Warm temperature suppresses plant systemic acquired resistance by intercepting the *N*-hydroxypipecolic acid immune pathway

Authors: Alyssa Shields[1], Lingya Yao[2], Jong Hum Kim[3,4,5], Wasan Mudher Abo Al-Timmen[1,6], Sha Li[7], Eric J. R. Marchetta[1], Vanessa Shivnauth[1], Tao Chen[7], Sheng Yang He[3,4], Xiufang Xin[2], and Christian Danve M. Castroverde[1,*]

Affiliations:

[1]Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada N2L 3C5

[2]National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

[3]Department of Biology, Duke University, Durham, North Carolina, USA 27708

[4]Howard Hughes Medical Institute, Duke University, Durham, North Carolina, USA 27708

[5]Department of Life Sciences, Pohang University of Science and Technology, Pohang University of Science and Technology, Pohang 37673, Republic of Korea

[6]Department of Biology, College of Science, University of Babylon, Babylon, Iraq

[7]State Key Laboratory of Agriculture Microbiology, Huazhong Agricultural University, Wuhan, China

*****Corresponding Author: dcastroverde@wlu.ca (ORCID: 0000-0002-9982-8451)

Running Title: Temperature regulation of plant SAR

Abstract

Climate warming influences disease development by targeting critical components of the plant immune system, including pattern-triggered immunity (PTI), effector-triggered immunity (ETI) and production of the central defence hormone salicylic acid (SA) at the primary pathogen infection site. However, it is not clear if and/or how temperature impacts systemic immunity. Here we show that pathogen-triggered systemic acquired resistance (SAR) in *Arabidopsis thaliana* is suppressed at elevated temperature. This was accompanied by global downregulation of SAR-induced genes at elevated temperature. Abolished SAR under warmer conditions was associated with reduced biosynthesis of the SAR metabolite *N*-hydroxypipecolic acid (NHP) in *Arabidopsis* and other plant species, as demonstrated by downregulation of NHP biosynthetic genes (*ALD1* and *FMO1*) and NHP precursor pipecolic acid (Pip) levels. Although multiple SAR signals have been shown previously, exogenous Pip was sufficient to restore disease protection at elevated temperature, indicating that heat-mediated SAR suppression is due to Pip-NHP downregulation. Along with *ALD1* and *FMO1*, systemic expression of the SA biosynthetic gene *ICS1* was also suppressed at warm temperature. Finally, we define a transcriptional network controlling thermosensitive NHP pathway via the master transcription factors CBP60g and SARD1. Our findings demonstrate that warm temperatures impact not only local but also systemic immunity by impinging on the NHP pathway, providing a roadmap towards engineering climate-resilient plant immune systems.

Keywords: Climate change, N-hydroxypipecolic acid, plant hormone, plant immunity, systemic acquired resistance, temperature

Main Text

Warming global temperatures due to climate change pose serious threats to the natural environment and human civilization (Altizer et al., 2016; Deutsch et al., 2018; Cavicchioli et al., 2019; Delgado-Baquerizo et al., 2020). In agricultural systems, increasing average temperatures are predicted to significantly reduce yields of the world's major crops (Zhao et al., 2017; Chaloner et al., 2021; Singh et al., 2023). As many crop varieties are bred to be grown outside the native growth ranges of their wild relatives, it is important to understand the impact

of elevated temperatures on plant growth, development, immunity and phenology. Together with other environmental factors, temperature profoundly affects various aspects of plant physiology, ranging from growth and development (Quint et al., 2016; Lippman et al., 2018; Hayes et al., 2020; Vu et al., 2021; Castroverde and Dina, 2021) to stress responses and immunity (Velasquez et al., 2018; Kim et al., 2021; Castroverde and Dina, 2021; Singh et al., 2023). Environmentally compromised immune signaling is associated with increased plant disease development, as postulated as the plant disease triangle (Stevens, 1960; Colhoun, 1973; Roussin-Léveillée et al., 2023).

Elevated temperatures target various components of the plant immune system (Velasquez et al., 2018; Cheng et al., 2019; Cohen and Leach, 2020; Kim et al., 2021), which encompass both cell surface (transmembrane) and intracellular immune receptors (Jones and Dangl, 2006; Zhou and Zhang, 2020; Kim and Castroverde, 2020; Ngou et al., 2022). Cell surface immune receptors recognize apoplastic immunogenic molecules, typically conserved pathogen-associated molecular patterns (PAMPs; Bigeard et al., 2015; Gust et al., 2017; DeFalco and Zipfel, 2021), while intracellular nucleotide-binding leucine-rich receptors (NLRs) perceive pathogen effectors and/or effector-induced host modifications (Cui et al., 2015; Jones et al., 2016; Saur et al., 2021). There is evidence that warm temperatures lead to reduced abundance, membrane localization or signaling of cell surface receptors (De Jong et al., 2002; Janda et al., 2019; Hilleary et al., 2020) and perturbed nuclear localization or activation of certain NLRs (Zhu et al., 2010; Mang et al., 2012; Cheng et al., 2013; Venkatesh and Kang, 2019).

Recent studies show that both PRR signaling and NLR signaling activate convergent downstream signaling, including increased cytoplasmic calcium influx, oxidative burst, phosphorylation cascades and production of defence hormone salicylic acid (SA), leading to overlapping defence gene expression and metabolism at the primary infection site (Pieterse et al., 2009; Ngou et al., 2021; Yuan et al., 2021). Previous research has shown that elevated temperature suppresses pathogen-induced biosynthesis of the central defence hormone salicylic acid (SA) (Malamy et al., 1992; Huot et al., 2017; Li et al., 2021; Kim et al., 2022; Rossi et al., 2023), which aligns with temperature modulation of other plant hormones (Franklin et al., 2011; Sun et al., 2012; Ibañez et al., 2018; Martínez et al., 2018; Ferrero et al., 2019; Bruessow et al., 2021; Castroverde and Dina, 2021). Apart from resistance at the local infection site, SA is also involved in systemic immunity known as systemic acquired resistance (SAR) (Fu and Dong, 2013; Zhang and Li, 2019; Kachroo and Kachroo, 2020; Lim et al., 2020; Peng et al., 2021). Primary infection and immune activation lead to SAR, which primes non-infected

systemic tissues against secondary infections (Cameron et al., 1994; Maldonado et al., 2002; Fu and Dong, 2013; Yu et al., 2013; Vlot et al., 2021; Zeier, 2021). SAR is widely conserved across the plant kingdom, leading to broad-spectrum host defences by rapidly generating mobile signals at the infection site and transporting them throughout the plant (Carella et al., 2016; Chen et al., 2018; Hartmann et al., 2018; Kachroo and Kachroo, 2020; Shine et al., 2022).

A major SAR signal is the immune-activating metabolite *N*-hydroxypipecolic acid (NHP) (Chen et al., 2018; Hartmann and Zeier, 2018; Hartmann et al., 2018). NHP has been shown to induce SAR, and blocking its biosynthesis results in SAR abolishment (Song et al., 2004; Mishina and Zeier, 2006; Jing et al., 2011; Navarova et al., 2012; Huang et al., 2020). NHP biosynthesis follows three consecutive enzymatic reactions (Hartmann and Zeier, 2018), involving the aminotransferase AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1; Zeier, 2013; Bernsdorff et al., 2016), the reductase SAR-DEFICIENT 4 (SARD4; Ding et al., 2016; Hartmann et al., 2017) and *N*-hydroxylase FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1), which converts pipecolic acid (Pip) to *N*-hydroxypipecolic acid (NHP; Chen et al., 2018; Hartmann et al., 2018). *ALD1*, *SARD4* and *FMO1* gene expression and NHP levels are induced systemically following pathogenic attack at a primary site (Song et al., 2004; Mishina and Zeier, 2006; Ding et al., 2016; Bernsdorff et al., 2016; Hartmann et al., 2017; Yildiz et al., 2021).

