Plant signaling pathways involved in stomatal movement under drought stress conditions

Abstract

Governed by environmental stimuli and internal signaling cascades, stomatal movement determines water use efficiency and CO\(_2\) assimilation for photosynthesis under normal and stressful conditions, most importantly under water scarcity. Manipulation of these signaling pathways is one way to optimize plant resilience/tolerance and performance under constantly changing environments. Oscillations and interactions between the internal and environmental cues make it difficult to determine the consequence of these signals. After drought stress perception, the activated molecules initiate the generation of redox, hormone, and chemical signals, the interactions of which control stomatal responses. Second messengers, proteins, and intermediate chemicals then react with these signaling molecules to positively or negatively transmit these signals through a series of molecular events. This review is aimed to discuss the step-by-step scheme of these signaling pathways to provide insight into these molecular events and to incentivize further studies on their unknown aspects for improved stomatal responses under various environmental conditions, particularly stress conditions, and specifically drought stress.

Keywords: plant signaling pathways, stomatal responses, drought stress, molecules, hormonal and chemical signals, ABA and anion (K\(^\text{−}\)) channels roles in stomatal movement

Introduction

Stomatal movement plays a critical role in water hydraulic conductivity and gas exchange capacity, which subsequently control nutrients uptake, temperature adjustment, CO\(_2\) assimilation, and thus growth, survival, and tolerance under water stress conditions Martin-St Paul et al.\(^1\). Opening and closing of stomata is caused by a variety of stimuli such as light/dark, ozone, CO\(_2\) concentration, humidity and multiple signaling networks generated by phytohormones (abscisic acid (ABA), ethylene (ETH), salicylic acid (SA), methyl jasmonate (MeJA), cytokinin (CK), auxin (AUX), brassinosteroid (BR)), redox (H\(_2\)O\(_2\) and other reactive oxygen species (ROS)), nitrous oxide (NO\(_\text{2}\)), enzymes (phospholipases C and D and their products; inositol 1,4,5-trisphosphate (InsP\(_3\)), phosphatidylinositol-4,5-biphosphate; farnesyltransferases, gamma-aminobutyric acid (GABA), protein kinases such as calmodulin-like20 (CaML20)), bioactive gas hydrogen sulfide (H\(_2\)S), bioactive lipids (sphingosine-1-phosphate, phospholipids derivatives, sphingolipids, fatty acids like some polyunsaturated ones, linolenic and arachidonic acid), etc. Blackman, Bhatia, Joon-Sang, Allen, Tanaka, Vahisalu, Xia, Misra, Marowa, Nazareno, Jin, Eisenach, Wu, et al.\(^2\)–\(^4\). In addition to the mentioned molecular signals, carbohydrate and polyamine metabolism Misra et al.\(^5\) and interactions between protein kinases such as Ca\(^{2+}\)-sensors and Sucrose non-fermenting Related protein Kinases (SnRK2s) may positively or negatively regulate ABA-dependent or independent stomatal movements Aubert Kulik, Bucholc, Kim, Maierhofer, Yoshida, Malcheska et al.\(^6\)–\(^9\). Besides hormones crosstalk, the combination of different stresses, duration, severity, repetition, Barrero, Berger, Zandkarimi et al.,\(^22\),\(^23\) circadian rhythms Grundy, Lee et al.,\(^25\),\(^26\) and sudden loss of hydraulic conductance that cause embolism Tombesi et al.,\(^27\) make their interpretations more difficult. Therefore, decoding these interactions and the process of embolism removal can be regarded as effective strategies to improve plant tolerance, recovery, and performance under stressful conditions. In 1974, Levitt\(^10\) reported that explosion of information on stomatal action led to a rejection of the old, classical theory of the mechanism proposed by Scurthur in 1932 and its replacement by Fujino\(^11\) report in 1959, describing the concept of active K\(^{+}\) ion transport. Many of the new facts, however, do not seem to fit into either concept, and neither provides a sufficiently detailed, step-by-step scheme to account for all the known facts. Therefore, Levitt\(^10\) provided enough information to support his report that is still valid and this information is now available for the proposal of a new scheme which embodies the best features of each of the above concepts and eliminates the inadequacies of each proposal on stomatal movement. The molecular mechanisms underlying this set of signal cross talks and interactions have not been fully discovered yet. Hence, this review article is aimed to provide more insights on this concept by reporting the relevant available literature on this subject.

