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Negative evidence on the transgenerational inheritance of defense priming in *Arabidopsis thaliana*

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Defense priming allows plants to enhance their immune responses to subsequent pathogen challenges. Recent reports suggested that acquired resistances in parental generation can be inherited into descendants. Although epigenetic mechanisms are plausible tools enabling the transmission of information or phenotypic traits induced by environmental cues across generations, the mechanism for the transgenerational inheritance of defense priming in plants has yet to be elucidated. With the initial aim to elucidate an epigenetic mechanism for the defense priming in plants, we reassessed the transgenerational inheritance of plant defense, however, could not observe any evidence supporting it. By using the same dipping method with previous reports, Arabidopsis was exposed repeatedly to *Pseudomonas syringae* pv *tomato* DC3000 (*Pst* DC3000) during vegetative or reproductive stages. Irrespective of the developmental stages of parental plants that received pathogen infection, the descendants did not exhibit primed resistance phenotypes, defense marker gene (*PR1*) expression, or elevated histone acetylation within *PR1* chromatin. In assays using the pressure-infiltration method for infection, we obtained the same results as above. Thus, our results suggest that the previous observations on the transgenerational inheritance of defense priming in plants should be more extensively and carefully reassessed. [BMB Reports 2022; 55(7): 342-347]

INTRODUCTION

Epigenetic inheritance is the transmission of a trait that reflects mitotically and/or meiotically stable changes in gene expression without alteration of DNA sequence. In eukaryotes, chromatin modifications such as post-translational modification of histones and cytosine methylation on DNA are dynamically regulated by developmental or environmental cues, and these marks can give rise to epigenetic memory of transcriptional state (1). Progressively, epigenetic information derived from environmental stimuli can be transmitted across generations enabling progeny to adapt to their surrounding environment (2).

Plants continuously withstand many environmental challenges and in the process adapt to various biotic and abiotic stresses through plastic responses. Against various pathogenic threats, plants develop sophisticated defense responses. As the first layer of plant defense, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is established when plants recognize highly conserved PAMPs by pattern-recognition receptors (PRRs; 3, 4), whereas the second layer of plant defense, effector-triggered immunity (ETI), involves highly variable resistance (R) proteins recognizing effector molecules secreted by pathogens (4, 5).

In addition to the local defense mechanisms at the infection sites, plants also induce defense priming systemically even in uninfected tissues, which allows more rapid and robust responses to secondary pathogen attacks. This systemic acquired resistance (SAR) depends on salicylic acid (SA) responses and confers long-lasting and broad-spectrum resistance against subsequent infections (4). It was reported that in the primed state, histone H3 lysine 9 and lysine 12 acetylation (H3K9K12Ac) and H3K4 di- and tri-methylation (H3K4me2/me3) levels are increased at some defense-gene loci compared to the non-primed state (6). However, the role of epigenetic modification of chromatin in SAR is not yet clear.

Recently, a few studies have reported that environmentally induced disease resistance in parents is transmitted to subsequent generations such that progenies obtain beneficial traits for responding to adverse environmental conditions. The priming state induced by SA-analog treatment or *Pst* DC3000 *avrRpt2* infection was transferred to the next generation, whereas the descendants that went through a stress-free generation returned to a non-primed state (7). On the other hand, Luna *et al*. (8) reported that the transgenerational effect of pathogen resistance obtained from repetitive *Pst* DC3000 infection was maintained over one stress-free generation. Induced resistance in parental plants by caterpillar herbivory, application of methyl jasmonate, or mechanical damage was also reported to be passed on to two subsequent generations (9). Transcriptional defense responses

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are routinely associated with epigenetic modifications. Although epigenetic modifications may serve as an epigenetic memory of transcriptional state (1), their role as heritable materials responsible for the transgenerational inheritance of defense priming and environment-induced traits is yet to be demonstrated. Nonetheless, cytosine methylation on DNA has been considered a favorable candidate (8-10) due to its maintenance mechanism through cell division.