Currently, the impact of elevated temperature on plant systemic immunity is underexplored. Although previous studies have shown that increased temperatures have a negative effect on several aspects of the plant immune system that occur in the local/primary sites of infection (like PTI, ETI and SA production), it is unknown if and how elevated temperature affect NHP-mediated plant immunity and the SAR response. In our study, we show that *e*levated temperature suppresses SAR-associated NHP biosynthetic gene expression and Pip production. Because it has been shown that the NHP immune pathway is redundantly controlled by the master transcription factors CALMODULIN-BINDING PROTEIN 60-LIKE G (CBP60g) and SAR-DEFICIENT 1 (SARD1) (Sun et al., 2015; Sun et al., 2018; Huang et al., 2020), we further found that warm temperature-suppressed plant systemic immunity is restored by constitutive *CBP60g/SARD1* gene expression or exogenous Pip application. Collectively, our study indicates that CBP60g and SARD1 control the temperature-vulnerability of *Arabidopsis* systemic immunity by regulating Pip/NHP biosynthesis.

Results

Warm temperature affects systemic acquired resistance in *Arabidopsis* plants

To determine if SAR is affected by temperature, lower leaves of *Arabidopsis* Col-0 plants were inoculated with the virulent bacterial pathogen *Pst* DC3000 as the primary challenge. Two days after primary infection, upper systemic leaves were inoculated with the pathogen *Pst* DC3000 as the secondary challenge. As shown in Figure 1a, primary pathogen infection expectedly lowered the systemic bacterial counts after secondary pathogen challenge at 23°C but not at 28°C in Col-0 plants. This indicates that *Arabidopsis* SAR due to local *Pst* DC3000 infection is effective at normal but not at elevated temperature, suggesting that temperature regulates plant systemic immunity.

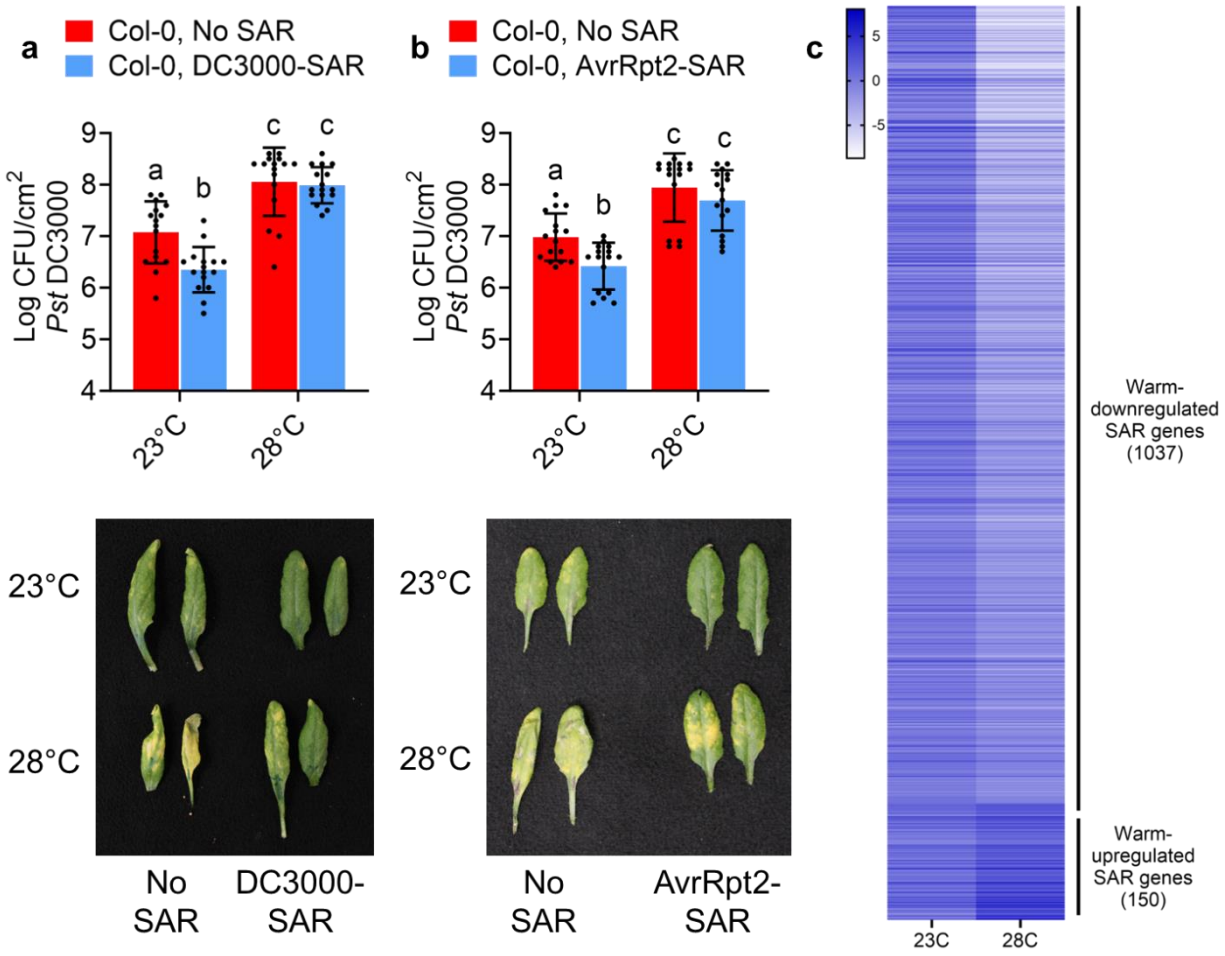

Fig. 1 *Arabidopsis* SAR is suppressed at elevated temperature.

(A, B) Lower leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock) and *Pst* DC3000 (OD600 = 0.02) in (A) or *Pst* DC3000/AvrRpt2 (OD600 = 0.02) in (B). Plants were then incubated at either 23°C or 28°C. Two days after primary local inoculation, upper systemic leaves were infiltrated with *Pst* DC3000 (OD600 = 0.001), and plants were incubated again at their respective temperatures (23°C or 28°C). Bacterial numbers (upper panels) and symptom expression photos (lower panels) were taken at 3 days post-inoculation (dpi) of systemic tissues. Data show the mean log CFU *Pst* DC3000/mL (± S.D.) and individual points (n=16 from 4 independent experiments) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letters. (C) Transcriptome analysis using SAR+ genes from Hartmann et al. (2018) interfaced with temperature-regulated genes from Kim et al. (2022). The number of SAR+ genes that are downregulated and upregulated at elevated temperature are shown (fold change cutoff > 2).

In addition to virulent pathogens, avirulent pathogens that induce local ETI can also induce SAR (Cameron et al., 1994; Zeier, 2021). We therefore tested whether SAR activated by the avirulent strain *Pst* DC3000/AvrRpt2, which activates RPS2-dependent immunity in Col-0 plants (Kunkel et al., 1993; Bent et al., 1994), is also impacted by warm conditions. As expected, SAR was induced at normal temperature (23°C) after primary *Pst* DC3000/AvrRpt2 infection (Figure 1b). Strikingly, systemic *Pst* DC3000/AvrRpt2 levels remained similar compared to mock treatment at elevated temperature (28°C), indicating that even ETI-induced SAR protection is also negatively affected in *Arabidopsis* Col-0 plants at higher temperature.

To further understand global SAR immune signaling, we analyzed a previously generated *Arabidopsis* SAR transcriptome after virulent *P. syringae* infection (Hartmann et al., 2018). We interfaced the SAR-induced (SAR+) genes from that study with our previously published temperature-regulated transcriptome (Kim et al., 2022) and categorized *Arabidopsis* SAR+ genes into downregulated or upregulated genes at elevated temperature. As shown in Figure 1c, 1037 SAR+ genes were downregulated, while 151 SAR+ genes were upregulated at elevated temperatures. This demonstrates that a significant majority of temperature-regulated SAR genes exhibit downregulated expression at elevated temperature, consistent with loss of systemic protection against secondary infection at 28°C (Figures 1a-b). Overall, our collective results indicate the *Arabidopsis* SAR induced by both virulent and avirulent pathogens are negatively impacted by warm temperatures.

