

Plants Salinity/Water Deficit Responses

Subjects: Agriculture, Dairy & Animal Science

Submitted by:  Ibrahim Tarbiyyah

Definition

Global development has generated a plethora of unfavorable and adverse environmental factors for the living organisms in the ecosystem. Plants are sessile organisms, and they are crucial to sustain life on earth. Since plants are sessile, they face a great number of environmental challenges related to abiotic stresses, such as temperature fluctuation, drought, salinity, flood and metal contamination. Salinity and drought are considered major abiotic stresses that negatively affect the plants' growth and production of useful content. However, plants have evolved various molecular mechanisms to increase their tolerance to these environmental stresses. There is a whole complex system of communication (cross-talk) through massive signaling cascades that are activated and modulated in response to salinity and drought. Secondary metabolites are believed to play significant roles in the plant's response and resistance to salinity and drought stress. Until recently, attempts to unravel the biosynthetic pathways were limited mainly due to the inadequate plant genomics resources. However, recent advancements in generating high-throughput "omics" datasets, computational tools and functional genomics approach integration have aided in the elucidation of biosynthetic pathways of many plant bioactive metabolites.

1. Introduction

Due to the plants' sessility and inability to migrate in nature, they are more prone to being affected by a great number of abiotic stresses such as salinity, drought, heat and radiation. Those abiotic stresses are considered major challenges for the development and growth of the plants and potentially influence the yield and quality of the useful products that provide medicinal or nutritional benefits to humans ^[1]. Hence, understanding the mechanisms by which plants react to abiotic stresses will aid in improving the quality and quantity of the rich and diverse natural products.

Many of these stressors induce elicitors that would eventually result in either negative or positive plant responses ^[2]. For instance, a positive influence has been proved by Crisosto ^[3] in a study on peaches; it showed that a higher fruit density was observed at lower levels of irrigation. On the contrary, abiotic stresses also have negative effects on the development and growth of the plant. For example, they can result in changing the reactive oxygen species (ROS) levels in the body and cause damage; these changes will force the plant to undergo programmed cell death (PCD) ^[4]. Other examples of negative effects of abiotic stresses are the osmotic and ionic stresses that occur when plants are exposed to increased levels of salinity and water shortage ^[5].

The plants tend to adapt to those abiotic stresses in order to survive and thrive ^[6]. There are many players that contribute to the process of resistance, including signaling molecules, DNA-binding proteins and an enormous number of stress-inducible genes. Those biological players participate in the complex orchestration of the plants' tolerance to stress at the molecular level. Stress-related molecules such as abscisic acid (ABA), jasmonic acid (JA) ^[7], ROS, calcium ions, a variety of transcription factors (TFs) and other regulatory and functional proteins all interact together in a spectacular manner in order to perform a remarkable adaptation to the abiotic stresses ^{[8][9]}. This communication between secondary messengers, phytohormones, DNA-binding proteins and stress-inducible genes is known as "cross-talk" ^{[9][10]}.

In response to abiotic stresses, such as salinity and drought, the genetic regulation also involves a complex organization of metabolic activities that involve the intracellular production of beneficial secondary metabolites. Those secondary metabolites facilitate the plant's defense mechanism by

conferring many biological activities such as protecting the plant against oxidative stress, and they are known as stress-protectant metabolites [11][12]. A number of studies have undisputedly determined that many secondary metabolites such as flavonoids, sesquiterpenes and phenolic acids have a substantial antioxidant activity role. Since the changing balance of the ROS in the plant occurs when plants are subjected to abiotic stress, these antioxidative secondary metabolites contribute to the defense mechanisms that the plant utilizes to overcome the harmful effects of stress [13][14][15]. Many plant-based secondary metabolites provide beneficial uses as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides and food additives [16][17]. There are many secondary metabolites with beneficial use as a dietary component or for medicinal purposes, including flavonoids (anthocyanins), alkaloids, terpenes and many others [11][12].

Throughout history, genetic manipulation techniques led to the improvement of the plant's production and quality of useful compounds and resistance to abiotic stresses. Plant natural products have been considered essential phytochemicals that have been used extensively in the field of medicine throughout human history. Some of the well-known plant-extract-derived medicines are morphine, quinine, colchicine, codeine and many others [18]. The anticancer drug that is known as Taxol is extracted from trees from the *Taxus* genus [12]. A remarkable number of studies on medicinal plants displayed crucial evidence that when they are subjected to abiotic stress, the accumulation of secondary metabolites is usually achieved [19][20][21][22]. As a result of these findings, the interest in plants' defense mechanisms and their secondary metabolite production has expanded and been focused on by many researchers.

2. Generic Pathways for Plant Response to Abiotic Stresses

According to our current knowledge about stress signaling pathways, the generic signaling pathway for any given abiotic stress can be divided into the following major steps: signal perception, signal transduction, stress-responsive gene expression and the activation of physiological and metabolic responses [23]. When plants are exposed to drought or salinity stresses, they produce signals that are perceived at the membrane level by ion channels, membrane receptors, receptor-like kinases and many other proteins. Upon perception, complex intracellular signals are then initiated and cause the generation of many messengers such as Ca^{2+} , reactive oxygen species (ROS), phytohormones and other stress signaling molecules [24][25][26]. Those signals are then transduced further inside the cell and interact with other molecules that will ultimately lead to the induction of the stress-induced gene that encodes for proteins that will particularly enhance the plant's tolerance to abiotic stresses directly or indirectly. The plant response to abiotic stresses can be described as the coordination of expression of genes that encode for a variety of products that contribute to the plant's adaptation to abiotic stresses [27][28]. Those products could carry out any function that supports the plant in the adaptation to the stress; they could be, for instance, secondary metabolites that carry out essential protective functions against abiotic stresses, transcription factors that facilitate the activation of second-level stress-inducible genes or any other functional or regulatory molecules [29].

