The Role of Salicylic Acid in Plant Defense Activation

Executive Summary

Salicylic acid (SA), a naturally occurring phenolic compound, stands as a pivotal and multifaceted plant hormone, orchestrating a plant's robust defense mechanisms against a diverse array of biotic and abiotic stressors. Recognized as the sixth plant hormone in 1992, SA's influence extends beyond its well-established role in mediating immunity against pathogens to actively mitigating the detrimental effects of environmental challenges such as chilling, drought, salinity, and heavy metals. This dual functionality positions SA as a central hub for overall plant adaptation and survival, underscoring a fundamental, integrated role in plant resilience that has been shaped by evolutionary pressures to integrate diverse environmental signals.

The intricate mechanisms governing SA involve sophisticated biosynthesis pathways, precise perception by dedicated receptors, and complex signal transduction networks that culminate in the induction of systemic acquired resistance (SAR), a whole-plant immune response. SA's interactions with other phytohormones, particularly jasmonic acid (JA) and ethylene (ET), are often antagonistic, representing a strategic allocation of defense resources tailored to specific threats. Understanding these sophisticated molecular processes and their broader physiological implications is increasingly vital for developing sustainable agricultural practices aimed at enhancing crop resilience and yield in a changing climate.

1. Introduction to Salicylic Acid as a Plant Hormone

Salicylic acid (SA), chemically known as 2-hydroxybenzoic acid, is a crucial naturally occurring phenolic compound that functions as a vital plant hormone. Its formal recognition as the sixth plant hormone in 1992 marked a significant advancement in the field of plant biology, shifting the scientific understanding of plant defense from a passive, localized reaction to an active, regulated, and systemic signaling process. This conceptual leap was built upon earlier foundational research, with observations of SA's role in disease resistance in tobacco plants dating back to 1979. This historical progression highlights a critical paradigm shift, establishing SA as a central signaling molecule that fundamentally altered perspectives on plant immunity and stress physiology.

While SA has secured a strong reputation as a vital defense hormone, mediating a plant's response to various pathogens including viruses, fungi, insects, and bacteria, its involvement extends far beyond immunity. SA actively participates in a plant's adaptive responses to diverse abiotic stresses, such as chilling, drought, salinity, heavy metals, and extreme heat, acting as a "therapeutic agent" for crops. This broad impact on stress tolerance underscores its integrated role in overall plant resilience.

Furthermore, SA's influence is well-established in various aspects of plant growth and development, impacting cell, tissue, and organ phenotypes. It plays a role in photosynthesis, transpiration, ion uptake, and transport, positioning it as an important growth regulator throughout the plant lifecycle. However, the role of SA is not monolithic across the entire plant.

Its content and dynamic during development can differ significantly between roots and shoots, depending on the species. For instance, basal SA levels in shoots can be 2–100 times higher than in roots, and the ratio between free and conjugated SA forms also varies. These differences can potentially lead to distinct SA functions in different organs. Observations regarding changes in root morphology in SA mutants are often contradictory, suggesting that SA's morphogenetic role, particularly in roots, is less understood and varies based on tissue, developmental stage, and species. This variability underscores the nuanced and context-dependent nature of SA's functions, requiring careful consideration of specific plant species and environmental conditions when discussing its diverse roles.

2. Biosynthesis and Metabolism of Salicylic Acid

The intricate processes by which salicylic acid is synthesized and metabolized within plants are critical for its precise regulation and function in defense. SA, a phenolic compound, is primarily derived from chorismate, a key intermediate in the shikimate pathway. Plants predominantly employ two main biosynthetic routes: the isochorismate synthase (ICS) pathway and the phenylalanine ammonia-lyase (PAL) pathway.

The **isochorismate (ICS) pathway** is generally recognized as the primary route for SA synthesis in many plant species, particularly in the model plant *Arabidopsis thaliana*, where it is the most significant contributor to SA accumulation. This pathway begins in the chloroplast, where chorismate is converted to isochorismate by the enzyme isochorismate synthase (ICS1). The resulting isochorismate is then transported from the chloroplasts to the cytosol via the ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) transporter, where it undergoes further modifications to yield bioactive SA. Upon pathogen perception, the expression of crucial SA biosynthetic genes, including *ICS1*, *EDS5*, and *PBS3*, is positively regulated by transcription factors such as SAR-Deficient 1 (SARD1) and Calmodulin Binding Protein 60g (CBP60g).

The **phenylalanine ammonia-lyase (PAL) pathway** also contributes to SA accumulation, though its importance varies across plant species. For instance, in rice, the PAL pathway appears to be more crucial for SA accumulation, while in soybeans, both the ICS and PAL pathways may contribute equally. The synthesis of SA occurs in both the chloroplast and cytosol, highlighting the compartmentalized nature of these processes. The variation in the predominant SA biosynthesis pathway across different plant species suggests an evolutionary adaptation. This redundancy or pathway preference might provide robustness against pathogen strategies that target specific biosynthetic enzymes, ensuring that SA production can still occur even if one pathway is compromised. This diversification of biosynthetic routes serves as a measure to avoid pathogen-mediated disablement of plant defense.