The *N*-hydroxypipecolic acid pathway is downregulated by elevated temperature

To determine the mechanism of how temperature regulates systemic immunity, we measured expression levels of genes required for biosynthesis of NHP, which is an immune-

activating metabolite crucial for SAR (Hartmann et al., 2018; Chen et al., 2018). As shown in Figure 2a, transcript levels of *ALD1* and *FMO1* in local (primary) leaves of *Arabidopsis* Col-0 plants at 1 day post-infection with *Pst* DC3000 were lower at 28°C than at 23°C. In agreement, pathogen-induced levels of the NHP precursor metabolite Pip were also lower at the warmer temperature (Figure 2b). Similar trends in warm temperature-suppression of NHP biosynthetic gene expression was observed in tomato and rapeseed plants (Figures 2c-d). Finally, as shown in Figure 2e, systemic (uninfected) leaves of *Arabidopsis* plants at 2 dpi exhibited lower *ALD1* and *FMO1* gene expression levels at 28°C compared to those at 23°C. Taken together, these results indicate that elevated temperature impacts NHP biosynthesis in various plants species, which is associated with loss of SAR at higher temperatures.

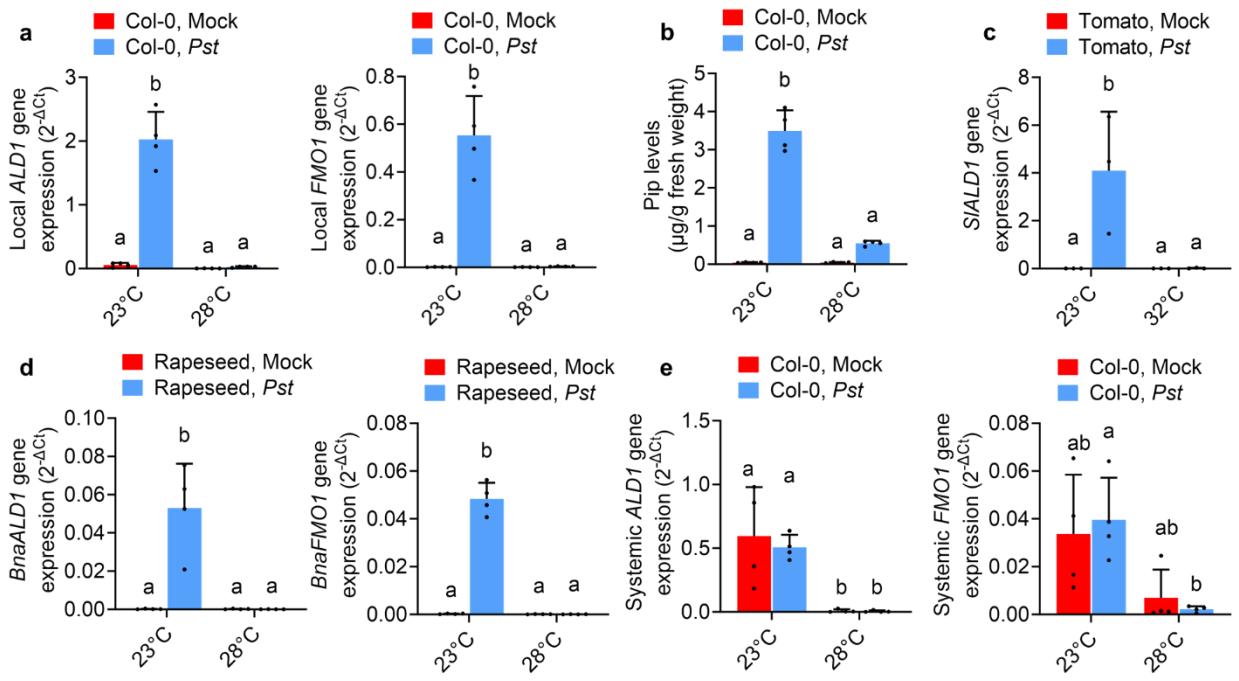

Fig. 2 Plant NHP biosynthetic gene expression and Pip production are suppressed at elevated temperature.

(A-B) Leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001). Plants in were then incubated at either 23°C or 28°C. (A) *ALD1* and *FMO1* transcript levels and (B) Pip levels of pathogen-inoculated tissues were measured at 1 day post-inoculation (dpi). (C) Leaves of four-week-old tomato cultivar Castlemart plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001). *SlALD1* transcript levels of pathogen-inoculated tissues were measured at 1 dpi. (D) Leaves of 4- to 5-week-old rapeseed cultivar Westar plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.0001). *BnaALD1* and *BnaFMO1* transcript levels of pathogen-inoculated tissues were measured at 1 dpi. (E) Lower leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.02). Plants in were then incubated at either 23°C or 28°C. *ALD1* and *FMO1* transcript levels in upper systemic tissues were measured at 2 dpi. Data show the means (± S.D.) and individual points (n=3 to 4) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letters. Experiments were performed at least two times with reproducible results.

Because SA is functionally linked and mutually amplified with Pip and NHP during plant systemic immunity (Hartmann and Zeier, 2019; Huang et al., 2020; Zeier, 2021; Shields et al., 2022), we also measured SA biosynthetic gene expression in upper, systemic leaves after primary pathogen challenge. As shown in Figures 3a and 3b, systemic expression of the SA biosynthetic gene *ICS1* (Wildermuth et al., 2001) was induced at 2 days after virulent *Pst* DC3000 or avirulent *Pst* DC3000/AvrRpt2 inoculation at 23°C. However, *ICS1* transcript levels in systemic tissues were comparable between mock and pathogen treatments at 28°C (Figures 3a-b), suggesting that elevated temperature downregulates SA accumulation systemically. Altogether, these results demonstrate that the SA biosynthetic pathway is suppressed at higher temperature similarly as NHP. This suggests that mutual amplification of these two important SAR-associated metabolites is negatively affected when temperatures increase.

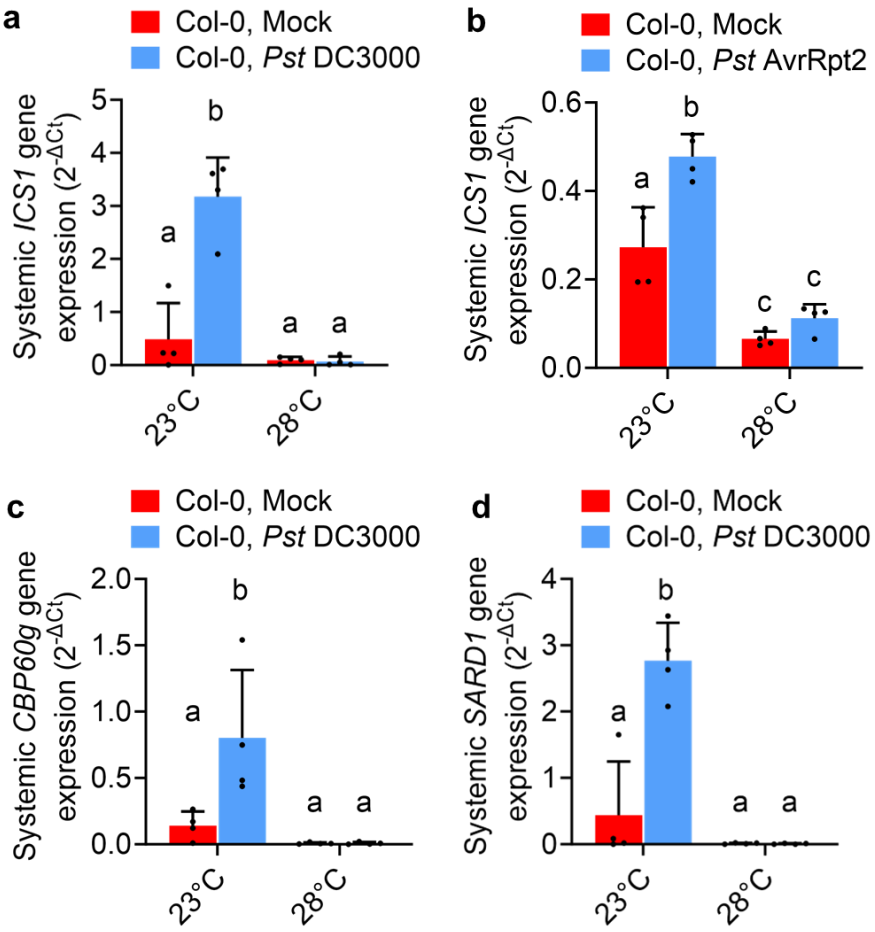

Fig. 3 Systemic SA biosynthetic gene expression is suppressed at elevated temperature.

