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## A ménage à trois: Salicylic acid, growth inhibition and immunity

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### ABSTRACT

Salicylic acid (SA) is a plant hormone almost exclusively associated with the promotion of immunity. It is also known that SA has a negative impact on plant growth, yet only limited efforts have been dedicated to explain this facet of SA action. In this review, we focus on SA-related reduced growth and discuss whether it is a regulated process and if the role of SA in immunity imperatively comes with growth suppression. We highlight molecular targets of SA that interfere with growth and describe scenarios where SA can improve plant immunity without a growth penalty.

### Salicylic acid, immunity and plant growth

Salicylic acid (**SA**) (see Glossary) is one of the key hormones of plant disease resistance [1]. In plants, SA is found at low basal levels that differ depending on plant organs and tissues. Adult arabidopsis (*Arabidopsis thaliana*) plants contain 1-10 nmol/g FW SA in rosettes [2] and around 350 pmol/g FW in roots [3]. SA content, however, rises to much higher levels upon encountering pathogens [4]. SA action in immunity is now understood; it acts by both binding to **NPR1** oligomers [5] and conditioning an increase in cytosolic reducing power [6]. These two events trigger NPR1 de-oligomerization, a process also involving thioredoxins (**TRX**) [7]. NPR1 monomers move to the nucleus where they interact with TGA transcription factors (Fig. 1) to induce transcriptional changes including the expression of *PATHOGENESIS-RELATED (PR)* genes encoding proteins with putative antimicrobial activities [8]. NPR3 and NPR4 are also SA receptors. They could act, however, even in the absence of NPR1 [9].

Upon activation, the SA pathway leads to plant resistance against pathogens [1]. Yet, SA accumulation has a strong negative impact on plant vegetative growth. This can be seen in arabidopsis following infection-induced SA accumulation [10] and from the stunted phenotype of arabidopsis grown on media containing SA [11, 12]. Besides, most mutants displaying basal SA over-accumulation are dwarfs (reviewed in [13]). By crossing these mutant lines with either *sid2* or *NahG* plants, it has been shown that this dwarfism is indeed due to SA. *Sid2* plants are deficient in *SALICYLIC ACID INDUCTION DEFICIENT 2*, an enzyme of SA biosynthesis; *NahG* plants express a bacterial salicylate hydroxylase (Fig. 1). In both lines, SA accumulation is abolished [14].

The threshold of SA sensitivity in terms of growth suppression is tissue- and species-dependent. In rice (*Oryza sativa*) seedlings, 1 mM exogenous SA did not produce any measurable inhibiting effect on shoot growth [15], whereas a concentration as low as 10  $\mu$ M SA inhibited root growth of arabidopsis seedlings [12, 16].

#### **SA as a growth inhibitor – an effect of a bioactive molecule**

The negative effects of SA on growth can be due to toxicity and/or biological regulations. The contribution of SA toxicity to growth suppression (if any) can be estimated by looking at equivalent effects of related molecules. **4-HBA**, a biologically inactive SA isomer with regards to immunity signaling [5], did inhibit arabidopsis root growth, but at a 10-fold higher concentration (200  $\mu$ M) with respect to SA-related inhibition [17]. Sinapic acid, like SA, is a plant-derived phenol carboxylic acid but it does not replicate SA growth effects: high levels (500  $\mu$ M) of sinapic acid in media promote arabidopsis germination and root growth [18]. Thus, from a chemical perspective there appears to be no reason why low micromolar amounts of SA should inhibit plant growth however this is clearly seen in arabidopsis [12, 16].

An interesting observation was made in algae where SA treatments did not inhibit but stimulated growth [19]. Vascular plants and algae share many basic metabolic activities (e.g. photosynthesis) yet, no NPR1 homologues are found in algal genomes [20] and SA, apparently, is not acting as a hormone in algae. Nevertheless, endogenous SA production was recently reported in *Chlorella* [21].

### **SA-induced growth suppression relies on a set of SABPs but not necessarily NPR1**

Intriguingly, to act as a signaling molecule in both immunity and growth suppression, SA does not necessarily rely on the same modules. While NPR1 and its paralogs (NPR3 and NPR4) are essential elements of the SA signaling pathway in immunity [9], they do not necessarily play a major role in growth retardation. SA can act in either a fully [12, 22] or partially NPR1 independent manner [23, 24] in growth suppression. The negative effects of SA on root development were not rescued in *npr1*, *npr3*, or *npr4* lines [12]. SA suppressed pollen tube growth independently of NPR proteins [25] and only in limited cases, knocking-out *NPR1* allowed reversion of the dwarf phenotype associated with SA over-accumulating mutants [13, 26]. The presence of NPR1, however, might act against SA-induced growth suppression because *npr1* seedlings show an aggravated growth suppression by high doses of SA [27]. SA, but not 4-HBA, inhibited the respiration of isolated mitochondria from tobacco cells [28]. This effect cannot be mediated by NPR1 because it is not located in mitochondria.

