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Abscisic acid and salicylic acid mediated defense: A biochemical review in agroecosystems

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Abstract
Plants in agroecosystems are continually exposed to a multitude of abiotic and biotic stresses that threaten their growth, productivity, and survival. To counter these challenges, plants have evolved intricate hormonal signaling networks, with Abscisic Acid (ABA) and Salicylic Acid (SA) emerging as central regulators of stress responses. ABA predominantly governs abiotic stress responses such as drought, salinity, and cold by modulating stomatal behavior, antioxidant defenses, and osmoprotectant accumulation. In contrast, SA orchestrates biotic stress resistance through the activation of systemic acquired resistance (SAR), hypersensitive response (HR), and expression of pathogenesis-related (PR) proteins. However, the relationship between ABA and SA is often antagonistic, particularly under simultaneous stress conditions, where plants must prioritize one signaling pathway over the other. This review comprehensively examines the biosynthesis, signaling pathways, enzymatic responses, and cross-regulatory mechanisms of ABA and SA. It further explores their molecular crosstalk, omics-based insights, field-level applications, crop-specific responses, and biochemical preparation protocols. Emphasis is placed on recent advances in gene expression analysis, enzyme assays, hormone quantification, and integrated stress management strategies. Through crop-wise case studies and practical insights, the paper highlights how a balanced understanding of ABA-SA interactions can inform breeding, biostimulant development, and precision agriculture. Ultimately, this review provides a holistic biochemical framework for leveraging phytohormonal defense to enhance crop resilience and sustainability in a changing climate.

Keywords: Abscisic acid, salicylic acid, plant stress response, abiotic stress, biotic stress

Introduction
Agriculture, the backbone of global food security, faces mounting pressures from climate variability, soil degradation, and biological invasions. Crops grown in agroecosystems—managed agricultural lands comprising cultivated plants, microorganisms, abiotic soil components, and external stressors—are perpetually challenged by both abiotic (drought, salinity, cold, heat) and biotic (pathogens, pests, and herbivores) stresses. To survive these unfavorable conditions, plants rely on a sophisticated and dynamic hormonal signaling network. Among the phytohormones, Abscisic Acid (ABA) and Salicylic Acid (SA) stand out as principal regulators of stress response and adaptation.

ABA, often referred to as the "stress hormone," plays a critical role in mitigating abiotic stresses. It mediates several physiological functions including stomatal regulation, osmotic balance, seed dormancy, and root architecture modification. Its levels increase rapidly under drought or salinity, triggering signal transduction cascades that activate defense genes and protective enzymes. In contrast, SA is renowned for its role in plant immunity. It is instrumental in the activation of Systemic Acquired Resistance (SAR), a defense mechanism conferring long-term protection against a broad range of pathogens. SA enhances the expression of Pathogenesis-Related (PR) proteins and facilitates localized hypersensitive response (HR), effectively containing pathogen spread.

Despite being predominantly associated with different stress types, growing evidence points to a complex and often antagonistic interplay between ABA and SA signaling pathways. For instance, during simultaneous exposure to drought and pathogen attack, plants must prioritize which hormonal pathway to activate, as activating both may lead to conflicting outcomes. ABA tends to suppress immune signaling to conserve water and energy, while SA attempts to mount a full-scale immune response.

This hormonal trade-off has significant implications for crop resilience and productivity in real-world farming environments, where multiple stresses often occur simultaneously.

Furthermore, ABA and SA influence diverse biochemical and molecular processes such as reactive oxygen species (ROS) generation, antioxidant enzyme activity, lipid peroxidation, and the expression of transcription factors (e.g., WRKY, NAC, and MYB families). These downstream effects modulate the plant's metabolic status, resource allocation, and overall physiological performance. The interplay of ABA and SA is thus central to how plants allocate resources between growth and defense—a critical balance in high-input, yield-driven agroecosystems.

Understanding this hormonal crosstalk is not merely of academic interest; it holds tangible benefits for sustainable agriculture. Manipulating ABA or SA levels through genetic engineering, hormonal priming, or exogenous application can significantly influence crop performance under adverse conditions. Precision agriculture and omics-based crop improvement strategies increasingly rely on insights from hormonal biology to design resilient cultivars. This biochemical review aims to elucidate the individual and integrated roles of ABA and SA in stress signaling, highlight recent experimental evidence, and discuss potential applications for improving agricultural sustainability.

The subsequent sections delve into the biosynthesis, signaling mechanisms, and practical implications of ABA and SA in crops. Special emphasis is placed on biochemical markers, stress-responsive enzymes, gene expression studies, and physiological trade-offs relevant to agroecosystem management. Tables and graphs are integrated throughout to visualize key concepts and experimental findings.

Biosynthesis and Metabolism of Abscisic Acid and Salicylic Acid

Abscisic Acid (ABA): Carotenoid-Derived Biosynthesis

Abscisic acid (ABA) is a 15-carbon sesquiterpenoid hormone synthesized via the carotenoid pathway. The primary site of ABA biosynthesis is in chloroplasts of vascular tissues, although roots, guard cells, and developing seeds also contribute significantly under stress conditions. The process begins with the conversion of β-carotene into zeaxanthin, followed by epoxidation to form violaxanthin and then neoxanthin. A crucial and rate-limiting step in ABA biosynthesis is catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED), which cleaves neoxanthin to produce xanthoxin. Xanthoxin is transported to the cytosol, where it undergoes a two-step conversion—first to abscisic aldehyde and then to ABA through the action of abscisic aldehyde oxidase (AAO).

Step	Enzyme	Product	Localization
1	Zeaxanthin Epoxidase	Violaxanthin	Plastid
2	NCED	Xanthoxin	Plastid
3	ABA Aldehyde Oxidase (AAO)	ABA	Cytosol

ABA catabolism primarily occurs via hydroxylation at the 8′-position, producing phaseic acid and dihydrophaseic acid, compounds with limited biological activity. This degradation is regulated by ABA 8'-hydroxylase, a cytochrome P450 monooxygenase, particularly active during stress recovery or growth resumption.

Transport of ABA from roots to shoots, or between cells, is mediated by specialized ATP-binding cassette (ABC) transporters such as AtABCG25 and AtABCG40 in Arabidopsis. These play critical roles in ABA signaling by ensuring hormonal availability in target tissues such as guard cells and vascular bundles.

Salicylic Acid (SA): Shikimate-Derived Biosynthesis

SA is synthesized via two major biosynthetic routes: the isochorismate synthase (ICS) pathway and the phenylalanine ammonia-lyase (PAL) pathway. In Arabidopsis thaliana, over 90% of pathogen-induced SA is produced via the ICS pathway.