Hormones crosstalk regulates stomatal movement during day/night cycle and under drought stress conditions

After perception of drought stress, abscisic acid (ABA) is synthesized in the plastids and transported into cytosols Xu et al.\(^12\) in roots (root tips, vascular bundles of roots) and stem vascular systems (phloem companion cells and xylem parenchyma cells next to the phloem sieve cells and xylem vessels, respectively; leaf veins and florescence stems), as well as leaf mesophyll, Malcheska, Koivai et al.,\(^13\),\(^14\) leaf cuticle, Wang et al.,\(^15\) and guard cells Koitai et al.,\(^16\) Methy1 jasmonate (MeJA), ABA and ethylene (ETH) are effectors of stomatal closure, however, their effects vary depending...
on their concentrations and combinations so that respectively ABA+ETH→MeJA→MeJA+ABA or MeJA+ETH→ABA or ETH→ABA+ETH exert stronger effects to induce stomatal closure Nazareno & Hernandez. Increased cytokinin (CK) (10^{-7} mol m^{-3}) to ABA (10^{-3} mol m^{-3}) ratio contributes to reversal ABA-stimulated stomatal opening Blackman & Davies. Tanaka and co workers study on Arabidopsis demonstrated that CK and auxin (AUX) hormones can act as negative ABA regulators, inhibiting ABA-induced stomatal closure by promoting ETH biosynthesis Tanaka et al.

However, because He et al. study showed that ETH can produce H_{2}O_{2} in light condition to stimulate stomatal closure, the effects of CK and AUX on inhibiting ABA-ETH-induced stomatal closure could possibly be attributed to their effects on hydrogen peroxide (H_{2}O_{2}) removal by CK and deactivation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to inhibit H_{2}O_{2} production by AUX as reported by Song et al. during the day, so not enhancing ETH production. Accordingly, during the day, CK and AUX counteract the effects of ABA and ETH to inhibit stomatal closure, while at night they cooperate with ETH at the requested concentrations for H_{2}O_{2} removal to facilitate stomatal opening. In either case, CKs and AUXs induce inhibitory effects on stomatal closure, He, Song et al. Ethylene (ETH) plays a dual function that mediates ultraviolet band (UV-B)-induced stomatal closure via peroxidase-dependent H_{2}O_{2} generation during the day He et al. and facilitates stomatal opening by H_{2}O_{2} removal in darkness Song et al. The effects of ETH on stomatal closure or opening depends on ETH concentration and its interactions with other phytohormones such as cytokinin, AUX, Tanaka et al. and brassinosteroid (BR) that result in the generation of different amounts of H_{2}O_{2} and nitrous oxide (NO) Shilay et al. Like ETH, 24-epibrassinolide (EBR), a natural form of BR, stimulate both stomatal closure and opening based on its concentration-dependent effect on hydrogen peroxide (H_{2}O_{2}) homeostasis and NO Xia, Shi et al. BR and EBR stimulate ETH synthesis, thereby activating G_{a} protein (G protein α-subunit), and ETH cooperatively with activated-Ga protein stimulate Atbrhöf-mediated H_{2}O_{2} and subsequent Nia1 (nitrate reductase 1)-catalyzed NO production and consequently induce stomatal closure Shi et al. In contrast to CK and AUX effects, SA and also tannin acid act as a positive regulator of ABA, promoting stomatal closure by stimulating ABA biosynthesis and possibly reducing the activity of phosphatase and ATPase enzymes Bhatia, Hao et al.