With an initial aim to elucidate an epigenetic mechanism for the transgenerational defense priming in Arabidopsis, we set up experimental schemes following the previous reports and tested transgenerational effects first. However, we could not obtain any evidence proving that defense priming is inherited across generations. Defense priming that was known to be induced by repetitive pathogen infection in parental plants was not transmitted to descendants regardless of the existence of gametes during the period of infection. Considering subtle differences in experimental conditions, the degree of pathogen stress perceived in parental plants was thought to be a candidate factor affecting the transmittance of induced resistance to progenies. Nonetheless, we claim that the heritability of defense priming across generations should be evaluated in a much stricter view, especially after considering plant-unique later differentiation of germline-cell lineages and meristem-derived organ development.

RESULTS

Experimental design to assess transgenerational defense priming in *Arabidopsis thaliana*

We used the standard ecotype of *Arabidopsis thaliana*, Columbia-0 (Col-0) accession, throughout this study. Parental Col-0 plants were either classified as N0 or P0 depending on the type of experimental treatments (Fig. 1). Plants were treated with 10 mM $MgCl_2$ (for N0) or *Pseudomonas syringae* pv *tomato* DC3000 (*Pst* DC3000) suspension in 10 mM $MgCl_2$ (for P0) a total of five times with 3-day (d) intervals by syringe infiltration (7) or dipping (8). To clarify true transgenerational defense priming from effects directly or indirectly given to gametes by the pathogen during dipping procedure, the infection by dipping was performed at two different developmental stages, prior to and after the onset of flowering (bolting) (Fig. 1). N1 and P1 plants were generated by self-pollinating N0 and P0 plants, respectively, and used for analyzing pathogen-resistance, *PR1*-expression, and H3Ac levels at the *PR1* locus (Fig. 1).

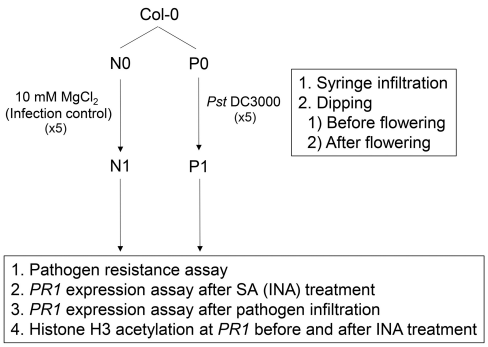

Fig. 1. Schematic diagram of the experimental design for this study. Parental plants, P0 plants, and their infection control N0 plants are all Col-0 ecotypes. P0 plants were inoculated with *Pst* DC3000 by two different methods, syringe-infiltration and dipping, a total of five times with 3-day (d) intervals for both methods. N0 plants were treated with 10 mM $MgCl_2$ in the same manner as with P0 plants. Dipping was performed before or after flowering. N1 and P1 plants in the next generation were produced from self-crossed N0 and P0 plants, respectively, and used for assays on defense priming.

Defense priming in parental plants was not inherited to descendants when parental plants were infected with bacterial pathogen before flowering

