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The diversity of salicylic acid biosynthesis and defense signaling in plants: Knowledge gaps and future opportunities

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Abstract

The phytohormone salicylic acid (SA) is known to regulate plant immunity against pathogens. Plants synthesize SA via the isochorismate synthase (ICS) pathway or the phenylalanine ammonia-lyase (PAL) pathway. The ICS pathway has been fully characterized using *Arabidopsis thaliana*, a model plant that exhibits pathogen-inducible SA accumulation. Many species including *Populus* (poplar) depend instead on the partially understood PAL pathway for constitutive as well as pathogen-stimulated SA synthesis. Diversity of SA-mediated defense is also evident in SA accumulation, redox regulation, and interplay with other hormones like jasmonic acid. This review highlights the contrast between *Arabidopsis* and poplar, discusses potential drivers of SA diversity in plant defenses, and offers future research directions.

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Salicylic acid biosynthesis, Pathogen defense, Populus, Thioredoxin, Redox signaling, SA-JA synergism.

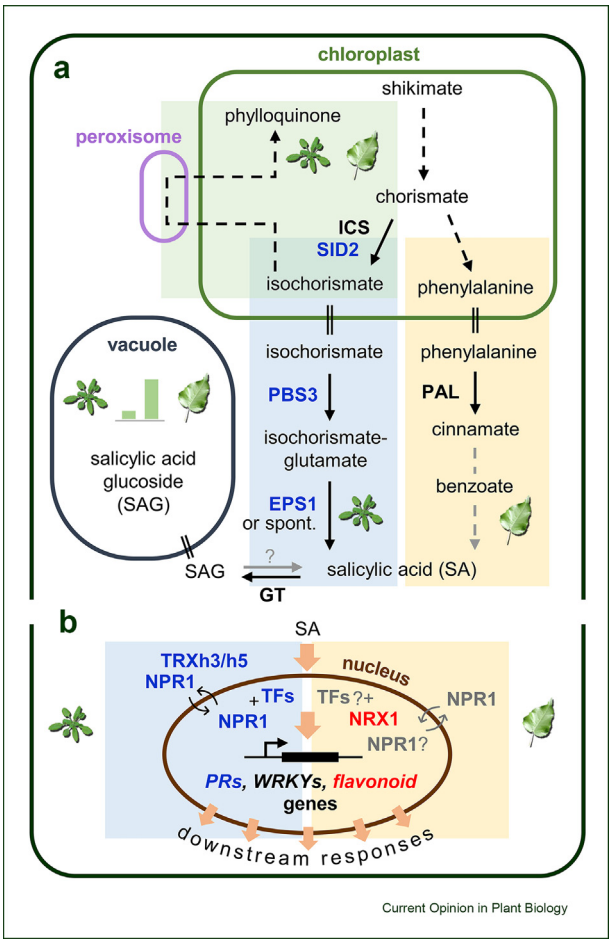
Introduction

Salicylic acid (SA) is a phytohormone and a central mediator of pathogen-induced plant defenses [1,2]. The discovery of SA has a storied past; it was first prepared from salicin, an analgesic produced in willow and poplar (family Salicaceae), and ultimately inspired the development of the blockbuster drug aspirin [3]. Nearly two centuries following its discovery, our understanding of SA biosynthesis in plants remains incomplete. Significant progress in understanding SA action in plant defense has been made using the powerful *Arabidopsis* model system. Translating these findings to crops and trees has not been straightforward, in part because of variation between species in SA biosynthesis, accumulation, and signaling. This review highlights recent advances in SA biosynthesis, redox regulation, and signaling crosstalk, with an added focus on the woody perennial *Populus* (poplar). Given the multifaceted roles of SA in plant responses to biotic and abiotic stresses, the underexplored diversity in SA biosynthesis and signaling presents a challenge to translational research for crop improvement in the face of climate change.

SA biosynthesis pathways: universal or lineage-specific?

Plants have evolved two distinct routes for synthesizing SA (Figure 1). Classical radiolabeling studies in diverse plant species support a biosynthetic origin of SA from cinnamic acid and benzoic acid [4,5]. This route shares common steps with the central phenylpropanoid pathway initiated by phenylalanine ammonia lyase (PAL) in the cytosol (Figure 1A). Despite this early finding, several biosynthetic enzymes leading to benzoic acid and SA remain hypothetical [6]. Major breakthroughs have come from forward genetic screens and functional characterization of *Arabidopsis* SA mutants. The chloroplastic AtICS1 (isochorismate synthase1, also known as SA INDUCTION DEFICIENT2 or SID2) is central to pathogen-induced SA synthesis [7]. This ICS pathway (Figure 1a) resembles the eubacterial synthesis of SA-derived siderophores [8], but the plant homolog

Figure 1



Diversity of SA biosynthesis and signaling. **a.** SA biosynthetic routes and vacuolar storage of SA conjugates. ICS and PAL pathways are shown in blue and yellow, respectively. The three Brassicaceae-specific proteins are shown in blue. Dashed lines indicate multi-step pathways and grey denotes unclarified steps. Ancestral ICS function in phylloquinone biosynthesis via chloroplast and peroxisome is shown in green box. **b.** Simplified representation of SA-sensitive redox signaling with key players shown. Responses reported for *Arabidopsis* and *Populus* are shown in red and blue, respectively.

for bacterial isochorismate-pyruvate lyase remained elusive. In 2019, two groups independently identified the missing link as AtGH3.12, an acyl adenylase family protein (also called AtPBS3 or avrPphB SUSCEPTIBLE3) involved in disease resistance [9,10]. AtGH3.12 is a cytosolic enzyme and catalyzes the conjugation of glutamate to isochorismate (IC) to form IC-glutamate [9,10] (Figure 1a). The IC-glutamate conjugate is then converted to SA either spontaneously or more efficiently by AtEPS1 (ENHANCED PSEUDOMONAS SUSCEPTIBILITY1), a BAHD acyltransferase-like protein [9,10].

