MPMI Vol. 35, No. 7, 2022, pp. 627–637, https://doi.org/10.1094/MPMI-11-21-0283-FI

e-Xtra*

Cold Exposure Memory Reduces Pathogen Susceptibility in *Arabidopsis* Based on a Functional Plastid Peroxidase System

Thomas Griebel,† Dominic Schütte, Alina Ebert, H. Hung Nguyen, and Margarete Baier

Plant Physiology, Dahlem Center of Plant Sciences, Freie Universität Berlin, Königin-Luise-Straße 12-16, 14195 Berlin, Germany

Accepted 24 March 2022.

Chloroplasts serve as cold priming hubs modulating the transcriptional response of *Arabidopsis thaliana* to a second cold stimulus for several days by postcold accumulation of thylakoid ascorbate peroxidases (tAPX). In an attempt to investigate cross-priming effects of cold on plant pathogen protection, we show here that such a single 24-h cold treatment at 4°C decreased the susceptibility of *Arabidopsis* to virulent *Pseudomonas syringae* pv. *tomato* DC3000 but did not alter resistance against the avirulent *P. syringae* pv. *tomato avrRPM1* and *P. syringae* pv. *tomato avrRPS4* strains or the effector-deficient *P. syringae* pv. *tomato* strain *hrcC*⁻. The effect of cold priming against *P. syringae* pv. *tomato* was active immediately after cold exposure and memorized for at least 5 days. The priming benefit was established independent of the immune regulator Enhanced Disease Susceptibility 1 (EDS1) or activation of the immune-related genes *NHL10*, *FRK1*, *ICS1* and *PR1* but required thylakoid-bound as well as stromal ascorbate peroxidase activities because the effect was absent or weak in corresponding knock-out-lines. Suppression of *tAPX* postcold regulation in a conditional-inducible *tAPX-RNAi* line led to increased bacterial growth numbers. This highlights that the plant immune system benefits from postcold regeneration of the protective chloroplast peroxidase system.

Keywords: *Arabidopsis thaliana*, ascorbate peroxidases, cold, immunity, memory, pathogen, priming, sAPX, susceptibility, tAPX

Plants have evolved sophisticated molecular networks that respond differently to simultaneous or sequentially experienced stress events than to single stress situations (Saijo and Loo 2020; Zhang and Sonnewald 2017). The combination of two sequential and transient stress events in which the exposure to a prior stress leads to earlier, faster, stronger, or more sensitive responses during the subsequent triggering stress defines a priming scenario (Crisp et al. 2016; Hilker et al. 2016). Although plants lack a nervous system and an antibody-based adaptive immune system, the plant capacity for a stress memory is evident and well described (Conrath 2011; Crisp et al. 2016; Gourbal et al. 2018; Hilker et al. 2016). The molecular priming memory is formed during a stress-free interphase (lag or memory phase), when primary stress responses are lost (Hilker et al. 2016). Such a priming memory, which subsequently modifies the response to a later triggering stimulus, can result from chromatin modifications but also be imprinted by preparatory formation or persistence of key signaling metabolites and proteins which are kept in an inactive form during the stress-free memory phase (Baier et al. 2019; Conrath 2011; Crisp et al. 2016). The priming and the subsequent stress events can be of the same nature (*cis*-priming) or differ from each other (*trans*-priming) (Hilker et al. 2016). The phenomenon of systemic acquired resistance (SAR) is an intensively studied example of priming in plants, in which a pathogen infection leads to improved and preactivated immune responses in distant, uninfected tissues (Conrath 2011). SAR requires long-distance signaling and provides long-lasting protection against a broad range of pathogens (Fu and Dong 2013; Shah and Zeier 2013). Pathogen-induced priming leads to a transcriptional reprogramming in uninfected plant tissues, including chromatin opening and modification and preactivation of immune-related genes (Baum et al. 2019; Gruner et al. 2013; Jaskiewicz et al. 2011).

In contrast to multiple and ternary stress concepts, the dual plant–pathogen interaction based on the plant innate immune system is broadly and conceptually understood (Albert et al. 2020; Jones et al. 2016; Lolle et al. 2020). Plants detect pathogens through recognition of pathogen-associated molecular patterns (PAMPs) by cell surface-exposed pattern recognition receptors (PRRs). PRR activation induces defense responses, summarized as PRR- or PAMP-triggered immunity (PTI), and is efficient against a broad range of pathogens (Albert et al. 2020). Host-adapted and virulent pathogens suppress PTI by secreting virulence proteins (so-called effectors) into the host cells with the aim to manipulate cellular physiology and to suppress innate immunity (Büttner 2016). This process strongly affects the susceptibility of the plant against pathogens and is designated accordingly as effector-triggered susceptibility (ETS) (Jones and Dangl 2006). A further layer of pathogen defense comprises intracellular nucleotide-binding leucine-rich repeat immune receptors (NLRs) that intercept the presence or activity of pathogen virulence effectors and initiate plant responses summarized as effector-triggered immunity (ETI) (Lolle et al. 2020). Two structurally different N-terminal domains, Toll-interleukin1 receptor-like and coiled-coiled, form two major groups of plant NLRs: TNLs and CNLs, respectively (Jacob et al. 2013). For instance, the CNL Resistance

†Corresponding author: T. Griebel; thomas.griebel@fu-berlin.de

Funding: This work was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation) grant CRC973/C4 and the Freie Universität Berlin.

*The *e*-Xtra logo stands for "electronic extra" and indicates that supplementary materials are published online.

The author(s) declare no conflict of interest.

to *Pseudomonas syringae* pv. *maculicola* 1 (RPM1) detects the presence of the bacterial effector avrRPM1 by sensing its virulence activity on the RPM1-Interacting Protein 4 (RIN4) (Mackey et al. 2002). An alternative scenario is described by immune activation through the TNL receptor pair Resistance to *Ralstonia solanacearum* 1 (RRS1) and Resistance to *P. syringae* 4 (RPS4) (Griebel et al. 2014). When the bacterial effector AvrRps4 from *P. syringae* pv. *pisi* is expressed in the otherwise virulent *P. syringae* pv. *tomato* DC3000, the TNL pair RPS4/RRS1 traces avrRPS4 interference by using the integrated WRKY domain in RRS1 as a decoy and trap and turns ETS into ETI (Hinsch and Staskawicz 1996; Le Roux et al. 2015; Sarris et al. 2015). Although the bacterial effectors and corresponding NLRs inducing ETI are numerous, all TNL receptors share the requirement of the nucleocytoplasmic immune regulator Enhanced Disease Susceptibility 1 (EDS1) for all identified signaling responses (Dongus and Parker 2021).

The outcome of a dual plant–pathogen interaction is determined at the genetic level and by the repertoire of available plant immune receptors. In addition, environmental factors such as light or temperature shape the plants' capacity to defeat pathogens (Roeber et al. 2021; Saijo and Loo 2020; Velásquez et al. 2018). Lower temperatures often lead to simultaneous activation of plant responses required for cold protection and pathogen resistance, indicating plant cross-tolerance of cold and pathogen defense (Saijo and Loo 2020). At the transcriptional level, even after a several-day-long cold period, reacclimation to precold conditions occurs fast and within hours (Byun et al. 2014; Zuther et al. 2015). Although a single 10-h cold exposure during the night phase is not sufficient to prime plant resistance against the hemibiotrophic bacterial pathogen *P. syringae* pv. *tomato* for several days (Wu et al. 2019), daily repetitive cold treatments of 1.5 h (in the dark) result in a memorized and enduring effect on in planta *P. syringae* pv. *tomato* growth numbers and a preactivation of PTI responses (Singh et al. 2014).

Ascorbate peroxidases (APX) scavenge H_2O_2 by using ascorbate as an electron donor (Groden and Beck 1979). *Arabidopsis thaliana* has nine nuclear-encoded *APX* genes that translate into isoforms with different subcellular localizations (cytosol, peroxisomes, chloroplasts, and mitochondria) (Ishikawa and Shigeoka 2008). Most plants have two active chloroplast APX, of which one is soluble in the stroma (sAPX) and one is anchored in the thylakoid membrane (tAPX) (Ishikawa and Shigeoka 2008; Miyake and Asada 1992; Pitsch et al. 2010). Chloroplastic APX differ from other APX by containing two additional sequence insertions (Pitsch et al. 2010; Wada et al. 2003). At the functional level, active chloroplast APX have a faster half-inactivation time (15 s) compared with cytosolic isoforms (>40 min) when the ascorbate levels are low (<10 μM) (Kitajima 2008). tAPX is part of a first layer to scavenge photosynthesis-generated H_2O_2 at the thylakoids, while sAPX provides downstream antioxidant protection in the stroma (Asada 1999). Chloroplastic APX activity is suggested to be central for increased abiotic stress tolerance by avoiding oxidative damage and supporting retrograde signaling (Caverzan et al. 2012; Maruta et al. 2016; Pandey et al. 2017). However, extreme phenotypes in loss-of-function mutants of single genes could not be observed, probably because of strong functional compensation by other antioxidant enzymes and low molecular weight antioxidants (Kangasjärvi et al. 2008; Maruta et al. 2016).