Based on the protein products of the stress-inducible genes, they can be divided into two functional categories: (1) genes that encode for products that directly affect the plant's tolerance to salinity and water deficit stresses, such as heat stress proteins (HSPs) or chaperones, LEA proteins, osmoprotectants, antifreeze proteins, detoxification enzymes and free-radical scavengers [30], and (2) stress-responsive genes that encode for protein products that influence the abiotic stress resistance by regulating the expression of other downstream target genes and modulate the signal transduction cascades. The latter category includes genes that may produce phytohormones such as abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and ethylene [7]. It also includes genes that encode for other signaling molecule products that contribute to the signaling cascade regulation of gene expression, such as the mitogen-activated protein kinases (MAPKs), calcium-dependent protein kinases (CDPKs) and a great number of stress-inducible transcription factors (**Figure 1**).

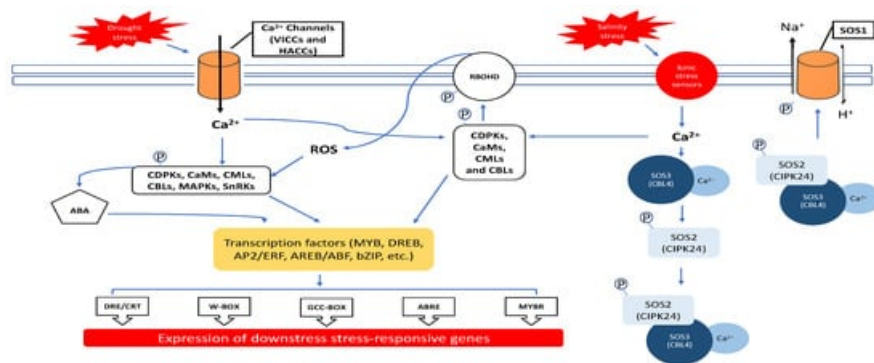


Figure 1. Generic pathway for plant response to salinity and drought stresses in *Arabidopsis thaliana* cell. Salinity and drought elicit Ca^{2+} signals through the SOS pathway to achieve ionic homeostasis. When the ions are increased, it is first perceived by the ionic stress sensors, and then the Ca^{2+} levels are increased. The Ca^{2+} binds to the SOS3 protein and then interacts with the SOS2 protein kinase. The Ca^{2+} -SOS3-SOS2 complex phosphorylates and activates the SOS1 membrane protein, which will cope with the ionic stress. This change in cytoplasmic Ca^{2+} level is sensed by calcium sensors (CDPKs, CaMs, CMLs and CBLs) that interact with their downstream signaling components, which could be RBOHD or transcription factors. The activation of RBOHD leads to the generation of ROS molecules, which in turn aid in the signaling mechanism through the interaction with many molecules such as phytohormones or several kinases. Those phytohormones (ABA) or the protein kinases interact with the stress-inducible TFs. Those stress-inducible TFs carry specific DNA-binding domains that bind to specific regulatory sequences found upstream of the stress-inducible gene and initiate transcription. Those genes will lead to several physiological and chemical changes that would enhance the plant's tolerance to salinity and drought stresses.

2.1. Ca^{2+} and ROS Regulation during Salinity and Drought Exposure

The mechanism of genetic regulation in plants upon the exposure to drought or salinity as an abiotic factor involves the activation of several intricate signaling transduction pathways. There are many intracellular signaling molecules that are involved in the plant's adaptation mechanism to salinity and drought stresses in which they interact together in a complex manner in order to produce a final protective role. Secondary messengers such as ROS and Ca^{2+} are considered well-known and significant signaling molecules that are active at the first steps of the plant's response to abiotic stresses [31].

2.2. Ca^{2+} Ions

As reported by many articles, the most important universal secondary messenger is calcium ions (Ca^{2+}). As mentioned before, salinity and drought stresses cause ionic and osmotic stresses for the exposed plant. Hence, the plants adapt to the stresses by retaining the cellular homeostasis by dealing with osmotic and ionic stresses. It was discovered that the Salt Overly Sensitive (SOS) genes are a primer mediator for the ionic hemostasis in plants. SOS3 gene encodes for a protein that acts as a receptor for cytosolic Ca^{2+} [32]. Increased levels of Ca^{2+} lead to the activation of the cascade of events that would activate the genes responsible for protecting the plant from salt stress. For instance, the SOS1 gene encodes for the plasma membrane Na^+/H^+ antiporter, and the SOS1 gene is mediated by the SOS2, which encodes for a calcium-dependent protein kinase (CDPK) [32][33][34]. The plasma membrane Na^+/H^+ antiporter aids in protecting the plant from salinity stress by maintaining the osmotic balance by compartmentalizing Na^+ and chloride into vacuoles or extruding the excessive toxic Na^+ to the apoplast or surrounding tissues [35][36][37][38]. Along with SOS, Ca^{2+} ions cooperate with other signaling molecules, including calcium-dependent protein kinases (CDPKs), which are considered very important phosphorylating agents in the plant response to abiotic stresses [39].

CDPKs play important roles at the cellular and molecular level in plants: increasing the resistance and adaptation to abiotic stress and modulating metabolic pathways [40]. The CDPKs play a significant role in

the biosynthesis of important organic molecules by phosphorylating and activating a variety of biosynthetic metabolic pathways. Allwood discovered that a key enzyme in the defense response against abiotic stress, known as the phenylalanine ammonia lyase (PAL) enzyme, could be phosphorylated by a CDPK isoform [41], indicating its connection to the secondary metabolite biosynthetic pathways and abiotic stress resistance.

