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Review

A Systematic Review on the Role of SnRK2 Gene in *Arabidopsis thaliana* Growth Stages under Abiotic Stresses

Elham Amjad [1, 2, †], Babak Sokouti [3, †, *], Solmaz Asnaashari [3, *], Siavoush Dastmalchi [3, 4, *]

1. Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; E-Mails: elham.amjad1996@gmail.com; amjad.e@ajums.ac.ir
2. Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; E-Mails: b.sokouti@gmail.com; asnaasharisolmaz@gmail.com; dastmalchi.s@tbzmed.ac.ir
4. School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

† These authors contributed equally to this work.

* **Correspondences:** Babak Sokouti, Solmaz Asnaashari and Siavoush Dastmalchi; E-Mails: b.sokouti@gmail.com; asnaasharisolmaz@gmail.com; dastmalchi.s@tbzmed.ac.ir

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Abstract

This systematic review examines the role of SnRK2 (Sucrose non-fermenting 1-Related protein Kinase 2) genes in *Arabidopsis thaliana* growth and responses to abiotic stresses. SnRK2 protein kinases are key components of abscisic acid (ABA) signaling and osmotic stress responses in plants. The review synthesizes findings from numerous studies on how different SnRK2 genes regulate *Arabidopsis* growth, development, and stress tolerance at various life stages. Key topics covered include SnRK2 functions under environmental stresses like drought, salinity, cold, and nutrient deficiency; SnRK2 roles in seed germination and early seedling growth; and applications of SnRK2 genes in developing transgenic *Arabidopsis* with enhanced

stress tolerance. The review highlights the complex regulatory networks involving SnRK2 kinases and their interactions with other signaling components like PP2C phosphatases and AREB/ABF transcription factors. Overall, this comprehensive analysis provides insights into the multifaceted roles of SnRK2 genes in modulating plant growth and stress adaptation, with potential applications for improving crop resilience. Further research directions are suggested to elucidate remaining questions about plants' SnRK2 functions and regulatory mechanisms.

Keywords
SnRK2; *Arabidopsis thaliana*; stress tolerance; abscisic acid (ABA) signaling; plant growth regulation

1. Introduction

Rockcress or *Arabidopsis* genus (family: Brassicaceae) is introduced as a small flowering plant and related to mustard and cabbage [1]. This genus is considered due to the presence of important species such as *Arabidopsis thaliana* (L.) Heynh.

A. *thaliana* is a small annual plant known as a weed native to Asia, Europe, and Africa. Due to the short life cycle and small genome (with 135 Mbp of DNA organized into five chromosomes), it is introduced as a popular model organism in plants' genetics and biology. It was the first genome-sequenced plant and was utilized as a standard tool for the evaluation of many plant traits [2-4]. A study of publications that have been published demonstrates the significant significance of doing research on the elements that cause plants to experience growth stress, such as fluctuations in temperature, salt in the soil, and drought, for the purpose of reducing the occurrence of unintended negative consequences, improve plant tolerance, and boost agricultural output [5, 6]. The accurate control of the transitions between stress responses and plant regrowth after recovery under favorable conditions is crucial for their survival and optimal development, and the molecular mechanisms involved in this progression are highly intriguing [7].

There are many aspects of plant development in which abscisic acid is essential as a hormone. It has a major impact on the plant's defense against various diseases and is one of the mechanisms responsible for the flexible responses to abiotic stressors [8]. This phytohormone is prompted in response to harmful environmental situations, and obtained signals inhibit growth and facilitate stress condition responses via the PYL family receptors, while in the optimal environmental conditions and with enough nutrients, the TOR (target of rapamycin) pathway stimulates plant growth [7]. Previous literature demonstrated that the mutual regulation among abscisic acid-dependent and TOR pathways is crucial for stress response and growth recovery after the elimination of stress [7, 9].

In response to biotic and abiotic stress, sucrose non-fermentation 1 (SNF1)-related protein kinase enzymes (SnRKs) stimulate protein phosphorylation pathways, which plays a significant role in maintaining plant productivity [10]. SnRK1, SnRK2, and SnRK3 are three proteins classified into three subfamilies [11]. The SnRK1 subfamily is one of the most fundamental players in the regulation of metabolic homeostasis in plants, and it plays an essential role in both the growth and development of plants, as well as the response of these plants to environmental changes [12]. This class of protein

kinase is the most similar group to SNF1 and AMPK in terms of structure and function [13]. In plant growth and development, the SnRK2 gene may regulate the abscisic acid signal transduction, osmotic stress reactions, and sugar metabolism and control plant growth and development [10]. An abiotic stress triggers the SnRK3 subfamily. Unlike SnRK1, the SnRK2 and SnRK3 subfamilies have more members [10]. Among these three subfamilies, SnRK2 has attracted more attention from researchers due to its performance and activity range [14]. In suitable conditions for the growth of plants, TORC1 kinase (target of rapamycin kinase complex I: TOR, Raptor, and LST8) stimulates growth and prevents the stress response via the phosphorylating of the abscisic acid receptor PYL and consequently inhibits the SnRK2 via PP2C (protein phosphatase type 2C). However, under environmental stress, abscisic acid binds to PYL and sequesters PP2C, which causes the activation of SnRK2. The active SnRK2 can phosphorylate the raptor, which leads to the TORC1 dissociation and growth inhibition [7]. Numerous genomic studies on SnRK2 protein levels on different plant models (such as *A. thaliana*) under various environmental stresses have been reported in previous research [14]. SnRK2 has been classified into three distinct groups: Group I includes kinases that are not activated through the abscisic acid pathway, Group II comprises kinases that are weakly activated or not activated via the abscisic acid pathway, and Group III includes kinase enzymes that are strongly activated by abscisic acid [11]. Therefore, the group III abscisic acid-dependent kinases have been studied more than others as the primary regulators of the plant responses to abscisic acid [11]. The direct phosphorylation of several downstream targets mediates the regulation of abscisic acid responses through the SnRK2 pathway. Group II members share some of the cellular functions with group III kinases, but these groups' contributions to abscisic acid-related responses are not clear [11]. Moreover, according to another previous definition, ten types of SnRK2 were identified in *Arabidopsis*, which were classified into three subgroups. Subgroup I are the SnRK2s that could be activated rapidly under osmotic stress. In contrast, subgroup II and III were reported to be stimulated by osmotic stress and abscisic acid, but subgroup III had a more noticeable role in abscisic acid responses. Subgroup III included SnRKs (2.6, 2.3, and 2.2) or SnRK2E, SnRK2I, and SnRK2D, respectively, that acted as the abscisic acid signaling positive regulators [15].

