMED2* (*PIN2)* and auxin influx carrier *AUXIN RESISTANT1* (*AUX1)*, which regulate auxin distribution, is associated with the regulation of root elongation in *Arabidopsis* plants [263]. *AUX1* and *PIN2* are apparently disrupted by ethylene signal that alters auxin distribution and transport in the roots. He et al. [264] suggests that auxin could be involved in aluminium-induced efflux of malic acid acting on anion channels. Thus, auxin/IAA transport could be a target for Al^{3+} toxicity tolerance if the modulation by ET is attenuated. However, considering several phytohormonal changes that occur during Al stress, molecular mechanisms associated with their interplay will require further

elucidation. Recent evidence that microRNAs are involved in Al stress tolerance [265] also provides new insights into understanding the mechanism of Al^{3+} tolerance in plants.

Overall, we expect that major advances in understanding physiological and molecular basis for Al tolerance will happen in the near future, considering that the pace at which new genes are being discovered has improved with new sequencing technologies. The future challenge for studying Al tolerance is the identification of new tolerance mechanisms. The discovery of the key molecular regulators, e.g., *ASR5*, which was recently shown to mediate Al-responsive gene expression to provide Al tolerance in rice, is an indication that several other mechanism of Al tolerance exist in plants. The blocking of Al^{3+} cell wall binding sites in rice may be one of the major mechanisms of aluminium tolerance that will need further investigation. Studies on barley, wheat and maize have shown variation in gene expression associated with variation in gene sequence, which would require further investigation to understand the regulatory networks affected by this sequence polymorphisms.

7.2.2. Advances in plant tolerance to iron toxicity

The problem of iron toxicity occurs in most wetland rice growing areas of the world, primarily in flooded acidic soils, inland and coastal swamps. Some of the irrigated lands in South and Southeast Asia, Africa and South America are affected [266]. In India alone, about 11.7 million hectares of land are affected by iron toxicity. In Burkina Faso, 300 ha of ferrous iron intoxicated soils were abandoned in the Valley du Kou in 1986, most of which remained uncultivated to date [267]. Iron toxicity is also becoming a major rice yield limiting factor in East Africa, including lowland rice cultivation areas of Uganda [268]. Yield losses in the range of 10% to 100% have been reported [266]. Moreover, toxicity at seedling and early vegetative stages can strongly affect plant growth and hinder development, and can result in complete crop failure.

Three major adaptation mechanisms are generally reported for Fe-toxicity tolerance. The details by which rice plants execute these processes and their molecular components are not yet fully understood, but there are some clues from various studies on rice and other plant species. For instance, plant tolerance by root oxidizing power is mediated by diffusion of molecular oxygen from the shoots to the roots through aerenchyma tissue and its subsequent release in the rhizosphere. Oxidation of Fe^{2+} in the rhizosphere results in the precipitation of insoluble iron oxides at the root surface, forming iron plaques. These iron plaques not only reduce Fe^{2+} concentration in the soil solution, but also form a physical barrier against further influx of Fe^{2+} into the roots.

Plant tolerance by retention of iron in the root or shoot involves compartmentalization. Nicotianamine (NA), Fe-NA complex transporters, *VIT* proteins, *FPN2*-like proteins, *MIT*- and *PIC1*-like proteins, organic acids, ferritins, Fe-sulphur and other heme proteins that can sequester Fe are all potential candidates for plant tolerance to excess iron through regulated storage and compartmentalization (Figure 6).