These studies fully elucidated the ICS pathway for SA biosynthesis in *Arabidopsis*, but several questions remain. Perhaps the most underappreciated fact is that all three biosynthetic genes in this pathway, *AtICS1*, *AtGH3.12*, and *AtEPS1*, are only found in the Brassicaceae family [10–12]. *AtICS1* is derived from a Brassicaceae-specific genome duplication, and its exon-intron structure, basal expression, and pathogen-inducibility all differ from *AtICS2* [7,13]. *AtICS2* resembles the ancestral gene involved in the biosynthesis of phylloquinone (vitamin K1) essential for photosynthetic electron transport [11**] (Figure 1a). The phylloquinone pathway has an endosymbiotic origin and is similar to eubacterial menaquinone (vitamin K2) biosynthesis [14,15]. Interestingly, pathway diversification at ICS also occurs in eubacteria, with EntC and MenF differentially involved in the biosynthesis of SA-derived siderophores and menaquinone, respectively [8,16]. Poplar, like many genome-sequenced plants, harbors a single *ICS* that is transcriptionally insensitive to abiotic stress and SA [11**,17]. These data strongly suggest recruitment of AtICS1 to SA biosynthesis as a lineage-specific event. The recent findings that downstream enzymes AtGH3.12 and AtEPS1 for SA biosynthesis are also Brassicaceae-specific [10,12,18] lend credence to the ICS pathway as a taxon-restricted evolutionary novelty rather than a conserved mechanism.

PAL pathway involvement in SA synthesis (Figure 1a) by many crop and tree species remains under-studied even where evidence for ICS involvement is at best equivocal. In barley, for instance, characterization of a homozygous *ics* mutant derived from fast neutron mutagenesis unequivocally demonstrated ICS involvement in phylloquinone, but not SA, biosynthesis [19*]. In the absence of large-scale mutant collections, CRISPR-based approaches can be used to generate targeted knockout mutants to aid functional characterization. Alternatively, a genome-wide association study (GWAS) in conjunction with metabolite and expression profiling of 300 unrelated *Populus tomentosa* individuals identified putative pathway intermediates and candidate genes for SA biosynthesis [20]. Although that analysis did not discriminate between ICS and PAL pathways for relevance to poplar SA synthesis, similar approaches with a refined focus should be fruitful in the quest for missing genes in the PAL pathway.

SA redox signaling: the devil is in the details

Arabidopsis SA defense signaling requires AtNPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1), a redox-sensitive master regulator and SA receptor [21]. Upon pathogen stimulation or SA treatment, AtNPR1 undergoes conformational changes for translocation into the nucleus where it interacts with other transcription factors to activate downstream defense gene expression [22] (Figure 1b). Redox modification of cysteine residues in both the protein-protein

interaction and the cryptic trans-coactivation domains of NPR1 is essential for its regulatory function [21,23,24]. For instance, mutations in the evolutionarily conserved Cys^{82} and Cys^{216} resulted in monomerization and nuclear localization of NPR1 in both *Arabidopsis* and rice (*Oryza sativa* L.) and constitutive activation of defense gene expression [25,26]. However, SA-induced AtNPR1 monomerization and nuclear import is catalyzed by thioredoxins AtTRXh3 and AtTRXh5 via thiol-disulfide exchange reactions at Cys^{156} [21]. Paradoxically, this AtNPR1-AtTRXh3/h5 interaction appears taxon-restricted because the regulatory Cys^{156} is found in a small minority of the Viridiplantae NPR gene family in Phytozome v13 (13 out of 181 members), all belonging to Brassicaceae. The rice ortholog OsTRXh2 implicated in OsNPR1-dependent disease resistance [26] was recently shown to be a direct target of a bacterial effector for proteasomal degradation, which dampens host immunity [27**]. *Nicotiana benthamiana* defense against barley stripe mosaic virus (BSMV) also depends on SA activation and signaling, but involves the AtTRXh1 ortholog NbTRXh1 [28**]. BSMV γb protein physically interacts with NbTRXh1 to weaken its reductase activity, thereby allowing viral spread [28**]. Whether NbNPR1 has a role is not known. In rice, pathogen-induced SA response can be independent of OsNPR1, as the transcription factor OsWRKY45 bypasses the NPR1-mediated defense [29,30]. Similarly, in *Populus*, *NPR1* is poorly expressed, insensitive to *in planta* or exogenous SA manipulations, and lacks a defined role in pathogen defense [17,31**].