As shown recently, 4-week-old *A. thaliana* Columbia-0 (Col-0) plants memorize a 24-h exposure (including day and night phase) to mild cold (4°C) for up to 7 days (van Buer et al. 2019). Such cold priming leads to modified activation of cold-stress-responsive genes during a second cold treatment and requires reincreasing expression of *tAPX* after cold priming (Bittner et al. 2020; van Buer et al. 2016, 2019). Consistently, conditional overexpression of *tAPX* establishes a cold memory in the absence of the priming cold treatment (van Buer et al. 2019). Levels of neither reactive oxygen species (ROS) nor ascorbate are altered in cold-primed plants 5 days after the end of the initial cold exposure compared with control plants (van Buer et al. 2016). Here, we investigated whether a single, memorized cold exposure lasting 24 h (including day and night phase) affects plants pathogen resistance against *P. syringae* pv. *tomato*, given that such a cold priming event specifically alters gene regulation during a second cold exposure or a high light treatment in a trigger-specific manner (Bittner et al. 2020, 2021; van Buer et al. 2016, 2019). Furthermore, we analyzed whether plastid APX contribute to cold priming against pathogens because tAPX was shown to be a cold priming mediator during a subsequent cold exposure (van Buer et al. 2016, 2019).

RESULTS

Cold exposure of 24 h reduces *Arabidopsis* immune susceptibility for up to 5 days in an EDS1-independent manner.

To study whether cold priming affects the plant immune response, we challenged cold-pretreated 4-week-old *Arabidopsis* Col-0 plants (4°C, 24 h) after a recovery period of 5 days by infiltration with different strains of the bacterial pathogen *P. syringae* pv. *tomato* DC3000 (Fig. 1A). In addition to the virulent *P. syringae* pv. *tomato*, avirulent *P. syringae* pv. *tomato* strains were used that additionally express either the bacterial effector avrRPM1 or avrRPS4. Both strains are recognized in planta by intracellular immune receptors and activate ETI immune responses (Grant et al. 1995; Hinsch and Staskawicz 1996). The nonvirulent *P. syringae* pv. *tomato* strain *hrcC*$^-$ is deficient of a functional type three secretion system (Roine et al. 1997) and provides information about the plant's resistance without the involvement of type three secretion system–dependent bacterial effectors. Cold-primed Col-0 plants (4°C, 24 h) showed significantly reduced titers of the virulent *P. syringae* pv. *tomato* strain 3 days after infiltration compared with naive control plants when the inoculation was performed 5 days after cold exposure (Fig. 1A). Bacterial titers of the avirulent ETI-inducing *P. syringae* pv. *tomato* strains *avrRPM1* and *avrRPS4* and the nonvirulent PTI-inducing strain *hrcC*$^-$ (Fig. 1A) did not differ in cold-primed and control plants. This indicates that, specifically, plant defense against virulent pathogens benefits from a prior cold exposure while plant resistance against avirulent and nonvirulent pathogens is not affected. Consistent with the results of Wu et al. (2019), *P. syringae* pv. *tomato* infections immediately (1 h) after 24 h of cold exposure (4°C) resulted in reduced bacterial growth in Col-0 (Fig. 1B). Our experiment showed that the effect lasted for 5 days, during which the plants did not experience cold (Fig. 1B). Hence, the priming effect of the 24-h cold treatment counteracted pathogen growth in the plants not only during the period when cold regulation of gene expression weakens out but also later, when the priming effect on the cold sensitivity was established (van Buer et al. 2019). Equal density of the starting inoculum and the infiltration efficiency was confirmed by measuring bacterial titers 3 h after plant infiltration in control and cold-primed plants (Supplementary Fig. S1).

To distinguish cold priming regulation from induced basal immunity and resistance responses, we included the strongly immune-compromised *eds1-2* null mutant (Bartsch et al. 2006) into our analyses. EDS1 is an essential molecular plant immune regulator that contributes to resistance mediated by cell-surface receptors and intracellular TNLs and, hereby, is an integral part of TNL-driven immune signaling in PTI, ETI, and SAR (Breitenbach et al. 2014; Dongus and Parker 2021). The enhanced susceptibility of *eds1-2* against *P. syringae* pv. *tomato* was significantly reduced compared with the level of control plants when the plants

were inoculated immediately or after 5 days of recovery from the cold treatment (72 and 65% reduction of bacterial titers, respectively) (Fig. 1B). Cold exposure and its memorization weakened plant susceptibility independent or downstream of EDS1-mediated immune signaling.

Cold response and recovery is functional in immune-impaired *eds1*.

Priming and memory concepts require the perception and response of a first (priming) stimulus which initiates the formation of a molecular memory for future stresses (Hilker et al. 2016). We compared initial cold response and recovery effects between Col-0 and *eds1-2* at the transcriptional level of selected genes directly after 24 h of exposure to 4°C (0 days) and after 3 days. For this analysis, we harvested plant leaves of the same size and developmental status as used for bacterial infiltrations. We selected four genes based on recent work on cold *cis*-priming: *Cold-Regulated Gene 15A* (*COR15A*; At2g42540), *Zinc Finger of Arabidopsis thaliana 10* (*ZAT10*; At2g27730), *Bon-Associated Protein 1* (I; At3g61190), and *Phenyl Ammonia Lyase 1* (*PAL1*; At2g37040) (van Buer et al. 2016). *COR15A,* which encodes a protein protecting the inner envelope of chloroplasts against freezing damage, is strongly induced in the cold and quickly (within 24 h) reset at optimal growth temperatures (Steponkus et al. 1998; Zarka et al. 2003). The same applies to the ROS-induced and pleiotropic stress-responsive genes *ZAT10*, *BAP1*, and *PAL1* (van Buer et al. 2016, 2019). Cold induction of *COR15A* and *ZAT10* reached a similar level in Col-0 and *eds1-2* at the end of the cold

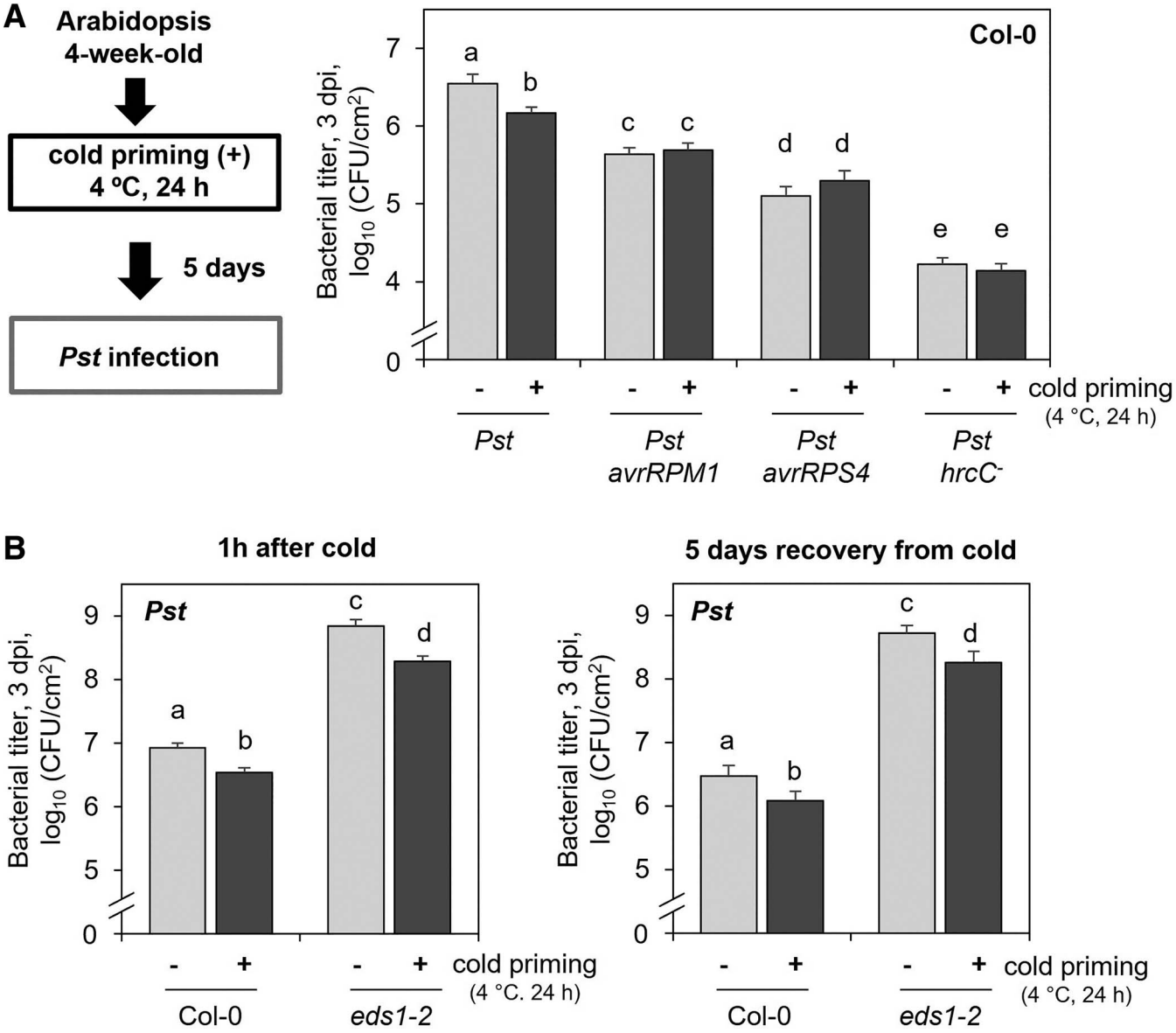

Fig. 1. Bacterial growth of *Pseudomonas syringae* pv. *tomato* (*Pst*) in Col-0 and *eds1-2* after a 24-h cold exposure. **A,** Four-week-old *Arabidopsis thaliana* plants were exposed to a 4°C cold priming treatment for 24 h (+). At 5 days after cold exposure, plants were infiltrated with *P. syringae* pv. *tomato* strains to determine the plant's level of resistance and susceptibility. Bacterial titers (log_{10}-transformed) of virulent *P. syringae* pv. *tomato*, avirulent *P. syringae* pv. *tomato avrRPM1* and *avrRPS4*, and the type-three-secretion-deficient *P. syringae* pv. *tomato hrcC*$^{-}$ strains in cold-primed (+) and control (−) Col-0 plants were determined at 3 days postinoculation (dpi). Bars represent means and standard errors calculated from three independent experiments, each with six to eight biological replicates using a mixed linear model. **B,** Bacterial titers of *P. syringae* pv. *tomato* (log_{10}-transformed) in cold-primed (+) and control plants (−) of Col-0 and *eds1-2* null mutants were determined 3 dpi. Plants were infiltrated with *P. syringae* pv. *tomato* (optical density at 600 nm = 0.001) 1 h (left panel) or 5 days (right panel) after the cold exposure. Bars represent means and standard errors calculated from five independent experiments each, with five to six biological replicates using a mixed linear model. Different letters above the bars denote statistically significant differences (adjusted $P < 0.05$; two-tailed *t* tests).

