Trends in Plant Science

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Spotlight

Too hot to defend: a tale of salicylic acid

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Extreme temperatures threaten plant immunity by suppressing the salicylic acid (SA) biosynthesis via unknown mechanisms. Kim *et al.* demonstrated that suppression of the SA pathway and plant immunity can be rescued by optimised expression of two master immune regulator(s), advancing our prospects for better protecting plants in a warming climate.

Heat tolerance versus plant immunity trade-off

An ongoing increase of the average global temperature as well as heat waves raise the risk of diseases that cause significant crop yield losses [1]. Thermoresilient crops with enhanced pest and disease resistance are imperative to produce crops with higher yields with minimal use of chemical pesticides. Salicylic acid (SA) is known for its multifaceted role in plant growth and defence [2]. One of the roles of SA is to mediate defence strategies against biotic and abiotic stress [3]. It acts as a signalling molecule to activate plant defence responses upon pathogen infection and triggers resistance against many plant pathogens [2]. Various studies have shown that plants grown at higher temperatures or exposed to a short period of heat wave can decrease the production of SA and thereby weaken the plants' defence against pathogens [4–6]. Therefore, it is important to understand the molecular mechanism behind heat-mediated suppression of the SA pathway because it could help (re)design thermoresilient crops.

In 2017, Huot *et al.* [4] showed that *Arabidopsis thaliana* Col-0 exposed to elevated temperature for a short period had significantly reduced SA accumulation and were more susceptible to *Pseudomonas syringae* pv. *tomato* (*Pst* DC3000). The same research group has now unravelled the molecular mechanism behind heat-mediated suppression of the SA pathway in *A. thaliana* and has shown that this suppression is conserved in both monocot [rice (*Oryza sativa*)] and dicot [tobacco (*Nicotiana tabacum*), rapeseed (*Brassica napus*), tomato (*Solanum lycopersicum*)] plants. They show that temperature sensitivity of the SA pathway has a significant impact on effector-triggered immunity (ETI), pattern-triggered immunity (PTI), and basal immunity [7]. Because ETI and basal immunity are required for protecting plants against pathogens, crop plants will be greatly affected by the warming climate and can become more susceptible to diseases.

SA accumulation in *A. thaliana* increases under chilling temperatures (5°C) [8] and decreases at elevated temperatures (28°C) [4]; therefore, it was hypothesised that the temperature sensitivity of SA production might be mediated by the thermosensing mechanism of the plant. Constitutively active thermosensors EARLY FLOWERING 3 (ELF3) and PHYTOCHROME B (phyB) lines in *A. thaliana* were tested. Although these plants were not thermoresponsive, pathogen-induced SA accumulation was still temperature-sensitive and susceptible to *Pst* DC3000. This is in line with the previous study in which they showed that knocking out four distinct PHYTOCHROME-INTERACTING FACTORS (*pif*) does not confer basal immunity to *Pst* DC3000 at elevated temperature [4]. Both of these studies indicate that temperature-modulated SA production is independent of the known thermosensors.

Biotic and abiotic stresses trigger the expression of the majority of genes involved in SA biosynthesis [3]. Therefore, the role of SA positive and negative regulators was tested at elevated temperature (28°C). Neither the overexpression of SA positive regulators (*ICS1*, *NPR1, TGA1*, *PAD4, EDS1*, or *WRKY75*) nor the knockout of SA negative regulators (*NPR3/4*, *CAMTAs*, and *BSMT1*) restored pathogen-induced SA and basal immunity [7]. Further on, transcriptomic analysis of Col-0 plants infected with *Pst* DC3000 at 23°C and 28°C identified suppression of immune regulators ***CBP60g***, ***SARD1*** (see Glossary), and their target genes at elevated temperature. *CBP60g* and *SARD1* are known to be involved in controlling the major plant immune responses, including the biosynthesis of SA [9,10]. The expression of *CBP60g* and *SARD1* was recently reported also to be suppressed under drought stress, leading to enhanced susceptibility to *Pst* DC3000 [11]. Therefore, the authors hypothesised that CBP60g and SARD1 act as the rate-limiting step and the primary target in heat-mediated suppression of the SA pathway. They confirmed this hypothesis by overexpressing *CBP60g,* which

Glossary

Biomolecular condensates: membraneless organelles of eukaryotic cells often formed by the physical process of liquid-liquid phase separation. Biomolecular condensates are involved in a wide range of processes, such as RNA metabolism, signal transduction, and gene regulation.