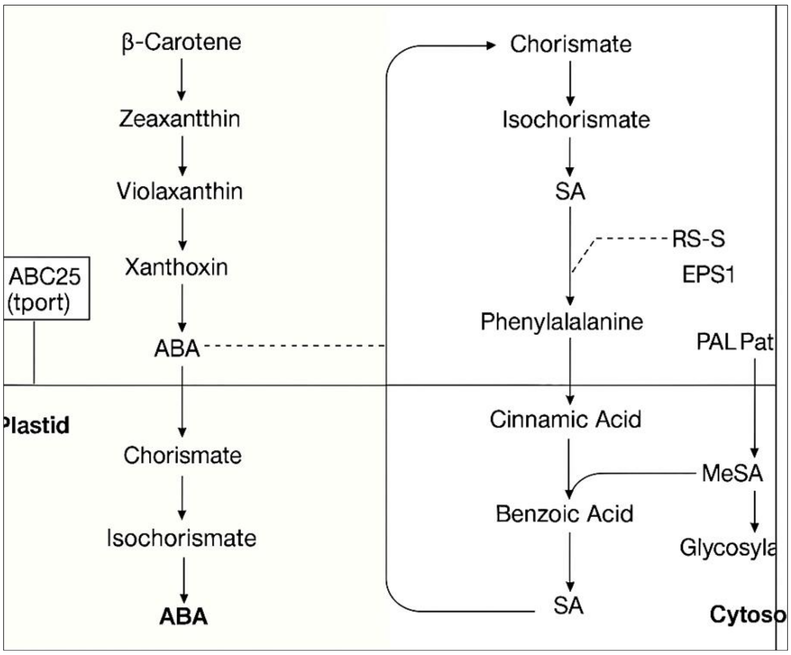
i) **Isochorismate Pathway**
Chorismate, produced through the shikimate pathway, is converted to isochorismate by isochorismate synthase 1 (ICS1), an enzyme located in chloroplasts. Isochorismate is subsequently converted to SA by the action of PBS3 and EPS1 enzymes, although this latter part is still under investigation.

ii) **Phenylalanine Ammonia-Lyase (PAL) Pathway**
Here, phenylalanine is deaminated by PAL to yield trans-cinnamic acid, which is converted to benzoic acid and eventually to SA. This pathway is more prominent under UV stress and wounding.

Pathway	Key Enzyme	Substrate	Final Product	Localization
ICS	ICS1	Chorismate	SA	Chloroplast
PAL	PAL	Phenylalanine	SA	Cytosol

SA can undergo various modifications, including glycosylation (to form SAG), methylation (to form MeSA), and conjugation with amino acids. These derivatives are not merely inactive forms; they play important roles in storage, signaling, and systemic defense. MeSA is a volatile signal involved in long-distance plant communication, while SAG serves as a storage form in vacuoles.

Hormonal Crosstalk: Metabolic Integration: The metabolic interplay between ABA and SA extends beyond simple antagonism. Their biosynthesis and catabolism are subject to reciprocal modulation under specific stress combinations. For example, high levels of ABA can inhibit PAL gene expression, thus suppressing SA biosynthesis, while elevated SA levels have been shown to downregulate NCED expression under certain pathogen attacks. This metabolic reprogramming reflects a tightly regulated system that optimizes plant survival based on prevailing environmental cues.



(Illustration can be added showing plastid-cytosol localization, enzymatic steps, and inhibition arrows)

Fig 1: Simplified Schematic of ABA and SA Biosynthetic Pathways and Points of Crosstalk

2.4 Genetic Regulation of Biosynthesis

Expression of NCED and ICS1 genes is highly inducible under stress. Transcription factors such as ABI5 (ABA-Insensitive 5) and NPR1 (Nonexpressor of Pathogenesis-Related Genes 1) act as master regulators of ABA and SA responses, respectively. Epigenetic modifications such as histone methylation and acetylation have also been implicated in controlling hormone biosynthetic genes, thus contributing to stress memory in plants.

Hormone	Master Gene	Induction Factor	Stress Type
ABA	NCED	Drought, Salinity, Cold	Abiotic
SA	ICS1, PAL	Pathogen Attack, UV	Biotic, Environmental

Environmental Regulation

Environmental cues such as temperature, light intensity, humidity, and pathogen density significantly influence hormonal biosynthesis. High temperature stress increases ABA biosynthesis in rice and maize by activating heat-shock factors that upregulate NCED. Similarly, SA biosynthesis is enhanced during pathogen recognition via PAMP-triggered immunity (PTI), where pathogen-associated molecular patterns activate the MAPK cascade, leading to PAL/ICS gene expression.

Roles in Stress Defense Mechanisms

In plant biology, the ability to tolerate adverse conditions hinges on intricate signaling networks that translate environmental stimuli into appropriate biochemical and physiological responses. Two key hormones, Abscisic Acid (ABA) and Salicylic Acid (SA), are central to this stress management system. Their actions span multiple levels of plant organization—from molecular to cellular, and ultimately, to whole-plant responses. Although their roles have traditionally been separated, with ABA governing abiotic stress and SA modulating biotic defense, contemporary research illustrates a more nuanced picture with significant overlap, coordination, and antagonism between these pathways.

Abscisic Acid is widely recognized as the core hormone responsible for mediating plant responses to abiotic stresses such as drought, salinity, and temperature extremes. Its synthesis increases rapidly in stressed tissues, especially in roots and leaves. One of the most immediate responses mediated by ABA is the closure of stomata, reducing transpirational water loss and conserving cellular hydration. This is achieved through signal transduction pathways that involve calcium ion flux, reactive oxygen species (ROS) generation, and the activation of anion channels in guard cells. Concurrently, ABA enhances the production of osmoprotectants such as proline and sugars, stabilizes membranes, and activates antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which mitigate oxidative stress induced by drought or salt accumulation.

Salicylic Acid, in contrast, functions as a central signal in plant immune responses against pathogenic microorganisms. Upon infection by biotrophic pathogens, SA accumulates rapidly at the infection site and triggers a localized hypersensitive response (HR), characterized by programmed cell death of infected cells to prevent pathogen spread. More importantly, SA activates systemic acquired resistance (SAR), a whole-plant immune state that protects uninfected tissues from subsequent attacks. This is facilitated by the induction of pathogenesis-related (PR) genes, including PR1, PR2, and PR5, whose protein products have antifungal, antimicrobial, and cell-wall-modifying functions. SA also plays a role in enhancing physical barriers, including cell wall lignification and callose deposition, which act to fortify plant tissues against invasion.

While ABA and SA are frequently studied in isolation, they often operate under overlapping stress conditions. For instance, a plant experiencing drought may simultaneously face pathogen exposure. In such cases, the interaction between ABA and SA becomes particularly critical. ABA signaling, through its promotion of stomatal closure, restricts pathogen entry but also suppresses SA-mediated immune responses by downregulating NPR1 and WRKY transcription factors. This antagonism reflects a resource allocation strategy in plants, where immediate water conservation is prioritized at the expense of immune defense. Conversely, SA can antagonize ABA signaling, reducing its suppressive effects and thereby favoring defense gene expression. The balance between these two hormones is thus highly context-dependent and mediated by transcriptional, post-transcriptional, and epigenetic regulation.

Experimental evidence from crop studies illustrates this trade-off with compelling clarity. In rice subjected to both water deficit and bacterial blight infection, exogenous application of ABA improved drought tolerance but resulted in larger lesion sizes due to suppressed immune gene expression. In contrast, SA-treated plants demonstrated enhanced disease resistance but exhibited lower drought survival due to prolonged stomatal opening and higher transpirational loss. These dual outcomes were further validated by hormone quantification and transcriptomic analysis, which revealed differential expression patterns of stress-related genes and transporters in response to ABA and SA treatments.

The influence of ABA and SA is also observable in their biochemical footprints. ABA enhances the activity of antioxidant systems and reduces malondialdehyde (MDA) accumulation—a marker of lipid peroxidation—under abiotic stress. SA, meanwhile, promotes a transient burst of ROS at infection sites, which acts as a secondary signal to activate downstream immune responses. Notably, these responses are fine-tuned by the spatiotemporal regulation of hormone levels, receptor sensitivity, and interaction with other hormonal signals such as jasmonic acid (JA), ethylene (ET), and auxins.

At the molecular level, ABA operates through the PYR/PYL/RCAR-PP2C-SnRK2 signaling module, leading to the activation of ABF and AREB transcription factors that modulate abiotic stress-responsive genes. SA exerts its effects through the NPR1-dependent pathway, which activates TGA and WRKY transcription factors critical for PR gene expression. These molecular cascades further contribute to phenotypic plasticity, enabling plants to adjust their metabolic state and structural integrity in response to environmental threats.