exposure and was reset to control rates within 3 days (Fig. 2). This is similar for *BAP1* regulation in Col-0 and *eds1-2*, although at an overall lower level in *eds1-2*. The conversion of L-phenylalanine to cinnamic acid by PAL1 is a key enzymatic step for a multitude of phenlypropanoids such as anthocyanins, flavonoids, hydroxycinnamates, and monolignols but also for the synthesis of basal amounts of the plant hormone salicylic acid (SA) (Ding and Ding 2020). However, pathogen-induced SA is mainly metabolized by iso-chorismate synthase 1 (ICS1), and its gene expression is strongly induced after pathogen attack (Hartmann and Zeier 2019). We could not detect a clear and significant upregulation of *ICS1* at the end of cold priming exposure and after 3 days of reacclimation; however, *ICS1* transcripts were rather reduced after 24 h at 4°C in *eds1-2* (Fig. 2).

In all of the samples, transcripts of SA-responsive immune marker gene *Pathogenesis-Related 1* (*PR1*) remained at low and basal levels and were not detectable. Overall, this analysis demonstrated that cold signaling during and after exposure to 4°C is perceived and processed in Col-0 and in plants lacking the central immune regulator EDS1. Although infections directly after cold exposure might benefit from overlapping with postcold deacclimation of gene expression, infections 5 days after priming required a molecular memory, because cold-induced genes were already reset for at least 2 days (Fig. 2) (Zuther et al. 2015). The low responsiveness of *ICS1* and *PR1* during and after the postcold phase distinguished cold priming-reduced susceptibility from SAR, in which a first infection leads to an upregulation of immunity genes (including *ICS1* and *PR1)* in the noninfected systemic tissue (Bernsdorff et al. 2016; Gruner et al. 2013; Hartmann and Zeier 2019). The *eds1-2* null mutant, which is impaired in establishing SAR (Breitenbach et al. 2014), showed reduced susceptibility in response to cold priming (Fig. 1). This indicates different regulation of SAR and cold-primed pathogen resistance.

Pathogen-induced transcript levels of selected *PTI* genes and SA signaling are independent from crosstalk with the cold priming memory.

Two recently published transcriptomic data sets showed that, 5 days after 24 h of cold exposure, gene expression patterns differ only marginally (Bittner et al. 2020, 2021). To differentiate transcriptional cold priming effects from preactivation of genes in *P. syringae* pv. *tomato*-induced SAR, we had a closer look at the transcript ratios of all genes from gene ontology (GO) group "SAR" (GO:0009627) at the end of the 5-day recovery phase from both data sets (Bittner et al. 2020; Bittner et al. 2021) and compared with data after 24 h cold exposure (Bittner et al. 2020) and with gene regulation data in a SAR-induced state (Gruner et al. 2013). Although genes from the GO term group "SAR" showed strong differential expression in the SAR data set, differential expression after 5 days recovery from cold exposure or after 24 h cold exposure (4°C) was weak or absent (Supplementary Fig. S2). This analysis confirmed that preactivation of SAR-related genes is not a central component reduced susceptibility by cold priming.

Next, we tested transcript levels of selected immune-related genes as indicators for a stronger and primed activation upon *P. syringae* pv. *tomato* infection. Transcriptome dynamics upon infections with virulent *P. syringae* pv. *tomato* are established rather late (between 16 and 24 h after infection), whereas ETI-inducing pathogens trigger mainly identical transcriptional changes already between 4 and 6 h (Mine et al. 2018). Hence, we tested the expression levels of PTI-triggered gene *NDR1/HIN1-like10* (*NHL10*, also known as *YLS9*; At2g35980) and mitogen-activated protein kinase-specific target gene *FLG22 Induced Receptor Kinase 1* (*FRK1*; At2g19190) (Boudsocq et al. 2010) 0, 6, and 24 h after *P. syringae* pv. *tomato* infection in leaves of cold-primed and control plants. At the time of pathogen infiltration, no cold-priming-related preactivation of *NHL10* and *FRK1* was detectable (Fig. 3). *NHL10* and *FRK1* were significantly induced 24 h after infiltration in Col-0 but remained at very low basal amounts in *eds1-2* (Fig. 3). Expression levels in cold-primed and control plants did not differ in time or intensity (Fig. 3). Next, we tested for cold-priming-responsive expression profiles of SA-biosynthetic *ICS1* and the SA-responsive *PR1*. *ICS1* and *PR1* were significantly induced at 24 h but not at 6 h after the pathogen treatment in Col-0 (Fig. 3). The induction levels did not differ between cold-primed and control samples (Fig. 3), indicating that pathogen-triggered SA production and signaling were not cold-primed and, therefore, not causative for the cold-reduced susceptibility. This conclusion is further supported by the requirement of functional EDS1 for a robust activation of SA-related immune pathways upon infection with virulent pathogens (Cui et al. 2017; Rietz et al. 2011). Cold priming did not affect *ICS1* and *PR1* levels downstream or independent of EDS1 because induced transcripts in Col-0 were absent or low in cold-pretreated and inoculated *eds1-2* (Fig. 3).

Postcold and *P. syringae* pv. *tomato*-triggered regulation of *tAPX* and *sAPX* transcripts.

Recently, tAPX was described for its role in establishing the memory that controls cold regulation of gene expression after cold priming for several days (van Buer et al. 2016, 2019). Hereby, postcold accumulation of *tAPX* transcripts was essential for the memory function (van Buer et al. 2016, 2019). In addition to tAPX, *Arabidopsis* expresses sAPX (Ishikawa and Shigeoka 2008), which evolved from the same ancestral gene as *tAPX* and still has a highly similar catalytic subunit (Pitsch et al. 2010). We compared regulation of *tAPX* and *sAPX* after cold exposure and after subsequent *P. syringae* pv. *tomato* infection in leaves of cold-primed and control plants of Col-0 and *eds1-2* (Fig. 4). Our data confirmed previously described postcold induction of *tAPX* in Col-0 (van Buer et al. 2016) and showed that this memory phase lasting process was also functional and significant in *eds1-2* (Fig. 4A). *sAPX* was upregulated during a 24-h cold phase of 4°C and quickly readjusted to precold levels at normal temperature (van Buer et al. 2016) (Fig. 4A). Cold-induced *sAPX* upregulation was less pronounced in *eds1-2* (Fig. 4A). Similar to cold exposure, infiltration of leaves with *P. syringae* pv. *tomato* reduced *tAPX* amounts in Col-0 between 3 and 24 h after infection in control and cold-primed plants (Fig. 4B). This effect was marginally less in *eds1-2* (Fig. 4B). At 24 h after *P. syringae* pv. *tomato* treatment, *sAPX* transcripts were enhanced in Col-0. Pathogen induction of *sAPX* was weaker in *eds1-2* and only marginal in cold-primed *eds1-2* (Fig. 4B). Therefore, our data reveal a regulatory similarity between cold- and pathogen-responsive cellular plant stress management: EDS1-dependent upregulation of *sAPX* and repressive regulation of *tAPX*. The pathogen-responsive diametrical regulation of *tAPX* and *sAPX* is further supported by other published transcriptomic studies; for example, after infiltration with *P. syringae* pv. *tomato* and ETI-inducing *P. syringae* pv. *tomato* strain *avrRPM1* (Mine et al. 2018) and EDS1-dependent ETI-inducing *P. syringae* pv. *tomato* strain *avrRPS4* (Bhandari et al. 2019) in the *P. syringae* pv. *tomato*-primed SAR state (Gruner et al. 2013); and detectable, even though less pronounced, after treatment with the bacterial PAMP flg22 peptide (Birkenbihl et al. 2017) (Supplementary Fig. S3).

Cold priming-reduced pathogen susceptibility requires plastid APX.