2.3. Reactive Oxygen Species (ROS)

During abiotic stresses, such as salinity and drought, overproduction of ROS results in perturbation of the cellular redox state and thus poses the threat of developing the toxic event that is known as oxidative stress. However, ROS are usually present at a low level in many organelles, and they act as significant secondary messengers in the plant's response to abiotic stresses and secondary metabolism. They are considered very effective secondary messengers due to their spatiotemporal flexibility [31].

There is a synergistic effect in the plant's defense system between Ca^{2+} and ROS signaling [42][43]. The respiratory burst oxidase homolog (RBOH) gene family encodes for an enzyme known as the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. This product enzyme is considered the major contributor of de novo synthesized ROS extracellularly. The RBOH gene is regulated and activated by the previously mentioned calcium-dependent protein kinase (CDPK), which facilitates its phosphorylation. Thus, in order to produce more ROS, Ca^{2+} levels ought to be increased. Vice versa, elevated levels of ROS lead to the increased concentration of Ca^{2+} ions by the interaction of the H_2O_2 and hydroxyl radicals with the Ca^{2+} -permeable cation channels such as the voltage-independent Ca^{2+} -permeable cation channels (VICCs) and the hyperpolarization-activated Ca^{2+} -permeable cation channels (HACCs). This results in a noticeable transient increase in the cytosolic Ca^{2+} , which is involved in several fundamental physiological and regulatory activities [26][44][45].

Similar to Ca^{2+} , ROS interact with other signaling molecules such as the MAPKs. The mitogen-activated protein kinase is one of many important signaling molecules that initiate cascades of events that are involved with transducing stress-related stimuli such as enhanced levels of ROS or calcium signaling. It was discovered that ROS upregulate the activity of the ANP1 kinase in Arabidopsis [46], and its functional homolog NPK1 in tobacco [43], which trigger the downstream MAPK cascades. Several molecular genetic experiments indicated that ANP1 is responsible for the regulation and activation of AtMPK3 and AtMPK6 [39]. The protein kinase products of MPK3/MPK6 are responsible for the direct phosphorylation of the transcription factors associated with the plant's defense system, such as WRKY and ERF. Many other studies have proved the association between ROS and MAPKs in several plant models, indicating their function in the plant's response to abiotic stresses [47][48].

3. Phytohormonal Regulation in Salinity and Water Deficit Stresses

Phytohormones, e.g., abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and ethylene [7], play critical regulatory tasks in the plant's response to abiotic stresses and metabolic pathways. Each hormone plays a variety of functions, and all together act in a concerted way rather than a stand-alone action. Generally, hormones are synthesized at low concentrations in the body of the plant, and they function as a controlling agent in different aspects of developmental and growth events throughout the plant's cycle of life [43].

3.1. Abscisic Acid

ABA contributes to a variety of regulatory networks involving plant's response to drought and salinity stresses that cause osmotic and ionic stresses. Under conditions of osmotic stress, abscisic acid is generally considered as a stress signaling hormone, and the expression of stress-responsive genes in plants is primarily regulated by ABA-dependent and ABA-independent pathways based on the cis-acting regulatory elements present in the promoter regions of the stress-responsive genes [49][50]. The cis-acting elements are regulatory sequences found at the promoter region of many genes, and they are important because they act as physical targets for many transcription factors in order to activate the downstream

target genes. The transcription factors usually carry a specific DNA-binding domain that binds to particular cis-regulatory elements [51]. In the ABA-dependent gene expression, the cis-acting element is the ABA-responsive element (ABRE) and involves the function of the ABA-dependent ABRE-binding protein/ABRE-binding factor (AREB/ABF) transcription factors that target the sequence known as G-box-like cis-acting element with the sequence of ACGTGG/TC [52].

Abscisic acid phytohormone regulates the activation of calcium channels that in turn lead to the activation of the previously mentioned enzyme NADPH oxidase, which has the ability to produce de novo ROS molecules. Moreover, ROS interacts with ABA and other hormones for the main purpose of defending the plant by regulating the signaling cascades and the expression of many stress-responsive genes. Such genes' products are antioxidant enzymes with ROS termination functions such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) [43].

3.2. Ethylene

Ethylene is a gaseous molecule and a well-studied plant phytohormone that carries out important molecular modulation responsible for enhancing the tolerance of the plant to abiotic stress and regulating several metabolic pathways for secondary metabolite biosynthesis. Under salinity and drought stresses, the accumulation of ethylene was observed in many plants. A considerable body of evidence suggests that ethylene functions by monitoring gene expression as a part of its signal transduction pathway. In the absence of ethylene, very important genes that contribute to the plant's response to stress will not be expressed; on the contrary, they will be degraded by E3 ubiquitin ligase [53][54]. Those genes are known as ethylene insensitive 2 (EIN2) and ethylene insensitive 3 (EIN3); they are two key intracellular ethylene-mediated gene regulators in the plant's defense response. With ethylene, EIN2 accumulates and thus facilitates the accumulation of the EIN3 proteins. The EIN3 proteins have a very important role in the genetic activation of a crucial stress-inducible transcription factor known as ethylene response factor 1 (ERF1) [53][55]. The ERF1 then assists as a transcription factor and recognizes a GCC cis-acting element in the promoter regions of ethylene-responsive genes and triggers transcription. This finding shows only one example of how ethylene as a plant hormone contributes to regulating gene expression in the plant's response to abiotic stresses such as drought and salinity.

3.3. Jasmonic Acid and MeJA

Plants produce jasmonic acid (JA) as a plant hormone to regulate a variety of plant processes, including response to abiotic stresses and regulation of the biosynthetic pathways of many secondary metabolites [12]. JA or JA-Ile signaling results in the activation of many TFs that regulate the expression of a great number of target genes through specific binding to particular cis-acting elements. Moreover, jasmonic acid and the methyl ester form of jasmonic acid (MeJA) have been proved to be able to act as an elicitor for the production of several secondary metabolites such as alkaloids, flavonoids, other phenolic compounds and many other compounds [56].