Mitogen-activated protein kinase (MAPK) phosphatases might positively or negatively regulate stomatal closure depending on the interactions between MAPKs and the effects of their isoform on H_{2}O_{2} and NO generation Li & Wang. Thus, understanding the hormone crosstalk, the influence of hormones on MAPK isoforms, and MAPKs interactions on redox status is required for accurate interpretation of their effects on stomata movement. Drought stress induces ionic (SO_{4}^{2-}), redox, and hormonal (brassinosteroid (BR), salicylic acid (SA)) signals and subsequently second messengers (Ca^{2+}, InsP_{3}) to regulate the transcription of important genes like the one encoding 9-cis-epoxycarotenoid dioxygenase (NCED), a key enzyme in ABA biosynthesis, Xia, Malcheska, Hao Estrada-Melo et al. In return, ABA activates anion and cation channels and regulate redox homeostasis-induced by NADPH oxidase activity. The NADPH oxidase-generated redox signals induce biosynthesis of ABA and consequently an ABA self-regulatory system is generated, which plays a fundamental role in regulation of many aspects of cellular processes and stress responses such as stomatal closure (Figure 1) Nazareno & Hernandez, Malcheska, Harb, Fujita, Castillo, et al. During the day, methyl Jasmonate (MeJA) interacts with ethylene (ETH) Nazareno & Hernandez or ABA to promote stomatal closure (Figure 1) Munemasa et al. In Arabidopsis, ABA-signalizing activates Ca^{2+}-dependent protein kinases (CDPKs), Ca^{2+} protein kinase (CPK), CPK3, CPK6, CPK4, and CPK11 to phosphorylates ABA-binding factor (ABF) transcription factor (TFs) regulating gene expression involved in MeJA biosynthesis Munemasa, Li et al. ABA-activated form of CPK6 induces genes expression encoding both MeJA and ETH biosynthesis, which, in return, activate NADPH oxidases to generate redox signaling. H_{2}O_{2} production is mediated by ETH- or ABA-mediated NADPH oxidases activity, while MeJA directly induces NO, and ROS generation (Figure 1) Munemasa Li, Hossain et al. Unlike ABA that induces H_{2}O_{2} generation by activating open stomata (OSTI) and subsequently activation of NADPH oxidases, MeJA is capable of inducing ROS and NO possibly through activation of NADPH oxidases by cyclic adenosine 5'-diphosphoribose (cADPR), the molecular mechanisms of which remains unknown Hossain et al. MeJA-induced cADPR function downstream of ABA-induced cyclic guanosine monophosphate (cGMP) to activate NADPH oxidases and subsequently generate ROS signaling (Figure 1). Reactive oxygen species (ROS) react with nitrous oxide (NO) to form reactive N species such as peroxynitrite (ONOO), which react with cyclic guanosine monophosphate (cGMP) to produce 8-nitro-cGMP and activates cyclic nucleotide-gated channel2 (CNGC2), to elevate cytosolic free Ca^{2+}[Ca^{2+}]_{cyt} in association with MeJA-induced cyclic adenosine 5'-diphosphoribose (cADPR), stimulating ABA-MeJA induced stomatal closure Hossain et al. Ca^{2+} channels are activated by 3',5'- cyclic adenosine monophosphate (cAMP), but not cGMP, the molecular mechanism of which remains elusive Hossain et al. MeJA/cADPR-generated ROS signaling respectively target CPK6 and Ca^{2+} channels that elevate [Ca^{2+}]_{cyt} and S-type anion channels to synergistically share ABA-MeJA-induced stomatal closure, but not in the absence of ABA (Figure 1) Hossain, Munemasa et al. Increased cytokinin (CK) (>10^{-6} mol m^{-3}) during the day, so not enhancing ETH generation in Arabidopsis Munemasa et al. coworkers showed that CPK6 was not responsible for ROS and NO production. Munemasa and coworkers showed that CPK6 was not responsible for ROS and NO generation in Arabidopsis Munemasa et al. ABA-CPK6 interaction mediates MeJA biosynthesis and then MeJA acts as a positive regulator of ABA to facilitate stomatal closure by:

a) Inducing the expression of genes encoding ABA biosynthesis, e.g., AtNCED3, or
b) Generating ROS and NO in an unknown way, which subsequently cooperatively with ABA restore CPK6 activation to open Ca^{2+} and S-type anion channels (Figure 1) Munemasa, Hossain, Daszkowska-Golec & Szergejko et al. The activation of Ca^{2+} channels is necessary for elevation of [Ca^{2+}]_{cyt} while the activation of Ca^{2+} sensor calmodulin (CaM) is necessary for MeJA-induced stomatal closure Munemasa et al. Thus, this review conclude that in the absence of ABA, when Ca^{2+} channels and Ca^{2+} sensor CaMs are not activated by ABA-induced [Ca^{2+}]_{cyt} elevation, the existence of stress-induced ETH is required Nazareno & Hernandez to function downstream of CPK6 signaling and/or to cooperatively with MeJA induce stomatal closure (Figure 1).
Roles of ABA in stomatal movement in response to drought stress

ABA perception rely on its binding to various receptors localized in cytosol, nucleus, chloroplasts, plasma membrane Xu, Castillo, Zhu, Li et al.31,43,50,51 Golgi bodies, and endoplasmic reticulum Jaffé et al.12 Heterotrimeric guanine (G) nucleotide-binding protein-coupled receptors (GCRs) consisting of α, β, and γ subunits function together in the G protein signaling pathway to regulate ABA responses in stomatal movement. AtGTC1 (Arabidopsis thalian GCR type Protein 1), AtGTC2, GRC1, and GRC2 interact with the G protein alpha subunit GPA1 (heterotrimeric Guanine nucleotide-binding Protein Alpha subunit 1) to mediate responses to multiple stresses, including drought, by regulating ABA signaling and the activity of many enzymes for stomatal movement. For instance, regulation of phosphatidylinositol-phospholipase C (PI-PLC) and phospholipase D (PLD) can be mediated by GCR1-GPA1 interaction Jaffé, Klingler, Apone, Pandey & Assmann, Urano, Chakraborty et al.52–57 In this context, understanding the influence of different ABA receptors, their interactions, and their target molecules are of critical importance for improved, resilient ABA-mediated signal processing and, thereby, stomatal conductance as occurs under optimal condition.

Rapidly up regulated by water stress, ABA Insensitive 1 (ABI1), ABA Insensitive 2 (ABI2) and Hypersensitive to ABA 1 (HAB1) genes encode clade A protein phosphatase 2Cs (PP2Cs), acting as negative regulators of ABA responses. Perception of ABA by its receptors (pyrabactin resistance1/pyrabactinresistance-like //regulatory components of ABA receptors; PYR/PYL/RCAR) sequesters ABA and form complexes that inactivate PP2Cs in an ABA-dependent manner Nazarenko & Hernandez, Malcheska, Harb, Fujita, Castillo, Geiger, et al.11,12,41–43,58,59 Inactivation of PP2Cs by the ABA-PYLs initiates the ABA-dependent signal transduction pathway where SnRK2-type protein kinases SnRK2.2, SnRK2.3 and particularly SnRK2.6/open stomata 1 (OST1) are released to positively transmit ABA signalosome Zhu.60 ABA signalosome phosphorylates SnRK2s and
subsequently activates a series of ion channels and ABA-responsive element binding protein (AREB)/ABA-binding factor (ABF) basic leucine zipper (bZIP) transcription factors (TFs) to regulate ABA-mediated stress responses such as stomatal movement. Nazarenno & Hernandez, Malchescia, Harb, Fujita, Castillo, Li, et al.11,12,41–43,51,59 ABA-induced Ca\(^{2+}\)-independent OST1 phosphorylates ABF3 to create a 14-3-3 binding motif required for the long-term regulation of gene expression, Sirichandra et al.41 suggesting that regulation of ABF3 and 14-3-3 binding site might be good candidates for the homeostasis of ABA signal to maintain optimal stomatal conductance. In this context, as the final step and perhaps the most effective approach to regulate stomatal movement, the interaction between anion channel regulators such as OST1-CPKs, OST1-SnRK2s, and OST1-Ca\(^{2+}\)-sensor proteins have the potential to improve the stomatal movement under both normal and stressful conditions. Regarding the activation of ion channels, ABA-induced OST1-YFP\(^{67}\) (fusion of the N-terminal half of the yellow fluorescent protein (YFP) to the C-terminus of OST1) phosphorylation activates slow anion channel (SLAC1) Maierhofer, Guzel Deger et al.44,45 and quickly activating anion channel1/alumium-activated anion channels (QUAC1/ALMT12) Eisenach et al.,13 allowing the release of anions, as well as facilitating potassium efflux by stimulating potassium efflux channels and inhibiting the activity of inward potassium channels, respectively Maierhofer, Guzel Deger et al.19,62 (Figure 1). However, OST1 is unable to activate slacte homolog 3 (SLAH3) Guzel Deger et al.42. Elevation of [Ca\(^{2+}\)]\textsubscript{i} activates some of the protein kinases which subsequently activate anion channels; thus, some of them can be regulated via Ca\(^{2+}\)-independent pathways.