To test whether *tAPX* is required not only for cold priming of ROS-responsive genes during cold triggering (van Buer et al. 2019) but also for beneficial responses during *P. syringae* pv. *tomato* infections, we included *tapx*- and *sapx*-knockout (KO)

lines (Kangasjärvi et al. 2008) in our analysis. Although altered phenotypes in both lines can be observed under stress conditions, both lines show wild-type-like phenotypes under normal and stable laboratory conditions, probably through compensation by other antioxidant compounds (Kangasjärvi et al. 2008; Maruta et al. 2010). Here, cold-primed (4°C, 24 h) and control plants of both KO lines and Col-0 were infiltrated with *P. syringae* pv. *tomato* 5 days after the priming stimulus. The bacterial titers measured in control plants revealed that *tAPX* did not contribute, per se, to the degree of plant pathogen susceptibility and basal resistance (Fig. 5A) and that *sapx*-KO lines were not significantly more resistant against *P. syringae* pv. *tomato* (Fig. 5A). The cold priming effect on reducing bacterial titers was strong in Col-0, weak in *tapx*-KO, and absent in *sapx*-KO

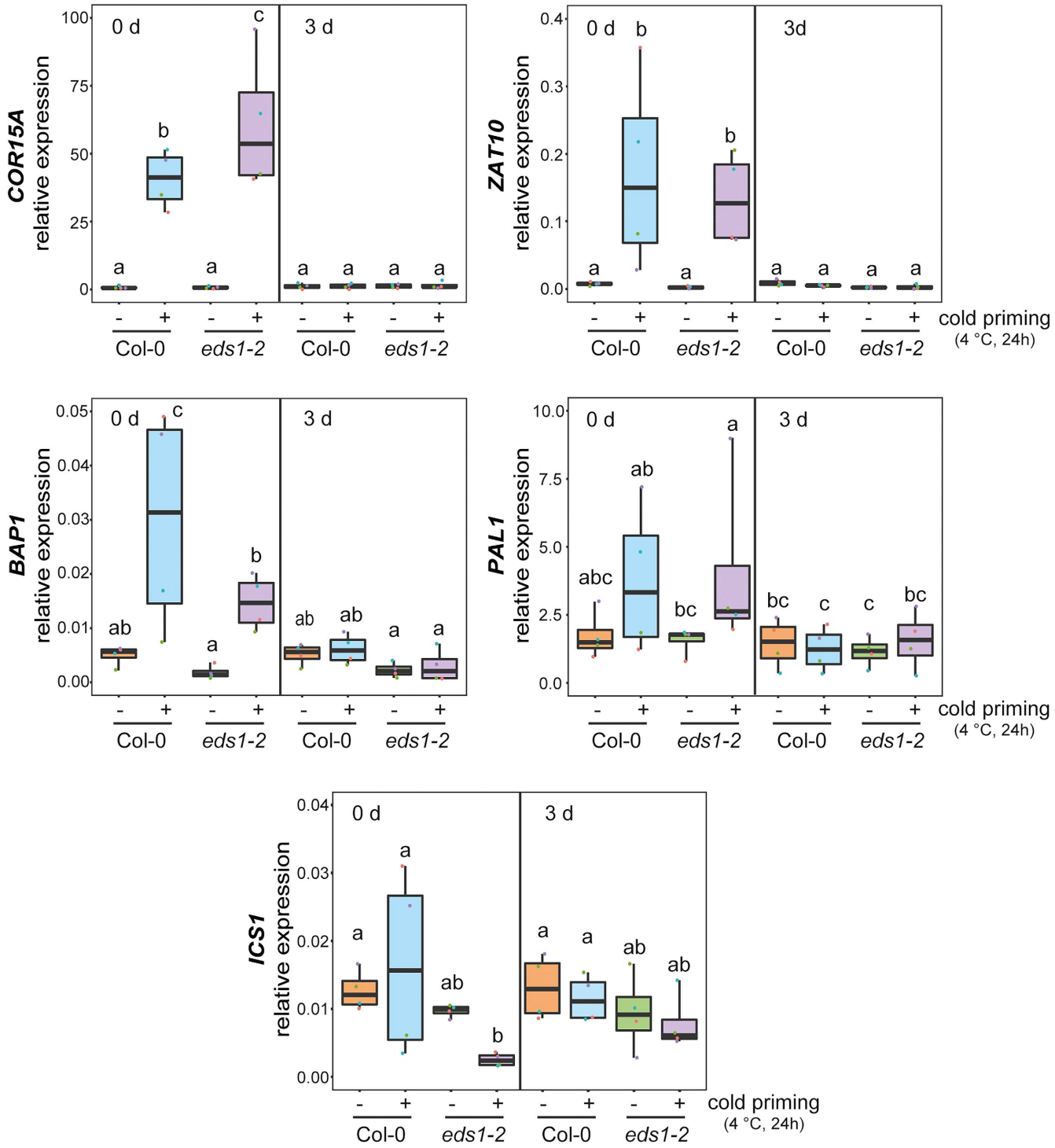

Fig. 2. Postcold expression of stress-responsive genes in Col-0 and *eds1-2*. Transcript levels of *COR15A*, *ZAT10*, *BAP1*, *PAL1*, and *ICS1* in leaves of Col-0 and *eds1-2* null mutants immediately (0 days) and 3 days after end of cold exposure (+; 4°C, 24 h) were determined by quantitative real-time PCR. Transcript levels in leaves harvested from control plants (−) of the same age are also shown and were determined in four independent experiments as relative expression to the geometric mean of three reference genes (*ACT2*, *YLS8*, and *RHIP1*). Different letters denote statistically significant differences (analysis of variance and least significant difference, $P < 0.05$).

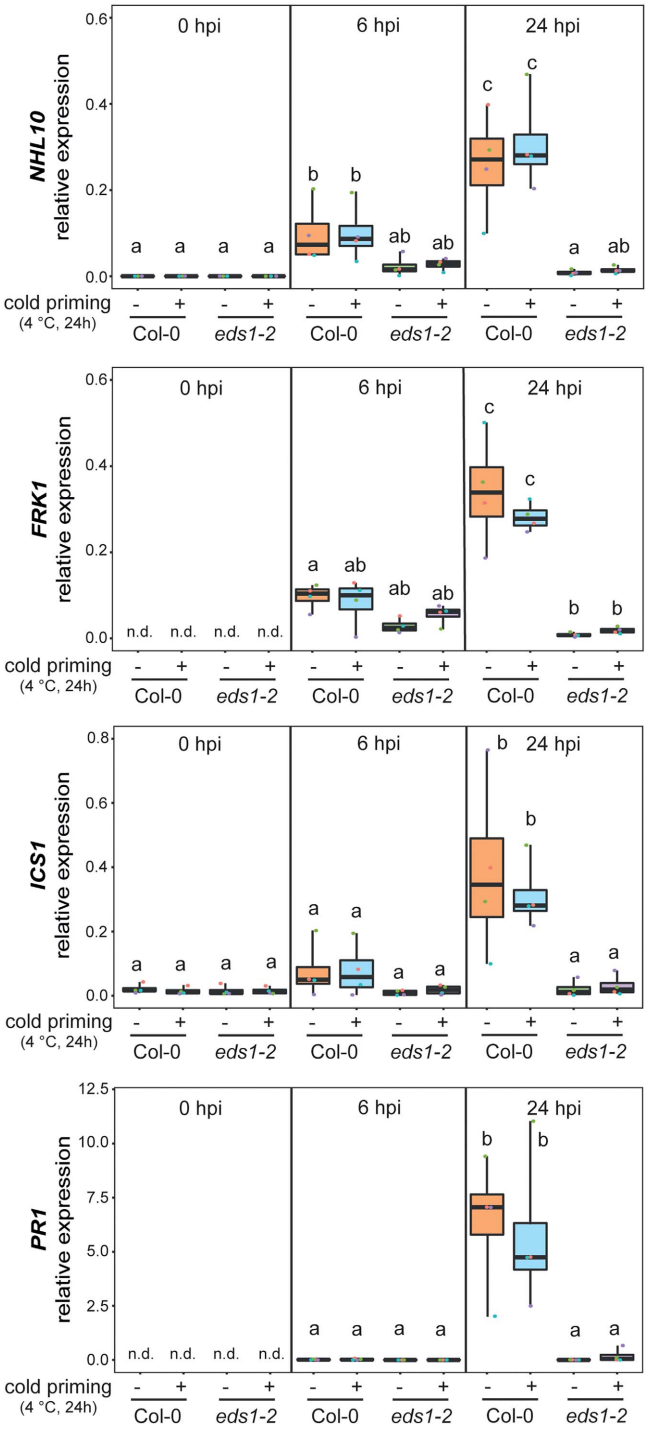

Fig. 3. Transcript levels of selected pathogen-responsive genes during *Pseudomonas syringae* pv. *tomato* infection upon cold priming. Transcript levels of pathogen-associated molecular pattern-triggered immunity marker genes *NHL10* and *FRK1*, salicylic acid (SA) biosynthetic *ICS1*, and SA-responsive *PR1* in leaves of Col-0 and *eds1-2* null mutants after infiltration with *P. syringae* pv. *tomato* (optical density at 600 nm = 0.005). Infiltrations were done 5 days after end of cold treatment with cold-primed plants (+; 4°C, 24 h) or control plants (–). Transcript levels were determined at the time of *P. syringae* pv. *tomato* infiltrations (0, 6, and 24 h postinoculation [hpi]) using quantitative PCR in four independent experiments as relative expression to the geometric mean of three reference genes (*ACT2*, *YLS8*, and *RHIP1*). Different letters denote statistically significant differences (analysis of variance and least significant difference, $P < 0.05$); n.d. = not detectable.