These SnRK2.6, SnR2.3, and SnR2.2 substrates are transcription factors responsible for the expression of genes responding to abscisic acid, as well as ion channels that control osmosis (e.g., anion channels (SLAC1) and potassium channels (KAT1) [15]. The activation of SnRK2s requires autophosphorylation, a procedure that is suppressed by group A of PP2Cs, which consists of HAB1, HAB2, ABI1, ABI2, and others [16].

The aim of our study is the assessment of previous studies and investigation of the role of SnRK2 in *Arabidopsis* under different stress conditions for gene modification to optimal use in plant species needed by communities.

In Table 1, we present a summary of the results obtained from the 37 papers that have been searched from the Scopus database, reviewed, and evaluated. The responses of *Arabidopsis* plant species to a variety of stress circumstances are addressed in the following way, which is classed as follows:

Table 1 SnRK2 kinases in *Arabidopsis* stress responses and signaling.

Category	Condition	Involved gene/receptor/hormone/fat on SnRK2 actions	Functional Mechanisms	Ref.
Abiotic Stress	low temperature	PCaP2	- PCaP2 gene expression increases under chilling stress - Overexpressing PCaP2 increases cold tolerance - Activates CBF-COR pathway and interacts with SnRK2s	[17]
	drought and salt	ABI1	- SnRK2.6 and ABI1 form an antagonistic complex in abscisic acid signalling	[16]
	drought	group-A PP2Cs (such as HAI1 and ABI1)	- SRK2D/E/I and AREB1 interact - Triple mutant shows reduced drought tolerance - Extremely impaired abscisic acid and loss of water stress-dependent gene expression	[18]
	low air humidity stress	ABI1, ABI2	- Two abscisic acid signaling pathways identified - Involves ABI1 and ABI2 regulation of SRK2E activity	[19]
	low air humidity stress	p44, p42	- Abscisic acid activates p42 and p44 protein kinases - SRK2E is crucial for stomatal closure under humidity changes	[20]
	drought, salt and cold	SE and HYL1	- SnRK2 kinases regulate miRNA biogenesis - Involved in pri-miRNA processing - Interact with SE and HYL1 proteins	[21]
	drought and salt	SCFAtPP2-B11	- 26S proteasome degrades SnRK2s - AtPP2-B11 affects SnRK2.3 stability - Abscisic acid promotes protein degradation	[22]
	drought	Transcription factor HAT1	- HAT1 negatively regulates abscisic acid synthesis in drought response	[23]
	drought	CDK8	- CDK8 regulates abscisic acid response - Interacts with RAP2.6 - Important for RNA polymerase II recruitment	[24]
	drought	Magnesium-chelatase H subunit	- Functional link between magnesium-chelatase and SnRK2.6/OST1 in abscisic acid response - Abscisic acid receptor interacts with OST1 - Impacts NO and ROS creation	[25]

	drought	Raf-like kinases	- Positive regulators of SnRK2 in osmostress signaling	[26]
	drought	AREB1, AREB2, ABF3 and ABF1 transcription factors	- ABF1 crucial for drought tolerance - Four AREB/ABFs are key transcription factors downstream of SRK2D/E/I - Quadruple mutant showed impaired gene expression	[27]
	salt	Phosphatidic acid	- SnRK2.4 and SnRK2.10 bind to phosphatidic acid-containing liposomes - Basic amino acids in domain 1 essential for binding - Overexpression reduces root growth	[28]
	drought and salt	Raf36 and Raf22	- Raf36 and Raf22 negatively regulate abscisic acid signaling - Under stress, abscisic acid-activated SnRK2s promote Raf36 degradation	[29]
Pathogenic stress	pathogenic infections	NPR1	- SnRK2.8 important in plant immune system responses - Mediates systemic acquired resistance (SAR) - Facilitates NPR1 nuclear import dependent on salicylic acid	[30]
Seed Germination and Growth	seed germination	BTB-A2.1, BTB-A2.2, and BTB-A2.3	- BTB-A2.1, BTB-A2.2, BTB-A2.3 negatively regulate abscisic acid signaling - Interact with and decrease SnRK2.3 stability	[31]
	seed germination	ABI5, AREB3	- Three SnRK2 protein kinases necessary for seed growth and dormancy control	[32]
	seed germination and seedling growth	KAPP	- KAPP enhances abscisic acid sensitivity - Interacts with multiple SnRK2 proteins	[33]
	early seedling	ABI3, ABI4, and ABI5	- RAV1 plays a key role in abscisic acid signaling during seed germination - Phosphorylated by SnRK2.2, SnRK2.3, and SnRK2.6	[34]
Hormonal and Molecular Crosstalk	sulfur starvation	-	- SnRK2.3 plays a key role in gene expression during sulfur starvation	[35]
	potassium deprivation	14-3-3χ, 14-3-3κ, 14-3-3ψ, Glyoxalase I, Adenosine kinase I, Ribose 5-phosphate isomerase, 60S acidic ribosomal protein P2	- SnRK2.8 overexpression enhances plant growth - Phosphorylates multiple proteins including 14-3-3 proteins and glyoxalase I	[36]
	Pladienolide B	-	- Pladienolide B enhanced in vitro activity of SnRK2.6	[37]
	Ethylene	-	- Ethylene prevents stomatal closure induced by methyl jasmonate and abscisic acid - Modulates guard cell anion channel activity	[38]

2. SnRK2 Function under Various Environmental Stress Conditions and Exposure to Stress-Inducing Compounds

Stress tolerance in plants is regulated by various proteins and molecular processes, and different types of stress can have distinct effects on plant growth and productivity (Figure 1).