In summary, the roles of ABA and SA in stress defense mechanisms represent an elegant orchestration of opposing yet complementary hormonal strategies. ABA equips the plant to endure harsh abiotic conditions by conserving water and stabilizing internal systems, while SA mobilizes immune defenses to eliminate pathogenic threats. Their interaction is governed by a tightly regulated biochemical dialogue that prioritizes plant survival through strategic trade-offs. Understanding this complex hormonal interplay not only provides insights into plant biology but also offers practical tools for enhancing crop resilience through breeding, genetic engineering, and the application of targeted agrochemicals.

ABA-SA Crosstalk and Antagonism

The signaling networks governed by Abscisic Acid (ABA) and Salicylic Acid (SA) are not independent but intersect at multiple molecular and biochemical junctures, often leading to antagonistic outcomes. Crosstalk between these two hormonal pathways plays a decisive role in determining the nature, timing, and intensity of stress responses in plants. This section explores the mechanisms underlying this hormonal interplay, highlighting how plants use this crosstalk to allocate limited resources between competing stress priorities, particularly in the context of real-world agroecosystems where simultaneous abiotic and biotic challenges frequently co-occur.

Under normal physiological conditions, both ABA and SA maintain relatively low basal levels in most plant tissues. However, exposure to stress leads to a swift surge in one or both hormones depending on the nature of the threat. When a plant encounters drought, osmotic imbalance, or cold stress, ABA biosynthesis is rapidly upregulated, primarily via the NCED gene family, leading to stomatal closure, antioxidant activation, and osmolyte accumulation. Conversely, pathogen invasion initiates SA biosynthesis through the ICS1 or PAL pathway, triggering systemic acquired resistance (SAR) and activating pathogenesis-related (PR) genes. When both stress types occur simultaneously, as is increasingly common under changing climatic conditions, the plant must resolve this hormonal conflict through a sophisticated system of antagonistic regulation.

The core of ABA-SA antagonism lies in transcriptional and post-transcriptional regulation. ABA-activated SnRK2 kinases phosphorylate transcription factors such as ABF/AREB, which bind to ABA-responsive elements (ABREs) in target gene promoters, thereby enhancing drought-responsive gene expression. In contrast, SA activates NPR1, a master regulator of SAR, which translocates to the nucleus upon SA accumulation and interacts with TGA transcription factors to induce PR genes. However, elevated ABA levels have been shown to repress NPR1-dependent gene expression, directly or via inhibition of WRKY and TGA factors. This inhibition reduces PR gene transcription, compromising immune responses during abiotic stress. Similarly, high SA concentrations can antagonize ABA signaling by repressing the expression or activity of PP2C phosphatases, which are key negative regulators of ABA pathways, leading to feedback disruption in ABA-controlled processes.

Empirical studies reinforce the biochemical reality of this antagonism. In Arabidopsis, mutants overexpressing ABA biosynthesis genes exhibit higher drought tolerance but show increased susceptibility to Pseudomonas syringae. The opposite is true in transgenic lines with elevated SA levels, which display strong immunity but suffer from reduced drought adaptability. In rice, wheat, and tomato, exogenous application of ABA during early pathogen infection was observed to reduce SA accumulation and PR gene expression, resulting in more severe disease symptoms. Conversely, SA-treated plants subjected to drought stress showed reduced stomatal closure and higher water loss, despite improved pathogen resistance. These findings underscore a fundamental physiological trade-off wherein

ABA prioritizes abiotic adaptation, while SA emphasizes immune competence, each at the expense of the other.

Beyond transcriptional control, ABA-SA antagonism is also mediated at the level of metabolic flux and enzymatic feedback. For example, increased ABA levels downregulate PAL gene expression, reducing SA biosynthesis. On the other hand, high SA concentrations can suppress NCED transcription or interfere with ABA signal transduction. Furthermore, post-translational modifications such as phosphorylation, ubiquitination, and redox-based regulation also influence the stability and activity of ABA and SA pathway components, further modulating their interaction dynamics. In some cases, cellular localization patterns shift in response to hormone levels, such as the nuclear translocation of NPR1 or the sequestration of SnRK2s, adding yet another layer of regulatory complexity.

A particularly interesting feature of ABA-SA crosstalk is its context-dependence. In certain cases, low to moderate levels of both hormones can act synergistically, especially in early stages of stress or under mild conditions. For instance, studies in maize and soybean have shown that co-application of sub-lethal doses of ABA and SA primes the plant for both abiotic and biotic challenges, resulting in enhanced tolerance without triggering full hormonal antagonism. This "primed" state is believed to involve epigenetic memory, where stress-responsive genes become more rapidly activated upon subsequent exposure. However, this synergistic effect is narrow in scope and dose-dependent. High concentrations of either hormone tend to override the other, reaffirming the dominance of antagonism at physiological and agronomic levels.

At the whole-plant level, the outcomes of ABA-SA antagonism manifest as phenotypic trade-offs. For instance, ABA-treated crops under dual stress scenarios often exhibit better wilting resistance and higher biomass retention but are more prone to disease outbreaks. SA-treated plants, on the other hand, show improved immune responses but reduced root-to-shoot ratios and decreased water-use efficiency. These traits have direct consequences for crop yield and quality, making ABA-SA crosstalk a crucial consideration in stress management strategies. In breeding programs, attempts to enhance both drought resistance and disease tolerance simultaneously have often failed due to this hormonal conflict, unless multi-gene pyramiding and stress-specific promoters are used to regulate hormone levels in a spatially and temporally controlled manner.

Recent advances in genomics and systems biology have begun to unravel the complex gene networks that mediate ABA-SA crosstalk. Transcriptome analyses reveal that hundreds of genes are co-regulated or differentially regulated by both hormones. These include not only classic stress markers but also transcription factors, kinases, transporters, and epigenetic regulators. Network modeling suggests the presence of hormone hubs—genes or nodes that act as integration points for multiple hormonal signals. Manipulating such hubs offers a promising approach to fine-tuning plant responses without triggering full-scale antagonism.

In the context of agroecosystems, the ability to balance ABA and SA signaling is critical for maintaining productivity under unpredictable environmental conditions. Precision agriculture technologies such as hormone biosensors, timed foliar sprays, and smart irrigation systems can be optimized using insights from hormonal crosstalk. Furthermore, the development of biostimulants and priming agents that modulate ABA or SA pathways in a controlled manner opens new avenues for stress mitigation. These strategies require a nuanced understanding of the ABA-SA antagonistic axis and its modulation by developmental stage, species type, and environmental stress severity.

In conclusion, ABA-SA crosstalk represents a strategic decision-making framework in plants, whereby resources are allocated based on the most immediate threat—whether it be water scarcity or pathogen invasion. The antagonism is not merely a biological constraint but a reflection of evolved survival priorities. Understanding and harnessing this biochemical tension is essential for designing resilient crops and sustainable agricultural systems in an era of increasing environmental uncertainty.

Enzyme Pathways and Biochemical Indicators

The stress response mechanisms regulated by Abscisic Acid (ABA) and Salicylic Acid (SA) are tightly linked to a range of enzymatic activities and biochemical markers. These downstream elements not only act as executors of hormonal signals but also serve as diagnostic tools for assessing plant stress status. Enzyme pathways activated or suppressed by ABA and SA govern essential processes such as reactive oxygen species (ROS) detoxification, membrane stability, osmotic regulation, and programmed cell death. Understanding these biochemical indicators is crucial for interpreting plant physiological responses and for designing targeted interventions in agricultural systems.