When SA acts independently of NPR1, it must rely on other molecular targets within pathways leading to growth suppression. Note that SA is a unique plant hormone in the way that it can bind to numerous proteins (SA binding proteins, **SABPs**) other than NPR proteins [29-31]. SA binding to SABPs, including NPR1 [5], appears to be SA specific because a clear preference is seen over the 4-HBA isomer [30, 32, 33]. SA-induced growth suppression is believed to rely on a set of SABPs but which one(s) is still waiting to be identified. Both confirmed and candidate SABPs [31] are important enzymes of primary metabolism including photosynthesis (Calvin cycle), and energy production (TCA cycle and glycolysis) (Fig. 2) therefore SA, by interfering with their activities, could have a direct effect on growth, an energy-requiring process. SA directly binds to the E2 subunit of the  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ KGDE2), a mitochondrial enzyme of the TCA cycle. In isolated tomato (*Solanum lycopersicum*) mitochondria,  $\alpha$ KGDE2 activity was reduced by almost 50% after SA pretreatment [32, 34]. Cytosolic and plastidial forms of glyceraldehyde 3-phosphate dehydrogenases (GAPDH) are important enzymes of glycolysis and photosynthesis, respectively. Several arabidopsis GAPDHs from both sub-groups directly bind SA *in vitro* [33]. SA, but not 4-HBA, inhibited *in vitro* chloroplastic GAP1 activity by interfering with cofactor (NADP) binding [30]. A high-throughput analysis [31] has identified dozens of SABPs (Fig. 2) that now await verification of the effect of SA on their enzymatic activities and/or functional properties.

It is tempting to speculate that growth inhibition could be prevented by cancelling out the potential negative effects on metabolism when SA binds to SABPs. Yet this could actually be a part of a plant's immune response as both  $\alpha$ KGDHE2 and GAPA1 have a negative role in immunity [34-36]. Interestingly, inhibition of SABP metabolic activity could be a side-effect to SA targeting non-canonical activities of such enzymes. In the case of GAPC1, SA treatment prevented its association with the negative RNA strand of the tomato bushy stunt virus, a condition required for virus replication [33].

### **SA reprograms the transcriptome and impacts the proteome**

Not all SA-induced metabolism changes are to be explained solely by direct interactions with SABPs. In arabidopsis, SA treatments profoundly reprogram the transcriptome (Fig. 3). This applies not only to *PR* genes implicated in immunity but also to genes of energy metabolism. By analyzing public repositories of microarray datasets, it can be seen that SA-treatment induces the expression of genes acting in the mitochondrial respiratory chain. This, for example, is the case for NADH dehydrogenase subunits 6 and 5; cytochrome c oxidase subunit 3; ATPase subunit 6 (array data GSE51626). On the contrary, SA downregulates the expression of light capturing proteins and the Calvin cycle enzymes acting in photosynthesis (Fig. 3).

Interestingly, SA can affect plant transcriptome in an NPR1-dependent and -independent manner. In NPR1-null plants, the expression of a distinct subset of genes is controlled by SA [27, 37]. It is thus tempting to speculate that genes regulated by SA independently of NPR1 are important for growth control and not for immunity. As an example, the expression of metabolic enzymes such as alcohol dehydrogenase 1 and  $\alpha$ -dioxxygenase 1 was similarly SA-upregulated in *npr1* and WT plants [37].

SA-induced transcriptomic changes are mirrored by proteome reprogramming. In arabidopsis, high endogenous SA was linked to proteome changes affecting hundreds of proteins [38]. In cucumber, SA-induced proteomic changes notably concerned dozens of enzymes of photosynthesis and energy metabolism. SA treatment led to the depletion of two TCA cycle enzymes, malate dehydrogenase and a putative  $\alpha$ KGDE whereas this treatment induced a strong accumulation of  $\alpha$ -1,4-glucanotransferase and CP12-2, proteins acting in starch degradation and regulation of Calvin cycle enzymes, respectively [39]. Interestingly, both

malate dehydrogenase and  $\alpha$ KGDE2 are SABPs. This raises the question as to whether SA binding controls their abundance via regulating proteolysis.

Cumulatively, these transcriptomic and proteomic changes are indicative of SA-dependent metabolic flux reprogramming. In *Brassica napus* leaves, SA-induced starch depletion paralleled by the accumulation of soluble sugars was linked to a higher expression of the sucrose biosynthesis enzyme sucrose phosphate synthase and that of  $\beta$ -amylase 1 and  $\alpha$ -amylase 3, both acting in starch degradation [40].

### **SA interplay with other hormones**

SA is known to interplay with other classes of plant hormones which may help explain its role in growth suppression. Below we describe recent observations associating SA with IAA (indole-3-acetic acid), ethylene (ET) and gibberellins.

#### *Indole-3-acetic acid*

SA and IAA accumulations appear to be regulated in an antagonistic manner. In cassava plants (*Manihot esculenta*) infected by the bacterial blight agent, a gene encoding a heat shock protein, MeHSP90.9, is upregulated. MeHSP90.9 acts as a dual transcriptional regulator of SA accumulation coupled to IAA depletion, via binding to MeSRS1 and MeWRKY20, respectively [41]. In arabidopsis, SA accumulation leads to a reduction of IAA biosynthesis (Fig. 4). This involves the inhibition of catalase 2 by SA which impacts tryptophan biosynthesis via TSB1 (tryptophan synthetase  $\beta$  subunit 1)[42]. SA negatively affects not only IAA accumulation but signaling. SA promotes the abundance of the AXR2 auxin repressor [43] and prevents IAA-stimulated lateral root development in a pathway implicating ARF7 [44]. This antagonism of SA with IAA may bring about some of the observed SA inhibiting effects. Indeed, inhibition of root gravitropism by SA was attributed to a loss of auxin asymmetric distribution [12].