Lower leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock), *Pst* DC3000 (OD600 = 0.02) or *Pst* DC3000 AvrRpt2 (OD600=0.02). Plants were then incubated at either 23°C or 28°C. (A-B) *ICS1* transcript levels were measured in systemic tissues using RT-qPCR at 2 days post-inoculation (dpi) with *Pst* DC3000 or *Pst* DC3000 AvrRpt2, respectively. (C-D) *CBP60g* and *SARD1* transcript levels were measured in systemic tissues at 2 dpi with *Pst* DC3000. Data show the means (± S.D.) and individual points (n=4) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letters. Experiments were performed two times with reproducible results.

Local or systemic application of exogenous Pip restores *Arabidopsis* immune priming at elevated temperature

Having observed that Pip levels were suppressed at elevated temperature, we hypothesized that exogenous supplementation with Pip may restore disease protection at 28°C if Pip production is the rate-limiting step at elevated temperature. We infiltrated leaves of *Arabidopsis* plants with mock or 1 mM Pip and then infected the same leaves with *Pst* DC3000. As shown in Figure 4a, pathogen levels were reduced after Pip treatment compared to mock treatment at 23°C, expectedly indicating that Pip is sufficient to induce immune priming. Remarkably, Pip-induced disease protection was maintained at 28°C (Figure 4a), which confirms that Pip-NHP production is the rate-limiting step in SAR at elevated temperature.

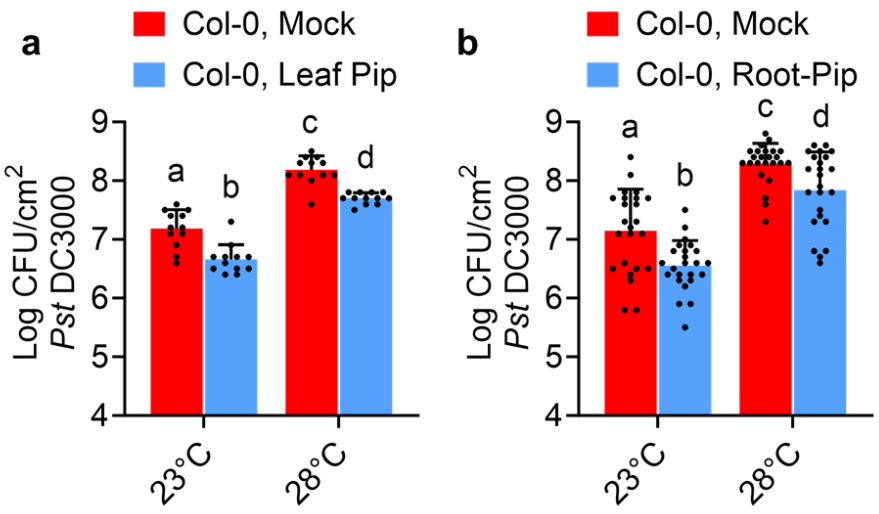

Fig. 4 Exogenous Pip treatment restores *Arabidopsis* immune priming at warm temperature.

Four-week-old *Arabidopsis* Col-0 plants were treated with mock or 1mM Pip solution by leaf-infiltration (A) or root-drenching (B). Plants were then incubated at either 23°C or 28°C. Two days after Pip treatment, leaves were infiltrated with *Pst* DC3000 (OD600 = 0.001), and plants were incubated again at their respective temperatures (23°C or 28°C). Bacterial numbers were quantified at 3 days post-inoculation (dpi). Data show the mean log CFU *Pst* DC3000/mL (± S.D.) and individual points (n=12 from 3 independent experiments in A; n=24 from 6 independent

experiments in B) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letters.

To determine if elevated temperature also affects systemic Pip-mediated disease protection, we irrigated *Arabidopsis* roots with mock or 1 mM Pip two days before infiltrating the leaves with *Pst* DC3000. Similar to the results with Pip-induced disease protection by leaf infiltration, we also observed reduced pathogen levels after Pip root irrigation at both 23°C and 28°C (Figure 4b). These results indicate that the temperature-suppression of the Pip-NHP pathway is governed at the level of biosynthesis and not systemic transport.

***CBP60g* and *SARD1* control temperature-vulnerability of the NHP-SAR pathway**

In addition to chemical supplementation, we next investigated if we could restore systemic immune responses genetically at warm temperatures. We recently showed that CBP60g and its functionally redundant paralog SARD1 control the temperature-sensitivity of plant basal resistance and pathogen-induced SA biosynthesis (Kim et al., 2022). We therefore investigated whether CBP60g and SARD1 also control temperature-sensitive systemic defence responses. As shown in Figure 3c-d, *CBP60g* and *SARD1* transcript levels are induced systemically after local *Pst* DC3000 challenge at 23°C, but this systemic induction is lost at 28°C. These suggest that the loss of systemic immunity at elevated temperature could be due to significantly decreased *CBP60g* and *SARD1* gene expression.

To show a causative role for CBP60g/SARD1 downregulation with NHP immune pathway suppression, we used plants constitutively expressing *CBP60g* (*35S::CBP60g*) or *SARD1* (*35S::SARD1*). As shown in Figures a-c, *35S::CBP60g* lines restored NHP biosynthetic gene expression (*ALD1, FMO1*) and Pip levels at 28°C in contrast to the wild-type Col-0 plants. Consistent with this, constitutive expression of the functionally redundant *SARD1* gene in *35S::SARD1* plants also led to restored expression of *ALD1* and *FMO1* at warm temperature (Figure 5d-e). Taken together, a major mechanism by which higher temperatures target plant systemic immunity and NHP biosynthesis is through the expression of the master immune transcription factor genes *CBP60g* and *SARD1*.

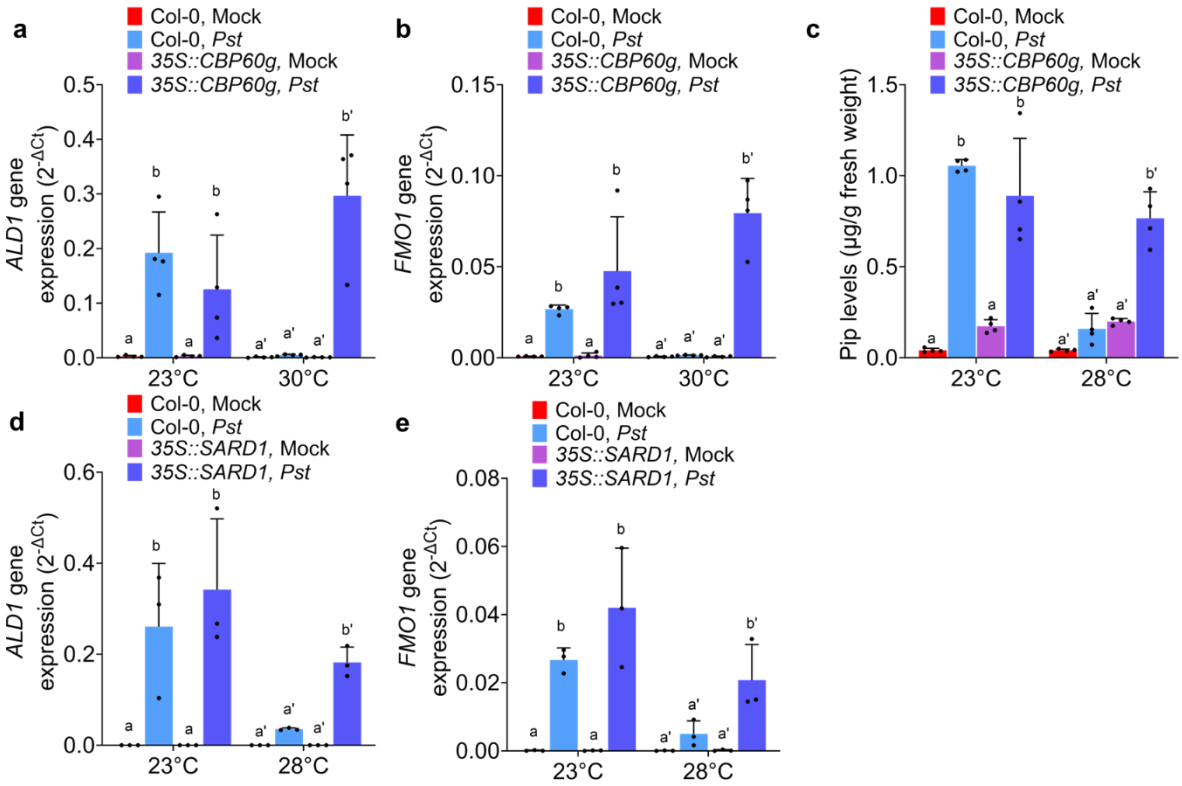

Fig. 5 CBP60g and SARD1 control the temperature-vulnerability of NHP-mediated immunity.