These studies support diversified redox signaling in SA-mediated defense depending on the species and/or cellular milieu, due in part to the functional multiplicity of plant TRX proteins [32,33]. For instance, AtTRXh5 but not its genome duplicate AtTRXh3 is involved in the *Arabidopsis* response to victorin, a fungal toxin [34]. *Populus* lacks *AtTRXh3/h5* orthologs but harbors a tandem array of *Nucleoredoxin1* (*NRX1*), a subfamily of the TRX superfamily [17]. Gene network modeling identified *PtaNRX1* as the only small redox protein family in poplar leaves that exhibits SA-dependent regulation [17]. The ortholog in cotton (*Gossypium barbadense*), GbNRX1, has been implicated in resistance against soil-borne pathogens via apoplast ROS modulation [35]. *Arabidopsis* AtNRX1 also modulates ROS scavenging; however, it acts as a negative regulator of disease resistance [36]. These data reinforce the complexity and diversity of redox signaling, as well as the challenges in translating results across species. With the advent of CRISPR technologies, precision gene editing at the allele, individual gene or gene family level can now be routinely achieved in a growing number of crop species. A recent study reported generation of null *nrx1* poplar mutants by CRISPR knockout of all seven tandemly duplicated genes [37]. Such mutants will be invaluable for functional characterization to address redundancy versus specificity of small redox protein (super)family members in SA signaling.

SA-JA interplay can be antagonistic and synergistic in plant defense

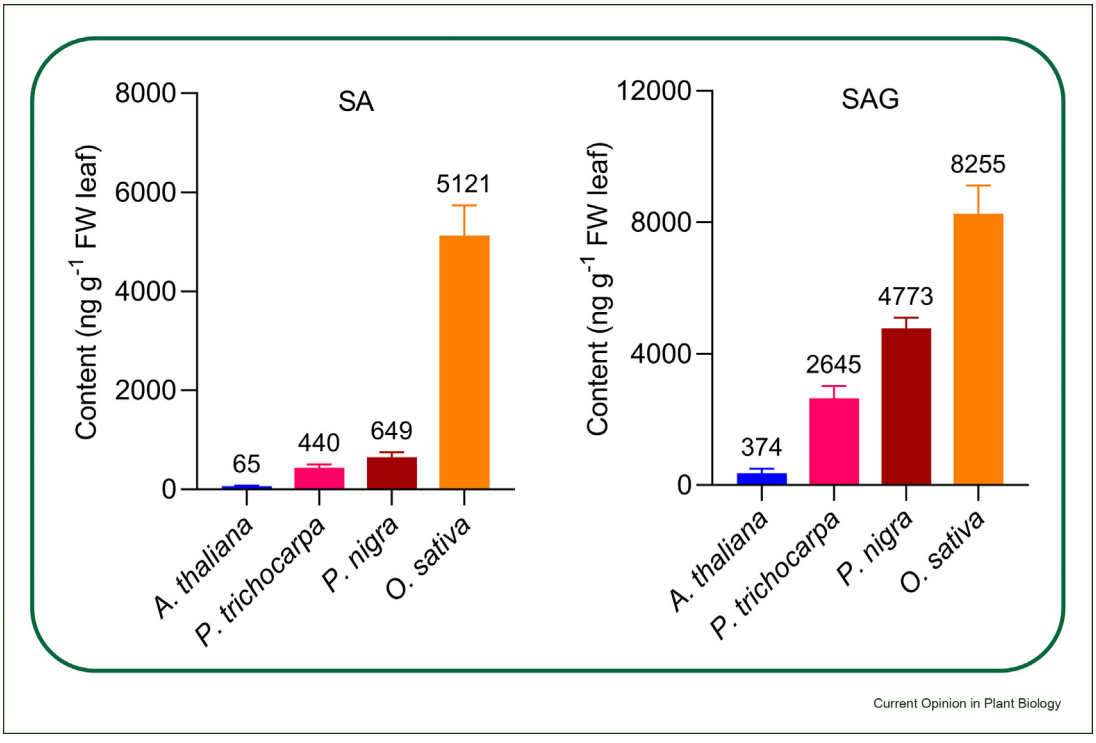
Research from *Arabidopsis* has established that SA-mediated defense is more effective against (hemi)-biotrophs while jasmonic acid (JA)-mediated defense is operative against necrotrophs. Furthermore, it is broadly accepted that these two hormones function in an antagonistic manner. Consistent with this dichotomy, certain pathogens have evolved virulence mechanisms that exploit the SA-JA antagonism to neutralize host defense [38,39]. The SA receptor AtNPR1 controls not only SA signaling but also SA-JA antagonism [40]. The JA receptor AtCOI1 (CORONATINE INSENSITIVE1) regulates JA-mediated inhibition of the SA pathway [41]. However, several recent studies suggest alternatives to SA-JA antagonism. For instance, the SA receptors NPR3 and NPR4 function oppositely to NPR1 [42] and can activate JA signaling during effector-triggered immunity with synergistic induction of both SA and JA defenses [43]. Under natural conditions, plants encounter numerous pathogens simultaneously, necessitating deployment of varied SA and JA defenses for ecological success. A meta-analysis of global transcriptome data revealed that a large number of defense genes activated by both biotrophy and necrotrophy are also induced by SA and JA [44]. Another study demonstrated spatial coordination of the SA and JA pathways during effector-triggered immunity in *Arabidopsis* [45]. The SA-JA interplay is likely modulated by additional factors, including the early signaling after pathogen recognition by the host, pathogen lifestyle, plant species and developmental stage, and resource availability, all of which deserve further research.