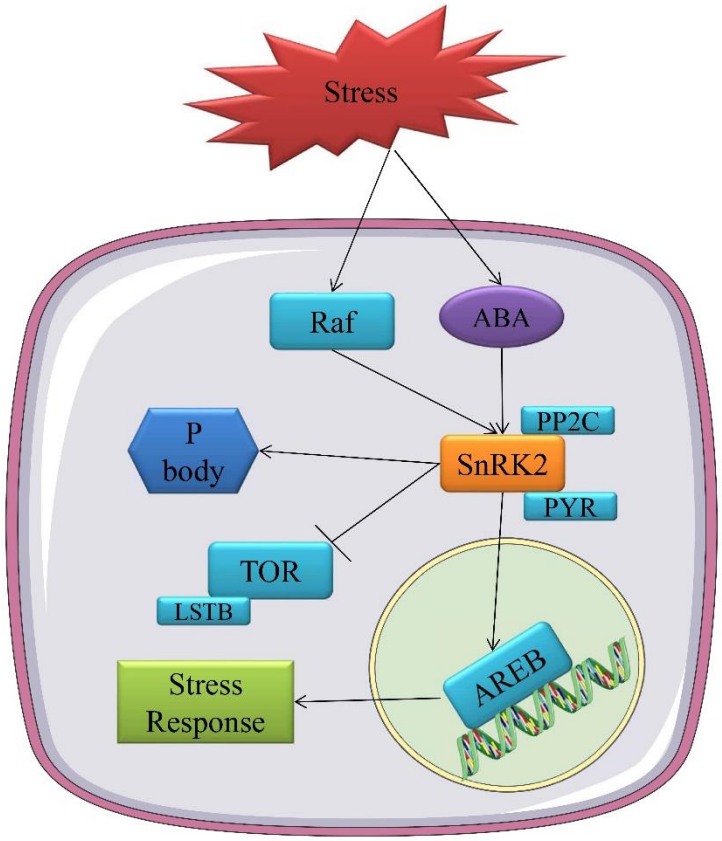

Figure 1 The functional performance of SnRK2 while facing stresses.

2.1 Seed Germination Stage and Early Seedlings

Abscisic acid is an essential phytohormone that controls several aspects of plant development, including seed germination, seed development, and stress tolerance. During the maturation of *A. thaliana* seeds, gene expression is regulated through ABI5 phosphorylation and other transcription factors (like AREB3). This regulation is accomplished by three abscisic acid-activated protein kinases, namely SRK2D, SRK2E, and SRK2I [32]. Seeds also were shown to need a kinase to dry out and remain dormant to survive. Due to PP2Cs' inhibition of SnRK2s' phosphorylation and activation, as well as the fact that these three kinases affect PP2Cs expression, it seems plausible that PP2Cs controls SnRK2s, ABI5s, and transcription factors (like AREB3) by fine-tuning their phosphorylation status [32].

An investigation into *A. thaliana* BTB-A2 genes revealed that BTB-A2.1, BTB-A2.2, and BTB-A2.3 were negatively regulated abscisic acid signaling, encoded proteins specific to the nucleus and cytoplasm and were highly expressed in common [31]. Disruption of these three genes suppresses seed germination, rather than a single or double mutant, reducing abscisic acid, which causes seed germination. Mutations of these three genes enhanced abscisic acid signaling, and abscisic acid

might increase the expression levels of these genes [31]. It has been demonstrated that this triple BTB-A2 protein complex physically interacts with SnRK2.3, lowers the stability of SnRK2.3, and reduces the amount of SnRK2.3 that causes abscisic acid hypersensitive germination, according to an investigation of protein-protein interaction [31]. BTB-A2.1, BTB-A2.2 and BTB-A2.3 are inhibitory proteins that inhibit abscisic acid signaling, so they were added to the seed germination process as negative regulators [31].

In separate research, *Arabidopsis* SnRK2 kinases are found to regulate early seedling development and seedling germination and phosphorylate the RAV1 transcription factor (related to ABI3/VP1) [34]. By regulating the expression of ABI3, ABI4, and ABI5 through RAV1, *Arabidopsis* RAV1 participated in abscisic acid signaling, and SnRK2s interfered with its role [34].

A research article published in 2019 revealed that KAPP (kinase-associated protein phosphatase) plays a key role in the interplay between protein kinases (SnRK2.2/2.3/2.6) and inhibits the germination and development of seeds in *A. thaliana* when abscisic acid is consumed [33].

In their study, Małgorzata et al. found that the abscisic acid core signaling pathway components, ABI1 of the PP2C type and SnRK2.6 kinase, controlled the MAPKKK18 kinase. By influencing MAPKKK18 protein turnover via the ubiquitin-proteasome pathway, ABI1 was able to reduce MAPKKK18 activity [39].