On the contrary, several dwarf lines of arabidopsis [45] and cucumber over-accumulate not only SA but also IAA [46] and IAA is accumulated in Pst DC3000-infected arabidopsis [42]. In addition, SA and IAA interplay in the control of root growth may not necessarily be antagonistic. Despite an apparent inhibiting effect on main root length, SA can selectively stimulate the formation of adventitious roots and the enlargement (maturation) of the root tip distal meristem at concentrations below 50  $\mu$ M [16]. These effects have been attributed to local

auxin accumulation and reprogramming of PIN-FORMED (PIN) auxin transporters. SA affected the expression but not the localization of PIN proteins: *PIN1-GFP* expression was enhanced while that of *PIN2-GFP* was repressed. Interestingly, these effects were spatially uncoupled from SA-induced *PR1:GUS* expression that was limited to the root tip maturation zone [16]. This supports the idea that SA activity in growth regulation does not necessarily use the same molecular machinery as in immunity.

A possible explanation of how SA, specifically at low concentrations, stimulates local auxin signaling has come from a study examining the same effect in cucumber. Based on *in vitro* enzymatic assays, SA was suggested to act as an alternative substrate of GH3.5 amido synthetase, thus preventing it from catalyzing the conjugation of IAA into IAA-Asp. In this way, SA would prevent active auxin pool depletion [47]. Similarly, GH3.5 from arabidopsis has been shown to use SA as a substrate [48], suggesting that this class of enzymes could have an important role in auxin (growth) and SA (immunity) interplay (Fig.4).

As already seen above, PIN transporters are components of the SA-IAA interplay. *In planta*, SA directly binds to PP2A phosphatase A subunits and inhibits total PP2A activity in an NPR1-independent manner [12]. This leads to the upregulation of PIN2 phosphorylation status, because it is a downstream target of PP2A (Fig. 4). SA treatment leads to the loss of PIN2-GFP polar localization and promotes its internalization in the root epidermis of arabidopsis [12]. SA could also affect PIN2 via another mechanism (Fig. 4). The presence of 100  $\mu$ M SA altered the distribution of PIN2-GFP in arabidopsis during a gravitropic response and this was associated with the transformation of the uniform PIN2 distribution into clusters within the plasma membrane of root cells [49]. Intriguingly, this effect was significantly alleviated in *rem1.2* null-mutants whereas overexpression of REM1.2 alone could bring about PIN2 clustering in the absence of SA [49]. REM1.2 is a member of the remorin 1 subfamily of lipid raft-regulatory proteins. By analyzing the results of a related study where SA induced plasmodesmata closure in a REM1.2- and REM1.3- dependent manner [23], the effects of SA on PIN2 distribution could be ascribed to changes in plasma membrane dynamics associated to an SA-induced promotion of lipid ordering and/or nanodomain assembly. Such observations may be connected to long-known effects of SA on the rapid production of signaling lipids in cell membranes [50] and therefore in a signaling process that is still to be incorporated within the conventional SA signaling pathway [51].

Interestingly, multiple evidences point out a role for SA in modulating auxin transport via a modification of PIN protein internalization via **clathrin endocytosis** [52].

### *Ethylene*

ET interplays with SA in the control of apical hook formation, a downward bending of the hypocotyl that is an important growth reaction to protect the apical meristem of germinating etiolated seedlings during skotomorphogenesis. Inhibition of arabidopsis apical hook formation by SA is NPR1-dependent and implemented by interfering with ET signaling [53]. It is suggested that following SA accumulation, NPR1 monomers shuttle into the nucleus where they bind EIN3, a key transcriptional factor of the ET signaling pathway, and inhibit its transcriptional activities resulting in the misregulation of *HOOKLESS1* [53], previously identified as a crucial element of apical hook formation [54]. Unexpectedly, the inhibiting effect of SA on apical hook formation was only retarded and not eliminated in *npr1* plants [53], suggesting the presence of compensatory pathways.

The same NPR1-EIN3 interaction was shown to be a part of a synergistic SA/ET interplay in the promotion of senescence [55]. In arabidopsis *ein3eil1* lines deficient in EIN3 and EIN3-like 1 (a close homolog), the promotion of senescence by SA (200  $\mu$ M) was cancelled. It would be interesting to know if the NPR1-EIN3 interaction has a role in growth suppression by SA. Interestingly, arabidopsis *ein3eil1* lines are SA-overaccumulators [56] yet they are not dwarfed [57].

### *Gibberellins*

A mechanism of plant growth inhibition by SA has been attributed to SA preventing the degradation of DELLA proteins [10] that are negative growth regulators in a gibberellic acid signaling pathway [58]. RGL3 is a DELLA protein and arabidopsis *rgl3* mutants accumulate SA and maintain better growth during a 10-day Pst DC3000 infection when compared to WT plants [10]. Interestingly, RGL3 directly interacts with EDS1, a positive regulator of SA-mediated immunity [59], and interferes with the putative transcriptional activity of EDS1 in the control of SA biosynthesis [10]. In this manner, RGL3 (and potentially other DELLA proteins) acts in conjunction with EDS1 in the control of SA-dependent immunity and growth trade-off.

## **SA modulates reactive oxygen species production and redox status signaling**



Reactive oxygen species (ROS) are important agents in both plant immunity and growth. SA modulates ROS production and cell redox status and affects transcription of antioxidant metabolism genes (Fig. 3). SA can counteract ROS damage because it generates an increase in cytosolic reducing power (**GSH:GSSG ratio**) [6, 60]. On the other hand, SA antagonizes catalase activity [42] and can stimulate a ROS burst by inhibiting the mitochondrial respiratory chain [61]. It should be noted that such contradictory SA effects do not necessarily occur in the same cells/organs.