In *Populus*, SA concentrations increase markedly upon infection by multiple pathogens, including biotrophs [31**], hemibiotrophs [46], and necrotrophs [47]. Interestingly, JA-metabolites also increased in response to the rust fungus *Melampsora larici-populina*, an obligate biotroph [31**,48**]. Complex SA and JA signaling interplay may be especially relevant in long lived perennials because biotic pressures are varied and must be coped with year-round. In a recent study, poplars with constitutively elevated SA were found to accumulate higher levels of JA metabolites while exogenous JA applications increased the content of SA [48**.] Transgenic perturbation of the Salicaceae-specific salicinoid biosynthesis by CRISPR-knockout of a glycosyltransferase in poplar also resulted in elevated accumulation of both SA and JA-metabolites [49*]. SA was shown to positively regulate the biosynthesis of flavonoid phytoalexins, such as flavan-3-ols in poplar [31**,48**] and sakuranetin in rice [50]. JA also increases these antimicrobial flavonoids in both species

[48**,51]. In rice, SA hydroxylase-knockout lines increased not only SA but also JA and sakuranetin contents, which resulted in broad-spectrum resistance against both hemibiotrophic and necrotrophic pathogens [52*].

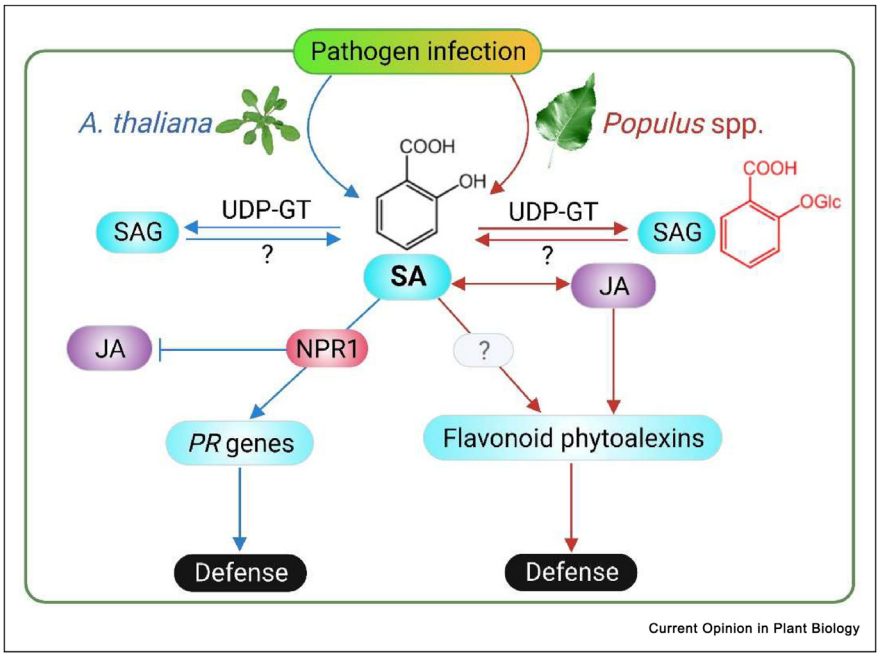
These examples suggest synergistic SA-JA interactions may be more common in poplar and rice than in *Arabidopsis*. While the underlying molecular mechanism awaits further investigation, variations in SA biosynthesis, homeostasis, and signaling pathways likely contribute to the diversity. The basal levels of SA in poplar and rice leaves are substantially higher than pathogen-induced SA accumulation in *Arabidopsis* [31**,53] (Figure 2). Compared to *Arabidopsis*, levels of SA-glucoside (SAG) stored in poplar and rice leaves are 10- and 20-fold higher, respectively (Figure 2). SAG can be hydrolyzed to SA without *de novo* synthesis under stressed conditions [54], though the responsible enzyme has not been characterized. Both SA and SAG (and the dihydroxybenzoate derivatives) can activate defense response [55,56]. The low-SA accumulator *Arabidopsis* relies on the pathogen-inducible ICS pathway for defense and often displays a trade-off with JA signaling. In contrast, woody perennials and field crops accrue substantial amounts of SA and SAG via the PAL pathway, which shares common precursors for the biosynthesis of several classes of phytoalexins co-regulated by SA and JA (Figure 3). Furthermore, several *Pathogenesis-related* (*PR*) genes are frequently considered as SA markers in *Arabidopsis* [2]. However, *PR* gene expression remained largely unchanged in poplars with multifold differences in SA levels or following exogenous applications with an SA analog [48**], and *PR* genes were absent in the poplar SA-responsive gene network modules [17]. Instead, *PR* transcript abundances correlated positively with pathogen growth in infected leaves across multiple poplar genotypes with varying levels of rust resistance [31**,48**]. This is consistent with the original discovery of PRs as abundant antimicrobial proteins following pathogen infection [57]. Variable SA-responsiveness of the expanded *PR1* gene family has also been reported in rice [58]. The different transcriptional responses of *PR* genes to SA and other chemical stimuli may reflect differential adaptive evolution between species.

Figure 2



Constitutive levels of SA and SAG in the leaves of *Arabidopsis*, poplar and rice. Hormone levels were determined using liquid chromatography coupled with tandem mass spectrometry as described previously [48**] using approximately 40 mg of fresh tissues. *A. thaliana* (ecotype Col-0) plants were grown under standard long day (16/8 h day/night cycle) growth conditions at 21°C, and four-week-old rosette leaves were collected for SA analysis. *P. trichocarpa* (genotype 262/263) and *P. nigra* (genotype Firtzlar2) were grown as described previously [48**]. Three youngest fully-expanded leaves from trees around 80 cm in height were collected for hormone analysis. *O. sativa* (cv. Nipponbare) seedlings were grown under the conditions described previously [62] and leaf samples were harvested from 4-week-old seedlings. Data represent mean with standard error (n = 5). Average levels of SA and SAG are indicated above the bars.