4. Transcriptional Regulation during Salinity and Drought Stresses

Transcription factors are regulatory proteins that recognize particular regulatory sequences of bases located upstream of the coding region of the gene and thus control the transcription process. Every transcription factor is identified by conserved and specific DNA-binding domains that are specific for several cis-acting elements. By binding to cis-regulatory sequences, these proteins facilitate either the activation or repression of the transcription of downstream genes. With regards to transcriptional regulation of the transcription factors in response to abiotic stress, studies have found that many regulatory transcription factors play essential roles in multiple abiotic stress responses by regulating a large spectrum of downstream genes; hence, these genes are known as stress-inducible or -responsive genes [57].

In the last few decades, considerable research has been conducted to identify and characterize various TFs involved in plant abiotic stress responses and metabolic pathways either in abscisic acid (ABA)-

dependent pathways or ABA-independent pathways, such as AP2/EREBP, MYB, bHLH, WRKY, NAC and bZIP [52][58]. *Arabidopsis thaliana* utilizes over 5% of its genome to code for over 1500 TFs, roughly 45% of which are from families that are only specific to plants [59]. In this review, detailed information regarding the transcriptional regulation of a variety of transcription factors, including the superfamilies AP2/EREBP, MYB and bHLH, is provided.

4.1. AP2/EREBP

The APETALA2/ethylene-responsive element binding protein (AP2/EREBP) family is a family of transcription factors thought to have a significant role in regulating gene expression at the transcription level [23][60][61]. These TFs have a highly conserved DNA-binding domain known as AP2/ERF DNA-binding domain. This AP2/ERF (APETALA2/ethylene-responsive element binding factor) is a domain consisting of 50–70 amino acids and is found in many proteins in the plant kingdom, such as *A. thaliana* ERF1, tobacco EREBPs (homolog of the ERF), *A. thaliana* AP2, *A. thaliana* C-repeat/dehydration-responsive element (DRE) binding factor 1 (CBF1 or DREB1) and DREB2 and *Arabidopsis thaliana* and maize abscisic acid (ABA)-insensitive 4 (ABI4) proteins [54][61].

Upon stresses, the AP2/EREBP superfamily are induced through cis-acting elements present at their promoter region. These regulatory elements include GCC box, ABRE, EBS, HSE, LRT and many other unknown binding sites that respond to a variety of abiotic stress stimuli such as AREBs, EIN3 and other transcription factors [61][62]. Thus, the transcriptional regulation pathways of this superfamily of transcription factor proteins are both ABA-dependent and -independent. For instance, promoter analysis studies on the DREB subfamily determined that it contains the most diverse cis-acting elements that are related to abiotic stresses, including ABRE, MeJA response, TCA, ERE, MBS, HSE, TC-rich and LTR motifs. This finding suggests that those transcription factor-encoding genes are the most frequently expressed and involved in the signal transduction pathways related to abiotic stresses [51][54]. It also suggests that this subfamily is regulated through ABA-dependent pathways due to the presence of the ABRE cis-acting element and through the ABA-independent pathway due to the availability of the other cis-regulatory elements.

The DREB 2 subfamily of the AP2/EREBP superfamily can regulate the expression of multiple responsive to dehydration (RD) genes by interacting with a GCC-like box called the DRE/CRT cis-acting element found at the promoter region of many stress-inducible genes in a variety of plant species (e.g., RD29A, RD17, ERD10) [63][64][65]. On the other hand, the ethylene-responsive factor (ERF) subfamily, which also belongs to the AP2/EREBP superfamily, activates an array of stress-responsive genes through the interaction with cis-regulatory sequences that are found upstream of the coding region of many stress-inducible genes such as the ethylene-response element (ERE) or GCC box with the core sequence of AGCCGCC, jasmonic acid- and elicitor-responsive element (JERE), coupling element 1 (CE1) and CT-rich element [6][55][66]. For instance, it was found that a certain ERF encoded by the ERF74 binds to the RbohD promoter region and triggers its expression [54][67] and hence participates in the defense mechanism against salinity and drought by generating ROS that act as signaling molecules. There are many other pieces of evidence suggesting the solid regulatory effect of the AP2/ERF family of TFs in the plant response to abiotic stresses [68].

4.2. MYB

The MYB family of transcription factors is one of the largest TF families in plants. A growing body of evidence has demonstrated the regulatory effects of the MYB family on many biological and biochemical activities such as defense and stress response regulation (Wu et al., 2019), primary and secondary metabolism regulation (Dubos et al., 2010; Wu et al., 2019) and the regulation of other plant-related developmental mechanisms. The MYB family of TFs is characterized by the presence of a conserved MYB DNA-binding domain.

A transcriptional regulatory network analysis of MYB TF family genes in rice was conducted by Smita et al

[69] in order to identify new links and characterize the interaction between the MYB family and the downstream target genes. In their study, they observed around 40 putative target genes for OsMYB that contain at least one MYB-binding region in their promoter. Out of the 40 target genes, 27 were found to have a high number of MYB-binding regions involved in drought-inducibility (MBS, CAACTG and TAACTG). This finding implies that the OsMYB genes are strongly involved and have great regulatory roles in drought response at the transcriptional level [69]. Studies have shown that AtMYB2, AtMYB74 and AtMYB102 MYB genes are upregulated by drought stress [70], which indicates that they play a role in the stress response regulation to protect the plant against the harmful effects of water deficit. Additionally, a study by Hasan et al. [71] investigated the possibility of MYB44 interacting directly with ABA signaling in cotton species. They concluded that the MYB44 gene encodes for a TF that interacts with ABA receptor proteins such as PYLs, which bind to PP2Cs. Hence, the MYB44 has been determined to be a regulator in the ABA-dependent signaling pathway that is related to the activation and repression of the SNF1-related protein kinases 2 (SnRK2s) in which it phosphorylates several stress-inducible genes. AtMYB20 enhances salt tolerance by repressing the expression of PP2Cs [71][72].