(Fig. 5A). Although cold triggering responses specifically required *tAPX* but not *sAPX* (van Buer et al. 2019), cold-primed pathogen susceptibility was supported by functionality of both plastid APX variants. Consequently, the cold memory that reduces pathogen susceptibility can be postulated to be more generally controlled by plastid APX activity than priming of the cold responsiveness. We have recently shown that cold-induced accumulation of *tAPX* transcripts affects cold regulation of cold-primable genes (van Buer et al. 2019). To test whether postcold *tAPX* affects plant susceptibility, we used an estradiol-responsive *tAPX-inducible RNA interference* (*iRNAi*) line (Maruta et al. 2012; van Buer et al. 2019). We suppressed postcold regulation of *tAPX* by spraying cold-primed plants after the initial cold exposure and, 4 days later, with an estradiol or a mock solution before *P. syringae* pv. *tomato* was infiltrated the following day. Both the estradiol and the mock treatment after cold exposure resulted in increased susceptibility in the Col-0 and *tAPX-iRNAi* lines compared with the cold-primed-only control group (Fig. 5B). However, the bacterial numbers were specifically enhanced by the estradiol treatment in cold-primed *tAPX-iRNAi* (Fig. 5B). To confirm the effect of the estradiol treatment on reducing *tAPX* transcript abundance in *tAPX-iRNAi,* we monitored the *tAPX* transcripts at the time point of infection (Supplementary Fig. S4). We concluded that the regeneration of the plastid antioxidant protection by the *tAPX* postcold induction supports plastid functions during a subsequent infection with *P. syringae* pv. *tomato*, which again results in a perturbation of plastid APX at the transcriptional level (Fig. 4; Supplementary Fig. S3). When *tAPX* transcript recovery was suppressed after the initial cold phase, plants infected with *P. syringae* pv. *tomato* showed increased pathogen susceptibility, as determined by bacterial numbers (Fig. 5B; Supplementary Fig. S4). We suggest that the regeneration of the *tAPX* pool and the restabilization of the plastid antioxidant protection after the cold stress phase provides benefits for plant fitness and defense during a subsequent pathogen infection.

DISCUSSION

When plants are exposed to simultaneously or sequentially occurring combined abiotic and biotic stress situations, responses often differ compared with single and individual stresses (Zhang and Sonnewald 2017). The outcome of different combined stresses can result in a trade-off situation or enable cross-tolerance (Saijo and Loo 2020). Cross-tolerance upon two sequentially applied stresses disconnected by a stress-free interphase, which enables recovery and requires memorization of the first stressor, is a characteristic feature of the *trans*priming phenomenon (Hilker et al. 2016). Here, we showed that a single 24-h cold exposure primed the susceptibility of *A. thaliana* Col-0 against the virulent plant pathogen *P. syringae* pv. *tomato* for up to 5 days (Figs. 1 and 5). This cold-priming-reduced pathogen susceptibility was independent from plant immunity pathways controlled by EDS1 but it required the chloroplast-located sAPX and tAPX (Fig. 5).

The immune system of *A. thaliana* benefits from the experience of cold temperatures. A single 24-h cold (4°C) exposure resulted in immediate decrease of pathogen susceptibility (shown by infection 1 h after cold), and was also robust for 5 to 8 days (shown by infection 5 days after cold) (Fig. 1), although the initial transcriptional cold response (e.g., *COR15A*, *ZAT10*, *BAP1*, and *PAL1*) was quickly reset to precold levels (Fig. 2). As shown recently by Wu et al. (2019), a shorter 10-h cold treatment applied during the night phase also decreases pathogen susceptibility in *A. thaliana*. However, the shorter night stimulus is only transiently memorized for up to 12 h (Wu et al. 2019). Repetitive application of seven 1-h cold periods (one treatment per day) equally results in reduced growth of *P. syringae* pv. *tomato* and entraines protection for

7 days (Singh et al. 2014). Our study showed that, already, a single cold treatment lasting 24 h, including day and night phase, primed and established a molecular stress memory lasting for 5 to 8 days (Fig. 1). Whereas repetitive cold treatments resulted in enhanced activation of PTI-responsive genes *FRK1* and *NHL10* upon triggering with type three secretion–deficient *P. syringae* pv. *tomato* strain *hrcC*$^{-}$ (Singh et al. 2014), the single 24-h cold priming stimulus did not reveal a priming pattern for *FRK1* and *NHL10* transcripts. Activation of *FRK1* and *NHL10* without priming signatures excludes a cross-stress memory formation similar to that with the repetitive cold stimuli and suggests a PTI-independent memory (Fig. 3).

In summary, our data showed that cold priming memory formation was independent from the central plant immune regulator EDS1: (i) bacterial growth was reduced in cold-primed *eds1* null mutants (Fig. 1), (ii) initial cold sensing in *eds1* was wild-type-like leading to *COR15A, ZAT10, BAP1*, and *PAL1* activation (Fig. 2), and (iii) EDS1-dependent transcript activation of selected immune-related genes (*NHL10, FRK1, ICS1*, and *PR1*) did not show a cold priming signature or an activation downstream of EDS1 (Fig. 3). Several EDS1-dependent responses have been well described for their effects in reducing plant susceptibility to virulent pathogens: EDS1, together with its homolog and heteromeric complex partner PAD4, boosts SA biosynthesis (Cui et al. 2017) and promotes tocopherol production in an SA-independent manner upon *P. syringae* pv. *tomato* infection (Stahl et al. 2019). Furthermore, EDS1 is required for the plant immune *cis*-priming SAR by contributing to signal generation in primary infected leaves and signal perception in the systemic uninfected tissues (Breitenbach et al. 2014). Based on the functionality of cold-priming-reduced susceptibility in *eds1-2*, we conclude that the molecular mechanisms of cold priming memory formation are independent from EDS1-controlled immune activation and are established regardless of SA production during *P. syringae* pv. *tomato* infection or SAR signaling. In addition, the indispensable requirement for EDS1 in TNL-mediated immunity (Dongus and Parker 2021; Griebel et al. 2014) excludes the possibility that (post)cold activation of TNL immune receptor signaling is causative for the reduced susceptibility in cold-primed plants. Several articles have described an induction of SA under low-temperature conditions (Kim et al. 2013, 2017). As during pathogen attack, cold-induced SA derives from the plastid isochorismate pathway but SA levels are not increasing before 7 days at 4°C (Kim et al. 2013, 2017). Cold exposure lasting 24 h was not sufficient to significantly increase *ICS1* transcript levels (Fig. 2) or enhance SA levels; these required a constant cold

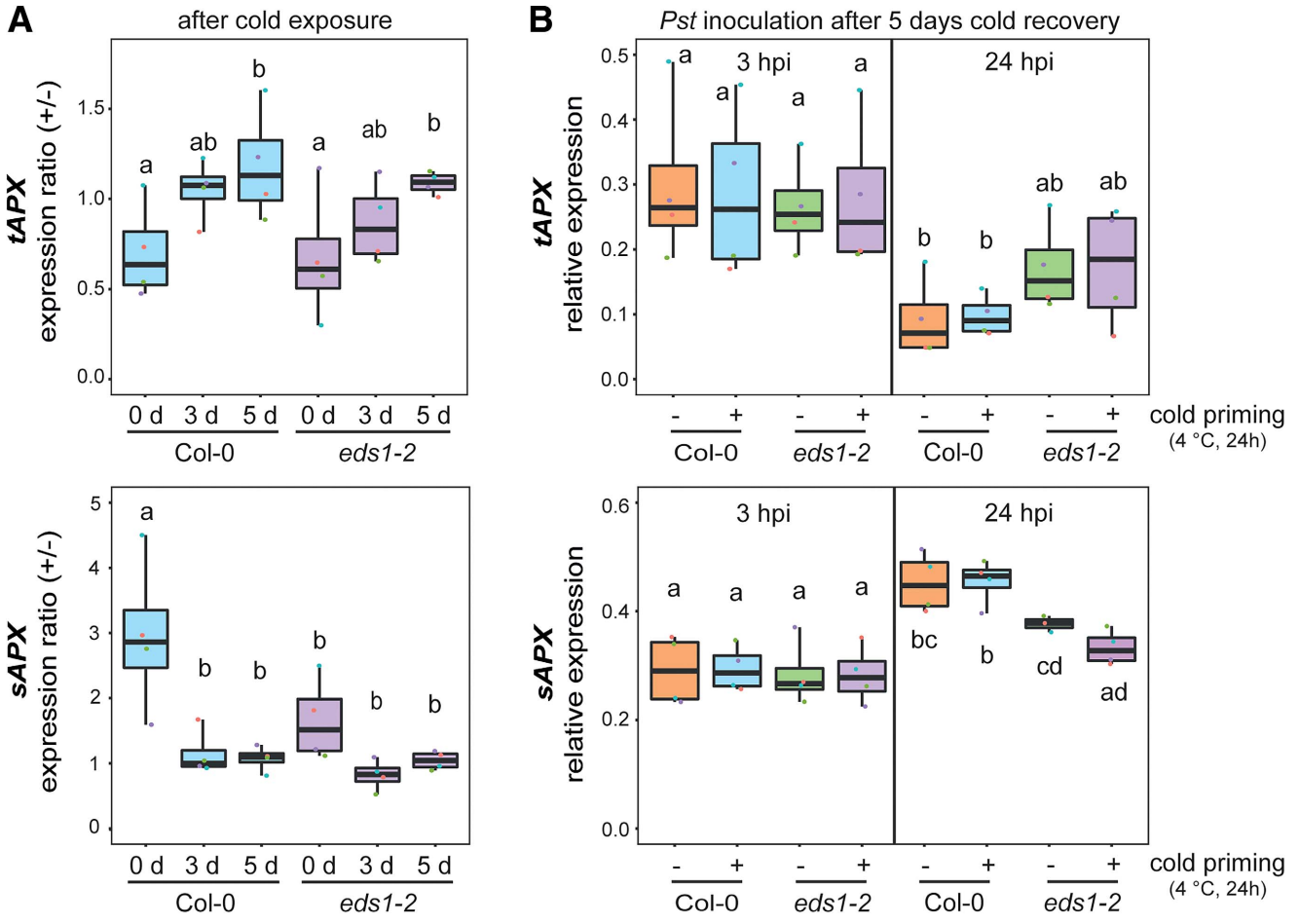

Fig. 4. Transcript regulation of plastid ascorbate peroxidases (APX) encoding APX soluble in the stroma (*sAPX*) and thylakoid-bound APX (*tAPX*) genes during postcold phase and after *Pseudomonas syringae* pv. *tomato* (*Pst*) infection. **A,** Postcold (4°C, 24 h) transcript levels of *tAPX* (upper panel) and *sAPX* (lower panel) in leaves of Col-0 and *eds1-2* null mutants immediately (0 d), 3 days (3 d), and 5 days (5 d) after the end of cold treatment. Transcript levels are shown as ratios from samples of cold-primed leaves (+) compared with samples from untreated control plants (−) of the same age. **B,** Transcript levels of *tAPX* (upper panel) and *sAPX* (lower panel) in leaves of Col-0 and *eds1-2* null mutants 3 and 24 h postinoculation (hpi) with *P. syringae* pv. *tomato* (optical density at 600 nm = 0.005) of cold-primed (+) or control (−) plants. Plants were infected 5 days after the end of the cold treatment. Transcript levels were determined in four independent experiments as relative expression to the geometric mean of three reference genes (*ACT2*, *YLS8*, and *RHIP1*). Different letters denote statistically significant differences (analysis of variance and least significant difference, $P < 0.05$).