In this context, it can be surmised that upon osmotic stress-induced [Ca\(^{2+}\)]\textsubscript{i} elevation and ABA signal stimulation, ABA receptor-phosphatase RCAR1 interacts with ABAH to inhibit its phosphorylation and negate its ABA negative regulatory effect and, then, RCAR1/PYR1-ABA1 integrative effect Geiger, Demir et al.18,63 activates specific calcium-dependent protein kinases (CPKs) such as CPK3,6,21, and 23, and calcineurin-B-like protein-calcineurin-B-like protein interacting protein kinases (CBL-CIPK) (e.g., CBL1, CBL9 and CIPK23) Maierhofer, Geiger, Guzel Deger et al.19,56,62 leading to the activation of both SLAC1 and SLAH3 Maierhofer, Guzel Deger et al.19,62. The above mentioned findings suggest that downstream of NO\(^{-}\)/CI signals, regulation of CPKs and the interaction between CBL-CIPK might be effective to improve stomatal movement by regulating SLAC1 and SLAH3. Differentially induced by drought, salinity and ABA treatment, Responsive to Dehydration 20 (RD20) TF, expressed mostly in non-seed tissues in aerial parts and in guard cells, regulates the expression of different Ca\(^{2+}\)-binding caloesein-like proteins (CLO) genes. Consequently, depending on their isoforms, CLO-encoded proteins differentially function as positive or negative regulators of ABA, stimulating stomatal closure or facilitating stomatal opening Aubert, Kim et al.41–43 Similarly, depending on the isoforms, Ca\(^{2+}\)-sensors Aubert, Kim et al.41,42 and RING E3 Ubiquitin Ligases Lee, Lim et al.,46,50 act as positive or negative regulators of ABA to differentially regulate stomatal responses. Overall, stress conditions and the isoforms and interactions between protein kinases, Ca\(^{2+}\)-sensor proteins, and E3 Ubiquitin Ligases should be taken into account for determining water use efficiency, plant/crop tolerance and performance under drought stress conditions.

As a positive co-regulator of ABA, root uptake of SO\(^{2-}\) and transport in xylem sap is recognized as an inductor of ABA synthesis in guard cells and, in cooperation with H\(_{2}\)S, promotes stomatal closure

Jin, Malchescia et al.12,13 SO\(^{2-}\) up regulates the expression of NCED3 gene involved in ABA biosynthesis, thereby activating QUAC1/ALMT12 anion channels (Figure 1) Malchescia et al.13. H\(_{2}\)S participates in stomatal closure by regulation of ABA and NO production as well as anion and, particularly, K\(^{+}\) channels activities. However, because of the contradictory results found up to now more investigations are required in order to fully understand the effects of factors responsible for SO\(^{2-}\) and H\(_{2}\)S functional diversities and their interactions on stomatal closure by regulation of ABA and NO production and activity of anion channels Jin et al.12. It has been hypothesized that like farnesyltransferases (FTases), expression of genes encoding enzymes involved in SO\(^{2-}\) biosynthesis/conversion such as sulfotransferases, phosphoadenosine-5’-phosphosulfate synthase Rath et al.46; Mueller & Shafqat\(^{65}\) 5’-adenylsulfate reductase, Setya et al.,51 cysteine desulphydrases (CDes) and O-acetyl-L-serine (thiol) lyses (OASTLs) has the potential to stimulate stomata movement in different ways Jin et al.12. Related sulfur metabolites like 3’-phosphoadenosine 5’-phosphate play a similar role in stomatal movement Ponsimirong et al.46. Regardless of ABA stimulation, future studies about the SO\(^{2-}\) and hydrogen sulfide (H\(_{2}\)S) interaction and their intermediary regulators may provide valuable information to show if SO\(^{2-}\) and H\(_{2}\)S have the capacity to directly activate anion channels similar to HCO\(_{3}\) effects and affect stomatal movement.