4.3. Basic Helix-Loop-Helix (bHLH)

The bHLH family of transcription factors is the second largest family in plants after the MYB family. In Arabidopsis, 162 members of bHLH have been identified, and 167 have been identified in rice plants [73]. As the name suggests, the bHLH family of transcription factors contains a highly conserved basic/helix-loop-helix unique structural domain consisting of two parts: basic amino acid and the helix-loop-helix region (HLH). Findings have determined that the bHLH protein predominantly attaches to the core DNA sequence motif called E-box (5-CANNTG 3), where N could be any nucleotide; the most common form of E-box is the G-box (CACGTG), which is also a binding sequence for the MYC family [74][75].

A growing body of evidence proves that the bHLH TFs are strictly involved in plants' response to a variety of abiotic stresses such as drought and salinity [74][76]. Cui et al. [77] conducted a transcriptome-wide expression analysis of bHLH genes from tea (*C. sinensis*) and revealed that a total of 39 CsbHLH were upregulated under the exposure of the plant to drought stress. Moreover, transcriptomic studies in Arabidopsis roots under salt exposure have shown that at least 15 bHLH genes are upregulated in response to the stress, which implicates their functional role in salt stress responses in plants. There is other evidence suggesting the crucial involvement of the bHLH transcription factor family in plants' response to abiotic stresses.

References

1. Mittler, R.; Blumwald, E. Genetic Engineering for Modern Agriculture: Challenges and Perspectives. *Annu. Rev. Plant Biol.* 2010, 61, 443–462.
2. Hodges, D.M.; Lester, G.E.; Munro, K.D.; Toivonen, P. Oxidative Stress: Importance for Postharvest Quality. *HortScience* 2004, 39, 924–929.
3. Crisosto, C.H.; Johnson, R.S.; Luza, J.G.; Crisosto, G.M. Irrigation Regimes Affect Fruit Soluble Solids Concentration and Rate of Water Loss of 'O'Henry' Peaches. *HortScience* 1994, 29, 1169–1171.
4. You, J.; Chan, Z. ROS Regulation During Abiotic Stress Responses in Crop Plants. *Front. Plant Sci.* 2015, 6, 1092.
5. Landi, S.; Hausman, J.-F.; Guerriero, G.; Esposito, S. Poaceae vs. Abiotic Stress: Focus on Drought and Salt Stress, Recent Insights and Perspectives. *Front. Plant Sci.* 2017, 8, 1214.
6. Patra, B.; Schluttenhofer, C.; Wu, Y.; Pattanaik, S.; Yuan, L. Transcriptional regulation of secondary metabolite biosynthesis in plants. *Biochim. Biophys. Acta (BBA) Bioenerg.* 2013, 1829, 1236–1247.
7. Kapazoglou, A.; Tsaftaris, A. Epigenetic Chromatin Regulators as Mediators of Abiotic Stress Responses in Cereals. In *Abiotic Stress in Plants—Mechanisms and Adaptations*; Available online: (accessed on 19 July 2021).
8. Jain, M.; Nagar, P.; Goel, P.; Singh, A.K.; Kumari, S.; Mustafiz, A. Second Messengers: Central Regulators in Plant Abiotic Stress Response. In *Abiotic Stress-Mediated Sensing and Signaling in Plants: An Omics Perspective*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 47–94.
9. Tiwari, S.; Lata, C.; Chauhan, P.S.; Prasad, V.; Prasad, M. A Functional Genomic Perspective on Drought Signalling and its Crosstalk with Phytohormone-mediated Signalling Pathways in Plants. *Curr. Genom.* 2017, 18, 469–482.
10. Kumari, S.; Panigrahi, K.C.S. Light and auxin signaling cross-talk programme root development in plants. *J. Biosci.*

2019, 44, 26.