Figure 3



Regulation of SA-mediated immunity in *Arabidopsis* and *Populus* against phytopathogens. In *Arabidopsis*, infection by biotrophic pathogens activates defense via the SA receptor NPR1, often with concomitant inhibition of JA signaling (blue lines). In poplar, both SA and JA pathways are induced simultaneously upon pathogen attacks, irrespective of their lifestyles (biotrophy or necrotrophy). Synergism between SA and JA pathways induces the accumulation of antimicrobial flavonoids, including catechins and proanthocyanidins (red arrows). Pathogen-inducible *PR* genes are not responsive to constitutively elevated SA in the absence of pathogens, and the involvement of NPR1 in poplar pathogen defense is unclear.

Conclusions and future directions

Multiple aspects of SA-mediated defense signaling from SA biosynthesis and homeostasis to redox modulation and hormonal crosstalk are more diverse than previously thought. Emerging data suggest that the key genes and regulators identified from the *Arabidopsis* model represent lineage-specific innovations and may not be directly translated across species. However, the SA signal relay and regulatory cascades defined in *Arabidopsis* provide a foundational framework to guide research efforts in crops. Elucidating missing steps in the PAL-mediated SA biosynthetic pathway will require integration of genetic, transcriptomic and metabolomic approaches as elegantly illustrated for the *Arabidopsis* ICS pathway [9,10]. For crop and tree species that lack genome-scale mutant collections, exploiting natural variations by GWAS and high-throughput phenotyping can be fruitful. This approach successfully identified multiple resistant and susceptible genes against the causal fungal pathogen of *Septoria* canker in poplar [59*]. Closing knowledge gaps in redox modulators and response markers will also be critical toward understanding SA signaling in diverse crop species. In the absence of SA mutants that have been instrumental in *Arabidopsis* research, ectopic expression of a bacterial SA synthase for constitutive (or inducible) accumulation of SA in non-model species can generate novel insights for downstream processes. This approach has been used in different poplar species to facilitate discovery of NRX1 as an alternative to TRXh in SA-responsive redox cascades [17], SA regulation of flavonoid phytoalexins [31**], and synergistic SA-JA crosstalk in pathogen defense [48**]. Given the multifaceted role of SA in biotic and abiotic defense as well as growth and development [60,61], taxon-specific fine-tuning and evolutionary novelties in SA synthesis, activation and signaling cascades are perhaps the norm. Exploiting this diversity can lead to additional molecular targets to aid breeding, genomic selection and/or CRISPR-based crop improvement efforts.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Raskin I: **Role of salicylic acid in plants**. *Annu Rev Plant Physiol Plant Mol Biol* 1992, **43**:439–463.

2. Vlot AC, Dempsey DMA, Klessig DF: **Salicylic acid, a multifaceted hormone to combat disease**. *Annu Rev Phytopathol* 2009, **47**:177–206.

3. Desborough MJR, Keeling DM: **The aspirin story – from willow to wonder drug**. *Br J Haematol* 2017, **177**:674–683.

4. Klämbt HD: **Conversion in plants of benzoic acid to salicylic acid and its β*d*-glucoside**. *Nature* 1962, **196**. 491-491.

5. El-Basyouni SZ, Chen D, Ibrahim RK, Neish AC, Towers GHN: **The biosynthesis of hydroxybenzoic acids in higher plants**. *Phytochemistry* 1964, **3**:485–492.

6. Widhalm Joshua R, Dudareva N: **A familiar ring to it: biosynthesis of plant benzoic acids**. *Mol Plant* 2015, **8**: 83–97.

7. Wildermuth MC, Dewdney J, Wu G, Ausubel FM: **Isochorismate synthase is required to synthesize salicylic acid for plant defence**. *Nature* 2001, **414**:562–565.

8. Serino L, Reimmann C, Baur H, Beyeler M, Visca P, Haas D: **Structural genes for salicylate biosynthesis from chorismate in *Pseudomonas aeruginosa***. *Mol Gen Genet* 1995, **249**: 217–228.

9. Rekhter D, Lüdke D, Ding Y, Feussner K, Zienkiewicz K, Lipka V, Wiermer M, Zhang Y, Feussner I: **Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid**. *Science* 2019, **365**:498–502.

10. Torrens-Spence MP, Bobokalonova A, Carballo V, Glinkerman CM, Pluskal T, Shen A, Weng J-K: **PBS3 and EPS1 complete salicylic acid biosynthesis from isochorismate in Arabidopsis**. *Mol Plant* 2019, **12**:1577–1586.

11. ** Yuan Y, Chung J-D, Fu X, Johnson VE, Ranjan P, Booth SL, Harding SA, Tsai C-J: **Alternative splicing and gene duplication differentially shaped the regulation of isochorismate synthase in *Populus* and *Arabidopsis***. *Proc Natl Acad Sci USA* 2009, **106**:22020–22025.
This study documented distinct roles for *Populus* and *Arabidopsis ICS* genes. The single-copy *Populus ICS* functionally complemented the *Arabidopsis* SA-deficient *sid2-2* mutant. *Populus ICS* was not stress-inducible, underwent extensive alternative splicing and exhibited an evolutionarily conserved function in phylloquinone biosynthesis. This contrasts sharply with the pathogen-inducible *Arabidopsis AtICS1*, which represents a novel isoform, derived from a Brassicaceae-specific duplication, for pathogen-elicited SA synthesis.