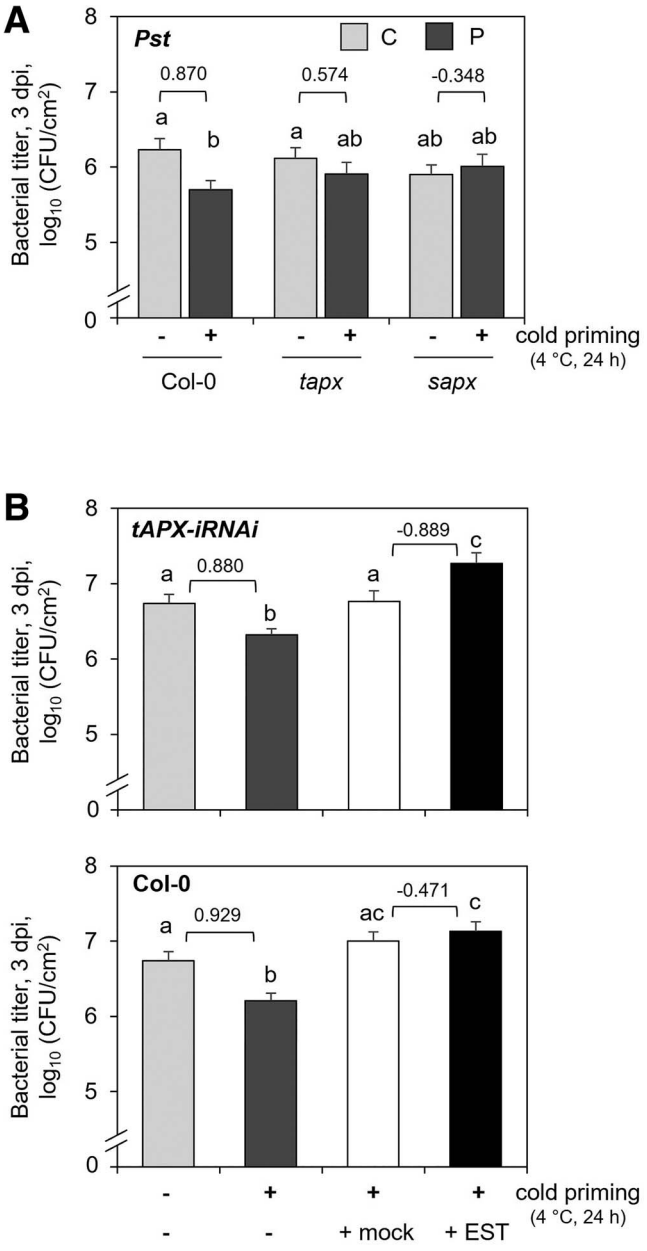

Fig. 5. Bacterial growth of *Pseudomonas syringae* pv. *tomato* (*Pst*) in cold-primed Col-0, thylakoid-bound ascorbate peroxidase (*tAPX*), and APX soluble in the stroma (*sAPX*) knock-out (KO) lines and after conditional repression of *tAPX*. **A,** Cold-primed (+) and control (−) Col-0, *sapx*-KO, and *tapx*-KO plants were infiltrated with *P. syringae* pv. *tomato* (optical density at 600 nm [OD_{600}] = 0.001) 5 days after cold exposure (4°C, 24 h). Bacterial titers (log_{10}-transformed) were measured 3 days postinoculation (dpi). Bars represent means and standard errors calculated from five independent experiments, each with five to six biological replicates using a mixed linear model. **B,** Cold-primed (+) and control (−) Col-0 and *tAPX-inducible RNA interference* (*iRNAi*) plants were infiltrated with *P. syringae* pv. *tomato* (OD_{600} = 0.001) 5 days after cold exposure (4°C, 24 h). For conditional repression of *tAPX* transcript in *tAPX-iRNAi*, plants were sprayed with a mock or an estradiol (EST) solution after the cold treatment and after 4 days. Bacterial titers (log_{10}-transformed) were measured 3 dpi. Bars represent means and standard errors calculated from four independent experiments, each with six to eight biological replicates using a mixed linear model. Different letters above the bars denote statistically significant differences within each graph (adjusted $P < 0.05$; two-tailed *t* tests). Numbers between two bars show the effect size between two means according to Cohen's d.

exposure of at least 1 week (Kim et al. 2013; Wu et al. 2019). In contrast to the independence of EDS1 during mild cold exposure (4°C, 24 h) and cold memory formation, induction and activation of the EDS1 complex at temperatures below 4°C negatively affected freezing tolerance in an SA-dependent manner (Chen et al. 2015).

Chloroplasts can be considered as an important cellular origin of cold sensing and priming: cold exposure supports chloroplast ROS production by an imbalance between the Calvin-Benson cycle and photosynthetic electron transport (Ensminger et al. 2006; Huner et al. 1993) and leads to reduced activation of chloroplast-to-nucleus redox-sensitive genes during a second cold phase (van Buer et al. 2016, 2019). tAPX, the main regulator of cold priming memory in chloroplasts, is transcriptionally activated during the postcold phase (van Buer et al. 2016, 2019) in an EDS1-independent manner (Fig. 4). Gene expression regulation upon cold triggering specifically benefits from tAPX activation but not from sAPX (van Buer et al. 2019). Bacterial growth rates in *tapx-KO* were wild-type-like and not affected, except for the missing cold memory response (Fig. 5). Similar to cold exposure, infections with virulent pathogens resulted in reduced *tAPX* transcript levels (Fig. 4). Conditional silencing of *tAPX* transcripts during the memory phase increased plant susceptibility and *P. syringae* pv. *tomato* titers during a subsequent infection (Fig. 5). Whereas cold priming responses during a second cold exposure were solely regulated by *tAPX* (van Buer et al. 2016, 2019), pathogen triggering of cold-primed plants also required functional *sAPX* for memory effects (Fig. 5). This suggests that the *sAPX* upregulation during the initial cold phase and the *tAPX* increase during the subsequent recovery phase contribute to cold-priming-reduced susceptibility against *P. syringae* pv. *tomato*. tAPX and sAPX jointly scavenge plastid ROS and, therefore, contribute to the plastid antioxidant protection and redox-mediated signaling and communication between cellular compartments. We assume that the regeneration of the plastid APX pool and the restabilization of the plastid antioxidant protection after the initial cold stress phase supports the plant's fitness and primes defense activation by altering cellular redox communication in response to a pathogen infection.

MATERIALS AND METHODS

Plant material and cultivation.

A. thaliana var. Col-0 plants, *eds1-2* null mutant (Bartsch et al. 2006), T-DNA knockout lines *tapx* and *sapx* (Kangasjärvi et al. 2008), and *tAPX-iRNAi* (Maruta et al. 2012) were used in this study. All lines are in the Col-0 background. Plants were cultivated in round pots (6 cm in diameter) containing a soil mixture (14:14:5) of Topferde (Einheitserde, Sinntal-Altengronau, Germany), Pikiererde (Einheitserde, Sinntal-Altengronau), and Perligran Classic (Knauf, Germany) supplemented with dolomite lime (Deutsche Raiffeisen-Warenzentrale, Germany) at 0.5 g $liter^{-1}$ and in a controlled environmental chamber with a day and night temperatures of 20 ± 2 and 18 ± 2°C, respectively; a cycle of 10 h of light (100 to 110 µmol photons m^{-2} s^{-1}; Lumilux Cool White fluorescence stripes, Osram, Germany) and 14 h of darkness; and a constant relative humidity of 60 ± 5% after stratification at 4°C for 2 days.

Cold treatments.

Cold treatments were performed as previously described (van Buer et al. 2016, 2019). Four-week-old plants were exposed to cold 2.5 h after onset of light by transferring them to a growth chamber with a constant temperature of 4 ± 2°C but otherwise identical aeration, illumination, and air humidity as in the 20°C chamber. After a continuous cold exposure for 24 h (comprising a full day and night phase), the plants were placed back to the 20°C

chamber, labeled, and randomized with the noncold-treated control plants.

Estradiol treatments.

For estradiol-sensitive conditional silencing of *tAPX* in *tAPX-iRNAi*, plants were sprayed with 100 µM estradiol (Roth, Karlsruhe, Germany) dissolved in 0.8% (vol/vol) dimethyl sulfoxide (DMSO) and supplemented with 0.01% (vol/vol) Tween 20 as described recently (Bittner et al. 2021; van Buer et al. 2019). Control plants were sprayed with a mock solution (0.8% [vol/vol] DMSO and 0.01% [vol/vol] Tween 20) without estradiol. The treatments were performed immediately after the cold exposure (= 5 days before bacterial infiltration) and repeated after 4 days.

Cultivation and inoculation of bacteria.