Leaves of four-week-old *Arabidopsis* Col-0 and *35S::CBP60g* (A-C) or *35S::SARD1* plants (D-E) were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001). Plants were then incubated at either 23°C or 28°C. (A) *ALD1* and (B) *FMO1* transcript levels were measured using RT-qPCR at 1 day post-inoculation (dpi) of pathogen-inoculated tissues of Col-0 and *35S::CBP60g* plants. (C) Pip levels were measured at 1 dpi in the same tissues. (D) *ALD1* and (E) *FMO1* transcript levels were measured at 1 day post-inoculation (dpi) of pathogen-inoculated tissues of Col-0 and *35S::SARD1* plants. Data show the means (± S.D.) and individual points (n=4 in A-C; n=3 in D-E), with experiments performed three times with reproducible results. Statistical analyses were conducted using two-way ANOVA with Tukey's Multiple Comparisons test, with statistically significant differences of means denoted by different letters.

Discussion

In this study, we showed that pathogen-induced SAR and the SAR-activating NHP pathway in *Arabidopsis* plants are sensitive to elevated temperatures. Local infection with both virulent or ETI-activating avirulent pathogens triggers SAR at normal temperatures but not at elevated temperatures. We showed that SAR regulation by temperature is caused by temperature-sensitive NHP pathway in local and systemic tissues. SAR-induced NHP biosynthetic gene expression and/or Pip levels are downregulated at elevated temperature. Even though multiple SAR signals have been proposed previously (Fu and Dong, 2013;

Návarová, et al., 2012; Hartmann et al., 2018; Wang, et al., 2018; Vlot et al., 2021; Zeier, 2021), we show here that exogenous supplementation with the NHP precursor Pip (locally and systemically) was sufficient to restore SAR at elevated temperature. This demonstrates a causative relationship between temperature-suppressed Pip-NHP pathway and SAR.

Because NHP primes systemic SA biosynthesis and immunity (Yildiz et al., 2021; Zeier, 2021), we also observed that systemic SA biosynthetic gene expression is downregulated by elevated temperature. SA induction is also important for SAR establishment since SA induction deficient *sid2* and SA-insensitive *npr1* mutants also have abolished SAR (Fu and Dong, 2013; Huang et al., 2020; Peng et al., 2021). Temperature-suppressed systemic SA pathway agrees with our previous study showing a negative impact of higher temperatures on local pathogen-induced SA biosynthesis and basal resistance (Huot et al., 2017; Kim et al., 2022). However, we found that Pip supplementation alone can rescue SAR at elevated temperature, suggesting that Pip-NHP signaling is sufficient in inducing SAR.

We previously showed that CBP60g and SARD1 controls the temperature-vulnerability of local pathogen-induced SA biosynthesis (Kim et al., 2022). Functionally redundant paralogs CBP60g and SARD1 are master transcription factors that directly target the promoters of numerous immunity-related genes (Wang et al., 2009; Zhang et al., 2010; Wang et al., 2011; Sun et al., 2015, Sun et al., 2018), including those important for SA biosynthesis (like *ICS1*) and NHP biosynthesis (like *ALD1* and *FMO1*). In this study, we further demonstrate that CBP60g and SARD1 control the temperature-regulation of NHP biosynthesis and systemic immunity. Expression of *CBP60g* and *SARD1* in systemic tissues are downregulated at elevated temperature, which is associated with suppressed NHP pathway. Restoring systemic *CBP60g* or *SARD1* expression using constitutively expressing *35S::CBP60g* or *35S::SARD1* plants rescues NHP biosynthetic gene expression and Pip levels under warming conditions.

Overall, changing climatic factors like elevated temperature have a broad and significant impact on the plant immune system, not only at local sites of infection (PTI, ETI, SA) but also on systemic immune priming in distal sites via the central SAR metabolite NHP. In this study, we determined that elevated temperature regulates SAR by influencing NHP pathway. We further demonstrated that this temperature-regulation of NHP-mediated SAR is also controlled by the temperature-sensitive master regulators CBP60g and SARD1, reminiscent of their roles in local immune responses (Kim et al., 2022). Not only does temperature affect the plant's ability to directly defend against pathogen attacks but also the plant's immune preparedness for future

infections. Our discoveries advance our understanding of how the plant immune landscape is regulated by a changing environment. This foundational mechanistic knowledge of the plant disease triangle is important to inform strategies in mitigating the negative impacts of warming temperatures on plant health and to provide a molecular roadmap towards engineering climate-resilient plants.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana Columbia-0 (Col-0), *35S::CBP60g* (Wan et al., 2012) and *35S::SARD1* seeds (Kim et al., 2022) were surface-sterilized with 70% ethanol for 10 minutes and rinsed with autoclaved water. Seeds were suspended in sterile 0.1% agarose and incubated in cold conditions (4°C) for three days. Seeds were sown into pots containing autoclaved soil mix – one-part Promix-PGX soil (Plant Products, Ancaster, Ontario), one-part Turface (Turface Athletics, Buffalo Grove, IL), and one-part Vermiculite Prolite (Therm-O-Rock, New Eagle, PA) – supplemented with 100mL of Miracle-Gro solution (The Scotts Company, Mississauga, ON). Tomato (*Solanum lycopersicum*) cultivar Castlemart plants were grown as described previously (Shivnauth et al., 2023). Rapeseed (*Brassica napus*) cultivar Westar plants were grown as described previously (Kim et al., 2022). Plants were grown in environmentally controlled chambers at 23°C with 60% relative humidity and 12h light [100 ± 20 µmol m^{-2} s^{-1}]/12 dark cycle.

Plant systemic immunity and SAR assays

Four-week-old *Arabidopsis* plants were covered with plastic domes to increase humidity and open the stomata 24 hours before infiltration, Plants were infiltrated with 0.25 mM $MgCl_2$ (mock), *Pst* DC3000 (OD600=0.02) or *Pst* DC3000/AvrRpt2 (OD600=0.02) in their lower leaves (Xin et al., 2013; Xin et al., 2018). Following infiltration, plants were further incubated in environmentally growth chambers at either 23°C or 28°C with identical relative humidity (60%) and lighting conditions (12h light/12 dark). For SAR disease assays, upper systemic leaves were or infiltrated with *Pst* DC3000 (OD600 = 0.001). Bacterial levels were quantified 3 days after systemic infiltration based on a previously published protocol (Huot et al., 2017; Kim et al.,

2022). Briefly, in planta bacterial extracts were plated on rifampicin-containing LM media and log colony forming units (CFUs) cm^{-2} were calculated.

Pip treatment and protection assay

Four-week-old *Arabidopsis* plants were infiltrated with mock solution or 1 mM Pip (Sigma) in their lower leaves. In parallel to direct Pip infiltration into leaves, plants were irrigated with 1 mL of mock solution or 1 mM Pip (Sigma) for root inoculations. Following infiltration or irrigation, plants were further incubated in environmentally growth chambers at either 23°C or 28°C with identical relative humidity (60%) and lighting conditions (12h light/12 dark). After two days, the Pip-treated leaves (for local Pip infiltration) or upper systemic leaves (for root irrigation) were further infiltrated with *Pst* DC3000 (OD600 = 0.001). Bacterial levels were quantified 3 days after systemic infiltration as stated in the previous section.