12. Holland CK, Westfall CS, Schaffer JE, De Santiago A, Zubieta C, Alvarez S, Jez JM: **Brassicaceae-specific Gretchen Hagen 3 acyl acid amido synthetases conjugate amino acids to chorismate, a precursor of aromatic amino acids and salicylic acid**. *J Biol Chem* 2019, **294**:16855–16864.

13. Garcion C, Lohmann A, Lamodiere E, Catinot J, Buchala A, Doermann P, Metraux JP: **Characterization and biological function of the ISOCHORISMATE SYNTHASE2 gene of Arabidopsis**. *Plant Physiol* 2008, **147**:1279–1287.

14. Basset GJ, Latimer S, Fatihi A, Soubeyrand E, Block A: **Phylloquinone (vitamin K1): occurrence, biosynthesis and functions**. *Mini Rev Med Chem* 2017, **17**:1028–1038.

15. Gu X, Chen I-G, Tsai C-J: **How do holoparasitic plants exploit vitamin K1?** *Plant Signal Behav* 2021, **16**, 1976546.

16. Müller R, Dahm C, Schulte G, Leistner E: **An isochorismate hydroxymutase isogene in *Escherichia coli***. *FEBS (Fed Eur Biochem Soc) Lett* 1996, **378**:131–134.

17. Xue L-J, Guo W, Yuan Y, Anino EO, Nyamdari B, Wilson MC, Frost CJ, Chen H-Y, Babst BA, Harding SA, *et al.*: **Constitutively elevated salicylic acid levels alter photosynthesis and oxidative state but not growth in transgenic *Populus***. *Plant Cell* 2013, **25**:2714–2730.

18. Westfall CS, Zubieta C, Herrmann J, Kapp U, Nanao MH, Jez JM: **Structural basis for prereceptor modulation of plant hormones by GH3 proteins**. *Science* 2012, **336**:1708–1711.

19. * Qin Y, Torp AM, Glauser G, Pedersen C, Rasmussen SK, Thordal-Christensen H: **Barley isochorismate synthase mutant is phylloquinone-deficient, but has normal basal salicylic acid level**. *Plant Signal Behav* 2019, **14**, 1671122.
This study describes functional characterization of a null mutant line for the single *ICS* gene in barley. Homozygous *ics* seedlings wilted after 10-days of germination, lacked phylloquinone, but contained wildtype-level SA. Wilted *ics* plants were rescued after spraying with 1,4-dyhydroxy-2-naphtoic acid, a phylloquinone precursor synthesized via the ICS pathway. Results suggest basal SA is synthesized via the PAL pathway, while *ICS* functions as a phylloquinone biosynthetic gene in barley.

20. Xiao L, Du Q, Fang Y, Quan M, Lu W, Wang D, Si J, El-Kassaby YA, Zhang D: **Genetic architecture of the metabolic pathway of salicylic acid biosynthesis in Populus**. *Tree Physiol* 2021, **41**:2198–2215.

21. Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X: **Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins**. *Science* 2008, **321**:952–956.

22. Withers J, Dong X: **Posttranslational modifications of NPR1: a single protein playing multiple roles in plant immunity and physiology**. *PLoS Pathog* 2016, **12**, e1005707.

23. Rochon A, Boyle P, Wignes T, Fobert PR, Després C: **The coactivator function of Arabidopsis NPR1 requires the core of its BTB/POZ domain and the oxidation of C-terminal cysteines**. *Plant Cell* 2006, **18**:3670–3685.

24. Boyle P, Le Su E, Rochon A, Shearer HL, Murmu J, Chu JY, Fobert PR, Després C: **The BTB/POZ domain of the Arabidopsis disease resistance protein NPR1 interacts with the repression domain of TGA2 to negate its function**. *Plant Cell* 2009, **21**:3700–3713.

25. Mou Z, Fan W, Dong X: **Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes**. *Cell* 2003, **113**:935–944.

26. Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Li Q, Yang D, *et al.*: **Functional analysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility†**. *Plant Biotechnol J* 2007, **5**:313–324.

27. ** Ji H, Liu D, Zhang Z, Sun J, Han B, Li Z: **A bacterial F-box effector suppresses SAR immunity through mediating the proteasomal degradation of OsTrxh2 in rice**. *Plant J* 2020, **104**:1054–1072.
This study identified OsTrxh2 as a direct target of the bacterial effector Xop1-mediated proteasomal degradation. OsTRXh2 is orthologous to AtTRXh3/h5. The authors presented evidence for OsTRXh2 reduction of OsNPR1 via the WCGPC catalytic domain, which is different from the mechanism proposed for AtTRXh3/h5. Degradation of OsTRXh2 weakened OsNPR1-mediated defense, but whether or not SA was involved awaits further research.

28. ** Jiang Z, Jin X, Yang M, Pi Q, Cao Q, Li Z, Zhang Y, Wang X-B, Han C, Yu J, *et al.*: **Barley stripe mosaic virus γb protein targets thioredoxin h-type 1 to dampen salicylic acid-mediated defenses**. *Plant Physiol* 2022, **189**:1715–1727.
This paper describes a counter-regulation between *N. benthamiana* NbTRXh1 and the γb protein of BSMV. NbTRXh1 is involved in SA-based defense against BSMV, and is itself a target of γb. Binding of γb dampened the reductase activity of NbTRXh1 to promote virulence, with unclear involvement of NPR1. The work supported participation of different TRX family members in SA-mediated immunity.