SA biosynthesis and accumulation are under tight regulatory control. SA levels are known to increase significantly in many plant-pathogen interactions following infection by viruses, fungi, insects, and bacteria. Exogenous application of SA can also effectively induce disease resistance. Genetic studies have revealed that loss-of-function mutations in positive regulators, such as ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4), lead to reduced SA accumulation and consequently, enhanced disease susceptibility. Conversely, mutations in negative regulators often result in increased SA accumulation and enhanced disease resistance. EDS1, a crucial positive SA regulator, interacts with PAD4 and SENESCENCE-ASSOCIATED GENE 101 (SAG101) to contribute to SA accumulation and basal defense. SA biosynthesis is also subject to negative feedback loops; SA-responsive transcription factors like WRKY70 and WRKY54 can rapidly inhibit the activity of SARD1 and

CBP60g, thereby reducing SA biosynthesis. Similarly, WRKY18 and WRKY40 directly negatively regulate the expression of *ICS1*, *EDS5*, and *PBS3* genes.
Once synthesized, SA undergoes various modifications, primarily to convert it into inactive derivatives, allowing for precise control and modulation of the defense signal. This extensive metabolic buffering system enables plants to rapidly adjust SA levels, preventing over-activation of metabolically costly defense responses and facilitating fine-tuned control over the intensity and duration of the immune signal.

- **Glucosylation:** SA can be conjugated with glucose to form SA glucoside (SAG) by enzymes such as SA GLUCOSIDE TRANSFERASE 1 (SAGT1), SAGT2, and UDP-DEPENDENT GLYCOSYLTRANSFERASE 76B1 (UGT76B1). SAG can be stored in large quantities in the vacuole, serving as a reservoir of inactive SA. NPR1 and NPR4 have been shown to regulate the expression of *UGT76B1*, thereby influencing SAG production.
- **Methylation:** SA can be methylated to methyl salicylate (MeSA) by BA/SA CARBOXYL METHYLTRANSFERASE 1 (BSMT1). MeSA is volatile, increasing its membrane permeability, and can be released from the plant to serve as a cue for plant-insect interactions. Notably, inactive MeSA can be reactivated back to SA by the methyl esterase activity of several *MES* genes, providing a mechanism for rapid signal modulation, allowing for quick amplification or attenuation as needed.
- **Amino Acid Conjugation:** This modification is also involved in SA catabolism.
- **Hydroxylation:** Hydroxylation of SA leads to the formation of 2,3- and 2,5-dihydroxybenzoic acid (2,3-DHBA and 2,5-DHBA). Enzymes like DOWNY MILDEW RESISTANT 6 (DMR6) and DMR6-LIKE OXYGENASE 1 (DLO1) catalyze this process, generating inactive forms of SA.

It is important to note that basal SA levels and the ratio between free and conjugated SA forms can differ significantly between shoots and roots, and also vary greatly among different plant species, further underscoring the dynamic and context-specific nature of SA metabolism.

3. Salicylic Acid Perception and Signal Transduction

The molecular mechanisms by which plants perceive salicylic acid and translate this perception into robust defense responses are central to plant immunity. The primary SA receptors identified are the NONEXPRESSOR OF PATHOGENESIS-RELATED GENES (NPR) proteins: NPR1, NPR3, and NPR4. The existence of multiple SA receptors and other SA-binding proteins (e.g., SABP1, SABP2, SABP3) suggests a robust and potentially redundant system for SA perception. This redundancy could be an evolutionary strategy to ensure defense activation even if one receptor is compromised by pathogens or environmental factors, thereby enhancing the system's resilience.
NPR1 stands as a central positive regulator of systemic acquired resistance (SAR) and overall plant immunity. It functions as a transcription coactivator, orchestrating the expression of numerous defense-related genes. NPR3 and NPR4, paralogues of NPR1, also act as SA receptors and have been shown to negatively regulate SAR. In unstressed plants, SA binding to NPR3 and NPR4, in conjunction with TGA2/5/6 transcription factors, can repress the expression of downstream defense genes. However, under high SA levels, their co-repressor activity is inhibited, releasing this repression and allowing defense activation.
The molecular mechanisms of SA perception and activation are intricate:

- **Direct SA Binding:** SA directly binds to NPR1, NPR3, and NPR4, establishing them as

bona fide SA receptors.