P. syringae pv. *tomato* DC3000, *P. syringae* pv. *tomato* strains carrying either the avirulence gene *avrRpm1* or *avrRPS4*, and type three secretion system–deficient *P. syringae* pv. *tomato* strain *hrcC*$^-$ were grown for 24 h at 28°4 C on nutrient-yeast extract glycerol agar (NYGA) solid medium containing the appropriate antibiotics. Bacterial cultures were suspended in 10 mM $MgCl_2$ and diluted to optical density at 600 nm (OD_{600}) of 0.001 for bacterial growth assays or 0.005 for gene expression analyses. The bacterial suspensions were infiltrated from the abaxial side into the leaves with a needleless syringe. For transcript analyses, control plants were mock treated with 10 mM $MgCl_2$. Bacterial inoculations were performed 3.5 h ± 0.5 h after onset of light and either 1 h or 5 days after the end of cold treatment, as indicated. The three youngest but fully-grown leaves of each plant were selected for infiltration.

Bacterial growth assays.

In planta bacterial titers were determined at the indicated time point after infiltration by combining three leaf discs for one sample and shaking in 10 mM $MgCl_2$ with 0.01% (vol/vol) Silwet L-77 at 28°C for 1 h. From each sample, a dilution series was spread in 15-µl spots on NYGA plates with appropriate antibiotics and incubated for 2 days at 28°C. CFU per leaf surface area were calculated for each sample. Statistical analysis of bacterial growth data was described previously (Tsuda et al. 2009). Log_{10}-transformed data from all independent experiments were analyzed using the lme4 package in the R environment and the following model was fitted to the data: $\log_{10} CFU_{gyr} = GY_{gy} + R_r + e_{gyr}$, where GY = genotype–treatment interaction, R = biological replicate, and e = residual. The mean estimates were used as the modeled log_{10}-transformed bacterial titers and were compared using two-tailed t tests. To correct for multiple hypothesis testing, the Benjamini-Hochberg method was applied.

Quantitative real-time PCR analysis.

For transcript analyses using quantitative real-time PCR (qRT-PCR), plant material was harvested from leaves of the same age and developmental status as the ones used for pathogen infiltrations. Each sample included leaves from at least two plants. Total RNA was extracted from frozen leaves using the Gene Matrix Universal RNA Purification Kit (EURx, Gdansk, Poland). cDNA was synthesized using the High-Capacity Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, U.S.A.) and Oligo dT16V primer according to the manufacturer's instructions using 1 µg of RNA for a 20-µl reaction. qRT-PCR assays were performed in technical triplicate on the CFX96 real-time system (Bio-Rad, Hercules, CA, U.S.A.) as described previously (van Buer et al. 2016) using SYBR Green (Sigma-Aldrich, Germany) and OptiTaq Polymerase (EURx) and a cycling program of 95°C for 5 min; followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and, finally, 72°C for 30 s. All qRT-PCR primers are listed in Supplementary Table S1. The cycle threshold (Ct) values were determined using the CFX Manager software and relative expression values (ΔCt) of genes of interest were analyzed against the geometric mean of the *actin 2* (*ACT2*), *yellow leaf specific protein 8*) (*YLS8*), and *RGS1-HXK1 interacting protein 1* (*RHIP*) transcript levels as reference. The relative expression ($2^{-\Delta Ct}$) was determined based on three technical replicates in four independently performed experiments. Box plots of the summarized data were generated using the R package gglpot2 and showed the median, the distance between the upper quartile ($q_n = 0.75$) and lower quartiles ($q_n = 0.25$), and the raw values of each experiment as dots. For statistical analysis, basic R environment and the agricolae package were used.

ACKNOWLEDGMENTS

We thank T. Romeis (Freie Universität Berlin/IPB Halle) for providing *P. syringae* strains, J. Parker (MPIPZ Cologne) for *eds1-2* mutant seeds and *P. syringae* pv. *tomato hrcC*$^-$, and U. Temp and E. Reifschneider for their technical assistance.

LITERATURE CITED

Albert, I., Hua, C., Nürnberger, T., Pruitt, R. N., and Zhang, L. 2020. Surface sensor systems in plant immunity. Plant Physiol. 182:1582-1596.

Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:601-639.

Baier, M., Bittner, A., Prescher, A., and van Buer, J. 2019. Preparing plants for improved cold tolerance by priming. Plant Cell Environ. 42: 782-800.

Bartsch, M., Gobbato, E., Bednarek, P., Debey, S., Schultze, J. L., Bautor, J., and Parker, J. E. 2006. Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in Arabidopsis immunity and cell death is regulated by the monooxygenase FMO1 and the Nudix hydrolase NUDT7. Plant Cell 18:1038-1051.

Baum, S., Reimer-Michalski, E.-M., Bolger, A., Mantai, A. J., Benes, V., Usadel, B., and Conrath, U. 2019. Isolation of open chromatin identifies regulators of systemic acquired resistance. Plant Physiol. 181:817-833.

Bernsdorff, F., Döring, A.-C., Gruner, K., Schuck, S., Bräutigam, A., and Zeier, J. 2016. Pipecolic acid orchestrates plant systemic acquired resistance and defense priming via salicylic acid-dependent and -independent pathways. Plant Cell 28:102-129.

Bhandari, D. D., Lapin, D., Kracher, B., von Born, P., Bautor, J., Niefind, K., and Parker, J. E. 2019. An EDS1 heterodimer signalling surface enforces timely reprogramming of immunity genes in Arabidopsis. Nat. Commun. 10:772.

Birkenbihl, R. P., Kracher, B., Roccaro, M., and Somssich, I. E. 2017. Induced genome-wide binding of three Arabidopsis WRKY transcription factors during early MAMP-triggered immunity. Plant Cell 29:20-38.

Bittner, A., Hause, B., and Baier, M. 2021. Cold-priming causes dampening of oxylipin biosynthesis and signalling during the early cold- and light-triggering response of *Arabidopsis thaliana.* J. Exp. Bot. 72: 7163-7179.

Bittner, A., van Buer, J., and Baier, M. 2020. Cold priming uncouples light- and cold-regulation of gene expression in *Arabidopsis thaliana.* BMC Plant Biol. 20:281.

Boudsocq, M., Willmann, M. R., McCormack, M., Lee, H., Shan, L., He, P., Bush, J., Cheng, S. H., and Sheen, J. 2010. Differential innate immune signalling via Ca^{2+} sensor protein kinases. Nature 464:418-422.

Breitenbach, H. H., Wenig, M., Wittek, F., Jordá, L., Maldonado-Alconada, A. M., Sarioglu, H., Colby, T., Knappe, C., Bichlmeier, M., Pabst, E., Mackey, D., Parker, J. E., and Vlot, A. C. 2014. Contrasting roles of the apoplastic aspartyl protease APOPLASTIC, ENHANCED DISEASE SUSCEPTIBILITY1-DEPENDENT1 and LEGUME LECTIN-LIKE PROTEIN1 in Arabidopsis systemic acquired resistance. Plant Physiol. 165:791-809.

Büttner, D. 2016. Behind the lines-actions of bacterial type III effector proteins in plant cells. FEMS Microbiol. Rev. 40:894-937.

Byun, Y. J., Koo, M. Y., Joo, H. J., Ha-Lee, Y. M., and Lee, D. H. 2014. Comparative analysis of gene expression under cold acclimation, deacclimation and reacclimation in Arabidopsis. Physiol. Plant. 152:256-274.

Caverzan, A., Passaia, G., Rosa, S. B., Ribeiro, C. W., Lazzarotto, F., and Margis-Pinheiro, M. 2012. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. Genet. Mol. Biol. 35:1011-1019.

Chen, Q. F., Xu, L., Tan, W. J., Chen, L., Qi, H., Xie, L. J., Chen, M. X., Liu, B. Y., Yu, L. J., Yao, N., Zhang, J. H., Shu, W., and Xiao, S. 2015. Disruption of the Arabidopsis defense regulator genes SAG101, EDS1, and PAD4 confers enhanced freezing tolerance. Mol. Plant 8:1536-1549.

Conrath, U. 2011. Molecular aspects of defence priming. Trends Plant Sci. 16:524-531.

Crisp, P. A., Ganguly, D., Eichten, S. R., Borevitz, J. O., and Pogson, B. J. 2016. Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics. Sci. Adv. 2:e1501340.

Cui, H., Gobbato, E., Kracher, B., Qiu, J., Bautor, J., and Parker, J. E. 2017. A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in Arabidopsis immunity. New Phytol. 213:1802-1817.

Ding, P., and Ding, Y. 2020. Stories of salicylic acid: A plant defense hormone. Trends Plant Sci. 25:549-565.

Dongus, J. A., and Parker, J. E. 2021. EDS1 signalling: At the nexus of intracellular and surface receptor immunity. Curr. Opin. Plant Biol. 62:102039.

Ensminger, I., Busch, F., and Huner, N. P. A. 2006. Photostasis and cold acclimation: Sensing low temperature through photosynthesis. Physiol. Plant. 126:28-44.

Fu, Z. Q., and Dong, X. 2013. Systemic acquired resistance: Turning local infection into global defense. Annu. Rev. Plant Biol. 64:839-863.

Gourbal, B., Pinaud, S., Beckers, G. J. M., Van Der Meer, J. W. M., Conrath, U., and Netea, M. G. 2018. Innate immune memory: An evolutionary perspective. Immunol. Rev. 283:21-40.

Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W., and Dangl, J. L. 1995. Structure of the Arabidopsis RPM1 gene enabling dual specificity disease resistance. Science 269: 843-846.

Griebel, T., Maekawa, T., and Parker, J. E. 2014. NOD-like receptor cooperativity in effector-triggered immunity. Trends Immunol. 35:562-570.