SA acts not only in the control of ROS production but also in redox status signaling to regulate target proteins (Fig. 2). To achieve this, SA relies on SABPs. SA inhibited the activities of several glutathione S-transferases (**GST**) of arabidopsis *in vitro* [32]. Interestingly, in the immunity pathway, NPR1 monomerisation involves TRX-h5/TRX-h3, both cytosolic TRXs acting in redox signaling [7]. Binding of SA to these proteins has not been established however chloroplastic TRX-m1 is a SABP [31]. Because chloroplastic TRXs are important regulators of photosynthesis (for a review see [62]), such characteristics of SA may help to explain its role in controlling growth. In GSTF8-null arabidopsis, the absence of this SABP brought about a deregulation of redox status and ROS levels and led to aberrations in root development [63]. A 2-day 10  $\mu\text{M}$  SA treatment profoundly stimulated **periclinal divisions** in endodermal cells of arabidopsis root tips thus promoting the formation of the middle cortex. This SA effect was accompanied by elevated levels of  $\text{H}_2\text{O}_2$  while it was almost completely blocked by concomitant application of 0.5  $\mu\text{M}$  KI, an  $\text{H}_2\text{O}_2$  scavenger. SA down-regulates *CAT2* and *CAT3* expression in roots, therefore it was suggested that SA stimulates periclinal divisions in root endodermal cells by preventing  $\text{H}_2\text{O}_2$  scavenging [24].

A positive role of SA in root development via the stimulation of ROS production was observed in rice [64]. *ABNORMAL INFLORESCENCE MERISTEM1* (*AIM1*) encodes a 3-hydroxyacyl-CoA dehydrogenase, an enzyme believed to be involved in SA biosynthesis. In *aim1* seedlings, that exhibit a strong reduction of basal SA, defects in root meristem activity were linked to a reduction in ROS and resulted in a short-root phenotype. Treatment with either SA or  $\text{H}_2\text{O}_2$  (both at 500  $\mu\text{M}$ ) could substantially rescue root growth of *aim1* plants.

**Genetic manipulation can lead to enhanced immunity that is not at the expense of growth – a glimmering hope for immunity-growth decoupling**

Basal SA-over accumulation is good for immunity yet it comes at a price; growth defects rendering dwarfed plants [46, 65]. That said, a mild SA over-accumulation of around 2-fold the basal level is enough to make plants more resistant to pathogens without sacrificing their growth. This is the case for arabidopsis mutants deficient in *DPE2F-like 1 (DEL1)* transcription factor where enhanced pathogens resistance was not accompanied by growth suppression [66, 67]. The same pattern was observed in plants deficient in *CAX1* ( $H^+/Ca^{2+}$  exchanger 1) [68, 69], plants overexpressing *OXIDATION RESISTANCE 2 (AtOXR2)* coding a mitochondrial protein putatively acting in alleviation of oxidative stress) [70, 71], and *ein3eil1* double mutant lines [56, 57]. To date, it should be noted that no direct link between the mentioned mutations and SA-associated immunity has been established.

There are examples where even higher levels of SA accumulation do not slow down arabidopsis growth. For instance, knocking out the *FITNESS* gene encoding a CCT domain-containing protein resulted in high (up to 7-fold) SA accumulation in arabidopsis but produced no negative growth effect [72]. Despite an activation of *PR1* expression, the pathogen resistance of *fitness* lines is yet to be proven. In a similar manner, pathogen resistance has not yet been shown for arabidopsis *LAZY1* deficient mutants that significantly over-accumulate SA (ca. 5-fold) [73]. Despite a slightly unusual shoot architecture with low hanging side branches and a retarded shoot gravitropic response [73], these plants do not exhibit stunted rosette size [73, 74]. So situations can occur where a negative impact of SA on growth is cancelled out. From an immunity perspective, SA signaling indeed can be overridden. SA, even at high concentrations, was not able to trigger *PR1* expression in arabidopsis grown in conditions of low red/far-red light (shade avoidance modeling) [75] and in phytochrome signaling deficient mutants [76].

### **Concluding Remarks and Future Perspectives**

Despite the fact that SA signaling in immunity is well studied and understood, we know much less about the role of SA in growth suppression. Yet such knowledge is urgently required because SA-stimulated pathogen protection linked to sustainable plant growth is an ongoing problem. Here we have demonstrated that SA action on growth suppression is a regulated process that has multiple causes: (i) SA can interfere with plant metabolism and could lead to less energy being produced and/or available for growth; (ii) SA can establish a crosstalk with hormones; (iii) SA has an impact on plant cell redox status.

Because growth suppression relies less on SA-NPR1 interactions than immunity does, we believe that deciphering the binding of SA to SABPs should be prioritized in studies aiming to explain the effects of SA on metabolism and/or growth inhibition. Owing to the fact that many SABPs are enzymes of primary metabolism, their implication in SA-dependent growth responses cannot be tested by conventional knock-out mutant studies. In order to counteract metabolic disturbances introduced by the mutation, such plants could be complemented with proteins in which point mutations abolish SA binding [30]. Controlling SA accumulation is also an important factor in growth-immunity trade-off, making this another important future research topic. In the majority of SA over-accumulation lines, no direct connection to the regulation of SA biosynthesis has been established [13, 77, 78]. Yet these mutants are promising templates for the engineering of resistant crops with emphasis on mutant lines where mild SA accumulation has a beneficial effect on resistance yet produce no growth defects. The absence of growth defects, however, is not the only trait that should be pursued. SA accumulation can compromise plant defense to necrotrophic fungal pathogens. This is the case for *ein3eil1* lines that are SA-over accumulators, not dwarfed yet show an induced defense only to biotrophic and not to necrotrophic pathogens [56, 79]. It should be noted that genetic manipulations affecting defense-growth tradeoffs may be specific to certain plant species thus hindering technology transfer. Arabidopsis *dnd1* (Defense No Death 1) mutants are pathogen-resistant, over-accumulate SA, and exhibit dwarfism [80], whereas such effects have only been partially replicated by silencing *DND1* orthologs in tomato and potato (*Solanum tuberosum*) [81]. In Arabidopsis, growth-immunity tradeoffs can be improved (Box 1). Conventional approaches for improving plant immunity (e.g. priming) can automatically diminish the detrimental effects of a long lasting SA accumulation on plant growth.