- **Redox Regulation and Monomerization:** Under non-stress conditions, NPR1 typically exists as a large cytoplasmic oligomer. Pathogen infection or SA treatment induces an oxidative burst, which is then followed by a reducing environment within the cell. This shift in redox state is believed to contribute to NPR1 monomerization and its subsequent translocation into the nucleus, a crucial step for its activity. This process involves *S-*nitrosylation and reduction catalyzed by thioredoxin H-type 3 (TRX-h3) and thioredoxin H-type 5 (TRX-h5). While this oligomer-monomer transit model has been widely accepted, recent findings have questioned its strictness, suggesting that NPR1 might be predominantly monomeric *in vivo* under certain conditions.
- **Nuclear Translocation:** Once monomerized, NPR1 translocates from the cytoplasm to the nucleus, where it can exert its transcriptional regulatory functions.
- **Cofactor Requirement:** A unique and significant molecular detail is the requirement of the transition metal copper for SA-binding to NPR1. Mutations in specific cysteine residues (Cys521 and Cys529) within NPR1's C-terminal transactivation domain not only disrupt its SA-binding capacity but also eliminate copper recruitment. This explains why many researchers initially struggled to identify NPR1 as an SA receptor, as the common laboratory chelator EDTA, when present in buffers, can preclude SA from binding to NPR1. This discovery implies that copper availability could directly impact the efficacy of SA-mediated defense, linking mineral nutrition to immune function.
- **Interaction with Transcription Factors:** In the nucleus, NPR1 interacts with members of the TGA transcription factor family (e.g., TGA2, TGA5, TGA6) to activate the expression of pathogenesis-related (PR) genes and other defense-related genes. NPR1 forms an "enhanceosome" with TGA2 on the promoter region of genes like *PR1*, facilitating their transcription. NPR1 also interacts with NIMIN proteins and WRKY transcription factors , which are crucial for fine-tuning the defense response.
- **SUMOylation:** The conjugation of Small Ubiquitin-like Modifier (SUMO) proteins to NPR1 is another regulatory mechanism that converts its association with transcriptional repressors to transcriptional activators, providing fine-tuned control over gene expression.
- **NPR1 Turnover:** The activity of NPR1 is also regulated at the protein stability level. "Spent" NPR1 is removed from gene promoters through polyubiquitination and subsequent proteasomal degradation, a process that is itself regulated by deubiquitinases like UBP6/7, which can prolong NPR1 longevity.

Beyond these core mechanisms, other key components and regulatory loops contribute to the SA signaling network. ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) is a critical positive SA regulator that interacts with PHYTOALEXIN DEFICIENT 4 (PAD4) and SENESCENCE-ASSOCIATED GENE 101 (SAG101). These proteins are essential for SA accumulation and for conferring basal defense against various pathogens. The expression of EDS1 and PAD4 is inducible by SA, suggesting positive feedback or signal amplification loops within the SA pathway. SARD1 and CBP60g are transcription factors that positively regulate the expression of SA biosynthetic genes, contributing to the initial surge in SA levels upon pathogen perception. WRKY and TGA transcription factors are dominant players in the early stages of the SA response; they not only activate late response genes but also participate in negative regulation of SA biosynthesis, forming crucial feedback loops. This intricate negative regulatory mechanism within the SA signaling pathway, including the repressive roles of NPR3/NPR4 in unstressed states, the negative feedback loops involving WRKY transcription factors on SA biosynthesis, and the regulated turnover of NPR1, highlights a sophisticated system for preventing over-activation of costly defense responses. This fine-tuning ensures that defense is

mounted efficiently only when necessary, balancing energy allocation between growth and defense.

NIM1-INTERACTING PROTEIN 3 (NIMIN3) can constitutively repress NPR1 activity in unstressed plants, while NIMIN2 can bolster NPR1-mediated signaling, showcasing a dynamic interplay in SA pathway regulation. While NPR1 is central, SA-dependent, NPR1-independent pathways also exist, with some being mediated by transcription factors like AtWhirly1. The molecular identities of other NPR1-independent pathways, often revealed through genetic analyses of lesion mimic mutants (LMMs), are largely unknown.

Table 1: Key Components of the Salicylic Acid Signaling Pathway

Component Name	Type	Primary Role in SA Pathway	Key Interactions/Mechanism	Relevant Snippet IDs
NPR1	Receptor, Coactivator	Central positive regulator of SAR; Activates defense gene expression	Binds SA; Undergoes redox-dependent monomerization; Translocates to nucleus; Requires copper cofactor; Interacts with TGA, NIMIN, WRKY TFs; SUMOylated; Regulated by ubiquitination	
NPR3/NPR4	Receptor, Regulator	Negative regulators of SAR; Repress defense genes in unstressed state	Binds SA; Inhibited by high SA levels; Interacts with TGA TFs	
EDS1	Regulator	Positive SA regulator; Basal defense against pathogens	Putative lipase; Interacts with PAD4, SAG101; Inducible by SA	
PAD4	Regulator	Positive SA regulator; Basal defense against pathogens	Putative lipase; Interacts with EDS1; Inducible by SA	
SARD1	Transcription Factor	Positive regulator of SA biosynthesis genes	Positively regulates ICS1, EDS5, PBS3 expression; Inhibited by WRKY TFs	
CBP60g	Transcription Factor	Positive regulator of SA biosynthesis genes	Positively regulates ICS1, EDS5, PBS3	