29. Shimono M, Sugano S, Nakayama A, Jiang C-J, Ono K, Toki S, Takatsuji H: **Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance**. *Plant Cell* 2007, **19**:2064–2076.

30. Matsushita A, Inoue H, Goto S, Nakayama A, Sugano S, Hayashi N, Takatsuji H: **Nuclear ubiquitin proteasome degradation affects WRKY45 function in the rice defense program**. *Plant J* 2013, **73**:302–313.

31. ** Ullah C, Tsai C-J, Unsicker SB, Xue L, Reichelt M, Gershenzon J, Hammerbacher A: **Salicylic acid activates poplar defense against the biotrophic rust fungus *Melampsora larici-populina* via increased biosynthesis of catechin and proanthocyanidins**. *New Phytol* 2019, **221**:960–975.
This paper shows the induction of SA in response to rust infection in poplar leaves. Elevated SA via exogeneous application of the SA analog benzothiadiazole or ectopic expression of a bacterial SA synthase in poplar trees activated both flavonoid biosynthetic genes and their regulatory genes, which resulted in the accumulation of antimicrobial flavan-3-ols.

32. Mata-Pérez C, Spoel SH: **Thioredoxin-mediated redox signalling in plant immunity**. *Plant Sci* 2019, **279**:27–33.

33. Jung Young J, Chi Yong H, Chae Ho B, Shin Mi R, Lee Eun S, Cha J-Y, Paeng Seol K, Lee Y, Park Jin H, Kim Woe Y, *et al.*: **Analysis of Arabidopsis thioredoxin-h isotypes identifies discrete domains that confer specific structural and functional properties**. *Biochem J* 2013, **456**:13–24.

34. Sweat TA, Wolpert TJ: **Thioredoxin h5 is required for victorin sensitivity mediated by a CC-NBS-LRR gene in *Arabidopsis***. *Plant Cell* 2007, **19**:673–687.

35. Li Y-B, Han L-B, Wang H-Y, Zhang J, Sun S-T, Feng D-Q, Yang C-L, Sun Y-D, Zhong N-Q, Xia G-X: **The thioredoxin GbNRX1 plays a crucial role in homeostasis of apoplastic reactive oxygen species in response to Verticillium dahliae infection in cotton**. *Plant Physiol* 2016, **170**:2392–2406.

36. Kneeshaw S, Keyani R, Delorme-Hinoux V, Imrie L, Loake GJ, Le Bihan T, Reichheld J-P, Spoel SH: **Nucleoredoxin guards against oxidative stress by protecting antioxidant enzymes**. *Proc Natl Acad Sci USA* 2017, **114**:8414–8419.

37. Chen Y-H, Sharma S, Bewg WP, Xue L-J, Gizelbach CR, Tsai C-J: **Multiplex editing of the *Nucleoredoxin1* tandem array in poplar: from small indels to translocations and complex inversions**. *bioRxiv* 2022, 505498. 2022.08.26.

38. El Oirdi M, El Rahman TA, Rigano L, El Hadrami A, Rodriguez MC, Daayf F, Vojnov A, Bouarab K: ***Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato**. *Plant Cell* 2011, **23**:2405–2421.

39. Zheng XY, Spivey NW, Zeng W, Liu PP, Fu ZQ, Klessig DF, He SY, Dong X: **Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation**. *Cell Host Microbe* 2012, **11**:587–596.

40. Nomoto M, Skelly MJ, Itaya T, Mori T, Suzuki T, Matsushita T, Tokizawa M, Kuwata K, Mori H, Yamamoto YY, *et al.*: **Suppression of MYC transcription activators by the immune cofactor NPR1 fine-tunes plant immune responses**. *Cell Rep* 2021, **37**, 110125.

41. Howe GA, Major IT, Koo AJ: **Modularity in jasmonate signaling for multistress resilience**. *Annu Rev Plant Biol* 2018, **69**:387–415.

42. Ding Y, Sun T, Ao K, Peng Y, Zhang Y, Li X, Zhang Y: **Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity**. *Cell* 2018, **173**:1454–1467.e1415.

43. Liu L, Sonbol F-M, Huot B, Gu Y, Withers J, Mwimba M, Yao J, He SY, Dong X: **Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity**. *Nat Commun* 2016, **7**, 13099.

44. Zhang N, Zhou S, Yang D, Fan Z: **Revealing shared and distinct genes responding to JA and SA signaling in Arabidopsis by meta-analysis**. *Front Plant Sci* 2020:11.

45. Betsuyaku S, Katou S, Takebayashi Y, Sakakibara H, Nomura N, Fukuda H: **Salicylic acid and jasmonic acid pathways are activated in spatially different domains around the infection site during effector-triggered immunity in *Arabidopsis thaliana***. *Plant Cell Physiol* 2018, **59**:8–16.

46. Ullah C, Unsicker SB, Reichelt M, Gershenzon J, Hammerbacher A: **Accumulation of catechin and proanthocyanidins in black poplar stems after infection by plectosphaerella populi: hormonal regulation, biosynthesis and antifungal activity**. *Front Plant Sci* 2019, **10**.