A key feature of stress physiology in plants is the generation of reactive oxygen species, including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•). These molecules are natural byproducts of photosynthesis and respiration, but their concentrations rise dramatically under both abiotic and biotic stress. While low levels of ROS serve as signaling molecules, excessive accumulation causes oxidative damage to lipids, proteins, and nucleic acids. ABA is known to enhance antioxidant defense systems, particularly during drought and salinity stress, by upregulating the expression and activity of key antioxidant enzymes. These include superoxide dismutase (SOD), which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide; catalase (CAT), which decomposes hydrogen peroxide into water and oxygen; and ascorbate peroxidase (APX), which reduces hydrogen peroxide using ascorbate as a substrate. This suite of enzymes works in concert to maintain redox homeostasis and protect cellular structures from oxidative injury.

Salicylic Acid, on the other hand, exhibits a dual role in ROS metabolism. At the onset of pathogen attack, SA induces a rapid and localized oxidative burst primarily mediated by NADPH oxidases. This burst is an essential component of the hypersensitive response (HR), leading to the formation of a protective boundary of dead cells around the infection site. While this localized ROS accumulation is beneficial for pathogen containment, it also imposes a risk of collateral damage to adjacent tissues. SA-mediated ROS signaling is often accompanied by the activation of specific peroxidases and the modulation of glutathione metabolism. In addition, SA influences the redox status of the NPR1 protein, which is maintained in an oligomeric, inactive form under non-stress conditions but becomes monomerized upon oxidative stress, enabling its translocation into the nucleus and activation of defense gene expression.

Membrane stability is another vital aspect of stress tolerance that is modulated by ABA and SA. One of the most widely used biochemical indicators of membrane damage is the concentration of malondialdehyde (MDA), a lipid peroxidation product formed when ROS attack unsaturated fatty acids in membrane lipids. Under abiotic stress conditions, ABA reduces MDA accumulation by bolstering the antioxidant enzyme system and enhancing the synthesis of compatible solutes like proline, which stabilize membrane structures. In contrast, SA may temporarily increase MDA levels during the hypersensitive response due to the deliberate promotion of oxidative cell death. Nevertheless, SA-induced membrane damage is often transient and spatially restricted, serving a strategic function in localized immunity.

Proline accumulation serves as another important biochemical indicator, particularly under ABA-mediated abiotic stress. As an osmoprotectant, proline helps maintain cellular osmotic balance, stabilizes proteins and membranes, and scavenges free radicals. Its biosynthesis from glutamate is catalyzed by pyrroline-5-carboxylate synthase (P5CS), a gene strongly upregulated by ABA signaling. SA may also influence proline levels, although its effect is less direct and more variable depending on the context. In some cases, SA-induced resistance has been correlated with mild proline accumulation, especially when biotic and abiotic stresses occur simultaneously.

Enzyme assays conducted in controlled experiments offer empirical support for these biochemical interactions. For instance, drought-stressed wheat plants treated with ABA showed a significant increase in SOD and CAT activity along with a reduction in MDA content compared to untreated controls. Similarly, SA-treated tomato plants infected with Fusarium oxysporum demonstrated elevated peroxidase and phenylalanine ammonia-lyase (PAL) activity, indicative of heightened defense metabolism. Such biochemical responses often precede visible symptoms and can thus serve as early warning indicators of stress adaptation success or failure.

A comparison of enzyme activities and biochemical markers under ABA and SA influence provides a useful perspective on their respective stress signatures:

Biochemical Indicator	ABA Response	SA Response	Functional Role
SOD	Strong induction	Moderate induction	Scavenges superoxide radicals
CAT	Strong induction	Low to moderate change	Decomposes hydrogen peroxide
APX	Moderate to strong induction	Moderate activation	Reduces H_2O_2 using ascorbate
POD (Peroxidase)	Slight to moderate	Strong induction during HR	Cell wall reinforcement, ROS metabolism
MDA	Reduced under ABA	Increased during HR, then normalized	Marker of lipid peroxidation
Proline	Significantly increased	Mild increase (variable)	Osmotic adjustment, ROS scavenging
PAL	Low impact	Strongly induced	Precursor for SA and phenolic defenses

These enzymatic and molecular responses also exhibit tissue specificity and temporal variation. For example, ABA-induced antioxidant enzymes are predominantly activated in leaves and roots within hours of drought onset, while SA-induced PR proteins and PAL activity are observed in vascular tissues during systemic resistance phases. Additionally, the magnitude and kinetics of these responses vary across species and cultivars, contributing to differential stress tolerance levels.

Advancements in high-throughput biochemical profiling now allow for the comprehensive assessment of stress-induced enzyme activities and metabolites. Techniques such as spectrophotometric assays, electrophoretic zymography, ELISA, and enzyme-linked immunosorbent assays provide quantitative measurements of enzymatic function. These methods, when integrated with transcriptomics and metabolomics, enable a systems-level understanding of plant stress physiology and hormonal integration.

In conclusion, the enzyme pathways and biochemical indicators regulated by ABA and SA serve as functional endpoints of hormone signaling and as diagnostic tools for plant stress responses. Their careful monitoring offers valuable insights into the effectiveness of defense strategies, enabling breeders, agronomists, and plant scientists to evaluate stress resilience in crops and to optimize the use of hormone-based treatments for improving agricultural productivity. As climate challenges intensify, the strategic use of these biochemical markers will become increasingly important for sustaining crop performance and food security.

Molecular and Omics-Based Evidence

The complexity of Abscisic Acid (ABA) and Salicylic Acid (SA) signaling in plant stress responses is underpinned by vast molecular networks that include receptors, kinases, transcription factors, transporters, and biosynthetic enzymes. With the advent of high-throughput omics technologies—genomics, transcriptomics, proteomics, and metabolomics—our understanding of how these hormones orchestrate plant responses to stress has expanded dramatically. These tools not only provide insights into the regulatory hierarchy of stress-responsive genes but also enable a systems-level interpretation of how ABA and SA act individually and in combination to modulate plant immunity and abiotic resilience.

At the transcriptional level, microarray and RNA-seq studies have elucidated numerous ABA- and SA-responsive genes that exhibit distinct and overlapping expression patterns. In Arabidopsis, the expression of over 2,000 genes is influenced by ABA during drought stress, many of which encode transcription factors such as AREB/ABF, DREB2A, MYB2, and NAC family members. These genes govern the activation of downstream targets involved in dehydration tolerance, ion transport, and ROS detoxification. Likewise, SA treatment induces a separate yet intersecting set of genes—most prominently, PR1, PR5, WRKY70, TGA factors, and ICS1—essential for pathogen defense, phenylpropanoid metabolism, and systemic signaling.

Transcriptomic comparisons have also revealed that crosstalk between ABA and SA pathways often occurs at key regulatory nodes. For instance, WRKY transcription factors—particularly WRKY40, WRKY70, and WRKY18—serve as integration points where antagonistic hormonal effects are modulated based on stress context. WRKY70 is induced by SA and repressed by ABA, thereby mediating a transcriptional switch between immune activation and drought adaptation. Furthermore, the expression of NPR1, the master regulator of SAR, is modulated by ABA both transcriptionally and post-translationally, highlighting the extent of hormonal influence over gene regulation.

Proteomic analyses complement transcriptomic data by confirming the accumulation and activity of hormone-responsive proteins. Comparative proteomics under ABA treatment in crops like wheat, rice, and maize has identified increased abundance of LEA (Late Embryogenesis Abundant) proteins, heat-shock proteins, and various antioxidative enzymes. In SA-treated plants, PR proteins, beta-glucosidases, and enzymes involved in lignin biosynthesis are commonly upregulated. These findings demonstrate that hormonal signals are translated into distinct proteomic signatures that underpin adaptive physiological responses.

Phosphoproteomics has further illuminated how ABA and SA initiate rapid signal cascades via reversible phosphorylation events. SnRK2 kinases, activated upon ABA perception through the PYR/PYL/RCAR-PP2C complex, phosphorylate downstream targets including transcription factors, ion channels, and metabolic enzymes. In contrast, SA-triggered responses involve mitogen-activated protein kinases (MAPKs) such as MPK3 and MPK6, which phosphorylate WRKY and TGA transcription factors to promote defense gene expression. These kinase cascades form the backbone of hormonal signaling and are often co-opted or inhibited by other hormonal or environmental cues, further illustrating their modular nature.