2.2 Low Temperature Stress

Low temperature or chilling stress refers to temperatures above freezing but within the range of 0 to 15°C. Low temperature affects all stages of plant growth and limits the distribution of plants worldwide. The capacity of plants to withstand cold stress is different; for example, plants of tropical and subtropical regions, such as rice and tobacco, can't survive at low temperatures, but *Arabidopsis* can resist and continue to grow [40]. Previous studies have shown that a series of cellular and physiological variations, such as membrane structure changes, photosynthesis, calcium signaling, and metabolism, occur in plants under low-temperature stress. These changes help plants adapt to low temperatures. Chloroplasts, which are the site of photosynthesis, are the first and most severe damaged organelle in these conditions [40, 41]. The researcher's Wang et al. found that when *Arabidopsis* plants were subjected to low-temperature stress, PCaP2 expression was significantly increased in several parts of the plant, including bulbs, roots, cotyledons, leaves, and flowers. The PCaP2-overexpressed plants showed increased tolerance in comparison to wild-type plants. Its RNAi and mutant forms negatively affected its sensitivity to low temperatures during seed germination, seedling development, and reproductive growth [17]. Furthermore, PCaP2 was reported as an affirmative regulator of the abscisic acid signaling pathway. CBF1, CBF3, and CBF-targeted COR genes expressed less than CBF2, but CBF2 expressed more when exposed to low temperatures. SnRK2 mutants of PCaP2 decreased transcription of SnRK2.2 and SnRK2.3, as well as downstream genes mediated by SnRK2s (ABF2, RD29A, KIN1, and KIN2). The cold or abscisic acid treatments, on the other hand, increased SnRK2.6, ABF1, ABF3, and ABF4 [17]. Activating gene expression through PCaP2 as a Ca2C-binding protein improved plant low-temperature tolerance in this study. Moreover, PCaP2 might induce low-temperature stress by destabilizing microtubules but not by seizing F-actin. Hence, PCaP2 promotes CBF- and SnRK2-mediated transcription, which in turn leads to low-temperature tolerance and the abscisic acid response [17].

2.3 Abiotic Stress

Various plant species develop sensitivity to environmental abiotic/osmotic pressures, such as drought and excessive salinity, by responding to stress. A phytohormone called abscisic acid, on the other hand, is an important component of the plant system and it plays an important role in its growth, cell division, and response to osmotic stress in the organism [27].

Phosphorylation of proteins is central to the signaling of abscisic acid and osmotic stress in higher plants. In *Arabidopsis*, these signaling pathways are regulated by the ABI1 and ABI2 protein phosphatase genes. Researchers have shown that SRK2E is crucial for signaling abscisic acid in response to water stress (i.e., water loss) [20]. *Arabidopsis* T87 cultivated cells were found to activate p44 and p42 protein kinases, respectively, by abscisic acid. *Arabidopsis* also activates one of these kinases, p44, which is found to be encoded in the SRK2E gene. Abscisic acid and low humidity stimulate one of these kinases. As a result of the srk2e mutation, stomatal closure was not maintained as quickly reduced air humidity levels resulted in the wilting phenotype. A number of abscisic acid-induced genes, including RD22 and RD29B, were also suppressed in SRK2E, which demonstrated that SRK2E is critical to the signaling of abscisic acid under water loss [20]. Researchers in the other research hypothesized that SRK2E/OST1/SnRK2.6's regulatory domain controlled stomatal closure in *Arabidopsis thaliana* by interacting with ABI1 and integrating signals from abscisic acid and osmotic stress [19]. They elaborated that the signal may integrate in two separate abscisic acid-dependent pathways under low air humidity stress conditions. As part of the abscisic acid-dependent route, domain II (pathway1) may be affected by abscisic acid that is created by low humidity. ABI1 binds to this area and may modulate the activity of SRK2E. Pathway 2 involves the activation of Abscisic acid via the adverse regulatory functions of ABI1 and ABI2. In particular, the abscisic acid-independent route modulated SRK2E activity via its action on domain I (pathway 3). The full closure of stomata cells was the result of a convergence of the three mechanisms already described [19].

Prior research has shown that the signaling *Arabidopsis* guard cells involved in the abscisic acid response have a functional connection with the magnesium-chelatase H subunit and SnRK2.6/OST1. Based on this study's findings, SnRK2.6/OST1 is an *Arabidopsis* guard cell abscisic acid signaling component that interacts with the abscisic acid receptor and acts downstream of the receptor [25].

In response to osmotic stress, members of the SnRK2 family respond physiologically by shutting stomata, which helps plants survive in dry environments [42]. The osmostress response of SnRK2 is influenced by *Arabidopsis* abscisic acid and free radical-reactive kinases from the B3 family of mitogen-activated kinases (MAPKKKs). It is important to note that leaves lose significant amounts of water when these pathways are disrupted by osmosis, as this interferes with the closure of the stomata [26]. Katata et al. found that Raf-like kinases are physically influenced by abiotic stress-responsive Raf-like kinases (as well as the *Arabidopsis* SnRK2, one of the proteins that are activated explicitly in drought conditions), leading to stomatal pores closing. They identified a subset of B3-MAPKKKs that *Arabidopsis* derived from subclass III SnRK2. Additionally, by phosphorylating S171 and S175, the Raf-like kinases activated the SRK2E loop in vitro [19, 26].