When considering improving plant immunity while not sacrificing growth, hopes of producing a “super-plant” should be dismissed. Indeed, in some immune response scenarios conservation of growth is impossible. As a survival strategy, plants initiate local cell death by inhibiting photosynthesis in a controlled manner to condition the ROS burst [82]. A downregulation of auxin signaling is a well-established plant defense strategy [83], namely in terms of limiting lateral root development that act as pathogen entry sites [44]. Specific defense reactions such as cell wall strengthening and production of antimicrobial proteins and/or metabolites will inevitably consume cell resources. To what extent these reactions divert energy from primary metabolism in pathogen-challenged plants awaits to be shown with metabolomics studies [84].

## Supplemental information

Supplemental information associated with this article can be found at doi:XXXXXXX'

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## **GLOSSARY**

**4-HBA:** 4-hydroxybenzoic acid, an inactive SA isomer

**Clathrin endocytosis:** An endocytic trafficking pathway implicating vesicles coated in clathrin proteins. It has a regulatory role in many cellular processes including hormonal signaling.

**DELLA:** Nuclear proteins that function as negative regulators of gibberellin signaling and contain a conserved amino acid motif (DELLA) in their N-terminal domains. Perception of gibberellin leads to proteasome-mediated degradation of DELLA proteins and de-repression of growth.

**ET:** Ethylene is an unsaturated hydrocarbon gas acting as a plant hormone and best known for its role in fruit ripening, flower opening and leaf abscission.

**GSH:GSSG:** A ratio between reduced and oxidized forms of glutathione which is an indicator of cell redox status and oxidative stress. In the oxidized form (GSSG) two glutathione molecules are bound by a disulfide bridge. In the reduced glutathione form (GSH) such disulfide bridges are cleaved.

**GST:** Glutathione S-transferases are enzymes catalyzing the conjugation of the reduced form of glutathione to proteins thus modifying their properties.

**IAA:** Indole-3-acetic acid, the most common auxin class plant hormone serving as a signaling molecule required for growth coordination and the development of plant organs

**NPR1:** NONEXPRESSER OF PR GENES 1, a transcriptional regulator acting as a master regulator within the plant defense signaling network

**Periclinal divisions:** Mitotic cell division occurring parallel to the tissue or organ surface

**ROS:** Reactive oxygen species, a general term for highly reactive chemical molecules formed due to the electron receptivity of O<sub>2</sub> and includes peroxides, superoxide, hydroxyl radical, and singlet oxygen



**SA:** Salicylic acid or 2-hydroxybenzoic acid, a plant hormone involved in plant growth and development, and plant defense against pathogens

**SABP:** Salicylic acid binding protein, a protein that directly binds SA. When SABP is an enzyme, this could alter its activity.

**SAR:** Systemic acquired resistance, a process that triggers the resistance of a whole plant following a local infection. The onset of SAR is controlled by different signaling molecules including a derivative of SA, methylsalicylate.

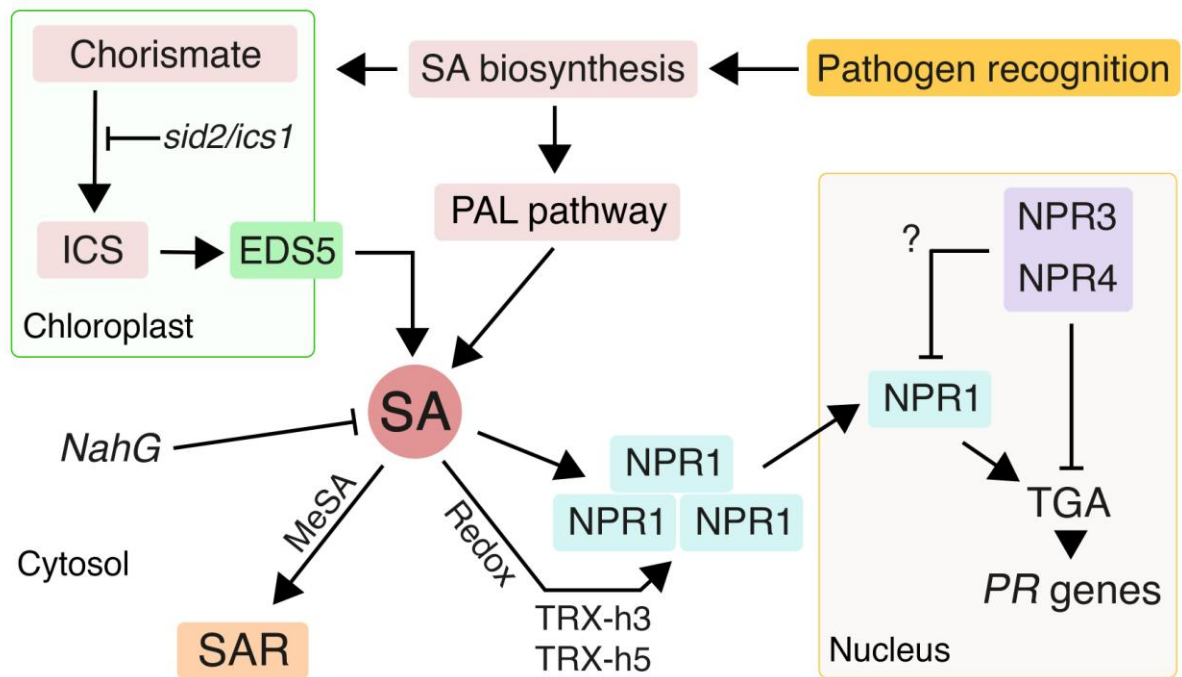
**TRX:** Thioredoxin, a class of ubiquitous proteins acting in redox signaling. A common mechanism of TRX action is by the reduction of protein disulfide bonds.