Gene expression analyses

Pathogen-infected leaves and upper systemic leaves at either 23°C or 28°C were harvested at 1 or 2 days after local infection, respectively. Tissues were flash-frozen in liquid nitrogen and stored at -80°C before total RNA extraction. Gene expression levels were quantified based on a previously published protocol (Huot et al., 2017; Kim et al., 2022) with slight modifications. RNA was extracted from flash-frozen plant tissues using the Qiagen Plant RNeasy Mini Kit (Qiagen, Toronto, ON) or TRIzol Reagent (Aidlab Biotech, China) according to the manufacturer's protocol. Resulting cDNA was synthesized using qScript cDNA super mix (Quantabio) or M-MLV reverse transcriptase (RT, Vazyme Biotech, China) based on manufacturers' recommendations. Real-time quantitative polymerase chain reaction (qPCR) was performed using PowerTrack SYBR Green master mix (Life Technologies) or iTaq™ Universal SYBR® Green Supermix (Bio-Rad, USA) with approximately 1.5-10 ng of template cDNA. Equivalently diluted mRNA without the qScript cDNA mix were used as negative controls. The resulting qPCR mixes were run using the Applied Biosystems QuantStudio3 platform (Life Technologies) or CFX96 Real-Time PCR Detection System (Bio-Rad, USA). The individual Ct values were determined for target genes and the internal control gene: *PP2AA3* for Arabidopsis; *SlACT2* for tomato and *BnaGDI1* for rapeseed (Huot et al., 2017; Kim et al., 2022; Shivnauth et al., 2023). Gene expression values were reported as $2^{-\Delta Ct}$, where ΔCt is Ct_{target}

$_{gene}$–$Ct_{internal\ control\ gene}$. qPCR was carried out with three technical replicates for each biological sample. Primers used for qPCR are shown in Supplementary Table 1.

Pip metabolite extraction and quantification

Pathogen-infected leaves at 23°C or 28°C were harvested at 1 day after infection. Pip extraction and quantification were performed based on previous reports with slight modifications (Yao et al., 2023). Approximately 100 mg leaf tissue was frozen and ground in liquid nitrogen, and Pip was extracted at 4℃ for 1h in 600 µL ice-cold extraction buffer (80% methanol in water, 0.1 g/L butylated hydroxytoluene). The extraction step was repeated twice, and a 1.2-mL supernatant was speed-dried in a vacuum centrifugal concentrator (Beijing JM Technology). The pellet was resuspended in 240 µL 30% methanol solution and diluted 10 times for quantification. Pip level was quantified using the AB SCIEX QTARP 5500 LC/MS/MS system. Selected ion monitoring (SIM) was conducted in the positive ES channel for Pip (m/z 130.0>84.0), which was done using a 20V collision energy and a 50V declustering potential. The instrument control and data acquisition were performed using Analyst 1.6.3 software (AB SCIEX), and data processing was performed using MultiQuant 3.0.2 software (AB SCIEX). Pip was separated with an ACQUITY UPLC BEH Amide column (1.7 µm, 2.0 x 100 mm, Waters) using the method in Supplementary Table 2. Pip levels were quantified by calculating the area of each individual peak and comparing it to standard curves. Reported Pip concentration was normalized by sample fresh weight (FW) in gram.

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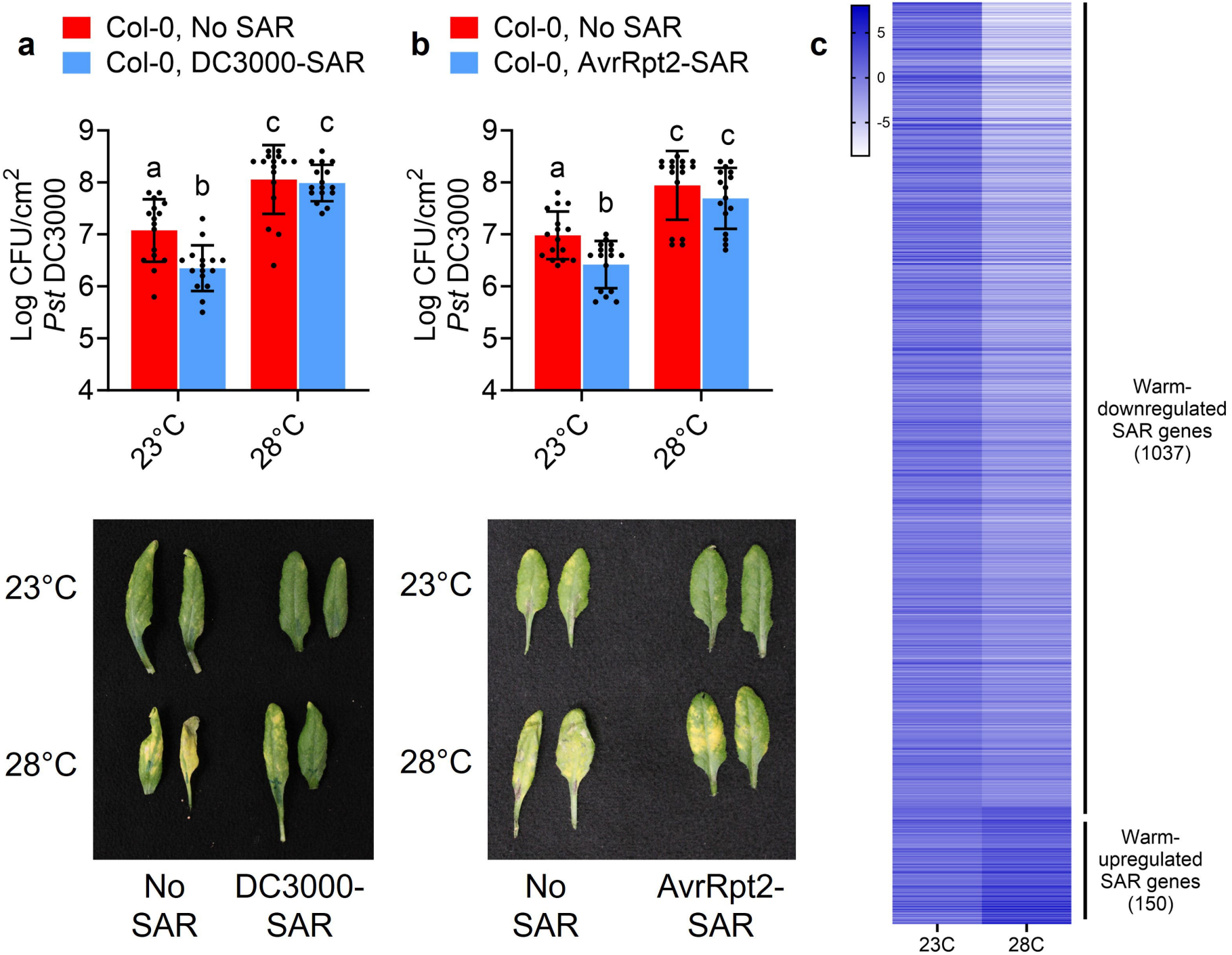
Authors' contributions