Metabolomics adds a final layer of understanding by profiling hormone levels, secondary metabolites, osmolytes, and antioxidants that accumulate during stress. Using GC-MS, LC-MS, and NMR platforms, researchers have identified ABA-responsive metabolite signatures that include raffinose, galactinol, and sugar alcohols involved in osmoprotection. SA-responsive profiles, on the other hand, often include phenolic acids, flavonoids, and benzoic acid derivatives associated with antimicrobial activity. These metabolic shifts not only protect plants directly but also influence hormone homeostasis by providing feedback to biosynthetic or degradation pathways.

Integrated omics studies have revealed the dynamic nature of hormonal crosstalk under complex stress scenarios. For example, dual stress experiments in tomato exposed to both salt stress and Fusarium infection showed differential regulation of over 1,500 genes, with 36% being ABA-inducible and 28% SA-inducible, and a subset responsive to both. These shared genes often encode calcium sensors, redox regulators, and transcription factors, suggesting that the plant's ability to "decide" between ABA- and SA-mediated responses depends on the integration of multifactorial cues at the omics level.

Emerging fields such as epigenomics and single-cell transcriptomics are beginning to shed light on the spatial and temporal resolution of ABA and SA signaling. DNA methylation and histone modification patterns around stress-responsive gene loci can modulate their accessibility and responsiveness to hormonal stimuli. For instance, drought-induced memory in rice has been associated with histone H3K4 trimethylation at ABA-responsive genes, resulting in faster re-induction upon recurring stress. Likewise, SA-induced priming of defense genes has been linked to chromatin remodeling complexes that enhance transcriptional reprogramming during subsequent infections. These epigenetic marks function as stress memory elements that allow plants to anticipate and prepare for future challenges based on previous experiences.

Gene co-expression network analysis (GCNA) and weighted gene correlation network analysis (WGCNA) have also been employed to identify regulatory hubs and master regulators. These computational approaches integrate gene expression profiles under varying hormonal treatments to construct interaction maps that highlight key nodes of ABA-SA crosstalk. Such hubs often include kinases, transporters, and transcription factors like ABI5, NPR1, and WRKY33, which can be targeted in genetic engineering or marker-assisted breeding programs to enhance stress tolerance.

Another promising development is the application of CRISPR-Cas gene editing to modulate ABA and SA pathways. Precise knockouts or upregulation of genes such as NCED (for ABA) and NPR1 or ICS1 (for SA) have already shown success in Arabidopsis and rice, demonstrating improved drought resistance or enhanced immunity depending on the edited pathway. However, challenges remain in balancing these modifications to avoid compromising one stress response while strengthening another. Omics-guided editing holds the promise of creating crop genotypes with optimized hormonal profiles for specific agroecological contexts.

In practical agricultural terms, omics-based insights are increasingly being translated into the development of hormone-responsive biomarkers for crop monitoring. Expression levels of key ABA/SA-inducible genes or proteins can be used as early indicators of stress in field diagnostics. Similarly, metabolite signatures can be profiled using portable sensors or biosensors to assess hormone levels and predict crop health status in real time. These advances support the growing field of precision agriculture, where molecular data inform on-ground decision-making regarding irrigation, fertilization, or pest management.

In conclusion, omics technologies have revolutionized our understanding of ABA and SA functions by providing high-resolution data on gene expression, protein dynamics, and metabolite accumulation. These datasets, when integrated, reveal the multidimensional nature of hormonal responses and their crosstalk in complex agroecosystem environments. The convergence of molecular biology and data science is thus enabling a more predictive, strategic, and sustainable approach to plant stress management in modern agriculture.

Field-Level Applications in Agroecosystems

The mechanistic insights into Abscisic Acid (ABA) and Salicylic Acid (SA) signaling have opened new avenues for translating hormonal science into real-world agricultural applications. In agroecosystems—where plants interact with a diversity of environmental variables, pathogens, and management practices—the practical manipulation of these hormones can enhance resilience, improve crop health, and stabilize yields. Field-level application of ABA and SA, whether through chemical treatments, biological agents, or genetic interventions, is increasingly gaining traction as a tool for sustainable crop management.

One of the most direct applications is through exogenous hormone application. Foliar sprays of ABA have been used to prime plants for drought and salt stress by inducing stomatal closure and activating antioxidant responses before the onset of stress. Several commercial formulations of ABA or ABA analogs, such as ProTone® and S-ABA, have shown success in crops like grapevine, tomato, and maize, particularly in semi-arid and rainfed farming systems. Similarly, SA-based sprays have been widely used to induce systemic acquired resistance (SAR) in crops exposed to fungal, bacterial, or viral pathogens. Acibenzolar-S-methyl (ASM), a functional SA analog, is commonly deployed in integrated pest management (IPM) programs in rice, wheat, citrus, and cucurbits, where it stimulates PR protein production and strengthens plant immunity without directly killing pathogens, thus reducing the risk of resistance development.

Seed priming with ABA or SA is another promising strategy in climate-resilient agriculture. Pre-sowing treatment of seeds with low concentrations of ABA has been shown to enhance germination under osmotic stress, improve seedling vigor, and modulate root architecture for better water uptake. SA priming, in contrast, enhances pathogen resistance and boosts antioxidant enzyme activity in early developmental stages. A synergistic priming approach, using both ABA and SA in optimized concentrations, has demonstrated improved drought tolerance and disease resistance simultaneously in crops like rice and soybean. These techniques are particularly beneficial in regions experiencing erratic rainfall, poor soil health, or pathogen carryover in soil.

Biological delivery systems for hormonal modulation are gaining popularity as environmentally friendly alternatives to synthetic applications. Certain plant growth-promoting rhizobacteria (PGPR), endophytic fungi, and mycorrhizal associations are capable of producing or modulating levels of ABA and SA within plant tissues. For example, Bacillus subtilis and Pseudomonas fluorescens strains have been reported to induce systemic resistance in crops by stimulating SA accumulation, while Azospirillum spp. can enhance drought tolerance by modulating endogenous ABA levels. Such microbial consortia can be incorporated into biofertilizers or biostimulants, offering a dual benefit of nutrient enhancement and stress priming.

Genetic engineering and marker-assisted breeding also offer field-level solutions for hormonal manipulation. Overexpression of key genes such as NCED (for ABA biosynthesis) or NPR1 (for SA-mediated immunity) has been successfully implemented in several crops. Transgenic tomato plants overexpressing LeNCED1 demonstrate increased drought tolerance through improved water-use efficiency, while rice lines engineered to express Arabidopsis NPR1 show broad-spectrum resistance to bacterial and fungal pathogens. Although concerns over regulatory approval and ecological consequences persist, gene-editing tools like CRISPR-Cas9 are enabling more precise and acceptable modifications, targeting stress-responsive genes without introducing foreign DNA.

Hormonal manipulation is also being embedded into broader precision agriculture frameworks. Advanced sensor technologies and remote-sensing tools are being developed to monitor plant hormonal status in real time. Near-infrared spectroscopy (NIRS), fluorescence imaging, and biosensor-based platforms are being deployed to detect hormonal fluctuations in field conditions. These tools can inform timely application of hormone treatments, irrigation scheduling, or pest control measures, reducing input costs and improving environmental sustainability.

Crop management practices such as mulching, deficit irrigation, and intercropping can indirectly influence ABA and SA dynamics. For example, deficit irrigation strategies that aim to trigger mild water stress can lead to moderate ABA accumulation, which preconditions plants for upcoming drought episodes. Intercropping with allelopathic species such as mustard or marigold has been shown to enhance SA production in neighboring crops, thereby contributing to pathogen suppression and improved soil health.