CBP60g and SARD1: calmodulin-binding protein 60g (CBP60g) and systemic acquired resistance deficient 1 (SARD1) are members of a plant-specific family of proteins that play an important role in plant innate immunity by regulating salicylic acid biosynthesis, signalling, and resistance to pathogens, as well as other types of biotic and abiotic stress. CBP60g binds to calmodulin, whereas SARD1 does not bind to calmodulin and operates in a Ca^{2+}-independent manner.

Liquid-liquid phase separation (LLPS): thermodynamic process whereby macromolecules come together to form a distinct liquid phase through a variety of interactions (e.g., RNA/protein–protein interactions or multivalent interactions between intrinsically disordered regions of proteins), acting as compartments that catalyse various biological reactions.

restored, at 28°C, the pathogen-induced expression of its target genes, such as SA biosynthetic genes (*ICS1* and *PBS3),* plant immunity-related genes (PTI related, e.g., *RLP23* and *LYK5*; ETI-related, e.g., *PAD4* and *EDS1*), plant defence compound-related genes (e.g., *PAD3* and *MYB51*), and SAR-related genes (pipecolic acid biosynthetic gene *ALD1*). Constitutive overexpression of *CBP60g* was associated with slight growth defects and delayed flowering; however, this trade-off between growth and defence could be minimised by expressing *CBP60g* in a pathogen-inducible system.

The molecular mechanism behind altered gene expression of *CBP60g* under elevated temperature was a puzzle to solve. The authors identified guanylate-binding protein (GBP)-like GTPase 3 (GBPL3) to be required for the expression of *CBP60g* on treatment with SA. GBPL3 is a positive regulator of SA, and it is involved in plant immunity [12]. It acts via **liquid-liquid phase separation (LLPS)**-driven **biomolecular condensates** together with Mediator and RNA polymerase II to form membraneless organelles called GBPL defence-activated condensates (GDACs),