C.D.M.C conceptualized and supervised the study. A.S. performed most of the experiments. L.Y.Y. performed the Pip metabolite measurements. J.H.K. performed gene expression experiments of *35S::SARD1* plants. W.M.A.A.T. and E.M. conducted experimental replicates of the SAR assays. V.S. conducted temperature experiments of tomato plants, extracted RNA and synthesized cDNA. S.L. and T.C. performed gene expression analyses of rapeseed plants. L.Y.Y., J.H.K. and S.L. were supervised by X.F.X., S.Y.H. and T.C., respectively. Everyone analyzed the data. A.S. and C.D.M.C. wrote the paper with input from all authors.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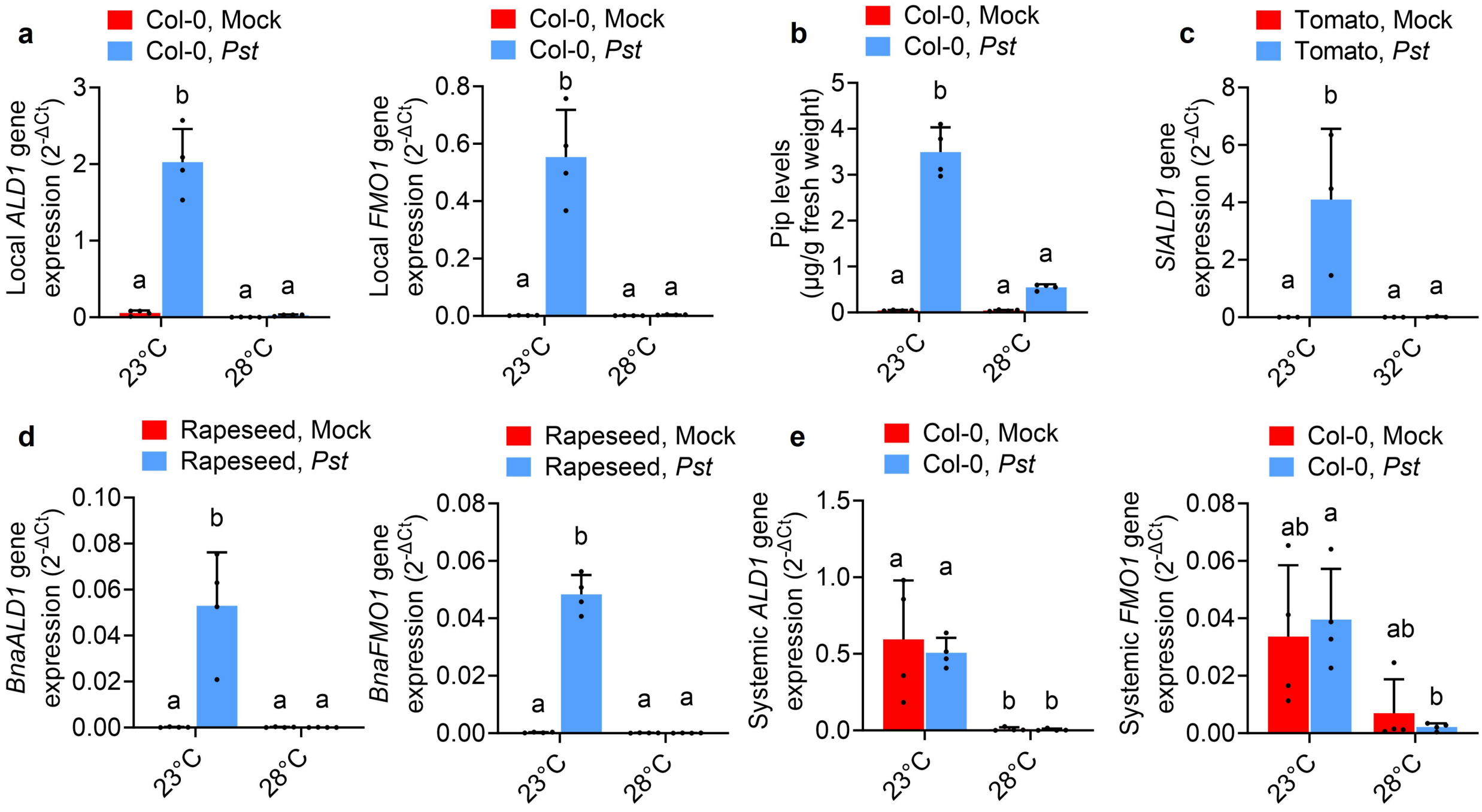
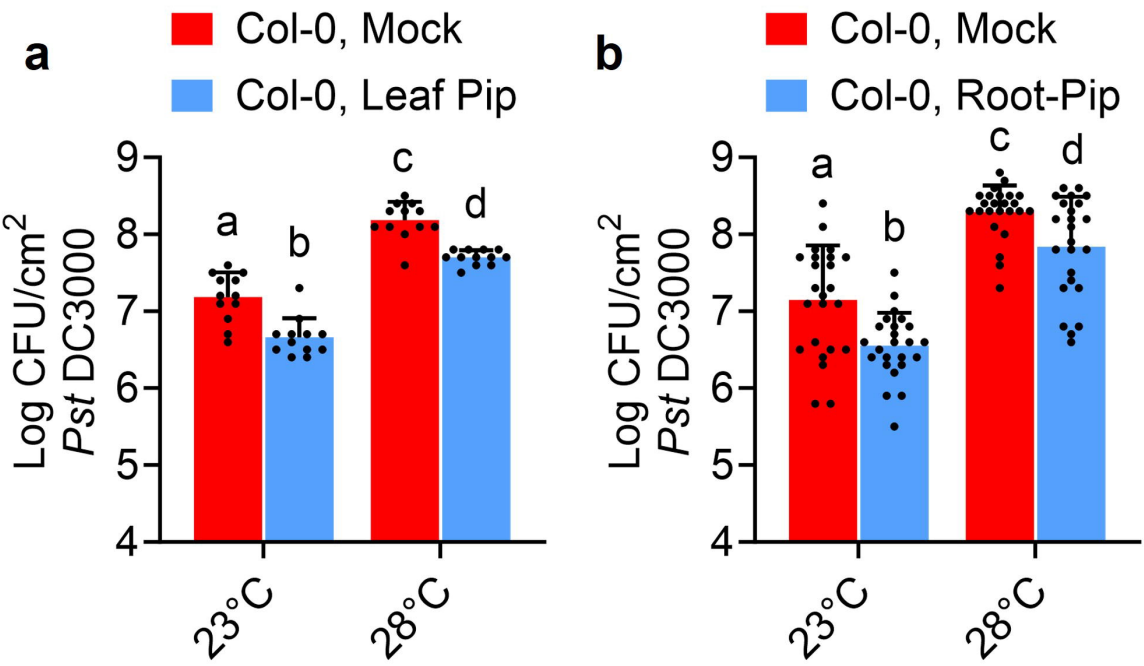
Col-0, No SAR
Col-0, DC3000-SAR
Log CFU/cm2 Pst DC3000
9
8
7
6
5
4
a
b
c
c
23°C
28°C
b
Col-0, No SAR
Col-0, AvrRpt2-SAR
Log CFU/cm2 Pst DC3000
9
8
7
6
5
4
a
b
c
c
23°C
28°C
23°C
28°C
No SAR
DC3000-SAR
23°C
28°C
No SAR
AvrRpt2-SAR
c
5
0
-5
Warm-downregulated SAR genes (1037)
Warm-upregulated SAR genes (150)
23C
28C


a
Col-0, Mock
Col-0, Pst
Local ALD1 gene expression (2^-ΔCt)
Local FMO1 gene expression (2^-ΔCt)
b
Pip levels (µg/g fresh weight)
c
Tomato, Mock
Tomato, Pst
SlALD1 gene expression (2^-ΔCt)
23°C
28°C
32°C
d
Rapeseed, Mock
Rapeseed, Pst
BnaALD1 gene expression (2^-ΔCt)
BnaFMO1 gene expression (2^-ΔCt)
e
Systemic ALD1 gene expression (2^-ΔCt)
Systemic FMO1 gene expression (2^-ΔCt)