Component Name	Type	Primary Role in SA Pathway	Key Interactions/Mechanism	Relevant Snippet IDs
			expression; Inhibited by WRKY TFs	
TGA TFs	Transcription Factor	Regulate SA-responsive gene expression; Crosstalk with JA/ET	Interact with NPR1 to activate PR genes; Repress JA/ET genes (e.g., ORA59)	
WRKY TFs	Transcription Factor	Regulate SA-responsive gene expression; Negative feedback on SA biosynthesis	Act downstream of NPR1; WRKY70/54 inhibit SARD1/CBP60g; WRKY18/40 negatively regulate ICS1, EDS5, PBS3; Repress JA genes	
UGT76B1	Enzyme	Converts SA to inactive SA glucoside (SAG)	Expression regulated by NPR1 and NPR4; Involved in SA homeostasis	
ICS1	Enzyme	Catalyzes first step of SA biosynthesis from chorismate	Chloroplast-localized; Key enzyme in primary SA pathway	
EDS5	Transporter	Transports isochorismate from chloroplast to cytosol	Multidrug and toxin extrusion family; Essential for SA biosynthesis	
BSMT1	Enzyme	Methylates SA to inactive methyl salicylate (MeSA)	Involved in SA inactivation and volatility	
DMR6/DLO1	Enzyme	Hydroxylates SA to inactive forms	Generates 2,3- and 2,5-dihydroxybenzoic acid	

4. Salicylic Acid in Plant Immunity: Specific Defense Responses

Salicylic acid plays a central and indispensable role in orchestrating plant immunity, activating a range of specific defense responses against biotic threats.

4.1. Systemic Acquired Resistance (SAR)

Systemic Acquired Resistance (SAR) is a cornerstone of plant immunity, representing a "whole-plant" resistance response that is induced following an initial localized exposure to a pathogen. This sophisticated, adaptive-like immune response in plants is often considered analogous to the innate immune system found in animals, demonstrating a system-wide protective mechanism. SAR confers broad-spectrum resistance against a wide array of pathogens, including bacteria, viruses, fungi, and various pests. Key characteristics of SAR include its systemic spread throughout the entire plant, even to tissues distant from the initial infection site. This allows the entire plant to be prepared to resist potential attacks, even in areas far from the primary infection site. Furthermore, SAR is long-lasting and establishes a form of immunological memory, enabling the plant to respond more robustly and swiftly to subsequent encounters with the same or related pathogens. This "memory" is a crucial conceptual understanding, elevating plant defense beyond simple, localized reactions to a systemic, remembered, and broad-spectrum protective mechanism. Additionally, SAR can confer cross-resistance, protecting against unrelated pathogens, which further broadens the plant's defense capabilities.

Salicylic acid is unequivocally a critical signaling molecule in the development and maintenance of SAR. Its levels dramatically increase in plant tissues upon pathogen infection, a pattern consistently observed across many species, including tobacco and cucumber. Exogenous application of SA or its synthetic analogs is sufficient to induce disease resistance and SAR in plants. Conversely, transgenic plants engineered to be unable to accumulate SA (e.g., those expressing the bacterial salicylate hydroxylase gene *NahG*, which inactivates SA by converting it to catechol) are severely impaired in their SAR response and unable to mount an appropriate defensive response to various pathogens. While SA is essential for SAR, it is thought not to be the sole mobile signal itself; rather, SAR is orchestrated through a collaborative effort between SA and other molecules like pipecolic acid.

A hallmark of SAR is the accumulation of pathogenesis-related (PR) proteins. These proteins serve as "marker genes" for SAR, being strongly induced as part of the systemic response. The diverse functions of PR protein families reveal that SA doesn't just trigger a generic defense but mobilizes a highly specialized and multi-faceted arsenal. This suggests a broad-spectrum, yet targeted, approach to pathogen neutralization, reflecting the diverse nature of plant pathogens. PR proteins belong to numerous different families, each possessing distinct antimicrobial properties and contributing to the plant's broad-spectrum defense.

4.2. Role in Pattern-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI)

SA's influence extends to the foundational layers of plant immunity, playing crucial roles in both Pattern-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI).

- **Pattern-Triggered Immunity (PTI):** Plants initiate PTI when they recognize conserved pathogen-associated molecular patterns (PAMPs) via plasma membrane-localized pattern recognition receptors (PRRs). SA signaling is indispensable for the full activation and effectiveness of PTI, with SA-insensitive receptor mutants showing compromised protection against bacterial pathogens.
- **Effector-Triggered Immunity (ETI):** Pathogens often evolve to deploy effector proteins that suppress PTI, leading to effector-triggered susceptibility (ETS). In response, plants

have evolved resistance (R) genes that encode proteins capable of recognizing these specific pathogen effectors. This recognition triggers ETI, which is typically a more robust and rapid defense response, often culminating in a localized programmed cell death known as the hypersensitive response (HR). Sufficient accumulation and proper perception of SA are essential for the robust activation of ETI.

4.3. Key SA-Responsive Genes and Proteins

Beyond the widely recognized PR proteins, SA activates a vast network of genes and proteins critical for both local and systemic immune responses. These include genes encoding components of the SA signaling pathway itself, such as *EDS1*, *PAD4*, *SAG101*, *ALD1*, *WRKY70*, and *SARD1*. The inducibility of many SA regulators by SA treatment suggests the presence of positive feedback or signal amplification loops within the SA pathway. This mechanism allows for a rapid and robust escalation of the defense response once a threat is detected, ensuring a strong and timely reaction. In *Arabidopsis*, SA induces downstream defense gene expression primarily through its receptors NPR1, NPR3/NPR4, and their interaction with TGA2/5/6 transcription factors.