### **Box 1. Hints for immunity-growth balancing**

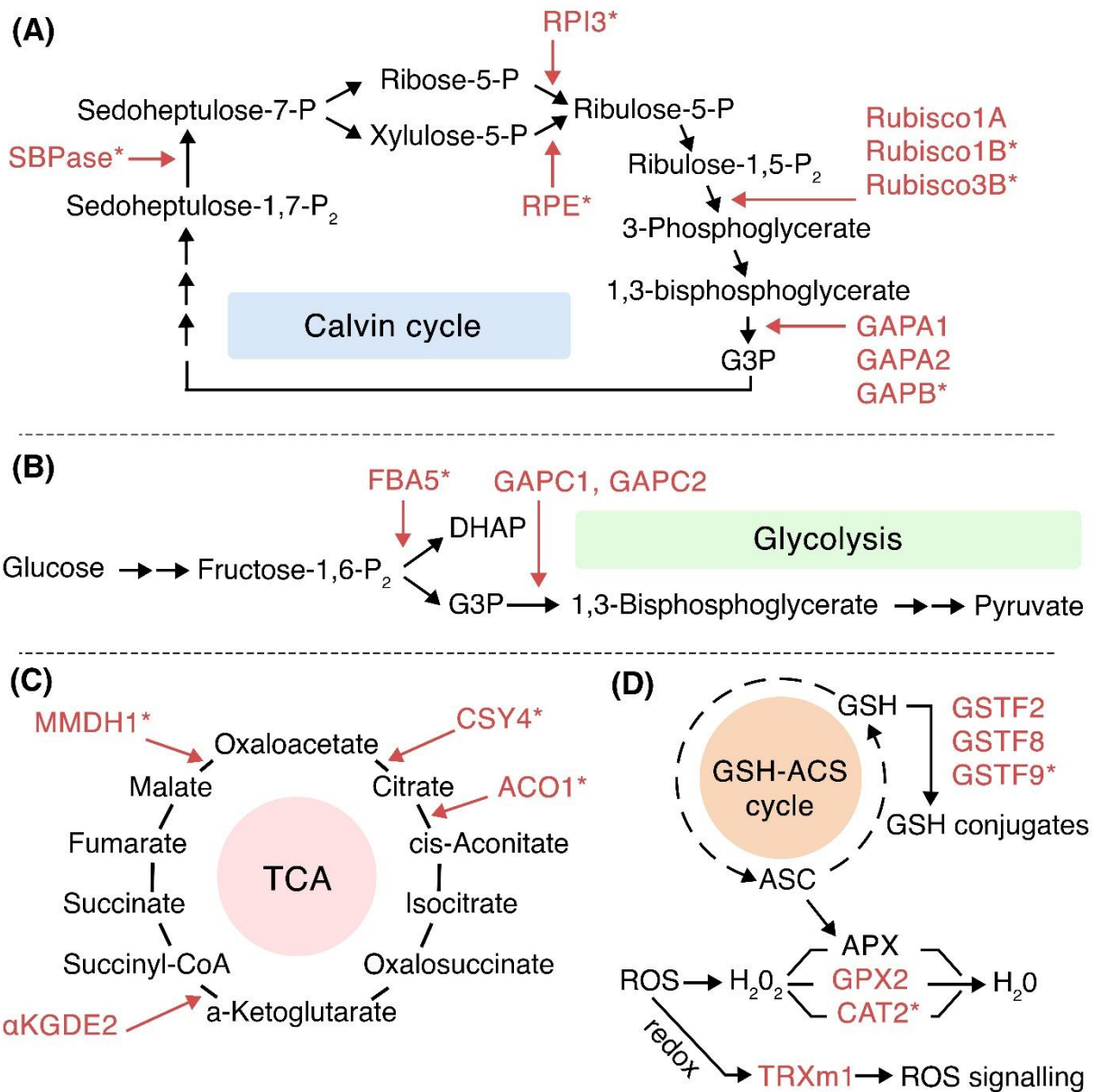
Some examples illustrate possibilities to manipulate growth-immunity balance in *arabidopsis*. *arabidopsis* genotype C24 accumulates high free SA levels in leaves (ca. 5 fold that of Col-0). Such plants have only mildly smaller rosettes and they produce the same amount of seed (~~in~~ ~~mg~~) as Col-0 plants [85]. Pathogen resistance of C24 *arabidopsis* was confirmed to be higher than that of Col-0 in multiple studies (for a review see [86]). It remains to be established which molecular modules allow C24 plants to perform better than Col-0 plants in growth-immunity trade-off.