In *Arabidopsis* , apoplasmic Fe is mostly found within the stele [269], suggesting that compartmentalization within the stele could restrict excess Fe from reaching the shoot during transportation towards the aerial parts. Fe^{2+} decreases could also occur in association with an

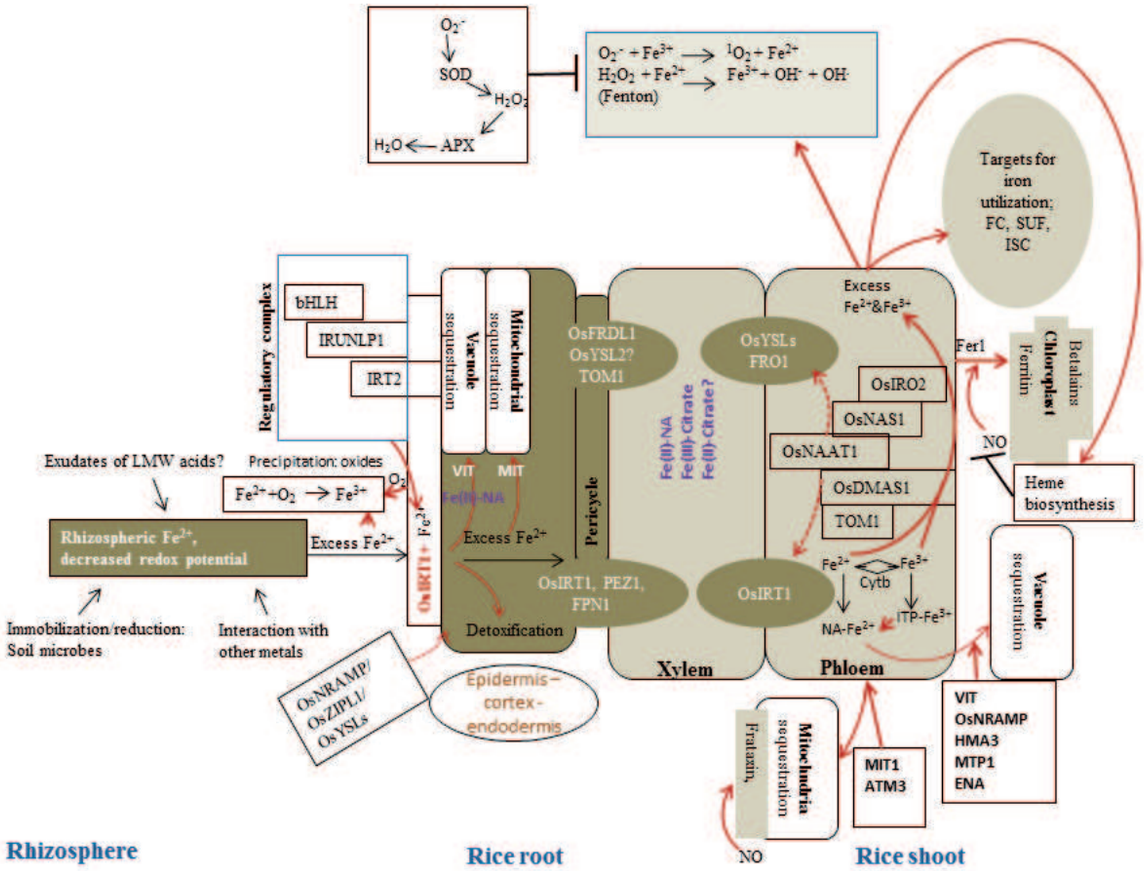


Figure 6. Iron transport in rice. Fe is taken up into the symplast by transporters in the epidermis (OsIRT1, OsNRAMP, OsZIPL1 and OsYSLs). Proteins encoded by bHLH, IRUNLP1 and IRT2 likely regulate the activities of the above transporters. Radial oxygen loss into the rhizosphere through aerenchyma cells detoxifies part of the excess iron forming insoluble Fe^{3+} at the root surfaces, a process referred to as exclusion. Excess Fe^{2+} travels through the symplastic space to the vasculature, bypassing the waxy Casparian strip on the endodermis. Prior to reaching the xylem, excess iron is retained in the root cell vacuoles, mitochondria and probably detoxified by organic acids within the root cells. Transport into the xylem is mediated by putative chelate effluxers: FRDL1, OsYSLs, TOM1, OsIRT1, PEZ1 and FPN1. In the xylem, iron is carried to the shoot through the transpiration stream either in the form of Fe^{3+} or in both Fe^{3+} and Fe^{2+} forms, and unloaded into the shoot, most likely by YSLs, FRO1 and OsIRT1 proteins. Within the phloem, the rate at which NA, DMA and ITP are synthesized, the kinetic stability of the complexes formed and the oxido-reduction system likely determines the iron speciation. Enzymes involved in NA, DMA and ITP synthesis, including OsIRO2, OsNAS1, NAAT1 and DMAS1, likely play a significant role in determining iron loading into the phloem. Genes encoding for putative iron effluxers from the phloem to storage organs (VIT, OsNRAMP, HMA3, MTP1, ENA, MIT1, ATM1) are co-regulated with IREG2/FPN2 and YSLs to limit potentially toxic iron in the cytosol, by compartmentalizing in the vacuoles, mitochondria, chloroplast and other non-characterized intracellular vesicles. In the chloroplasts, Fe excess probably promotes NO production. NO is probably involved in activation of the transcription factor (TF) cascades responsible for the regulation of Fe uptake, homeostasis and for the tuning of cellular metabolism, including increased synthesis of ferritins and betalains in chloroplasts and frataxins in the mitochondria. Because NO also triggers the synthesis of ROS, heme biosynthesis likely occurs to compartmentalize excess iron and to limit NO production. Alongside heme biosynthesis, the potent antioxidant system involving SOD and APX probably scavenge and detoxify the excess ROS. Also presented are targets of iron utilization, which could reduce iron overload. This includes synthesis of ferrochelatase (FC) for heme biosynthesis, mitochondrial iron-sulphur cluster (ISC) and plastid-localized sulphur utilization factors (SUF).