PR proteins are a diverse group, each contributing specialized functions to the plant's defense arsenal. Examples of PR protein families and their primary functions include:

Table 2: Major Pathogenesis-Related (PR) Protein Families Induced by Salicylic Acid

PR Family	Domain Classification	Key Proteins/Examples	Primary Function in Plant Defense	Relevant Snippet IDs
PR-1	IPR034111, IPR001283	PR-1a, PR-1b, PR-1c, MhPR1	Antifungal (CAP), sterol-binding antimicrobial properties	
PR-2	(GH17)	β-1,3-Glucanases	Cleaves β-1,3-glucans; Antifungal properties	
PR-3	IPR016283	Chitinase types I, II, IV, V, VI, VII	Endochitinase; Degrades fungal cell walls	
PR-4	IPR001153	Barwin domain chitinase I/II	Antifungal and chitinase activity	
PR-5	IPR001938	Thaumatin-like, MhPR5	Antifungal activity	
PR-6	IPR000864	Potato protease I	Proteinase inhibitor	
PR-7	(Subtilisin-like)	Tomato endoproteinase P69	Endoproteinase activity	
PR-8	(GH18)	Cucumber chitinase, MhPR8	Chitinase III activity	
PR-9	(Haem peroxidase III)	Tobacco lignin-forming peroxidase	Peroxidase activity	

PR Family	Domain Classification	Key Proteins/Examples	Primary Function in Plant Defense	Relevant Snippet IDs
PR-10	IPR024949, IPR000916	Parsley "PR-1"	Ribonuclease-like activity	
PR-11	(GH18)	Tobacco chitinase V	Chitinase activity	
PR-12	IPR008176	Radish Rs-AFP3	Plant Defensin	
PR-13	IPR001010	Arabidopsis THI2.1	Thionin	
PR-14	IPR000528	Lipid transfer proteins	Shuttling of phospholipids and fatty acids	
PR-15	IPR001929	Barley OxOa	Germin; Oxalate oxidase	
PR-16	IPR001929	Barley OxOLP	Germin-like	
PR-17	IPR007541	Tobacco NtPRp27	Late blight resistance(?)	

This comprehensive list illustrates that SA-induced defense is not a single, generic response but a multi-pronged strategy involving various specialized proteins, each contributing to the plant's robust and adaptable defense system against a wide array of threats.

5. Crosstalk with Other Phytohormones in Defense Networks

Plant defense responses are not governed by salicylic acid alone; they involve complex and dynamic interactions, often antagonistic, with other phytohormones. This intricate crosstalk allows plants to fine-tune their defense strategies based on the nature of the threat.

5.1. Antagonistic Interactions with Jasmonic Acid (JA) and Ethylene (ET)

The SA-mediated defense response primarily targets biotrophic pathogens, such as *Pseudomonas syringae*, which establish feeding relationships with living host tissue by producing nutrient-absorbing structures while keeping the host alive. In contrast, the jasmonic acid (JA) and ethylene (ET) pathways are crucial for defense against necrotrophic pathogens (e.g., *Botrytis cinerea*), which invade and kill host cells to extract nutrients. These pathways are also involved in responses to herbivory and wounding. Due to these distinct functional roles, the SA and JA/ET defense networks are typically mutually antagonistic; the activation of one pathway often inhibits signaling via the other. This mutual antagonism represents a critical resource allocation strategy, as plants must prioritize their defense responses based on the specific pathogen lifestyle. Activating one pathway often comes at the cost of suppressing the other, preventing the plant from expending energy on inappropriate or conflicting defense mechanisms, thereby highlighting the evolutionary pressure for efficient resource management in defense.

The mechanisms of SA-mediated repression of JA/ET pathways are multifaceted:

- **Transcriptional Repression:** SA primarily represses ET/JA signaling at the gene

transcriptional level.

- **WRKY Transcription Factors:** The SA-induced WRKY transcription factor WRKY70 has been shown to suppress JA-induced *PDF1.2* expression. WRKY70 and its homologue WRKY54 also actively repress genes within the JA signaling network, acting as key crosstalk nodes.
- **TGA Transcription Factors:** Class-II TGA transcription factors (TGA2, TGA5, and TGA6), which are positive regulators of the NPR1-dependent SA-signaling pathway, also exert both positive and negative roles in the ET/JA-signaling pathway. These TGAs are required for SA-mediated repression of the ET/JA-response and can bind to the promoter of *ORA59*, a master regulator of the ERF-branch of the JA/ET pathway.
- **ROXY19:** The SA-inducible plant-specific glutaredoxin *ROXY19* (also known as *GRX480* or *GRXC9*) physically interacts with clade II TGA factors and strongly represses ET/JA-induced *ORA59* and *PDF1.2* expression in a TGA-dependent manner.
- **ORA59 Protein Degradation:** Some evidence suggests that SA may repress ET/JA signaling through the degradation of the transcriptional activator ORA59 protein itself, rather than just its gene expression.
- **NPR1 as an Integrator:** NPR1, the master regulator of SA signaling, is identified as an essential integrator for SA-ET/JA crosstalk. It is required for the SA-induced expression of *WRKY70* and *ROXY19*, which in turn repress the ET/JA pathway. Intriguingly, NPR1's nuclear translocation, while crucial for its SA-mediated response, is not required for its role in SA-ET/JA crosstalk, suggesting a cytoplasmic function in this process. This highlights NPR1's multifaceted importance beyond just SA signal transduction, indicating it is a central regulatory hub that processes and integrates multiple hormonal signals to fine-tune the overall immune response.
- **Epigenetic Regulation:** SA may influence JA signaling by inducing transcription-repressive epigenetic marks, such as trimethylation of lysine 27 in histone H3, on JA-responsive defense genes like *PDF1.2*. Histone acetyltransferases (HAT) and deacetylases (HDA) are also involved in regulating the ET/JA-signaling pathway, suggesting potential points of SA-mediated control.