In order to minimize SA action in growth suppression, a more vigorous immune response resulting in a rapid “victory” over the pathogen is prioritized. One way to achieve this is to have more elements of immune signaling ready at hand (a phenomenon known as priming). Rice plants that over-express NPR1, either constitutively or, preferably, by using specific infection-inducible promoters, showed promising results of producing resistance without a growth penalty [87]. Yet, high NPR1 expression has never been selected as an evolutionary beneficial trait and therefore drawbacks might be expected. Hence, NPR1 over expression promotes SA-induced leaf senescence in *arabidopsis* [55]. In accordance with such an idea of a “more vigorous immune response”, an interesting trait observed in F<sub>1</sub> hybrids is a more rapid SA accumulation upon infection. *arabidopsis* F<sub>1</sub> hybrids obtained from crossing Col-0 with either Sei-0 or Ler ecotypes, showed a significant increase in Pst DC3000 resistance compared to parental ecotypes. In both cases, hybrids accumulated on average 2-fold more SA after a day of Pst DC3000 infection while no basal SA over-accumulation was detected [4]. Moreover, the effect of heterosis *per se* was found to be connected, at least in part, to diminished basal SA content and signaling response(s) in *arabidopsis* [88]. These molecular aspects of heterosis and corresponding epigenetic patterns are yet to be fully disclosed. Like hybrid plants, mutants that do not over-accumulate basal SA levels yet exhibit potentiated SA defenses upon infection are also promising templates for biotechnological engineering (e.g. *rgl3* mutants) [10].

### **Figures and legends**

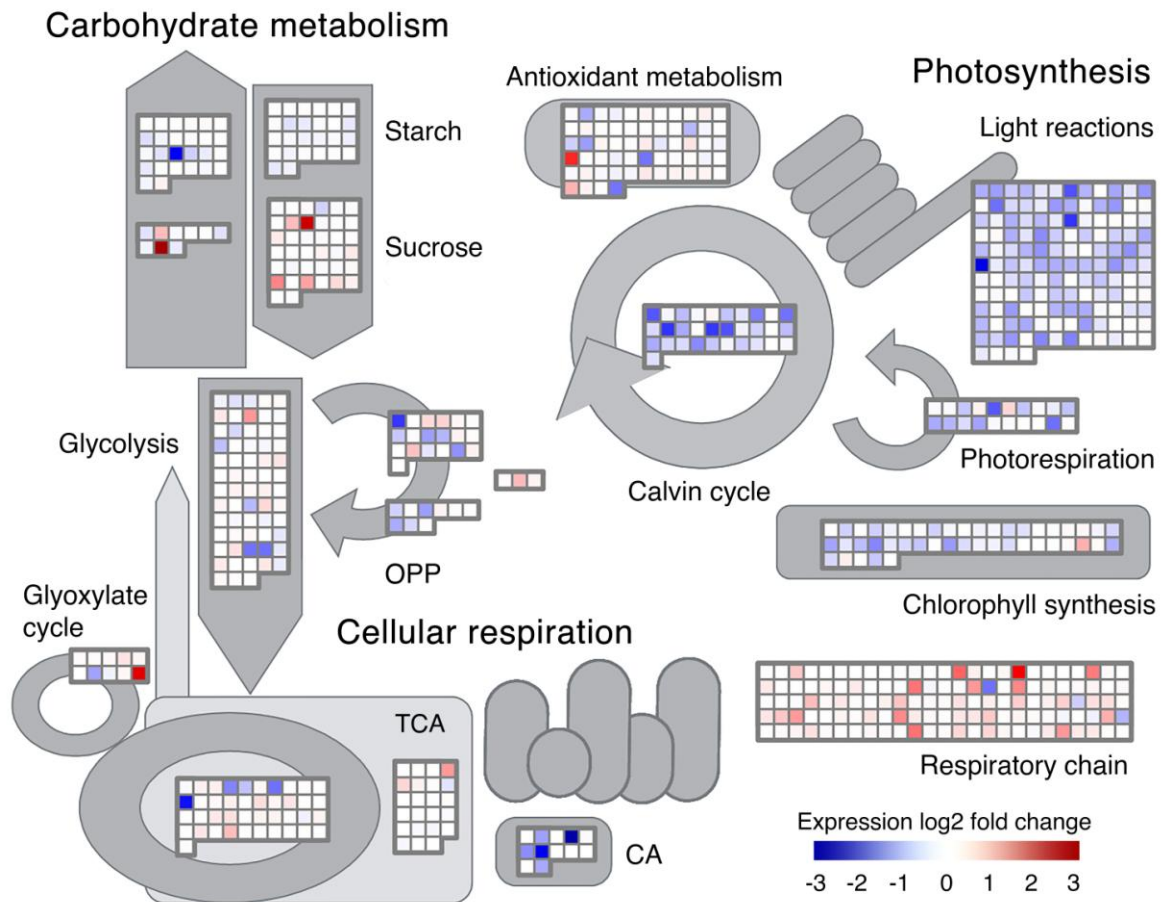


**Figure 1. SA synthesis and pathogen response signaling.** SA synthesis is triggered as a reaction to pathogen recognition; SA can be synthesized via two pathways referred to according to their characteristic intermediates; isochorismate (ICS) or phenylalanine (PAL). The ICS pathway is initiated in chloroplasts where ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) acts as an exporter of ICS to the cytosol for the final SA synthesis steps [89]. The ICS pathway is required for pathogen-induced SA production in arabidopsis since it is abolished by mutating isochorismate synthase *SID2/ICS1* [90]. SA can be converted to inactive catechol by 1-hydroxylases; enzymes identified in bacteria, fungi and plants [91]. Heterologous expression of a bacterial SA 1-hydroxylase gene, *NahG*, is used as a tool to deplete SA levels in plants. In the SA signaling cascade, NPR1 monomerisation requires SA binding to NPR1 and a redox regulation involving cytosolic thioredoxins (TRX-h3, TRX-h5) and leads to the expression of *PR* genes via NPR1-TGA transcription factor interactions. NPR3 and NPR4 proteins (paralogs of NPR1) also bind SA yet act as transcriptional co-repressors [9, 92] and, arguably, have a role in NPR1 degradation [93]. SA produced at the local infection site leads to whole-plant systemic acquired resistance (**SAR**) and involves SA methylation to produce volatile methylsalicylate (MeSA) [1].