Groden, D., and Beck, E. 1979. H_2O_2 destruction by ascorbate-dependent systems from chloroplasts. Biochim. Biophys. Acta Bioenerg. 546: 426-435.

Gruner, K., Griebel, T., Návarová, H., Attaran, E., and Zeier, J. 2013. Reprogramming of plants during systemic acquired resistance. Front. Plant Sci. 4:252.

Hartmann, M., and Zeier, J. 2019. N-hydroxypipecolic acid and salicylic acid: A metabolic duo for systemic acquired resistance. Curr. Opin. Plant Biol. 50:44-57.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hincha, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M. C., Rolff, J., Romeis, T., Schmülling, T., Steppuhn, A., van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E., and Kopka, J. 2016. Priming and memory of stress responses in organisms lacking a nervous system. Biol. Rev. Camb. Philos. Soc. 91:1118-1133.

Hinsch, M., and Staskawicz, B. 1996. Identification of a new Arabidopsis disease resistance locus, RPs4, and cloning of the corresponding avirulence gene, avrRps4, from *Pseudomonas syringae* pv. *pisi.* Mol. Plant-Microbe Interact. 9:55-61.

Huner, N. P. A., Öquist, G., Hurry, V. M., Krol, M., Falk, S., and Griffith, M. 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth. Res. 37:19-39.

Ishikawa, T., and Shigeoka, S. 2008. Recent advances in ascorbate biosynthesis and the physiological significance of ascorbate peroxidase in photosynthesizing organisms. Biosci. Biotechnol. Biochem. 72:1143-1154.

Jacob, F., Vernaldi, S., and Maekawa, T. 2013. Evolution and conservation of plant NLR functions. Front. Immunol. 4:297.

Jaskiewicz, M., Conrath, U., and Peterhänsel, C. 2011. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. EMBO Rep. 12:50-55.

Jones, J. D. G., and Dangl, J. L. 2006. The plant immune system. Nature 444:323-329.

Jones, J. D. G., Vance, R. E., and Dangl, J. L. 2016. Intracellular innate immune surveillance devices in plants and animals. Science 354: aaf6395.

Kangasjärvi, S., Lepistö, A., Hännikäinen, K., Piippo, M., Luomala, E.-M., Aro, E.-M., and Rintamäki, E. 2008. Diverse roles for chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses. Biochem. J. 412:275-285.

Kim, Y., Park, S., Gilmour, S. J., and Thomashow, M. F. 2013. Roles of CAMTA transcription factors and salicylic acid in configuring the low-temperature transcriptome and freezing tolerance of Arabidopsis. Plant J. 75:364-376.

Kim, Y. S., An, C., Park, S., Gilmour, S. J., Wang, L., Renna, L., Brandizzi, F., Grumet, R., and Thomashow, M. F. 2017. CAMTA-mediated regulation of salicylic acid immunity pathway genes in Arabidopsis exposed to low temperature and pathogen infection. Plant Cell 29:2465-2477.

Kitajima, S. 2008. Hydrogen peroxide-mediated inactivation of two chloroplastic peroxidases, ascorbate peroxidase and 2-cys peroxiredoxin. Photochem. Photobiol. 84:1404-1409.

Le Roux, C., Huet, G., Jauneau, A., Camborde, L., Trémousaygue, D., Kraut, A., Zhou, B., Levaillant, M., Adachi, H., Yoshioka, H., Raffaele, S., Berthomé, R., Couté, Y., Parker, J. E., and Deslandes, L. 2015. A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. Cell 161:1074-1088.

Lolle, S., Stevens, D., and Coaker, G. 2020. Plant NLR-triggered immunity: From receptor activation to downstream signaling. Curr. Opin. Immunol. 62:99-105.

Mackey, D., Holt, B. F., 3rd, Wiig, A., and Dangl, J. L. 2002. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell 108:743-754.

Maruta, T., Noshi, M., Tanouchi, A., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T., and Shigeoka, S. 2012. H_2O_2-triggered retrograde signaling from chloroplasts to nucleus plays specific role in response to stress. J. Biol. Chem. 287:11717-11729.

Maruta, T., Sawa, Y., Shigeoka, S., and Ishikawa, T. 2016. Diversity and evolution of ascorbate peroxidase functions in chloroplasts: More than just a classical antioxidant enzyme? Plant Cell Physiol. 57:1377-1386.

Maruta, T., Tanouchi, A., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T., and Shigeoka, S. 2010. Arabidopsis chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. Plant Cell Physiol. 51:190-200.

Mine, A., Seyfferth, C., Kracher, B., Berens, M. L., Becker, D., and Tsuda, K. 2018. The defense phytohormone signaling network enables rapid, high-amplitude transcriptional reprogramming during effector-triggered immunity. Plant Cell 30:1199-1219.

Miyake, C., and Asada, K. 1992. Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. Plant Cell Physiol. 33:541-553.

Pandey, S., Fartyal, D., Agarwal, A., Shukla, T., James, D., Kaul, T., Negi, Y. K., Arora, S., and Reddy, M. K. 2017. Abiotic stress tolerance in plants: Myriad roles of ascorbate peroxidase. Front. Plant Sci. 8:581.

Pitsch, N. T., Witsch, B., and Baier, M. 2010. Comparison of the chloroplast peroxidase system in the chlorophyte *Chlamydomonas reinhardtii*, the bryophyte *Physcomitrella patens*, the lycophyte *Selaginella moellendorffii* and the seed plant *Arabidopsis thaliana.* BMC Plant Biol. 10:133.

Rietz, S., Stamm, A., Malonek, S., Wagner, S., Becker, D., Medina-Escobar, N., Vlot, A. C., Feys, B. J., Niefind, K., and Parker, J. E. 2011. Different roles of enhanced disease susceptibility1 (EDS1) bound to and dissociated from phytoalexin deficient4 (PAD4) in Arabidopsis immunity. New Phytol. 191:107-119.

Roeber, V. M., Bajaj, I., Rohde, M., Schmülling, T., and Cortleven, A. 2021. Light acts as a stressor and influences abiotic and biotic stress responses in plants. Plant Cell Environ. 44:645-664.

Roine, E., Wei, W., Yuan, J., Nurmiaho-Lassila, E. L., Kalkkinen, N., Romantschuk, M., and He, S. Y. 1997. Hrp pilus: An hrp-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000. Proc. Natl. Acad. Sci. U.S.A. 94:3459-3464.

Saijo, Y., and Loo, E. P. 2020. Plant immunity in signal integration between biotic and abiotic stress responses. New Phytol. 225:87-104.

Sarris, P. F., Duxbury, Z., Huh, S. U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S. B., Wirthmueller, L., Menke, F. L. H., Sohn, K. H., and Jones, J. D. G. 2015. A plant immune receptor detects pathogen effectors that target WRKY transcription factors. Cell 161:1089-1100.

Shah, J., and Zeier, J. 2013. Long-distance communication and signal amplification in systemic acquired resistance. Front. Plant Sci. 4:30.

Singh, P., Yekondi, S., Chen, P.-W., Tsai, C.-H., Yu, C.-W., Wu, K., and Zimmerli, L. 2014. Environmental history modulates Arabidopsis pattern-triggered immunity in a HISTONE ACETYLTRANSFERASE1-dependent manner. Plant Cell 26:2676-2688.

Stahl, E., Hartmann, M., Scholten, N., and Zeier, J. 2019. A role for tocopherol biosynthesis in Arabidopsis basal immunity to bacterial infection. Plant Physiol. 181:1008-1028.

Steponkus, P. L., Uemura, M., Joseph, R. A., Gilmour, S. J., and Thomashow, M. F. 1998. Mode of action of the COR15a gene on the freezing tolerance of *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. U.S.A. 95:14570-14575.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., and Katagiri, F. 2009. Network properties of robust immunity in plants. PLoS Genet. 5:e1000772.

van Buer, J., Cvetkovic, J., and Baier, M. 2016. Cold regulation of plastid ascorbate peroxidases serves as a priming hub controlling ROS signaling in *Arabidopsis thaliana.* BMC Plant Biol. 16:163.

van Buer, J., Prescher, A., and Baier, M. 2019. Cold-priming of chloroplast ROS signalling is developmentally regulated and is locally controlled at the thylakoid membrane. Sci. Rep. 9:3022.

Velásquez, A. C., Castroverde, C. D. M., and He, S. Y. 2018. Plant–pathogen warfare under changing climate conditions. Curr. Biol. 28:R619-R634.

Wada, K., Tada, T., Nakamura, Y., Ishikawa, T., Yabuta, Y., Yoshimura, K., Shigeoka, S., and Nishimura, K. 2003. Crystal structure of chloroplastic ascorbate peroxidase from tobacco plants and structural insights into its instability. J. Biochem. 134:239-244.

Wu, Z., Han, S., Zhou, H., Tuang, Z. K., Wang, Y., Jin, Y., Shi, H., and Yang, W. 2019. Cold stress activates disease resistance in *Arabidopsis thaliana* through a salicylic acid dependent pathway. Plant Cell Environ. 42:2645-2663.

Zarka, D. G., Vogel, J. T., Cook, D., and Thomashow, M. F. 2003. Cold induction of Arabidopsis CBF genes involves multiple ICE (inducer of *CBF* expression) promoter elements and a cold-regulatory circuit that is desensitized by low temperature. Plant Physiol. 133:910-918.

Zhang, H., and Sonnewald, U. 2017. Differences and commonalities of plant responses to single and combined stresses. Plant J. 90:839-855.

Zuther, E., Juszczak, I., Lee, Y. P., Baier, M., and Hincha, D. K. 2015. Time-dependent deacclimation after cold acclimation in *Arabidopsis thaliana* accessions. Sci. Rep. 5:12199.