The antagonism is reciprocal; activation of the ET/JA pathway also represses the SA response. For example, deletion of the JA receptor COI1 and the JA-responsive MYC branch leads to increased SA accumulation and enhanced resistance to biotrophic pathogens. This reciprocal suppression means that increased SA levels can enhance susceptibility to necrotrophic pathogens, while a deficiency in SA may have no significant impact or only affect resistance at the primary infection site.

A critical aspect of this co-evolutionary arms race is that some pathogens can actively exploit the SA-JA/ET crosstalk to their advantage. For instance, *Pseudomonas* bacteria secrete coronatine (COR), a JA-Ile mimic, which activates the JA response. This activation, through the MYC branch, leads to the expression of NAC transcription factors (*ANAC019*, *ANAC055*, and *ANAC072*), which directly repress *ICS1* (a key SA biosynthesis gene) and activate *BSMT1* (involved in SA methylation), thereby reducing SA accumulation. Similarly, some necrotrophic pathogens, like *Botrytis cinerea*, can exploit the SA pathway to cause disease by suppressing the JA signaling system, for example, by secreting a virulence factor β-(1,3)(1,6)-D-glucan that activates the SA pathway. This manipulation reveals that pathogens have evolved mechanisms not just to evade or suppress defense, but to actively interfere with the plant's own signaling pathways to induce susceptibility, which has significant implications for developing durable crop resistance.

5.2. Synergistic Interactions and Complex Interplay

While antagonism is a dominant theme, interactions between SA and other hormones are not exclusively repressive. Treatment with low concentrations of both SA and JA has been reported to result in synergistic expression of both the SA target gene *PR1* and the JA marker gene *PDF1.2*. This suggests that the nature of the crosstalk can be concentration-dependent and context-specific, allowing for nuanced responses depending on the precise environmental cues and pathogen pressure.

Other phytohormones, including abscisic acid (ABA), auxin, brassinosteroids (BR), gibberellic acid (GA), cytokinins (CK), strigolactones (SL), and signaling molecules like nitric oxide (NO), are also involved in plant immune responses and often modulate the core SA-ET/JA pathways. For example, brassinosteroids can exhibit complex roles, sometimes inducing broad-spectrum disease resistance independently of SA, or conversely, attenuating SA-induced gene expression. SA itself has been shown to influence BR signaling, further illustrating the intricate web of hormonal interactions that fine-tune plant defense.

Table 3: Summary of Salicylic Acid Crosstalk with Jasmonic Acid and Ethylene

Hormone Pathway	Primary Pathogen Type	Interaction Type	Key Molecular Mechanisms of Crosstalk	Consequences for Plant Defense	Relevant Snippet IDs
SA	Biotrophs (e.g., *Pseudomonas syringae*), Hemibiotrophs	Antagonistic with JA/ET	SA-induced WRKY TFs (WRKY70) repress JA genes; SA-induced TGA TFs repress JA/ET genes (e.g., *ORA59*); SA-induced ROXY19 represses JA/ET genes; SA may promote ORA59 protein degradation; NPR1 integrates crosstalk (cytoplasmic function)	Enhances resistance to biotrophs; Can increase susceptibility to necrotrophs; Strategic resource allocation	
JA/ET	Necrotrophs (e.g., *Botrytis cinerea*), Herbivores, Wounding	Antagonistic with SA	JA pathway (via COI1-JAZs, MYC branch, NAC TFs)	Enhances resistance to necrotrophs/herbivores; Can increase	

Hormone Pathway	Primary Pathogen Type	Interaction Type	Key Molecular Mechanisms of Crosstalk	Consequences for Plant Defense	Relevant Snippet IDs
			represses SA biosynthesis (*ICS1*) and promotes SA inactivation (*BSMT1*)	susceptibility to biotrophs; Pathogens can exploit this crosstalk (e.g., Coronatine, β-glucan)	
SA & JA	(Various)	Synergistic (low conc.)	(Mechanisms less understood, but observed at low hormone concentrations)	Simultaneous activation of both SA and JA target genes (e.g., *PR1*, *PDF1.2*)	

This table illustrates the complex interplay, where the plant strategically prioritizes defense responses based on the specific threat, often at the expense of other pathways, a testament to the efficient management of costly defense resources.

6. Context-Dependent Roles of Salicylic Acid in Plant Tolerance

The role of salicylic acid in plant tolerance responses to both biotic and abiotic stresses is not uniform but highly context-dependent, varying significantly with the specific plant species, the type and severity of the stressor, and other environmental factors. This variability underscores the complexity of plant stress physiology and the need for nuanced approaches in agricultural applications.