SRK2D/E/I is a primary substrate transcription factor for gene expression associated with abscisic acid signaling during development, according to a phosphoproteome analysis on *Arabidopsis thaliana*. In response to osmotic stress, these transcription factors were identified as ARB1, ARB2, ABF3, and ABF1 as the bZIP transcription factor [27]. Based on an analysis of the abscisic acid-

dependent gene expression in *Arabidopsis thaliana*, ABF1 was functionally homologous to AREB1, AREB2, and ABF3. The areb1 areb2 abf3 mutant plants showed less abscisic acid sensitivity in primary root development and more drought sensitivity, even though ABF1 is expressed lower than the other three AREB/ABF transcription factors. Furthermore, genome-wide transcriptome investigations found that the quadruple mutant mostly exhibited damage to the expression of SRK2D/E/I downstream genes. These genes include those participating in osmotic stress responses and acquired tolerance, including LEA proteins and transcription factors [27]. The in vitro study also showed that the AREB1 protein was phosphorylated by ABA-activated SnRK2s (SRK2D/SRK2.2, SRK2E/SRK2.6, and SRK2I/SRK2.3 (SRK2D/E/I)) [18]. In planta models, SRK2D/E/I and AREB1 co-localized and interacted in nuclei, according to these results. According to their study, triple mutants of the SRK2D, SRK2E, and SRK2I genes showed a significant improvement in drought tolerance and a marked reduction in abscisic acid susceptibility, which were also found to be significantly different from single or double mutants. In contrast to the other single and double mutants, SRK2D/E/I triple mutants showed an upregulation of jasmonic acid sensitive and blooming genes when subjected to loss of water stress, severely damaging gene expression associated with abscisic acid stress and water loss, including transcription factors. SRK2D/E/I and AREB/ABF triple mutants also showed a pattern of expression dependent on abscisic acid for downregulating certain genes. This finding supports the theory that SRK2D/E/I regulates abscisic acid signaling by AREB/ABFs [18]. There was a significant reduction in gene expression associated with group-A protein phosphatase 2C (PP2C) and dehydration-responsive late embryogenesis abundant (LEA) proteins in the SRK2D/E/I triple mutants. As a result, SRK2D interacts with group-A PP2Cs like HAI1 and ABI1, suggesting that SRK2D/E/I and group-A PP2Cs modulate abscisic acid signaling. Accordingly, SRK2D/E/I acted as the major regulators [18]. Researchers added a novel regulatory mechanism layer in 2017 that might dynamically modify abscisic acid signaling. By promoting the degradation SnRK2.3 in *A. thaliana*, they found that SCFAtPP2-B11 altered abscisic acid signaling [22]. At its core, the SCF complex is a ubiquitin ligase, and its many subunits work together to transport active ubiquitin to specific proteins. As well as modulating the stability of LEA (Late embryogenesis abundant) proteins under drought conditions, *Arabidopsis* AtPP2-B11 acts as a substrate receptor for SCF type E3 ligases. APP2-B11 also regulates plant responses to salt stress, affecting annexin1 protein levels [22, 43].

A publication on Cyclin Dependent Kinase 8 (CDK8) showed its central role in abscisic acid signaling and drought responses. According to their research, CDK8 is connected to RAP2.6 and SnRK2.6. It also positively affects abscisic acid signaling and drought stress response in *Arabidopsis thaliana* [24]. By revealing the interaction between CDK8 and SnRK2.6 and RAP2.6, they enhanced the expression of RD29A and COLDREGULATED 15A (COR15A) promoters by binding to GCC or dehydration-responsive element (DRE) elements. The researchers also found that CDK8, as well as RAP2.6, was important for abscisic acid-induced RAP2.6 expression and upregulating abscisic acid-responsive genes. This suggests that CDK8 may connect SnRK2.6-mediated abscisic acid signaling to RNA polymerase II, which in turn promotes a rapid transcriptional response to abscisic acid and drought stress signals [24].

Another study from 2017 found that when stress conditions were present in *Arabidopsis* plants, the accumulation of miRNA decreased when functionally redundant members of SnRK2 kinases—the main components of abscisic acid and osmotic stress signaling—were inactivated [21]. In addition, the study above demonstrated that SnRK2 kinases were critical for maintaining a steady-state level of HYL1 protein in plants subjected to osmotic stress. The ability of SnRK2 kinases to

phosphorylate SE and HYL1 proteins was shown in an in vitro experiment, indicating that these proteins were microRNA processing components [21]. According to the results, SnRK2 kinases play an essential role in miRNA accumulation regulation and a link between microRNA production and abscisic acid signals. In light of this experimental evidence, it may be concluded that various environmental stressors stimulate the SnRK2 kinases, reprograming gene expression by manipulating miRNA synthesis and controlling transcription factors [21]. In a separate work, Tan et al. demonstrated that the HAT1 transcription factor, which they inserted as a substrate for the SnRK2.3 kinase, may inhibit drought-induced abscisic acid production and signaling in *Arabidopsis thaliana* [39]. Abscisic acid is a drought-responsive molecule inhibited by homeodomain-leucine zipper protein II (HD-ZIP II). According to this study, SnRK2.3 kinase, a positive signaling molecule associated with abscisic acid signaling, phosphorylated HAT1 and destabilized its binding activity. During drought stress and abscisic acid treatment, the levels of HAT1 protein were reduced, whereas overexpression of SnRK2.3 suppressed the phenotypes associated with HAT1 overexpression (HAT1OX) [23].

As a result of salt stress, the SnRK2.4 and SnRK2.10 protein kinases tend to activate independently of abscisic acid in an uncommon manner. These two protein kinases were first found in a search for phosphatidic acid-binding proteins; later, they were shown to have a functional role in root development when exposed to salt stress. In their study, Julkowska et al. focused on how SnRK2.4 interacts with SnRK2.10 via its phosphatidic acid binding domain and how this influences SnRK2.4's function in plants. In addition, they demonstrated that root growth was influenced by the functioning of the SnRK2.4 phosphatidic acid binding domain [28].