Roles of anion channels and gated or guard cell outward rectifying outward K\(^{+}\) channel in stomatal movement in response to drought stress

The influx of Ca\(^{2+}\) from plasma membrane and tonoplast into cytoplasm and anions (Cl\(^{-}\), NO\(^{-}\), SO\(^{2-}\), Ma\(^{2+}\)) influx from cytoplasm and acidic lumen by the cotyledon by H\(^{+}\) influx depolarizes the plasma membrane of guard cells giving rise to voltage-activation of the K\(^{+}\)-release channel gated or guard cell outward rectifying outward K\(^{+}\) (GORK) and deactivation of K\(^{+}\) influx channels. Inhibited influx along with enhanced efflux of K\(^{+}\) cause guard cell turgor loss and stomatal closure (Figure 1) Marowa, Jin, Malchescia et al.10,12,43, Daszkowska-Golec & Szarejko43; Zhang et al.42. At low concentration, de-protonated form of malic acid functions as a signaling molecule that activates the vacuolar Cl\(^{-}\) inward rectifying channel ALMT9 and causes enhanced Cl\(^{-}\) uptake during stomatal opening Eisenach et al.13. Phosphorylation by protein kinases or phosphatases regulate the activity of plasma membrane H\(^{-}\)-ATPase in guard cells, facilitating stomatal closure Daszkowska-Golec & Szarejko43; Haruta et al.43 ABA, ABA-induced H\(_{2}\)O \_Zhang, Planes et al.44,51 Phosphatidic acid (PA) Camoni et al.39 and Ca\(^{2+}\)-Kinoshita et al.43 all inhibit the activity of H\(^{-}\)-ATPase. CR1-GPA1 and Phospholipase Dα1/δ (PLd1-XLDP) genes are involved in plant growth and development processes and responses to abiotic stresses and ABA signaling. They are involved in ABA-induced stomatal closure likely by inducing ROS accumulation and signaling, affecting GORKs activity Pandey & Harb, Assmann, Chakraborty, Chen, Zhao, Uraji, et al.41,54,57,59 PLDα1 interact with G protein (GPA1; Gα subunit of a heterotrimeric GTP-binding protein) and 14-3-3 protein to regulate cell signaling and metabolism in plants Mishra, Hong et al.43,51 While PLDα1 positively transmit ABA signals to induce stomatal closure, PLDδ1-GPA1 interaction suppresses ABA signaling to facilitate stomatal opening so that ABA signal in gpα1 and plδ1 single knockout mutants is not strong enough to inhibit stomatal opening, but GPA1 signal does not affect stomatal closure Mishra et al.47 PLD-produced PA induce stomatal closure by firstly binding to the AtRbohD and AtrbohF-N-terminus and stimulating


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ROS production, secondly by activating protein kinases (e.g., H+-ATPase, protein kinase C, and MAPKs), and thirdly by suppressing ABI1. PDLδ1-produced PA interacts with ABI1 at 1:1 ratio and, thereby, inhibits ABI1 translocation from the cytosol to the nucleus and accordingly stimulates stomatal closure; however, it does not inhibit stomatal opening Mishra, Hong et al. Under salinity and drought stresses, intracellular Ca$^{2+}$ ([Ca$^{2+}$]) elevation stimulates PDLδ1-mediated PA production which subsequently interacts with SnRKs and regulates activity of vacuolar H+-ATPases. These proton pumps help to maintain the proton gradient that drives Na+/H+ antiporter activity Hong, Bargmann et al. Increased expression of Arabidopsis PDLδ1 in canola promoted stomata movement and, thus, improved water status, biomass accumulation and yield by enhancing drought tolerance Lu et al. Enhancing tolerance to both drought and salinity, PDLδ1 and PLDδ8 cooperatively regulate ABA signaling in guard cells, but their functions do not completely overlap Uraj et al.