11. Mazid, M.; Khan, T.A.; Mohammad, F. Effect of abiotic stress on synthesis of secondary plant products: A critical review. *Agric. Rev.* 2011, 32, 172–182.
12. Akula, R.; Ravishankar, G.A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* 2011, 6, 1720–1731.
13. Edreva, A.; Velikova, V.; Tsonev, T.; Dagnon, S.; Gesheva, E. Stress-Protective Role of Secondary Metabolites: Diversity of Functions and Mechanisms. *Gen. Appl. Plant Physiol.* 2008, 34, 67–78.
14. Khan, A.L.; Hussain, J.; Hamayun, M.; Gilani, S.A.; Ahmad, S.; Rehman, G.; Kim, Y.-H.; Kang, S.-M.; Lee, I.-J. Secondary Metabolites from *Inula britannica* L. and Their Biological Activities. *Molecules* 2010, 15, 1562–1577.
15. Macías-Rubalcava, M.L.; Fernández, R.E.S. Secondary metabolites of endophytic *Xylaria* species with potential applications in medicine and agriculture. *World J. Microbiol. Biotechnol.* 2017, 33, 15.
16. Ivănescu, B.; Burlec, A.; Crivoi, F.; Rosu, C.; Corciovă, A. Secondary Metabolites from *Artemisia* Genus as Biopesticides. *Molecules* 2021, 26, 3061.
17. Rafińska, K.; Pomastowski, P.; Wrona, O.; Górecki, R.; Buszewski, B. *Medicago sativa* as a source of secondary metabolites for agriculture and pharmaceutical industry. *Phytochem. Lett.* 2017, 20, 520–539.
18. Jennings, D.W.; Deutsch, H.M.; Zalkow, L.H.; Teja, A.S. Supercritical extraction of taxol from the bark of *Taxus brevifolia*. *J. Supercrit. Fluids* 1992, 5, 1–6.
19. Hussein, R.A.; El-Anssary, A.A. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. *Herb. Med.* 2019.
20. Bettaieb, I.; Hamrouni, I.; Bourgou, S.; Limam, F.; Marzouk, B. Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. *Acta Physiol. Plant.* 2011, 33, 1103–1111.
21. Shen, Q.; Lu, X.; Yan, T.; Fu, X.; Lv, Z.; Zhang, F.; Pan, Q.; Wang, G.; Sun, X.; Tang, K. The jasmonate-responsive Aa MYC 2 transcription factor positively regulates artemisinin biosynthesis in *Artemisia annua*. *New Phytol.* 2016, 210, 1269–1281.
22. Twilley, D.; Rademan, S.; Lall, N. A review on traditionally used South African medicinal plants, their secondary metabolites and their potential development into anticancer agents. *J. Ethnopharmacol.* 2020, 261, 113101.
23. Wang, H.; Wang, H.; Shao, H.; Tang, X. Recent Advances in Utilizing Transcription Factors to Improve Plant Abiotic Stress Tolerance by Transgenic Technology. *Front. Plant Sci.* 2016, 7, 67.
24. Fedrizzi, L.; Lim, D.; Carafoli, E. Calcium and signal transduction. *Biochem. Mol. Biol. Educ.* 2008, 36, 175–180.
25. Miller, G.; Shulaev, V.; Mittler, R. Reactive oxygen signaling and abiotic stress. *Physiol. Plant.* 2008, 133, 481–489.
26. Singh, R.; Parihar, P.; Singh, S.; Mishra, R.K.; Singh, V.P.; Prasad, S.M. Reactive oxygen species signaling and stomatal movement: Current updates and future perspectives. *Redox Biol.* 2017, 11, 213–218.
27. Ambawat, S.; Sharma, P.; Yadav, N.R.; Yadav, R.C. MYB transcription factor genes as regulators for plant responses: An overview. *Physiol. Mol. Biol. Plants* 2013, 19, 307–321.
28. Bouaziz, D.; Pirrello, J.; Charfeddine, M.; Hammami, A.; Jbir, R.; Dhieb, A.; Bouzayen, M.; Gargouri-Bouzid, R. Overexpression of StDREB1 Transcription Factor Increases Tolerance to Salt in Transgenic Potato Plants. *Mol. Biotechnol.* 2012, 54, 803–817.
29. Matsui, A.; Ishida, J.; Morosawa, T.; Mochizuki, Y.; Kaminuma, E.; Endo, T.A.; Okamoto, M.; Nambara, E.; Nakajima, M.; Kawashima, M.; et al. Arabidopsis Transcriptome Analysis under Drought, Cold, High-Salinity and ABA Treatment Conditions using a Tiling Array. *Plant Cell Physiol.* 2008, 49, 1135–1149.
30. Ciarmiello, L.F.; Woodrow, P.; Fuggi, A.; Pontecorvo, G.; Carillo, P. Plant Genes for Abiotic Stress. In *Abiotic Stress in Plants—Mechanisms and Adaptations*; InTech: Rijeka, Croatia, 2011.
31. Feno, S.; Butera, G.; Vecellio Reane, D.; Rizzuto, R.; Raffaello, A. Crosstalk between Calcium and ROS in Pathophysiological Conditions. *Oxid. Med. Cell. Longev.* 2019, 2019, 9324018.
32. Köster, P.; Wallrad, L.; Edel, K.H.; Faisal, M.; Alatar, A.A.; Kudla, J. The Battle of Two Ions: Ca²⁺ Signalling against Na⁺ Stress. *Plant Biol.* 2019, 21, 39–48.
33. Huang, F.; Luo, J.; Ning, T.; Cao, W.; Jin, X.; Zhao, H.; Wang, Y.; Han, S. Cytosolic and Nucleosolic Calcium Signaling in Response to Osmotic and Salt Stresses Are Independent of Each Other in Roots of Arabidopsis Seedlings. *Front. Plant Sci.* 2017, 8, 1648.
34. Quintero, F.J.; Ohta, M.; Shi, H.; Zhu, J.-K.; Pardo, J.M. Reconstitution in Yeast of the Arabidopsis SOS Signaling Pathway for Na⁺ Homeostasis. *Proc. Natl. Acad. Sci. USA* 2002, 99, 9061–9066.
35. Goel, P.; Bhuria, M.; Sinha, R.; Sharma, T.R.; Singh, A.K. Promising Transcription Factors for Salt and Drought Tolerance in Plants. In *Molecular Approaches in Plant Biology and Environmental Challenges*; Springer: Berlin/Heidelberg, Germany, 2019; pp. 7–50.
36. Guan, B.; Hu, Y.; Zeng, Y.; Wang, Y.; Zhang, F. Molecular Characterization and Functional Analysis of a Vacuolar Na⁺/H⁺ Antiporter Gene (HcNHX1) from *Halostachys Caspica*. *Mol. Biol. Rep.* 2011, 38, 1889–1899.
37. Osakabe, Y.; Arinaga, N.; Umezawa, T.; Katsura, S.; Nagamachi, K.; Tanaka, H.; Ohiraki, H.; Yamada, K.; Seo, S.U.; Abo, M.; et al. Osmotic Stress Responses and Plant Growth Controlled by Potassium Transporters in Arabidopsis. *Plant Cell* 2013, 25, 609–624.