alkalization of apoplastic pH, which reduces Fe^{2+} mobility and chemical stability [269]. Alkalization has been reported to be modulated by ethylene [270], suggesting additional role

of ethylene in regulating Fe^{2+} besides its role in aerenchyma formation. Tissue tolerance of Fe toxicity is mediated by detoxification of free radicals. In rice, expression of several genes involved in oxidative stress control, including peroxidases, glutathione transferase (GST) and cytochromes, was upregulated in roots and shoots in response to excess Fe [271]. Similar trends were observed at the protein and enzymatic activity levels of the same genes. Excess iron was reported to induce the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in the leaf sheath and laminae, respectively, in a tolerant variety from *Oryza glaberrima* [272]. The activity of glutathione reductase and peroxidase (POD) was also reported to increase in rice leaf segments exposed to excess iron [273]. Fang et al. [274] also showed that lipid peroxidation resulting from Fe toxicity was inhibited by free radical scavengers such as mannitol and GSH. Moreover, the differential expression of anti-oxidant enzyme activities (SOD, APX, CAT, GR and DHR) was observed between rice varieties contrasting in tolerance of Fe toxicity [275].

Several genetic studies also reflect that iron toxicity tolerance is a complex quantitative trait controlled by a large number of rather small effect quantitative trait loci (QTLs), indicating the involvement of multiple tolerance mechanisms. For instance, Wu et al. [276] identified QTLs for leaf bronzing and shoot dry weight on chromosome 1 and 8, explaining 10–32% of the phenotypic variation. Interestingly, QTLs associated with enzymatic activity of anti-oxidants in rice leaves were detected in the same region [277]. Similarly, Fukuda et al. [278] detected a region on chromosome 3 responsible for high shoot iron content in a susceptible variety, which co-localize with the QTL previously identified by Shimizu et al. [279] for the same trait. Co-localization of most of these QTLs was captured in an integrative genetic map reflecting mapping studies from different conditions of Fe toxicity [277], which substantiates on recurrent chromosomal regions identified in several QTL studies.

A major limitation of iron toxicity tolerance studies, however, is that most of the QTLs associated with iron toxicity tolerance have not been furthered to cloning of tolerance genes. It is thus critical to devote some effort to fine-map the few, but consistent QTLs mentioned herein in order to increase precision and accelerate candidate gene identification. Subsequently, functional validation of several genes identified in microarray studies will need to be explored. Exploring allelic variation of these genes in contrasting genotypes and evaluating the promising alleles in well designed and efficient phenotyping experiments would provide a basis for their use in marker-assisted breeding (MAB) for Fe-toxicity tolerance.