There are ways of regulating stomatal movement at molecular level independently of ABA, and one of the most interesting of these regulating mechanisms is the one starred by a protein family involved in the post-transcriptional regulation of gene expression known as Glycine-rich RNA-binding proteins (GR-RBPs or GRP) Kwak, Kim et al. GRP2 and GRP4, two members of the eight members of the GR-RBP family found in Arabidopsis, have an impact on seed germination, seedling growth, and stress tolerance of Arabidopsis plants under cold, salt, and dehydration stress conditions Kwak, Kim et al. The functional role and mechanism of action of GR-RBP7 has been investigated in relation to plant response to abiotic stress using a transgenic approach. It has been observed by Histochemical analyses of transgenic plants transformed with a genetic construction with the promoter of GR-RBP7 fused with GUS (GR-RBP7PRO:GUS) that GR-RBP7 is highly expressed in guard cells. GRP7 action affects stomatal opening under drought and salinity conditions, and stomatal closing under cold and freezing, indicating that GRP7 exerts a negative effect on the first two types of abiotic stresses and a positive impact on the second one Kim et al. GRP7 over expression does not affect stomatal closure induced by ABA, suggesting that stomatal opening and closure regulated by GRP7 occur in an ABA-independent manner. No differences in seed germination or seedling growth were found between wild-type, GRP7-overexpressing transgenic plants and grp7 mutants when treated with ABA further supporting the hypothesis that GRP7 affects stomatal opening and closing in an ABA-independent manner. GR-RBPs are involved in post-transcriptional gene expression, so it is conceivable that GR-RBP7 interacts with messenger RNAs (mRNAs) of genes closely related to stomatal movements in guard cells and in this manner it is able to modulate the mRNA processing and folding, with different results on stomatal movement depending on the nature of the stressful condition Kim et al.

Concluding remarks
Manipulation of the signaling pathways for optimizing plant resilience/tolerance and performance under constantly changing environment was the focus of this review. This review postulated that after drought stress perception, the activated molecules initiate the generation of redox, hormone, and chemical signals, the interactions of which control stomatal responses. It further reported that the second messengers, proteins, and intermediate chemicals then react with these signaling molecules to positively or negatively transmit these signals via a series of molecular events. Moreover, this review discussed the step-by-step scheme of these signalling pathways to provide insight into these molecular events and to incentivize further studies on their unknown aspects for improved stomatal responses under various environmental conditions, particularly stress conditions, and specifically drought stress. Downstream of ABA signal, ET-MeA homeostasis, OST1-CDPKs, OST1-SnRK2s, and OST1-Ca$^{2+}$-sensor proteins, CPKs, CBL-CPK, E3 Ubiquitin Ligases, and PLDs act as the final effectors to regulate anion and cation channels activity and, thereby, Stomatal movement. Comparing the variations in signaling pathways under stressful and optimal conditions, regulation and perhaps co-regulation of these effectors require further investigations in order to find the most effective approaches in alleviating the disruptive effects of drought stress on Stomatal movement that is probably the most important factor in Stomatal gas exchange (water vapor and CO$_2$, efflux and influx), and consequently photosynthesis and plant/crop yield. Revealing the molecular steps of molecular signals, this review provides insights into the crosstalk signals and changes in the mode of actions of signaling molecules that vary depending on the activity of different signaling pathways and the interactions between signaling molecules with the target molecules and, thereby, facilitates the correct interpretation of the signaling events involved in Stomatal movement under normal and drought stress conditions. Consequently, the correct interpretation of the signaling events would help the breeders to choose the most effective strategy for engineering drought tolerant plants with optimized Stomatal responses.

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Conflict of interest
The author declares there is no conflict of interest.

References


43. Li S, Han X, Yang L, et al. Mitogen-activated protein kinases and