38. Yang, L.; Liu, H.; Fu, S.M.; Ge, H.M.; Tang, R.J.; Yang, Y.; Wang, H.H.; Zhang, H.X. Na⁺/H⁺ and K⁺/H⁺ Antiporters AtNHX1 and AtNHX3 from Arabidopsis Improve Salt and Drought Tolerance in Transgenic Poplar. *Biol. Plant.* 2017, 61, 641–650.
39. Liu, Y.; He, C. Regulation of Plant Reactive Oxygen Species (ROS) in Stress Responses: Learning from AtRBOHD. *Plant Cell Rep.* 2016, 35, 995–1007.
40. Shkryl, Y.N.; Veremeichik, G.N.; Bulgakov, V.P.; Zhuravlev, Y.N. Induction of Anthraquinone Biosynthesis in *Rubia Cordifolia* Cells by Heterologous Expression of a Calcium-Dependent Protein Kinase Gene. *Biotechnol. Bioeng.* 2011, 108, 1734–1738.
41. Allwood, E.G.; Davies, D.R.; Gerrish, C.; Ellis, B.E.; Bolwell, G.P. Phosphorylation of Phenylalanine Ammonia-lyase: Evidence for a Novel Protein Kinase and Identification of the Phosphorylated Residue. *FEBS Lett.* 1999, 457, 47–52.
42. Demidchik, V.; Shabala, S. Mechanisms of Cytosolic Calcium Elevation in Plants: The Role of Ion Channels, Calcium Extrusion Systems and NADPH Oxidase-Mediated “ROS-Ca²⁺ Hub.” *Funct. Plant Biol.* 2018, 45, 9–27.
43. Raja, V.; Majeed, U.; Kang, H.; Andrabi, K.I.; John, R. Abiotic Stress: Interplay between ROS, Hormones and MAPKs. *Environ. Exp. Bot.* 2017, 137, 142–157.
44. Rout, G.R.; Das, A.B. Molecular Stress Physiology of Plants. *Mol. Stress Physiol. Plants* 2013, 1–440.
45. Yang, Y.; Sornaraj, P.; Borisjuk, N.; Kovalchuk, N.; Haefele, S.M. Transcriptional Network Involved in Drought Response and Adaptation in Cereals. In *Abiotic Biotic Stress Plants—Recent Advances and Future Perspectives*; InTech: Adelaide, Australia, 2016; pp. 3–30.
46. Sinha, A.K.; Jaggi, M.; Raghuram, B.; Tuteja, N. Mitogen-Activated Protein Kinase Signaling in Plants under Abiotic Stress. *Plant Signal. Behav.* 2011, 6, 196–203.
47. Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive Oxygen Gene Network of Plants. *Trends Plant Sci.* 2004, 9, 490–498.
48. Nakagami, H.; Kiegerl, S.; Hirt, H. OMTK1, a Novel MAPKKK, Channels Oxidative Stress Signaling through Direct MAPK Interaction. *J. Biol. Chem.* 2004, 279, 26959–26966.
49. Nakashima, K.; Tran, L.P.; Van Nguyen, D.; Fujita, M.; Maruyama, K.; Todaka, D.; Ito, Y.; Hayashi, N.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Functional Analysis of a NAC-type Transcription Factor OsNAC6 Involved in Abiotic and Biotic Stress-responsive Gene Expression in Rice. *Plant J.* 2007, 51, 617–630.
50. Xiong, L.; Zhu, J.-K. Regulation of Abscisic Acid Biosynthesis. *Plant Physiol.* 2003, 133, 29–36.
51. Jangale, B.L.; Chaudhari, R.S.; Azeez, A.; Sane, P.V.; Sane, A.P.; Krishna, B. Independent and Combined Abiotic Stresses Affect the Physiology and Expression Patterns of DREB Genes Differently in Stress-Susceptible and Resistant Genotypes of Banana. *Physiol. Plant.* 2019, 165, 303–318.
52. Todaka, D.; Takahashi, F.; Yamaguchi-Shinozaki, K.; Shinozaki, K. ABA-Responsive Gene Expression in Response to Drought Stress: Cellular Regulation and Long-Distance Signaling; Elsevier Ltd.: Amsterdam, The Netherlands, 2019; Volume 92.
53. Cho, Y.-H.; Lee, S.; Yoo, S.-D. EIN2 and EIN3 in Ethylene Signalling. *Annu. Plant Rev. Online* 2018, 44, 169–187.
54. Xie, Z.; Nolan, T.M.; Jiang, H.; Yin, Y. AP2/ERF Transcription Factor Regulatory Networks in Hormone and Abiotic Stress Responses in Arabidopsis. *Front. Plant Sci.* 2019, 10, 1–17.
55. Zhang, L.; Li, Z.; Quan, R.; Li, G.; Wang, R.; Huang, R. An AP2 Domain-Containing Gene, ESE1, Targeted by the Ethylene Signaling Component EIN3 Is Important for the Salt Response in Arabidopsis. *Plant Physiol.* 2011, 157, 854–865.
56. De Geyter, N.; Gholami, A.; Goormachtig, S.; Goossens, A. Transcriptional Machineries in Jasmonate-Elicited Plant Secondary Metabolism. *Trends Plant Sci.* 2012, 17, 349–359.
57. Liu, L.; White, M.J.; Macrae, T.H. Functional Domains, Evolution and Regulation. *Eur. J. Biochem* 1999, 257, 247–257.
58. Fujita, Y.; Yoshida, T.; Yamaguchi-Shinozaki, K. Pivotal Role of the AREB/ABF-SnRK2 Pathway in ABRE-mediated Transcription in Response to Osmotic Stress in Plants. *Physiol. Plant.* 2013, 147, 15–27.
59. Riechmann, J.L.; Heard, J.; Martin, G.; Reuber, L.; Jiang, C.-Z.; Keddie, J.; Adam, L.; Pineda, O.; Ratcliffe, O.J.; Samaha, R.R. Arabidopsis Transcription Factors: Genome-Wide Comparative Analysis among Eukaryotes. *Science (80-)* 2000, 290, 2105–2110.
60. Liu, C.; Zhang, T. Expansion and Stress Responses of the AP2/EREBP Superfamily in Cotton. *BMC Genom.* 2017, 18, 118.
61. Phukan, U.J.; Jeena, G.S.; Tripathi, V.; Shukla, R.K. Regulation of Apetala2/Ethylene Response Factors in Plants. *Front. Plant Sci.* 2017, 8, 1–18.
62. Dietz, K.-J.; Vogel, M.O.; Viehhauser, A. AP2/EREBP Transcription Factors Are Part of Gene Regulatory Networks and Integrate Metabolic, Hormonal and Environmental Signals in Stress Acclimation and Retrograde Signalling. *Protoplasma* 2010, 245, 3–14.
63. Cheng, L.B.; Yang, J.J.; Yin, L.; Hui, L.C.; Qian, H.M.; Li, S.-Y.; Li, L.-J. Transcription Factor NnDREB1 from Lotus Improved Drought Tolerance in Transgenic Arabidopsis *Thaliana*. *Biol. Plant* 2017, 61, 651–658.
64. Li, X.; Liang, Y.; Gao, B.; Mijiti, M.; Bozorov, T.A.; Yang, H.; Zhang, D.; Wood, A.J. ScDREB10, an A-5c Type of DREB Gene of the Desert Moss *Syntrichia Caninervis*, Confers Osmotic and Salt Tolerances to Arabidopsis. *Genes* 2019, 10,