The direct substrates of SnRK2 were found to be *Arabidopsis* group C Raf-like protein kinases, which a newly published research showed adversely control abscisic acid signaling [29]. Previous studies have shown that *Arabidopsis* Raf36 protein may interact with several SnRK2s. Based on the results of the reverse genetic and biochemical analysis, Raf36 was shown to adversely control abscisic acid responses during postgermination growth, to have its N-terminus directly phosphorylated by SnRK2s, and to have its destruction facilitated by abscisic acid. Further, Raf22 and Raf36 have redundant roles in controlling abscisic acid reactions [29].

2.4 Pathogenic Attack

When plants are exposed to pathogenic infections, such as those caused by bacteria and fungi, they produce both general and specific defensive mechanisms to avoid the development of illness [44]. After a pathogenic infection, plants use pattern recognition receptors in their cells to recognize pathogen-associated molecular patterns (PAMP) on invading pathogen surfaces. In plants, PAMP-triggered immunity (PTI) becomes their first line of defense and immunity [45]. Pathogens now manufacture PTI inhibitors to suppress PTI and introduce effectors, which are infectious agents. Injecting effectors into plant cells activates effector-triggered immunity. The hypersensitive reaction then triggers programmed cell death at the infection site to stop the spread of the virus [30, 45]. Another wave of the immune response, often known as systemic acquired resistance (SAR), occurs in distant tissues when plant cells recognize pathogens and PAMPs. Instead of inducing cell death, systemic acquired resistance causes infected local and distant tissues to produce salicylic acid. While a large quantity of salicylic acid builds up quickly in diseased tissues, its production is modestly stimulated in distant tissues during SAR [30]. As salicylic acid builds up, it mediates a number of

antimicrobial functions and induces genes that code for proteins involved in disease [46]. Among the key regulators of plant disease resistance, NPR1 is essential in elemental resistance, resistance-dependent resistance, induced systemic resistance, acquired systemic resistance, and induced systemic resistance. It mediates salicylic acid-triggered SAR and enhances the DNA-binding and transcriptional regulatory effects of TGA transcription factors by interacting with them. These TFs induce genes involved in disease [30, 47]. In a study conducted by Lee et al., salicylic acid-independent systemic signals were able to produce a gene that encoded SnRK2.8 in *A. thaliana* cells infected with pseudomonas syringae pv tomato DC3000/avrRpt2 (Pst DC3000/avrRpt2). In the process of SAR, this gene phosphorylates the NPR1. The nuclear import of NPR1 was dependent on its phosphorylation by SnRK2.8. However, salicylic acid was found to be necessary for the induction of SARs mediated by SnRK2.8, even though salicylic acid did not play a role in transcription or activation of SnRK2.8. Salicylic acid signals and SnRK2.8-mediated phosphorylation of NPR1 were both found to activate NPR1 during systemic immunity development in *A. thaliana* [30]. According to the findings of another study, the phosphorylation of the Pseudomonas effector AvrPtoB by *Arabidopsis* SnRK2.8 is essential for establishing bacterial pathogenicity [48]. According to this article, effectors alter the host's metabolism and immunology so that infections may reap the benefits. For the various effectors to function, host kinases needed to phosphorylate them. This research found that the effector AvrPtoB, which is found in all strains of Pseudomonas syringae, enhanced bacterial pathogenicity by acting as an E3 ubiquitin ligase. Furthermore, SnRK2.8 was essential for AvrPtoB virulence function, which includes preventing bacterial colonization, inhibiting callose deposition, and targeting the plant defense regulator NPR1 and its receptor FLS2 [48].

2.5 Hormonal and Molecular Crosstalk in Stress Adaptation

2.5.1 Pladienolide B

Streptomyces platensis Mer-11107 is the original source of the anti-cancer drug pladienolide B, which has structural similarities to macrolides [49-51]. To improve abscisic acid reactions, pladienolide B is known to decrease messenger RNA splicing [37]. The effects of pladienolide B on *A. thaliana* SnRK2.6 were studied in research that was only recently published. The study's findings revealed that pladienolide B increased the in vitro activity of SnRK2.6, whilst SnRK2.2 did not exhibit any response [37]. To activate the kinase, the scientists postulated that conformational changes brought about by the interaction of SnRK2.6 with bound pladienolide B were responsible. Consequently, pladienolide B's capacity to activate SnRK2.6 kinase led to a better comprehension of plant stress signaling networks and uncovered novel approaches that may be used to create more resilient agricultural varieties [37].

2.5.2 Ethylene

Among other hormones, ethylene plays a key role in regulating different genetic networks of plants as a principal hormone, regulating their growth, development, and senescence [52]. In plants, ethylene operates via a network of signaling pathways that activate a family of transcription factors called ethylene response factor (ERF) genes [53].

The crosstalk of signal between ethylene and jasmonate is important for a suitable maintenance of the plant development and defense responses. In *A. thaliana* guard cells, ethylene signaling

suppressed both abscisic acid and jasmonate signaling. A study conducted by Munemasa et al. During the experiments, *A. thaliana* rosette leaves were treated with either ethephon (an ethylene-releasing compound) or 1-aminocyclopropane-1-carboxylic acid (an ethylene precursor) for over 2 hours in order to determine if ethylene inhibits abscisic acid and guard cell methyl jasmonate signaling. The findings showed that pretreatment with these two ethylene producers caused a temporary closure of the stomata. In contrast, both of them could inhibit the methyl jasmonate-induced stomatal closure [38]. According to their findings, ethylene inhibits methyl jasmonate-induced stomatal closure in *A. thaliana* by modulating the activities of guard cell slow-type anion channels independent of the OST1/SnRK2.6 pathway [38]. Ethylene signal activation by 1-aminocyclopropane-1-carboxylic acid or ethephon inhibited abscisic acid-induced stomatal closure [54, 55]. However, 1-aminocyclopropane-1-carboxylic acid treatment did not inhibit OST1/SnRK2.6 kinase activation by abscisic acid [38]. The abscisic acid-activated protein kinase plays an important function in the signaling of guard cells to abscisic acid, OST1/SnRK2.6 [20, 56]. The SnRK2.6 was first described by Zheng et al. as a proper regulator for *Arabidopsis* seed oil production. Moreover, it could assist in the regulation of sucrose metabolism and the growth of *Arabidopsis* [57].