To explore if defense priming in parental plants can be trans-

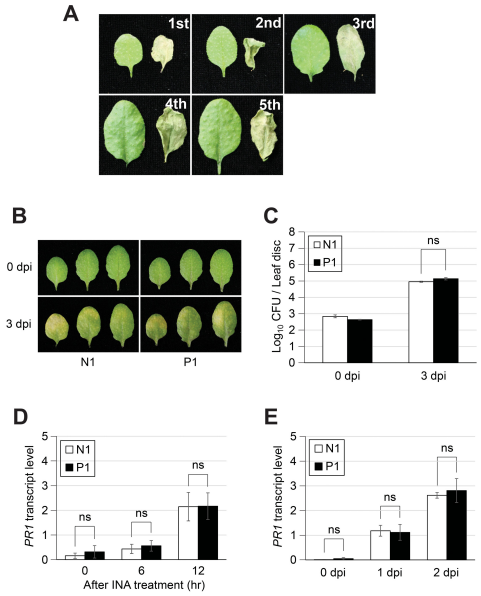

Fig. 2. Assays to test transgenerational defense priming in the descendants of plants infected with *Pst* DC3000 by syringe-infiltration. (A) Representative leaves of 3-week (w)-old N0 (left) and P0 (right) plants pressure-infiltrated with 10 mM $MgCl_2$ (N0) or *Pst* DC3000 suspension (P0). Infiltration was performed five times with 3-d intervals and the pictures were taken after 24 hr of each infiltration. (B) Representative leaves of N1 and P1 plants at 0 and 3 days post-infection (dpi). (C) Assays for bacterial cell growth using N1 and P1 plants at 0 and 3 dpi. Values are the means ± SE of three biological replicates. 'ns' means a statistically no significant difference in a Student's *t*-test. (D, E) RT-qPCR analyses of *PR1* transcript levels in N1 and P1 plants after INA treatment (D) or *Pst* DC3000 infection (E). Means ± SE of three biological replicates are shown after normalization to *Ubiquitin 10* (*UBQ10*). Plants were grown on soil for 4 w under 12L/12D (B, C, E) or on MS media for 2 w under 16L/8D (D) and were treated with *Pst* DC3000 suspension at OD_{600} = 0.001 (B, C, E) or 0.3 mM INA (D).

mitted to their descendants, the leaves of N0 and P0 plants were pressure-infiltrated as described in the Materials and Methods section and Fig. 1. P0, but not N0, leaves showed an obvious disease symptom, which became severer as the number of infections increased (Fig. 2A), indicating that pathogen infection was successful. N1 and P1 plants, which are the descendants of N0 and P0, respectively, were obtained and tested for their resistance to *Pst* DC3000. At 3 days post-infection (dpi), we found no significant differences in phenotypic disease symptoms in leaves (Fig. 2B) or in bacterial cell growth (Fig. 2C) between the N1 and P1 plants. As defense priming was previously reported to be associated with the priming of SA-responsive genes at the molecular level (11), we studied the effect of pathogen infection to parental plants on the priming of defense-gene expression in descendants using *PR1*, a well-known SA-response marker gene. *PR1* transcript levels increased substantially after the treatment of 2,6-dichloroisonicotinic acid (INA; synthetic SA analog) after 12 hours (hr), indicating that INA was properly treated (Fig. 2D). However, the basal levels and the levels of *PR1* transcript after INA treatment were not significantly different between the N1 and P1 plants (Fig. 2D). Furthermore, when avirulent *Pst* DC3000 *avrRpt2* was used for P0 infection and subsequent P1 preparation, the induction pattern of *PR1* by INA in the P1 plants was not different from that of the N1 plants (Supplementary Fig. 1).

To more directly address the transgenerational defense priming at the molecular level, we also analyzed *PR1* expression after pathogen infection. Consistent with the results of leaf disease symptoms and bacterial cell growth, *PR1* transcript levels in the N1 and P1 plants were not significantly different from each other at both 1 dpi and 2 dpi, although the levels were clearly increased after pathogen infection (Fig. 2E). Thus, all the results from the infiltration studies indicated that the experience of parental plants with bacterial-pathogen infection did not affect the defense priming of descendants.

Defense priming in parental plants was not inherited to descendants when parental plants were infected with bacterial pathogen after flowering

The results from our infiltration study described above were not consistent with a previous study (8) which reported a transgenerational defense priming after parental plant infection with *Pst* DC3000. Differences in experiments between the two studies were in the methods of inoculation (syringe-infiltration in our study vs. dipping in Luna *et al*. (8)) and the developmental stages of plants at infection time. Although we completed repeated infections before the onset of flowering, Luna *et al*. (8) infected parental plants by dipping across flowering stages; from before-flowering to after-flowering stages during the repeated dipping procedure. Therefore, we suspected a possibility that the defense priming observed in the next generation might have been resulted from the infection of the gametophytes of the parental generation.