47. Li Y-x, Zhang W, Dong H-x, Liu Z-y, Ma J, Zhang X-y: **Salicylic acid in *Populus tomentosa* is a remote signalling molecule induced by Botryosphaeria dothidea infection**. *Sci Rep* 2018, **8**, 14059.

48. ** Ullah C, Schmidt A, Reichelt M, Tsai C-J, Gershenzon J: **Lack of antagonism between salicylic acid and jasmonate signalling pathways in poplar**. *New Phytol* 2022, **235**:701–717.
This study demonstrated the induction of SA and JA metabolites upon rust infection using multiple poplar genotypes with varying levels of rust resistance. Synergism between SA and JA was also reported, as high SA also induces JA and vice-versa. Unlike *Arabidopsis*, *PR* gene induction is not associated with elevated SA in poplar. The absence of SA-JA antagonism resulted in the accumulation of flavonoid phytoalexins and protected trees against rust.

49. * Gordon H, Fellenberg C, Lackus ND, Archinuk F, Sproule A, Nakamura Y, Köllner TG, Gershenzon J, Overy DP, Constabel CP: **CRISPR/Cas9 disruption of UGT71L1 in poplar connects salicinoid and salicylic acid metabolism and alters growth and morphology**. *Plant Cell* 2022, **34**:2925–2947.
This paper characterized the poplar UDP-dependent glycosyltransferase UGT71L1 involved in salicinoid biosynthesis. The *UGT71L1* mutants accumulated lower levels of salicinoids and displayed abnormal growth. Interestingly, the *UGT71L1* knockout lines contained higher SA and JA levels than wildtype, supporting a potential synergistic link between these two hormone pathways in poplar.

50. Duan L, Liu H, Li X, Xiao J, Wang S: **Multiple phytohormones and phytoalexins are involved in disease resistance to Magnaporthe oryzae invaded from roots in rice**. *Physiol Plantarum* 2014, **152**:486–500.

51. Miyamoto K, Enda I, Okada T, Sato Y, Watanabe K, Sakazawa T, Yumoto E, Shibata K, Asahina M, Iino M, *et al.*: **Jasmonoyl-l-isoleucine is required for the production of a flavonoid phytoalexin but not diterpenoid phytoalexins in ultraviolet-irradiated rice leaves**. *Biosci Biotechnol Biochem* 2016, **80**:1934–1938.

52. * Liang B, Wang H, Yang C, Wang L, Qi L, Guo Z, Chen X: **Salicylic acid Is required for broad-spectrum disease resistance in rice**. *Int J Mol Sci* 2022:23.
In this study, the role of SA hydroxylases (OsSAH) in SA homeostasis was demonstrated in rice. Perturbation of *OsSAH* by overexpression or knockout altered both SA and jasmonates, suggesting a positive link between SA and JA. Elevated SA and JA levels are also associated with enhanced pathogen resistance via the accumulation of flavonoid phytoalexins.

53. Raskin I, Skubatz H, Tang W, Meeuse BJD: **Salicylic acid levels in thermogenic and non-thermogenic plants**. *Ann Bot* 1990, **66**:369–373.

54. Malamy J, Hennig J, Klessig DF: **Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection**. *Plant Cell* 1992, **4**:359–366.

55. Hennig J, Malamy J, Grynkiewicz G, Indulski J, Klessig DF: **Interconversion of the salicylic acid signal and its glucoside in tobacco**. *Plant J* 1993, **4**:593–600.

56. Kawano T, Tanaka S, Kadono T, Muto S: **Salicylic acid glucoside acts as a slow inducer of oxidative burst in tobacco suspension culture**. *Zeitschrift Fur Naturforschung C - A J Biosci* 2004, **59**:684–692.

57. Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, Legrand M, Fritig B: **Plant 'pathogenesis-related' proteins and their role in defense against pathogens**. *Biochimie* 1993, **75**:687–706.

58. Mitsuhara I, Iwai T, Seo S, Yanagawa Y, Kawahigasi H, Hirose S, Ohkawa Y, Ohashi Y: **Characteristic expression of twelve rice PR1 family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180)**. *Mol Genet Genom* 2008, **279**:415–427.

59. * Muchero W, Sondreli KL, Chen J-G, Urbanowicz BR, Zhang J, Singan V, Yang Y, Brueggeman RS, Franco-Coronado J, Abraham N, *et al.*: **Association mapping, transcriptomics, and transient expression identify candidate genes mediating plant-pathogen interactions in a tree**. *Proc Natl Acad Sci USA* 2018, **115**:11573–11578.
The study demonstrated the power of GWAS mapping with a large population (~1000) of undomesticated poplar trees for identification of candidate resistance as well as susceptible genes against invasive diseases. A similar approach can also be applied for metabolic or signaling pathway investigations.

60. Rivas-San Vicente M, Plasencia J: **Salicylic acid beyond defence: its role in plant growth and development**. *J Exp Bot* 2011, **62**:3321–3338.

61. Peng Y, Yang J, Li X, Zhang Y: **Salicylic acid: biosynthesis and signaling**. *Annu Rev Plant Biol* 2021, **72**:761–791.

62. Lahari Z, Ullah C, Kyndt T, Gershenzon J, Gheysen G: **Strigolactones enhance root-knot nematode (*Meloidogyne graminicola*) infection in rice by antagonizing the jasmonate pathway**. *New Phytol* 2019, **224**:454–465.