It has previously been found that methyl jasmonate does not activate the guard cell OST1/SnRK2.6 kinase. Despite the fact that this kinase is essential for the induction of stomatal closure in cells, prior studies showed that methyl jasmonate does not activate this kinase in our cells [38, 58].

The reactive oxygen species (ROS) in guard cells may act as signal integrators, combining the signals from ethylene, jasmonate, and abscisic acid. In this study, the ethylene pathway was found to inhibit the jasmonate and abscisic acid pathways while slowing the activity of the slow-type anion channels [38].

2.5.3 Nutrient-Deprived Conditions

Sulfur Starvation. Throughout a plant's life cycle, sulfur is recognized as an essential component. This crucial macronutrient is mainly taken up from the soil in the form of sulfate, and then it is transferred to the plasmids in the leaves for absorption into organic compounds [59]. Proteins, vitamins, coenzymes, glutathione, and ferredoxin are just a few examples of biomolecules that include sulfur, which is essential for molecular reduction and detoxifying xenobiotics and heavy metals [35]. According to the study conducted by Kimura et al. on *A. thaliana*, four out of seven SnRK2 genes (SNRK2.1/2.3/2.42.6) showed an improvement in transcript levels when sulfur starvation and O-acetyl-l-serine were applied. The SnRK2.3 protein kinase also played an essential part in responding to sulfur deficiency, regulating gene expression and facilitating metabolism [35].

Potassium Deprivation. Potassium is one of the several mineral elements that plants need for development; nevertheless, among all these minerals, potassium has the most significant effect on qualitative traits [60]. Potassium is known to have several critical activities, such as activating enzymes, protecting cell membrane electrical potential gradients, regulating turgor and osmosis, and ensuring balanced water transport in plants [60]. One research from 2007 found that when potassium levels were low, the expression of SnRK2.8 was downregulated, which may have something to do with the dramatic stunting of *Arabidopsis* growth. Overexpression of SnRK2.8 also promoted this species' development. Table 1 shows the seven sites phosphorylated by SnRK2.8; proteomic investigations have verified this. Potential targets may include proteins with roles in

metabolism, translation, or control of metabolism, all of which may be related to growth in general. So, the SnRK2.8 kinase cascade may regulate the growth-inhibiting effects of low-nutrient plant conditions by coordinating the activities of many proteins involved in metabolism [36].

2.6 Transgenic Applications

Modifying the plant cells' DNA by genetic engineering techniques leads to the appearance of transgenic plants (Table 2). This modification or gene combination in plants aims to get the possible useful products with improved shelf life and quality, increased yield, and improved pest, cold, heat, and drought tolerance towards different biotic and abiotic stresses. Additionally, transgenic plants can express foreign proteins with therapeutic and industrial values [61].

Table 2 Studies of transgenic *Arabidopsis* models under salt stress condition.

No.	Type of SnRK2 cloning gene	*Arabidopsis* affected gene	Observed effects*	Ref.
1	Black cottonwood young shoots, *Populus trichocarpa* were used for the PtSnRK2 cDNA (PtSnRK2.5 and PtSnRK2.7 genes) cloning	-	- PtSnRK2.5 and PtSnRK2.7 overexpression could improve the salt stress tolerance in *A. thaliana*. - The *PtSnRK2.5* and *PtSnRK2.7* transgenic seedlings presented considerably greater survival rates than control group. - The overexpression of PtSnRK2.7 could widely influence the transcriptome in response to salt stress.	[62]
2	*Fraxinus velutina velutina* mature leaves were used for the full length FvSnRK2182 gene cloning	upregulation of the genes encoding the ion transport–related proteins (including SOS1, HKT1, NHX1, and AKT1); upregulation of the expression of the genes functioning downstream of SnRK2 in the abscisic acid signaling pathway (ABF2, ABF3, AREB4, and Rboh,) (under salt stress)	- The heterologous expression of the FvSnRK2182 in *Arabidopsis* improved salt tolerance by improving the root growth and ion homeostasis. - The heterologous expression in *Arabidopsis* caused the greater content of auxin throughout the germination of seeds and growth of seedlings, and led to the increasing the long of primary roots and the number of lateral roots. - The transgenic lines of *Arabidopsis* could better tolerate the NaCl and/or abscisic acid treatments and produced a higher biomass than the wild type *Arabidopsis.*	[63]
3	SnRK2 subfamily of maize (ZmSAPK1)	ZmSPK1 (a member of plant SnRK2 subfamily in maize) was subcloned into the plant expression vector pGreen0029 under control of the cauliflower mosaic virus 35S promoter and transferred into *Arabidopsis* by Agrobacterium tumefaciens	- ZmSPK1 enhanceى salt tolerance in transgenic lines. - Overexpression of ZmSPK1 increased the fresh and dry weights under salt stress.	[64]
4	ZmSAPK8 (SnRK2 gene from maize)	RD29A, RD29B, RAB18, ABI1, DREB2A, and P5CS1	- The ZmSAPK8 heterologous overexpression in *Arabidopsis* could considerably support salt stress tolerance. - Under salt stress situation, ZmSAPK8-overexpressed transgenic plants displayed greater rate of germination and content of proline, low leakage of electrolyte, and greater survival rate than the wild type.	[65]

			- The results specified that transgenic plants showed improved transcription of the stress-related genes, RD29A, RD29B, RAB18, ABI1, DREB2A, and P5CS1, under high-salinity situations.	
5	SnRK2 Homologs in Gossypium and GhSnRK2.6	-	- The GhSnRK2.6 Overexpression enhanced the transgenic *Arabidopsis* salt tolerance.	[66]

* The **"observed effects"** refer to the outcomes or results noted in the study, indicating that the overexpression of specific genes, such as PtSnRK2.5 and PtSnRK2.7, enhanced the plant's tolerance to environmental stressors. This suggests that the modified plants demonstrated improved resilience or adaptability to challenging conditions compared to non-modified plants.