However, the field application of ABA and SA is not without challenges. Hormone stability, cost-effectiveness, delivery mechanisms, and timing of application are critical parameters that determine efficacy. ABA is light-sensitive and expensive to synthesize, making large-scale field use prohibitive unless analogs or biosynthesis inducers are used. SA, while more stable and cheaper, can lead to growth retardation or yield penalties if overapplied, particularly under non-stress conditions. Therefore, precise calibration of dose, timing, and environmental conditions is essential for success. Formulation science plays a key role here—controlled-release nanoemulsions, hydrogels, and encapsulated beads are being developed to enhance hormone delivery and stability in field conditions.

Another layer of complexity arises from hormone crosstalk with other signaling pathways, such as those of jasmonic acid, ethylene, gibberellins, and cytokinins. Field conditions are rarely limited to single stress types, and co-occurring stresses can produce unpredictable interactions. For example, ABA application in a pathogen-infested field may suppress SA-induced immunity, leading to unintended susceptibility. Hence, a systems-based approach is required where hormone applications are synchronized with plant developmental stages, environmental conditions, and other agronomic inputs.

Success stories from field trials demonstrate the feasibility of hormone-based interventions in commercial agriculture. In arid regions of India, foliar application of ABA analogs in chickpea improved pod retention and yield under terminal drought. In China, SA sprays in rice paddy fields led to significant reduction in sheath blight and blast incidence without increasing pesticide use. In Europe, seed priming with SA improved germination and disease resistance in barley and sugar beet, offering yield advantages of 10-15% under moderate biotic pressure. These case studies underscore the practical potential of ABA and SA manipulation when integrated into holistic crop management programs.

In summary, field-level applications of ABA and SA represent a convergence of molecular understanding and agronomic innovation. Whether through direct application, microbial modulation, or genetic enhancement, these hormones offer powerful tools for improving crop performance under diverse environmental challenges. As the global agricultural landscape becomes increasingly constrained by climate change, population pressure, and resource limitations, harnessing the strategic power of ABA and SA could redefine crop resilience and sustainable farming practices for the future.

Case Studies: Crop-Wise Review

The roles of Abscisic Acid (ABA) and Salicylic Acid (SA) in plant defense are not uniform across species. Their effectiveness varies depending on the crop's genetic background, environmental context, and type of stress encountered. Empirical data from field and controlled studies reveal that ABA and SA function through both conserved and crop-specific mechanisms. This section presents crop-wise case studies to illustrate how these hormones mediate defense responses, enhance resilience, and influence agronomic outcomes in important agricultural species.

Rice (*Oryza sativa*)

Rice, a staple food crop for over half the world's population, is highly susceptible to both drought and biotic stressors such as *Xanthomonas oryzae* pv. oryzae (causing bacterial blight) and *Magnaporthe oryzae* (causing blast disease). Under drought conditions, ABA accumulation in rice roots and leaves leads to stomatal closure, reduced transpiration, and the activation of dehydration-responsive genes such as OsLEA3, OsDREB2A, and OsbZIP23. Exogenous ABA treatments have been shown to enhance drought tolerance by increasing root depth and osmotic adjustment, although often at the cost of reduced tiller number and panicle length.

Conversely, SA plays a pivotal role in activating resistance against bacterial and fungal pathogens. Application of SA or its analogs has been shown to induce PR proteins (e.g., OsPR1a, OsPR10), phenylalanine ammonia lyase (PAL) activity, and oxidative bursts associated with hypersensitive response (HR). However, simultaneous induction of ABA and SA often results in antagonistic outcomes. In one study, rice plants primed with ABA showed reduced lesion size under drought but greater susceptibility to bacterial blight due to suppressed SA-responsive genes. On the other hand, SA-primed plants displayed enhanced resistance to pathogens but reduced photosynthetic efficiency under water stress, confirming the ABA-SA trade-off.

Wheat (*Triticum aestivum*)

Wheat is frequently challenged by abiotic stresses like heat and drought during the grain-filling period, as well as diseases such as rusts (*Puccinia* spp.) and Fusarium head blight. ABA treatments during pre-anthesis stages in wheat have been associated with increased antioxidant enzyme activities and reduced membrane damage, thereby improving grain filling and thousand-kernel weight under drought conditions. Wheat varieties with higher endogenous ABA levels, such as C306, show better stress adaptation compared to susceptible varieties like PBW343.

SA contributes to resistance against stripe rust (*Puccinia striiformis*) by enhancing PAL activity and upregulating PR gene expression. Field trials have demonstrated that SA sprays can reduce disease severity by 30-50% when applied during the early booting stage. However, excessive SA accumulation has been linked to chlorosis and premature senescence, highlighting the importance of dosage and timing.

Tomato (*Solanum lycopersicum*)

Tomato serves as a model crop for studying hormonal interactions due to its susceptibility to multiple stressors. ABA biosynthesis mutants in tomato, such as sitiens, display impaired drought tolerance, indicating the hormone's essential role in water stress adaptation. Exogenous ABA application improves water retention and fruit yield under limited irrigation. However, it also suppresses basal immunity by downregulating SA-mediated PR genes, making the crop more vulnerable to pathogens like Botrytis cinerea.

SA, on the other hand, induces strong resistance to both necrotrophic and biotrophic pathogens. Application of SA or BTH (a SA analog) activates a range of defense genes (PR1, PR2, PAL) and leads to increased accumulation of antimicrobial phenolics and callose deposition. The integration of SA treatment in tomato greenhouse production has been shown to reduce the incidence of Fusarium wilt by over 40%. In cultivars with enhanced SA signaling (e.g., overexpressing NPR1), disease resistance is improved, although sensitivity to osmotic stress remains a limitation.

Maize (*Zea mays*)

In maize, drought and oxidative stress during the flowering stage critically impact yield. ABA accumulation is central to mitigating these effects by regulating stomatal conductance, antioxidant defenses, and kernel development. Field applications of ABA analogs during tasseling have led to improved ear size, pollen viability, and grain yield under terminal drought. Moreover, the expression of ABA-related genes such as ZmNCED and ZmPP2C is strongly correlated with water-use efficiency.

Maize resistance to pathogens such as Fusarium verticillioides and Cercospora zeae-maydis also involves SA signaling. SA treatments enhance lignin deposition, increase peroxidase activity, and reduce disease severity. However, maize's inherent SA levels are lower compared to other dicots, and external SA application must be carefully timed to avoid adverse effects on growth. Hybrid varieties with improved hormone sensitivity or transporter efficiency are currently under development.

Soybean (*Glycine max*)

Soybean is vulnerable to both drought and root diseases such as Phytophthora root rot. ABA-mediated responses include enhanced root growth, improved hydraulic conductivity, and upregulation of genes such as GmAREB1 and GmDREB2. Soybean genotypes with naturally higher ABA content tend to perform better under low soil moisture. However, exogenous ABA application in excess can delay flowering and pod filling, making precise regulation essential.

SA-induced resistance is particularly effective against Phytophthora sojae. SA priming in soybean has been associated with increased PR gene expression, PAL activity, and callose formation. Moreover, SA-based seed treatments improve early seedling vigor and resistance to damping-off diseases caused by Pythium spp. Combined use of SA and PGPR (e.g., *Bradyrhizobium japonicum*) further enhances disease resistance and nodulation, showing promise for sustainable soybean production.

Grapevine (*Vitis vinifera*)

In grapevine, ABA is heavily involved in fruit ripening and abiotic stress tolerance. It regulates sugar accumulation, anthocyanin synthesis, and drought-induced senescence. ABA sprays are commonly used to improve berry color and firmness, particularly in red varieties like Cabernet Sauvignon and Shiraz. However, such applications must be managed carefully to avoid over-ripening and desynchronization of sugar and acid balance.