65. Yang, G.; Yu, L.; Zhang, K.; Zhao, Y.; Guo, Y.; Gao, C. A ThDREB Gene from *Tamarix Hispida* Improved the Salt and Drought Tolerance of Transgenic Tobacco and *T. Hispida*. *Plant Physiol. Biochem.* 2017, 113, 187–197.
66. Sharoni, A.M.; Nuruzzaman, M.; Satoh, K.; Shimizu, T.; Kondoh, H.; Sasaya, T.; Choi, I.R.; Omura, T.; Kikuchi, S. Gene Structures, Classification and Expression Models of the AP2/EREBP Transcription Factor Family in Rice. *Plant Cell Physiol.* 2011, 52, 344–360.
67. Yao, Y.; He, R.J.; Xie, Q.L.; Zhao, X.H.; Deng, X.M.; He, J.B.; Song, L.; He, J.; Marchant, A.; Chen, X. ETHYLENE RESPONSE FACTOR 74 (ERF74) Plays an Essential Role in Controlling a Respiratory Burst Oxidase Homolog D (RbohD)-dependent Mechanism in Response to Different Stresses in *Arabidopsis*. *New Phytol.* 2017, 213, 1667–1681.
68. Hongxing, Z.; Benzhong, Z.; Bianyun, Y.; Yanling, H.; Daqi, F.; Wentao, X.; Yunbo, L. Cloning and DNA-Binding Properties of Ethylene Response Factor, LeERF1 and LeERF2, in Tomato. *Biotechnol. Lett.* 2005, 27, 423–428.
69. Smita, S.; Katiyar, A.; Chinnusamy, V.; Pandey, D.M.; Bansal, K.C. Transcriptional Regulatory Network Analysis of MYB Transcription Factor Family Genes in Rice. *Front. Plant Sci.* 2015, 6, 1–19.
70. Dubos, C.; Stracke, R.; Grotewold, E.; Weisshaar, B.; Martin, C.; Lepiniec, L. MYB Transcription Factors in *Arabidopsis*. *Trends Plant Sci.* 2010, 15, 573–581.
71. Hasan, M.M.U.; Ma, F.; Islam, F.; Sajid, M.; Prodhan, Z.H.; Li, F.; Shen, H.; Chen, Y.; Wang, X. Comparative Transcriptomic Analysis of Biological Process and Key Pathway in Three Cotton (*Gossypium* Spp.) Species Under Drought Stress. *Int. J. Mol. Sci.* 2019, 20, 2076.
72. Cao, Y.; Li, K.; Li, Y.; Zhao, X.; Wang, L. MYB Transcription Factors as Regulators of Secondary Metabolism in Plants. *Biology* 2020, 9, 61.
73. Dong, Y.; Wang, C.; Han, X.; Tang, S.; Liu, S.; Xia, X.; Yin, W. A Novel BHLH Transcription Factor PebHLH35 from *Populus Euphratica* Confers Drought Tolerance through Regulating Stomatal Development, Photosynthesis and Growth in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 2014, 450, 453–458.
74. Sun, X.; Wang, Y.; Sui, N. Transcriptional Regulation of BHLH during Plant Response to Stress. *Biochem. Biophys. Res. Commun.* 2018, 503, 397–401.
75. Wang, R.; Zhao, P.; Kong, N.; Lu, R.; Pei, Y.; Huang, C.; Ma, H.; Chen, Q. Genome-Wide Identification and Characterization of the Potato BHLH Transcription Factor Family. *Genes* 2018, 9, 54.
76. Wang, L.; Xiang, L.; Hong, J.; Xie, Z.; Li, B. Genome-Wide Analysis of BHLH Transcription Factor Family Reveals Their Involvement in Biotic and Abiotic Stress Responses in Wheat (*Triticum Aestivum* L.). *3 Biotech* 2019, 9, 236.
77. Cui, X.; Wang, Y.-X.; Liu, Z.-W.; Wang, W.-L.; Li, H.; Zhuang, J. Transcriptome-Wide Identification and Expression Profile Analysis of the BHLH Family Genes in *Camellia Sinensis*. *Funct. Integr. Genom.* 2018, 18, 489–503.

Keywords

salinity;drought;abiotic stress;phytohormones;ROS;signal transduction;cross-talk;secondary metabolite;transcriptome analysis