Studies conducted by Song et al. indicate that *Populus trichocarpa*, when produced heterologously, may overproduce Torr. & A. Gray observed that *A. thaliana* exhibited improved stress tolerance when exposed to SnRK2 genes (i.e., PtSnRK2.5/2.7). Compared to the wild type, the overexpression of this gene significantly improved survival rates under salt stress, as evidenced by the preservation of root elongation and chlorophyll [62]. Also, even in a typical growth environment, transcriptome analysis showed that overexpressing PtSnRK2.7 might influence genes involved in stress metabolism, including those involved in lipid and flavonoid metabolism. When PtSnRK2.7 was overexpressed, several metabolic genes were downregulated, including those associated with anion transport [62]. In their study, Song et al. demonstrated that under drought conditions, the overexpression of specific genes in *A. thaliana*, including RD29A, COR15A, AtGolS3, DREB1A, and PKS18, was not constitutively induced. Instead, these genes were upregulated when AtSnRK2.8 was overexpressed [62, 67].

Researchers from the same lab found that *Arabidopsis* exhibited better salt tolerance through root development and improved ion homeostasis induced by *Fraxinus velutina* (FvSnRK2182) when it was homologously expressed with the SnRK2 gene. With a heterologous expression of auxin, *Arabidopsis* germination and seedling growth were characterized by increased auxin content, which resulted in longer root lengths and lateral roots [64]. Also, transgenic *Arabidopsis* lines outproduced wild-type *Arabidopsis* in terms of biomass and tolerance to NaCl and abscisic acid treatments. Under salt stress conditions (NaCl), the transgenic lines' shoots showed reduced Na^+ concentrations and greater K^+ contents; the genes for AKT1, HKT1, NHX1, and SOS1 and genes encoding ions-transport related proteins were significantly elevated in these conditions. In transgenic lines, genes downstream of SnRK2 (RBoh, ABF2, AREB4, and SnRK2) significantly increased expression when salt stress conditions were applied [63].

The other study showed that ZmSPK1, a member of the plant SnRK2 subfamily in maize, increased salt tolerance in transgenic *Arabidopsis*. After subclonation of ZmSPK1 and transferring into *Arabidopsis*, the transgenic species showed better growth, higher seedling fresh and dry weight, increased proline content and superoxide dismutase (SOD) activity under salt treatment conditions, while the content of malondialdehyde (MDA) and the relative electric conductivity of transgenic

Arabidopsis was kept to a relatively lower level. Therefore, the results suggested ZmSPK1 as an important agent salt tolerance establishment [64]. The ZmSAPK8 gene was discovered and characterized in maize in 2011 by Ying et al. Transgenic *Arabidopsis* plants overexpressing the gene showed salt tolerance, probably because the downstream signaling pathways were improved. According to the study, ZmSAPK8 constitutive overexpression of *Arabidopsis* plants under normal conditions did not slow their growth. This gene has thus been proposed as a critical salt-tolerant gene for crops [65]. Another study reported that overexpression of SnRK2 homologs in Gossypium (GhSnRK2.6) increased salt tolerance in transgenic *Arabidopsis*. This research examined the salt tolerance of transgenic *Arabidopsis* by transforming the GhSnRK2.6 gene into *Arabidopsis*. Under different salt concentrations, fresh weight was compared between transgenic and wild-type *Arabidopsis*. As observed, all transgenic lines were heavier than wild plants regarding fresh weight when exposed to salt. Wild-type plants were inhibited by increased salt concentration, but transgenic plants were not, causing their fresh weights to remain unchanged [66].

3. Conclusions

The review highlights the critical role of SnRK2 genes in *Arabidopsis thaliana*'s growth regulation and abiotic stress responses. These genes are central to ABA signaling and osmotic stress adaptation, integrating various pathways to balance plant development and stress tolerance.

Key findings include SnRK2's involvement in diverse stress responses, seed germination, and early seedling development. Transgenic studies suggest potential applications in crop improvement. While significant progress has been made, further research is needed to understand the specific roles of individual SnRK2 members fully, their interactions with other signaling networks, and their potential for improving stress tolerance in crops. Overall, SnRK2 genes represent a promising avenue for enhancing plant resilience to environmental stresses, with implications for developing more robust crops in changing climate conditions.

Abbreviations

PP2C	protein phosphatase 2C
LEA	late embryogenesis abundant
MAPKKKs	mitogen-activated kinases
CDK8	Cyclin Dependent Kinase 8
COR15	COLDREGULATED 15A
DRE	Dehydration-responsive Element
HD-ZIP II	homeodomain-leucine zipper protein II
HAT1OX	HAT1 overexpression
PAMP	pathogen-associated molecular patterns
PTI	PAMP-triggered immunity
SAR	systemic acquired resistance
Pst DC3000/avrRpt2	Pseudomonas syringae pv tomato DC3000/avrRpt2
ERF	ethylene response factor
ROS	reactive oxygen species
SOD	superoxide dismutase
MDA	malondialdehyde
GhSnRK2.6	SnRK2 homologs in Gossypium

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Author Contributions

EA carried out the gathering and analyzing of studies. SA participated in the design of the study and drafted the manuscript. BS and SD conceived of the study and participated in its design and coordination as well as the analyses of data. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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