SA, meanwhile, confers resistance against downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*). Preharvest SA treatments enhance phenolic content and antioxidant levels in grape skins, improving postharvest shelf life and wine quality. SA also interacts positively with UV-B exposure, further boosting stilbene synthesis—an important group of antimicrobial compounds in grapes.

Comparative Summary

Crop	ABA Role	SA Role	Outcome of ABA-SA Crosstalk
Rice	Drought tolerance, root deepening	Pathogen resistance, PR gene activation	Trade-off in combined drought-pathogen stress
Wheat	Antioxidant response, yield preservation	Rust resistance, SAR activation	Timing critical; excessive SA can cause senescence
Tomato	Stomatal control, drought resilience	Broad-spectrum pathogen defense	ABA can suppress SA-mediated immunity
Maize	Flowering resilience, antioxidant protection	Improved disease resistance via lignification	SA must be optimized due to inherent low levels
Soybean	Root architecture, water-use efficiency	PR induction, enhanced nodulation	Co-priming with microbes is highly effective
Grapevine	Ripening control, drought-induced senescence	Mildew resistance, phenolic synthesis	ABA for quality, SA for disease protection

In summary, these case studies demonstrate that the application and regulation of ABA and SA are highly crop-specific. What works for one species or variety may not yield similar outcomes in another due to differences in hormone sensitivity, transport capacity, receptor abundance, and gene expression profiles. Understanding these nuances is vital for designing crop-specific strategies that leverage hormonal signaling for stress mitigation. As research advances, integrating hormonal knowledge with genotype selection, agronomic practices, and technological interventions will enable precision crop management tailored to each species' hormonal landscape.

Biochemical Preparation and Analytical Protocols

The study and application of Abscisic Acid (ABA) and Salicylic Acid (SA) in plant defense research require rigorous biochemical preparation, quantification, and analysis of hormone concentrations, enzyme activities, gene expression, and metabolite profiles. Reliable detection of these hormones and their associated markers is essential for understanding stress responses, validating physiological observations, and guiding field-level interventions. This section outlines standardized protocols used to extract, quantify, and interpret ABA and SA activity and associated biochemical markers in both research and applied settings.

Quantification of endogenous ABA and SA levels is typically performed using chromatographic techniques, owing to their sensitivity, accuracy, and specificity. High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS/MS), and Gas Chromatography-Mass Spectrometry (GC-MS) are widely used for hormone profiling. Sample preparation begins with homogenization of fresh plant tissue (usually 100-500 mg of leaf or root) in cold extraction solvents such as 80% methanol or acetonitrile containing 1% formic acid. Antioxidants like butylated hydroxytoluene (BHT) are often added to prevent hormone degradation. Internal standards (e.g., deuterated ABA or 2-hydroxybenzoic acid) are spiked into the extract to ensure quantification accuracy.

After centrifugation and filtration, the supernatant is passed through solid-phase extraction (SPE) columns to purify the target compounds. Eluted fractions are dried under nitrogen gas and reconstituted in the appropriate mobile phase before injection into the HPLC or LC-MS/MS system. ABA is detected at 254 nm using a C18 reversed-phase column, whereas SA and its conjugates are typically separated on phenyl columns due to their aromatic nature. LC-MS/MS allows the simultaneous quantification of both free and conjugated forms (e.g., SA glucosides and methyl esters) with high specificity, based on their molecular ion and fragment ion transitions.

Enzyme activity assays are indispensable for evaluating the functional outcomes of hormonal signaling. Superoxide dismutase (SOD) activity is commonly measured by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), with absorbance read at 560 nm. Catalase (CAT) activity is assayed by monitoring the decomposition of hydrogen peroxide at 240 nm, while ascorbate peroxidase (APX) activity is measured by the decline in absorbance at 290 nm as ascorbate is oxidized. Peroxidase (POD) activity, particularly relevant to SA responses, is measured using guaiacol as a substrate, with absorbance at 470 nm.

For lipid peroxidation, malondialdehyde (MDA) levels are quantified using the thiobarbituric acid reactive substances (TBARS) assay. In this method, MDA reacts with thiobarbituric acid (TBA) under acidic and high-temperature conditions to form a pink chromogen, which is measured at 532 nm. Proline content, an important osmoprotectant in ABA-mediated drought responses, is typically assessed using the Bates method, in which proline forms a red complex with ninhydrin in acidic solution, measured at 520 nm.

Gene expression analysis provides molecular insight into hormone-induced signaling cascades. Total RNA is extracted from plant tissues using TRIzol reagent or column-based kits, followed by DNase treatment and quantification using a spectrophotometer or fluorometer. Reverse transcription is then performed using oligo-dT or random hexamer primers to synthesize complementary DNA (cDNA). Quantitative real-time PCR (qRT-PCR) is carried out using SYBR Green or TaqMan probes to quantify the expression levels of key hormone-responsive genes such as NCED3, RD29A, ABI5 (for ABA), and PR1, NPR1, WRKY70 (for SA). Housekeeping genes like Actin, GAPDH, or EF1α are used as internal controls for normalization. Fold changes are calculated using the $2^{-\Delta\Delta Ct}$ method, providing a quantitative measure of gene regulation under stress or treatment conditions.

For protein-level validation, Western blotting is employed to detect key signaling proteins such as SnRK2 (ABA pathway) or NPR1 and TGA transcription factors (SA pathway). Plant protein extracts are separated by SDS-PAGE, transferred to PVDF membranes, and probed with specific primary antibodies, followed by HRP-conjugated secondary antibodies. Chemiluminescent detection allows

semi-quantitative assessment of protein expression and post-translational modifications.
Metabolite profiling is crucial for understanding the downstream biochemical consequences of hormonal treatments. Primary metabolites such as sugars, organic acids, and amino acids are extracted in aqueous or methanol-based solvents and analyzed using GC-MS or LC-MS. Secondary metabolites such as phenolic acids, flavonoids, and lignins—particularly important in SA-mediated defense—are extracted using acidified methanol and quantified by spectrophotometric or chromatographic methods. Total phenolic content is estimated using the Folin-Ciocalteu reagent, with gallic acid as a standard.
In field conditions, portable tools such as enzyme test kits, colorimetric strips, and biosensors are increasingly being used for rapid detection of hormonal and biochemical indicators. ABA and SA test kits based on ELISA (enzyme-linked immunosorbent assay) enable detection in leaf sap or xylem extracts with high throughput. Similarly, ROS levels and antioxidant capacity can be monitored using handheld fluorometers and conductivity meters. These tools provide practical advantages for on-site diagnostics, especially in precision agriculture and stress management trials.
Preparation of hormone solutions for exogenous applications also requires careful consideration. ABA stock solutions are typically prepared in ethanol or DMSO and diluted in water with surfactants (e.g., Tween-20) for foliar spraying. SA is often applied in aqueous solutions buffered at neutral pH. Optimal concentrations vary by species and developmental stage, but common field-use ranges are 10-100 µM for ABA and 50-500 µM for SA. Overdosing can lead to phytotoxicity, growth inhibition, or hormonal imbalances, underscoring the importance of pre-application calibration.
Finally, statistical analysis and replication are essential components of biochemical experimentation. All assays should be conducted in biological triplicates, and standard curves or calibration plots must be constructed for each analyte. Analysis of variance (ANOVA) and post hoc tests (e.g., Tukey's HSD) are typically used to determine the significance of differences between treatments. Data visualization using bar graphs, heatmaps, or principal component analysis (PCA) further enhances interpretation and communication of results.