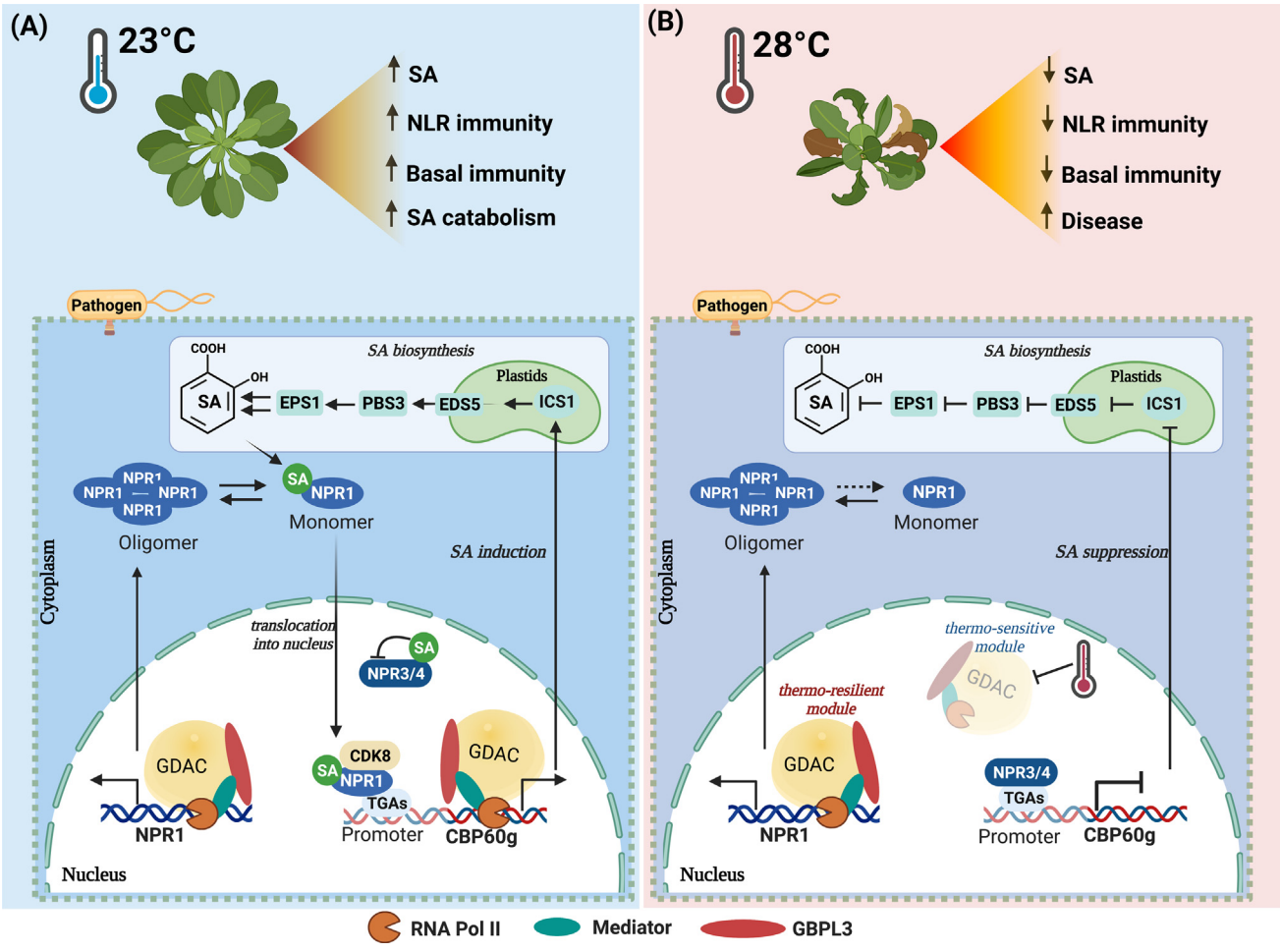

Figure 1. Model depicting crosstalk of salicylic acid (SA) pathway under control conditions (23°C with pathogen infection) and at elevated temperature (28°C with pathogen infection). (A) Under control conditions, pathogen infection causes GBPL defence-activated condensates (GDACs) to drive the expression of target genes such as CBP60g. CBP60g regulates the expression of SA biosynthetic genes and plant immunity-related genes. Higher SA levels induce monomerisation of NPR1, and the monomeric NPR1 travels to the nucleus and derepresses SA pathway genes that were suppressed by NPR3/4 through direct interaction with TGA transcription factors and CDK8 mediator. (B) Under elevated temperature with pathogen infection, there are two distinct subpopulations of GDACs: (i) temperature-sensitive and (ii) temperature-resilient modules. In temperature-sensitive modules, the recruitment of GDACs to CBP60g is impaired, leading to suppression of SA biosynthesis and plant immunity-related genes, resulting in compromised immunity. In temperature-resilient modules, GDACs drive expression of target genes, including NPR1. Due to lower levels of SA, monomerisation and translocation of NPR1 to the nucleus is reduced, and NPR3/4 continue to act as negative regulators of SA pathway genes, resulting in compromised immunity at elevated temperature. (Image created with www.biorender.com.)

which drive the expression of its target genes [12]. Using confocal imaging, the authors showed that the number of GDACs per nucleus significantly decreased for benzothiadiazole (SA analogue)-treated plants at elevated temperature and reversibly appeared back at 23°C. Further on, ChIP-qPCR revealed that GBPL3 binding to certain target loci such as the promoters of *CBP60g* and *SARD1* was significantly reduced at elevated temperature but not to other target loci such as *NPR1*. Overall, they show that elevated temperature has a specific effect on the recruitment of GBPL3 and other proteins important in the SA pathway to the promoter of *CBP60g* (Figure 1).

Concluding remarks and future perspectives

Kim *et al.* identified immune regulator CBP60g as the rate-limiting step in SA production at elevated temperature. They highlighted the importance of biomolecular condensates (GDACs) in controlling the expression of *CBP60g* and its possible roles as a regulatory node for responding to changing temperature. The authors show that temperature sensitivity of the SA pathway is probably shared by many plants, which may have a wider implication for developing plants with higher immunity toward pests and pathogens. This paper describes a significant advancement in our understanding of plant immunity in a warming climate. Yet, there are a few questions that need to be further explored: (i) How do CBP60g, Mediator, and suppression of SA production, under elevated temperature, affect the plant immunity against necrotrophic pathogens that exploit SA to promote disease development? (ii) Does the GBPL3-CBP60g module restore basal and ETI-mediated pathogen immunity in other crop species? (iii) Does CBP60g also play a role in SA production during chilling stress? (iv) What is the molecular mechanism behind the distinct behaviour of two subpopulations of GDACs? The answers to these questions would enhance our understanding of plant responses to the warming climate and pave the way for developing climate-resilient crops.

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Declaration of interests

No interests are declared.

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