a
Col-0, Mock
Col-0, Leaf Pip
Log CFU/cm2
Pst DC3000
9
8
7
6
5
4
a
b
c
d
23°C
28°C
b
Col-0, Mock
Col-0, Root-Pip
Log CFU/cm2
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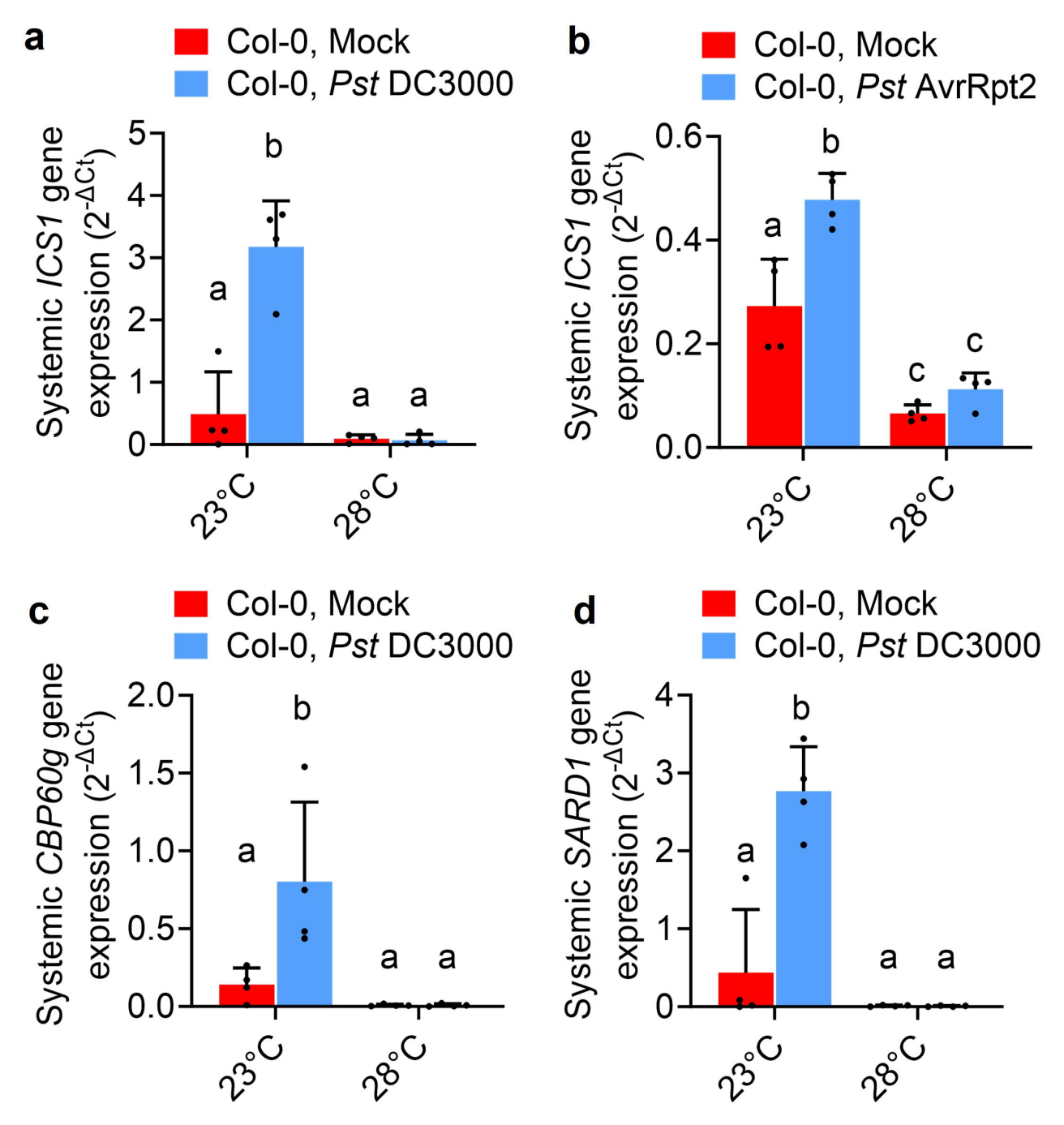
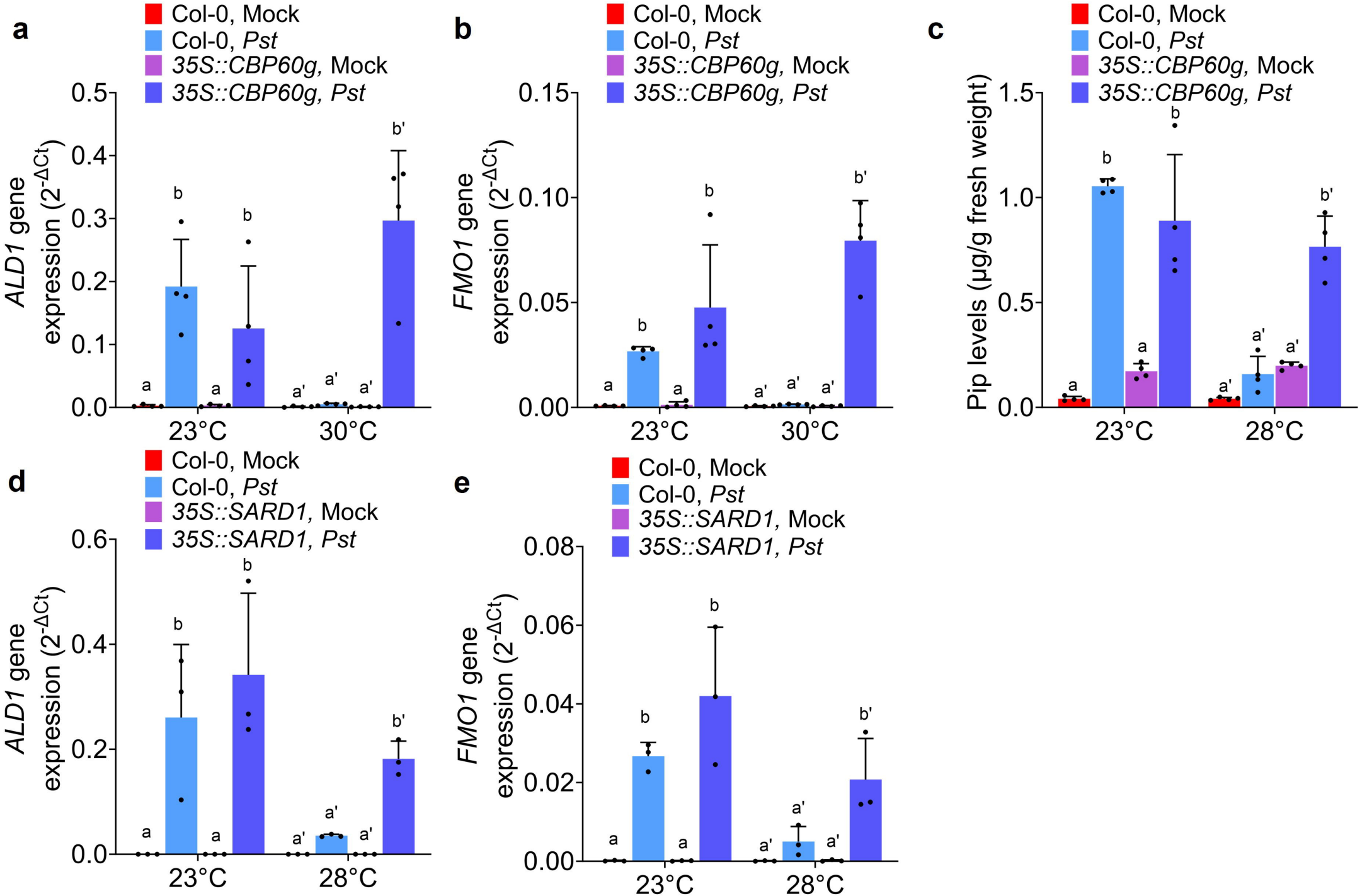
9
8
7
6
5
4
a
b
c
d
23°C
28°C


a
Col-0, Mock
Col-0, Pst DC3000
Systemic ICS1 gene expression (2-ΔCt)
5
4
3
2
1
0
b
a
a
a
23°C
28°C
b
Col-0, Mock
Col-0, Pst AvrRpt2
Systemic ICS1 gene expression (2-ΔCt)
0.6
0.4
0.2
0.0
b
a
c
c
23°C
28°C
c
Col-0, Mock
Col-0, Pst DC3000
Systemic CBP60g gene expression (2-ΔCt)
2.0
1.5
1.0
0.5
0.0
b
a
a
a
23°C
28°C
d
Col-0, Mock
Col-0, Pst DC3000
Systemic SARD1 gene expression (2-ΔCt)
4
3
2
1
0
b
a
a
a
23°C
28°C


a
Col-0, Mock
Col-0, Pst
35S::CBP60g, Mock
35S::CBP60g, Pst
ALD1 gene expression (2^-ΔCt)
0.5
0.4
0.3
0.2
0.1
0.0
23°C
30°C
b
FMO1 gene expression (2^-ΔCt)
0.15
0.10
0.05
0.00
c
Pip levels (µg/g fresh weight)
1.5
1.0
0.5
0.0
28°C
d
35S::SARD1, Mock
35S::SARD1, Pst
0.6
e
0.08
0.06
0.04
0.02