Discussion

The intricate roles of Abscisic Acid (ABA) and Salicylic Acid (SA) in mediating plant responses to environmental and biological stresses reflect an evolutionary adaptation to multifactorial challenges in terrestrial ecosystems. In agroecosystems, where environmental variability and pathogen pressure intersect with anthropogenic practices, the ability to modulate hormonal defense pathways becomes crucial for sustaining productivity and resilience. This review has underscored the distinct and overlapping functions of ABA and SA, highlighting the biochemical, molecular, and field-level implications of their actions. However, understanding these hormones in isolation offers only a partial view; the complexity of their crosstalk, dose-dependent interactions, and trade-offs must also be contextualized within broader plant physiological and ecological frameworks.
ABA has emerged as the central coordinator of abiotic stress responses, particularly drought, salinity, and temperature extremes. It regulates a well-defined set of physiological processes—stomatal closure, osmotic adjustment, antioxidant defense, and gene expression—that collectively enhance water-use efficiency and cellular stability. The consistent upregulation of NCED genes and downstream effectors such as SnRK2 kinases under abiotic stress demonstrates the robustness of ABA's signaling network. In multiple crop species, exogenous application of ABA or overexpression of ABA-related genes has translated into measurable agronomic benefits, including delayed wilting, improved root growth, and yield stability under water-limiting conditions. However, this stress tolerance often comes at the cost of suppressed immune function and growth retardation, especially when ABA is applied at inappropriate developmental stages or in pathogen-prone environments.
SA, by contrast, governs plant immunity, particularly resistance to biotrophic pathogens, through the activation of systemic acquired resistance (SAR) and the expression of pathogenesis-related (PR) proteins. Its role in priming the plant's immune system for future attacks—without incurring the energetic cost of full defense activation—is a key advantage of SA-mediated signaling. This priming effect has been successfully harnessed in field applications, especially in integrated pest management (IPM) programs. Yet, the deployment of SA in stress-prone agroecosystems must be handled with caution, as excessive SA accumulation can exacerbate oxidative stress, interfere with hormonal homeostasis, and lead to growth inhibition.
A recurring theme throughout the literature is the antagonistic interaction between ABA and SA, particularly under simultaneous abiotic and biotic stress. This antagonism appears to be an evolutionary compromise, wherein the plant prioritizes the most immediate or life-threatening challenge. Under drought, ABA signaling dominates to conserve water and maintain turgor, even if it means downregulating immune defenses. Conversely, during pathogen attack, SA pathways override ABA effects to mount a rapid defense response, even at the expense of increased water loss or reduced photosynthesis. The transcriptional suppression of SA-responsive genes by ABA (e.g., PR1, NPR1) and vice versa (e.g., NCED repression by SA) exemplifies this biochemical balancing act.
From a crop improvement perspective, this hormonal trade-off presents both a challenge and an opportunity. Enhancing one pathway often compromises the other, necessitating fine-tuned regulation for optimal performance under dual stress conditions. Emerging strategies such as tissue-specific expression of hormonal genes, inducible promoters, and controlled hormone-release formulations offer a way to overcome this limitation. For example, expressing ABA biosynthesis genes predominantly in roots and SA-responsive genes in leaves could spatially separate the hormonal responses, preserving both drought tolerance and immune competence. Additionally, the use of hormone analogs or priming agents at sub-lethal concentrations has shown promise in inducing a low-level defense state without triggering full antagonism.
The integration of omics data—transcriptomic, proteomic, and metabolomic—into hormonal studies has further refined our understanding of ABA and SA responses. These data reveal that hormonal effects are not binary but exist along a continuum of interactions modulated by dosage, tissue type, developmental stage, and environmental context. Key

transcription factors such as WRKY70, MYB44, and TGA5 have been identified as convergence points where hormonal signaling integrates with other environmental cues. Moreover, post-translational modifications, such as phosphorylation and redox changes, add additional layers of control that fine-tune hormone responses at the protein level.

Epigenetic mechanisms also contribute to the modulation of hormone signaling pathways. Histone modifications and DNA methylation patterns can "prime" stress-responsive genes for faster or stronger activation upon repeated exposure. This stress memory, particularly well-documented in SA-mediated immunity, offers a biological foundation for durable resistance in plants and opens new avenues for breeding crops with enhanced resilience.

In the field, variability in hormone responsiveness among different cultivars adds yet another dimension to the discussion. Genotypic differences in ABA and SA sensitivity, receptor density, and signal transduction efficiency can lead to significant differences in stress outcomes. For instance, drought-tolerant rice varieties often exhibit higher ABA accumulation and stronger antioxidant responses, while disease-resistant varieties may show faster or more sustained SA-induced PR gene expression. Identifying and selecting for such traits using molecular markers or genomic selection could accelerate breeding for multi-stress resilience.

However, deploying hormone-based strategies in agriculture must also consider environmental sustainability, economic feasibility, and regulatory compliance. While ABA and SA applications have demonstrated efficacy in controlled experiments, their field use must account for environmental degradation, cost of synthesis, and potential off-target effects on non-target organisms. Biologically derived or microbially synthesized hormones present a more sustainable alternative to synthetic formulations and are likely to gain favor in organic and low-input farming systems.

In conclusion, the role of ABA and SA in plant defense extends beyond their individual biochemical pathways; it encompasses a broader regulatory network that integrates environmental signals, internal physiological states, and evolutionary priorities. Their coordinated action, antagonism, and synergy define the plant's capacity to survive, adapt, and thrive in complex and changing environments. Future research must focus on unraveling this hormonal matrix with even greater precision, using systems biology, artificial intelligence, and synthetic biology tools. The ultimate goal is to develop predictive models and agronomic strategies that allow for real-time modulation of hormone responses, ensuring both yield stability and environmental compatibility in modern agriculture.

Conclusion

Abscisic Acid and Salicylic Acid represent two of the most pivotal hormonal regulators in plant biology, orchestrating complex and highly adaptive responses to abiotic and biotic stresses, respectively. Their roles in agroecosystems extend from mediating cellular defense mechanisms to influencing whole-plant physiology and crop yield stability. ABA acts primarily as a guardian against water-related stresses by promoting stomatal closure, activating antioxidant systems, and inducing osmoprotectants, while SA mobilizes the plant's immune system, activating pathogenesis-related genes and triggering systemic acquired resistance.

The biochemical and molecular distinctions between these hormones are well-established; yet, the increasingly recognized crosstalk between ABA and SA, particularly under multifactorial stress conditions, reflects the real-world complexity of plant-environment interactions. This antagonistic yet context-dependent interplay poses a significant challenge for crop management—highlighting the need for precise and context-aware hormonal modulation.

Advances in molecular biology, omics technologies, and precision agriculture have significantly enhanced our understanding of ABA and SA pathways. Tools such as qRT-PCR, LC-MS/MS, proteomics, and gene-editing technologies have allowed researchers to map these signaling networks with unprecedented resolution. Field applications—ranging from seed priming to foliar sprays and microbial inoculants—demonstrate that hormonal interventions can be successfully translated from the lab to the farm, provided their use is tailored to specific crop species, growth stages, and environmental conditions.

However, as agriculture faces mounting pressures from climate change, declining soil fertility, and evolving pathogen threats, a deeper integration of hormonal biology with systems-level crop management is needed. Strategic manipulation of ABA and SA—whether through breeding, biotechnology, or biostimulant formulations—must be approached with a balance of scientific rigor, agronomic practicality, and ecological awareness.

In summary, ABA and SA are not merely biochemical messengers but central agents of plant decision-making in stress environments. Their nuanced control over survival strategies offers both challenges and opportunities for modern agriculture. By continuing to unravel their pathways and interactions, and by leveraging these insights through targeted applications, we can enhance crop resilience, secure food production, and foster a more sustainable future for agroecosystems worldwide.

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