8. Conclusions and perspectives

In this chapter, we have attempted to present the recent advances in crop tolerance to abiotic stresses. Various strategies used by plants to counteract stress, and some success in identifying genomic regions associated with plant tolerance is presented. Interestingly, plants have evolved common regulatory networks in response to abiotic stresses. For instance, drought, salt and cold stress induce calcium influx to activate the downstream second messengers to yield different or similar responses. Calcium influx channels at the membrane (e.g., the recently

reported *hyper-osmolality induced [Ca $^{2+}$] increases 1* (*OSCA1*) from *Arabidopsis thaliana* that is gated by hyper-osmotic stress [280]) act in concert with the membrane-located NADPH-oxidase Respiratory burst oxidase Homolog (RboH), generating apoplastic ROS. Intracellular transduction is conveyed by calcium-binding proteins (e.g., CBLs/CIPKs, CDPKs and calcineurins), a MAP-Kinase cascade and phytohormones (e.g., ABA, ET, JA and SA), which apparently act as integrators of early signals. Depending on the relative temporal patterns of these upstream signals, the activity of TFs and their interacting proteins will decipher specific combinations of genes required to be expressed to boost enzymatic or protein reaction levels necessary to counter the stress perceived. These proteins largely contribute to adaptive response in most plants, e.g., production of compatible osmolytes that helps to reinstall turgidity during drought and synthesis of LEA proteins that prevent protein precipitation. Other examples include chelation/sequestering of ions into cellular compartments in response to toxic elements, induction of anti-oxidative enzymes, induction of molecular chaperones and adaptive regulation of plant hormones. These adaptive strategies and the molecular components involved provide potential molecular genetic targets for enhancing abiotic resistance in crops.

However, many challenges still lie ahead. For example, the regulation of signaling cascades, especially how plants can discriminate the signaling components, and even their specific combinations, to activate specific downstream biological processes for a given stress. A frequent manifestation has been the case of ethylene controversial role in abiotic stress response. Whether the negative regulations associated with ethylene represent a plant strategic mechanism to prime the subsequent useful reaction remains to be confirmed. Also, temporal and specific differences in activation of upstream signaling components will need to be explored to help in identifying molecular components essentially required to counter a given stress. Moreover, the specific downstream components for which much of the studies have been conducted, e.g. transcription factors, transmembrane proteins, transporters, enzymes for osmolyte biosynthesis, hormonal regulators, ROS scavengers and other traits that have been shown to play major roles in plant response to stress, will need classification according to their aptitude and functional significance in response to a given abiotic stress. Morpho-physiological traits associated with stress tolerance would also substantially reinforce the successes in molecular biology if addressed to a greater extent. The use of models for predicting gene effects, particularly when combining multiple traits, will also find greater application in dissecting G × E interactions and will help breeders to improve target varieties. Thus, there is need to integrate molecular tools with precise high-throughput phenotyping and biochemical analysis to confirm the consistency of various molecular findings, and to realize the full benefits of molecular biology in selecting genotypes that are stably tolerant under a given stress, considering the interaction with various environments. Here, we emphasize stresses that have been commonly reported in literature, which would provide a basis for understanding other minor stresses. We also refer to the chapter on biotic stresses and the numerous interactions in signaling pathways and expressions of resistance and tolerance on molecular level towards abiotic and biotic stress in plants. Additional background information can also be found in excellent reviews and references therein.

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Author details

Geoffrey Onaga[1] and Kerstin Wydra[2*]

*Address all correspondence to: kerstin.wydra@fh-erfurt.de

1 National Crops Resources Research Institute, Kampala, Uganda

2 Erfurt University of Applied Sciences, Faculty of Landscape Architecture, Horticulture and Forestry, Erfurt, Germany

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