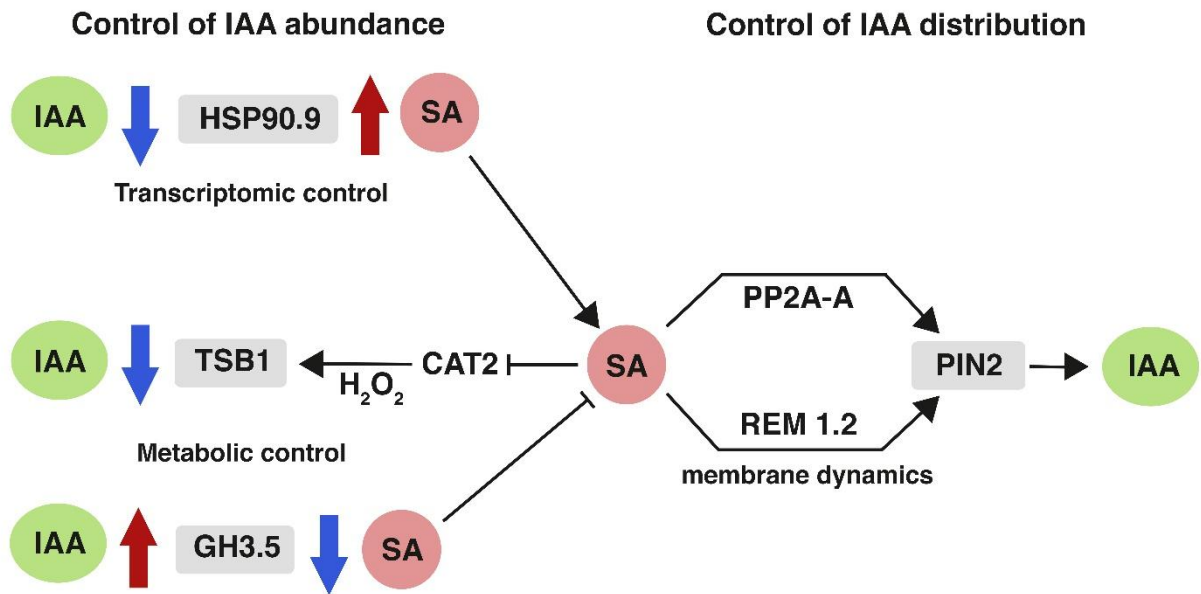


**Figure 2. Key plant energy metabolism enzymes and reactive oxygen species (ROS) pathway proteins that are SABPs.** Simplified representations of metabolic pathways are as follows: (A) Calvin cycle; (B) glycolysis; (C) tricarboxylic acid cycle (TCA); (D) ascorbate-glutathione cycle. SABPs are in red script. DHAP, Dihydroxyacetone phosphate; G3P, Glyceraldehyde 3-phosphate; RPI, ribose-5-phosphate isomerase; RPE, Ribulose-5-phosphate-3-epimerase; SBPase, Sedoheptulose-1,7-bisphosphatase; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; GAPA1, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) subunit A1; GAPB, GAPDH subunit B; GAPC1, GAPDH subunit C1; FBA, Fructose-bisphosphate aldolase;  $\alpha$ KGDHE2, Alpha-ketoglutarate dehydrogenase E2 subunit; ACO, Aconitate hydratase; MMDH, Mitochondrial malate dehydrogenase; CSY, Citrate synthase; TRXm, Thioredoxin m-type; APX,

Ascorbate peroxidase; GPX, Glutathione peroxidase; CAT, Catalase; ASC, Ascorbate; GSH, Glutathione; GSTF, Glutathione S-transferase class Phi; \*, candidate SABP, a protein identified as a SABP in high-throughput analyses but not (yet) confirmed as one in dedicated biochemical assay.



**Figure 3. SA-induced transcriptomic changes in energy metabolism pathways.** The image was generated by Mapman [94] using array data (GSE51626) obtained from Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>). Two week-old arabidopsis Col-0 plants were treated with 2 mM SA for 24 h. Fold-changes (log<sub>2</sub>) in transcript levels between SA-treated and non-treated plants are shown as a heatmap. CA, carbonic anhydrases; OPP, oxidative pentose phosphate pathway; TCA, tricarboxylic acid cycle. For numeric values of gene expression changes see online Supplementary Table S1.



**Figure 4. Interplay between SA and IAA.** In cassava plants, IAA and SA synthesis is regulated in an antagonistic manner by Heat Shock Protein HSP90.9 acting as a dual transcriptional regulator of SA accumulation coupled to IAA depletion, via binding to MeSRS1 and MeWRKY20, respectively [41]. In arabidopsis, accumulation of SA leads to catalase 2 (CAT2) inhibition and consequent accumulation of H<sub>2</sub>O<sub>2</sub>; downstream this conditions a reduction of IAA synthesis by negatively affecting tryptophan synthetase  $\beta$  subunit 1 (TSB1) [42]. Yet, SA can promote IAA signaling by acting as a substrate of GH3.5 that prevents depletion of the active IAA pool [47]. SA also affects auxin transport via PIN2 by binding and inhibiting PP2A-A [12] and/or an unknown mechanism involving REM1.2, a protein involved in membrane dynamics [49].