Variations Across Different Plant Species: SA's impact is observed across a wide range of plant species, with specific outcomes varying:

- ***Arabidopsis*:** SA is critical for immune signaling, eliciting the hypersensitive response (HR) and activating systemic acquired resistance (SAR). However, high air humidity can suppress SA accumulation and signaling, compromising immunity.
- **Maize:** SA can reduce dry matter and leaf area index decrease, alleviate chloroplast disruption, and promote antioxidant enzyme activity under high-temperature stress. For salt stress, SA significantly enhances grain yield.
- **Wheat:** SA priming enhances drought tolerance by upregulating antioxidant defense and glyoxalase systems, improving seedling establishment, photosynthetic performance, and membrane permeability. Under combined drought and heat, SA levels correlate with increased amino acids. For salt stress, SA alleviates growth inhibition and increases nutrient acquisition.
- **Rice:** SA regulates stomatal aperture under salt and drought stresses and contributes to increased chlorophyll content and improved yield in bacterial blight disease.
- **Tomato:** Exogenous SA significantly reduces cadmium accumulation and alters its distribution.
- **Ginger:** SA attenuates salinity-induced growth inhibition by regulating ionic balance and

the antioxidative system.

- **Canola:** Co-application of *Pseudomonas putida* and SA develops stress tolerance and improves plant growth under drought.
- **Common Bean (*Phaseolus vulgaris*):** SA with kinetin or calcium improves growth traits, photosynthetic pigments, carbohydrate content, nitrogenous constituents, antioxidant enzyme activities, and proline accumulation under nickel and/or lead stress.
- **Safflower (*Carthamus tinctorius L.*):** Exogenous SA improves salinity response by increasing beneficial compounds and decreasing proline.
- **Sugarcane (*Saccharum officinarum L.*):** Sett priming with SA improves salinity tolerance during early development.
- **St John's Wort:** Exogenous SA applications alleviate salinity stress via physiological and biochemical changes.
- **Willow (*Salix babylonica*):** While willow extract enhances salinity tolerance, the benefits are not solely due to SA content, as pure SA did not yield the same effects.
- **Camellia oleifera:** SA levels decline during drought, but drought-treated groups show stronger antioxidant capacity, water regulation, and drought protection.
- **Oregano:** SA improves Photosystem II (PSII) efficiency under moderate drought stress.
- **Soybean (*Glycine max L.*):** Exogenous SA and kinetin reduce malondialdehyde (MDA), hydrogen peroxide (H2O2), proline, and leaf electrolyte leakage under waterlogging stress, enhancing antioxidant enzyme activity.
- **Finger Millet (*Eleusine coracana L.*):** SA supplementation significantly reduces nickel toxicity and increases growth and mineral concentration in Ni-treated plants.
- **Strawberry:** FaSnRK1α mediates SA pathways to enhance resistance to *Botrytis cinerea*.
- **Grapevines:** JA and SA application reduces injury from pests and the number of eggs laid by *Drosophila suzukii* females.

Variations Across Different Environmental Conditions: SA's role in abiotic stress tolerance is also highly dependent on the specific stressor:

- **Drought Stress:** SA alleviates drought stress by promoting seedling growth, enhancing antioxidant capacity, regulating water balance, promoting stress-related gene expression, and regulating physiological metabolism.
- **Salt Stress:** SA alleviates salt stress through ion balance regulation, antioxidant effects, and hormone level regulation. It promotes root growth, increases chlorophyll content and photosynthetic efficiency, and reduces transpiration.
- **Heavy Metal Stress:** SA alleviates heavy metal toxicity by enhancing antioxidant enzyme activities, reducing reactive oxygen species (ROS), and modulating non-enzymatic antioxidant metabolites. However, high concentrations of SA can also be toxic.
- **High-Temperature Stress:** SA alleviates heat stress by regulating ROS homeostasis, accumulating antioxidants, and participating in epigenetic and hormonal signaling.
- **Low-Temperature Stress (Cold/Chilling/Freezing):** SA regulates plant metabolism and induces the biosynthesis of cold-resistant proteins, improving tolerance to low temperatures. It promotes the activation of the antioxidant system, preserves cell membrane stability, regulates gene expression, and promotes low-temperature signal production.
- **Combined Stresses:** SA can exert regulatory or alleviative effects on multiple stresses simultaneously, which is beneficial for plant tolerance responses. For example, under combined drought and heat stresses, SA levels were correlated with increased amino acid levels in certain citrus plants.

SA also interacts with other signaling molecules and plant hormones like jasmonic acid (JA),

ethylene (ETH), auxin, abscisic acid (ABA), and nitric oxide (NO). These interactions can be synergistic or antagonistic, influencing plant defense responses. For instance, SA can inhibit the synthesis of ETH and the methylation of JA, reducing the plant's response to adversity. The specific mechanisms and outcomes of SA's role in stress tolerance are therefore highly context-dependent, varying with the plant species, the type and severity of the stress, and the interplay with other signaling molecules.

7. Agricultural Applications and Future Prospects

The profound role of salicylic acid in plant defense activation and stress tolerance has significant practical implications for agriculture and crop protection. Exogenous application of SA has emerged as a promising strategy to enhance crop resilience and yield. SA improves resistance to drought and various environmental stresses, enhances plant hardiness, flowering, and fruit yield, and critically aids in Systemic Acquired Resistance (SAR). It is often referred to as a "therapeutic agent" for crops due to its protective effects against abiotic stresses such as drought, salinity, heavy metal toxicity, and extreme heat. SA can also improve plant growth and developmental stages, potentially elevating photosynthesis, chlorophyll pigments, and stomatal regulation, while reducing oxidative injuries from reactive oxygen species (ROS).

The development of plant activators targeting the SA signaling pathway, such as benzothiadiazole derivatives, represents a significant advancement in crop protection. These compounds offer a promising alternative to conventional fungicides because they prime the plant's innate immune system to induce broad-spectrum disease resistance without directly inhibiting pathogen proliferation. Key advantages of these SA-targeting activators include prolonged defense activity, lower effective dosages, and a negligible risk of pathogen resistance development. For example, the synthetic fungicide acibenzolar-S-methyl (BSA) is not directly toxic to pathogens but acts by inducing SAR in crop plants, effectively controlling some plant diseases, as demonstrated in Honeycrisp apples where it reduced fungicide applications while maintaining disease control.

Historically, a major challenge in implementing SA-based disease management in agriculture has been the observed trade-off between SA-induced disease resistance and suppressed plant growth. Increased SA levels, while enhancing defense, typically divert resources away from growth, leading to reduced biomass yields. This limitation has hindered widespread practical applications, as genetic modification to increase SA often resulted in stunted plants.

However, recent breakthroughs offer a path to overcome this trade-off. Researchers have demonstrated a method to separate growth suppression from the defense response by modifying specific cold-regulated genes that negatively respond to elevated SA levels. By "severing" the SA-responsiveness of these genes, plants were able to maintain normal growth even with elevated SA levels, while retaining enhanced disease resistance. This innovative approach, initially demonstrated in poplar trees and later in the model plant *Arabidopsis*, opens the door to developing climate-resilient crops that can simultaneously achieve higher yields and improved disease resistance. The team is actively expanding this research to other crops like alfalfa, testing its ability to grow with limited water and nutrient supply, promising to generate crops that are robust against multiple environmental challenges.

Future prospects in SA-mediated plant defense focus on a deeper understanding of its intricate regulatory networks and crosstalk. For instance, if the antagonistic crosstalk between SA- and ET/JA-signaling pathways could be disconnected, plants might be able to defend against both biotrophic and necrotrophic pathogens simultaneously without the current trade-offs. This could

involve genetically engineering the SA-mediated signaling pathway to control the ET/JA-signaling pathway, potentially priming plants for necrotrophs after biotroph infection. Modulating both positive and negative regulatory factors of these signaling pathways are promising areas for future study to develop better defensive plants and fulfill the increasing food needs of a growing global population in the face of climate change.

Conclusions

Salicylic acid is unequivocally a central and indispensable phytohormone in plant defense, orchestrating a complex and dynamic immune system that enables plants to respond to a wide spectrum of biotic and abiotic stresses. Its recognition as a key signaling molecule fundamentally transformed the understanding of plant immunity, moving it from a passive barrier to an active, regulated, and systemic adaptive response.

The intricate biosynthesis pathways, precise perception mechanisms involving NPR proteins and their cofactors like copper, and the sophisticated signal transduction networks, including redox regulation and interaction with diverse transcription factors, highlight the molecular elegance underlying SA's functions. The existence of multiple receptors and extensive metabolic buffering systems for SA modification underscores an evolutionary drive for robustness and fine-tuned control, ensuring that costly defense responses are activated efficiently and proportionally to the threat.

A defining characteristic of SA's role in defense is its participation in a complex hormonal crosstalk, particularly its mutual antagonism with the jasmonic acid and ethylene pathways. This antagonism represents a strategic resource allocation mechanism, allowing plants to prioritize defense against specific pathogen lifestyles (biotrophs vs. necrotrophs) and manage energy trade-offs between growth and defense. The observation that pathogens can actively exploit this crosstalk for their own benefit further emphasizes the ongoing co-evolutionary arms race and the need for sophisticated plant breeding strategies.

The context-dependent nature of SA's effects across various plant species and environmental conditions underscores the complexity of plant stress physiology. However, this variability also presents opportunities for targeted agricultural applications. The development of SA-targeting plant activators offers a sustainable alternative for disease management, reducing reliance on conventional pesticides. Furthermore, recent breakthroughs in uncoupling SA-induced defense from growth suppression hold immense promise for engineering climate-resilient crops that maintain high yields while possessing enhanced resistance to diverse stressors.

In conclusion, SA's multifaceted roles in plant defense activation, its intricate molecular mechanisms, and its complex interactions with other phytohormones collectively represent a sophisticated biological system. Continued research into these nuanced networks will be crucial for developing innovative strategies to enhance crop productivity and ensure global food security in a rapidly changing world.

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