To address this possibility, first, we started an infection by dipping using 3-week (w)-old parental plants that were at vegetative stage and repeated dipping four-more times with 3-d intervals between the repeats. As shown in Fig. 3A, there was no sign of bolting or flowering even at 3 d after the final infection by dipping, eliminating any chances of pathogen-inducing effects on reproductive cells. Similar to the results from the pressure-infiltration study (Fig. 2), the N1 and P1 plants did not show distinguishable phenotypic susceptibilities (Fig. 3B) nor significant differences in bacterial cell growth (Fig. 3C) after *Pst* DC3000 infection. Although the transcript levels of *PR1* were elevated, the N1 and P1 plants did not show significantly different *PR1* induction patterns or amplitudes upon INA treatment (Fig. 3D) and pathogen infection (Fig. 3E).

The parental plants were then infected by dipping after bolting (Fig. 4A). Surprisingly, the N1 and P1 descendants of these infected parental plants were not distinguishable in their pathogen resistance as represented by phenotypic susceptibility (Fig. 4B), bacterial cell growth (Fig. 4C), and *PR1* induction patterns after either INA treatment (Fig. 4D) or pathogen infection (Fig. 4E). Luna *et al*. (8) reported that the effect of repetitive pathogen attack in the parental generation could be transmitted to the

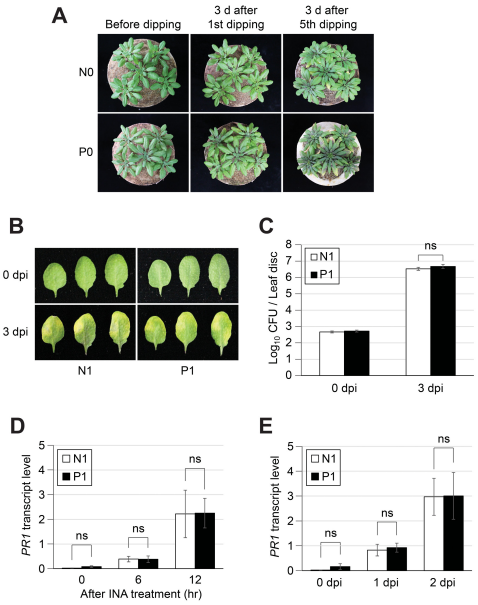

Fig. 3. Assays to test transgenerational defense priming in the descendants of plants infected with *Pst* DC3000 by dipping before flowering. (A) N0 and P0 plants were grown under 12L/12D. Dipping was performed with 10 mM $MgCl_2$ (N0) or *Pst* DC3000 (P0) five times with 3-d intervals. The first dipping was carried out when plants were 3-w-old. (B) Representative leaves of N1 and P1 plants at 0 and 3 dpi. (C) Assays for bacterial cell growth using N1 and P1 plants at 0 and 3 dpi. Values are the means ± SE of three biological replicates. 'ns' means a statistically no significant difference in a Student's *t*-test. (D, E) RT-qPCR analyses of *PR1* transcript levels in N1 and P1 plants after INA treatment (D) or *Pst* DC3000 infection (E). Values were normalized to *UBQ10*. Means ± SE of three biological replicates are shown.

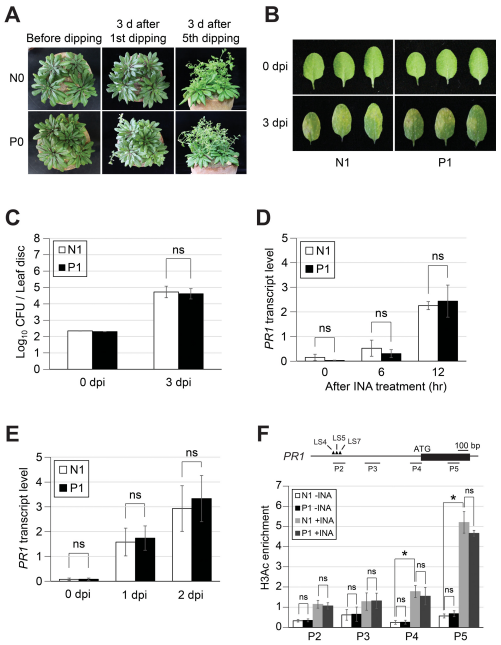

Fig. 4. Assays to test transgenerational defense priming in the descendants of plants infected with *Pst* DC3000 by dipping after flowering. (A) N0 and P0 plants grown under 12L/12D. Dipping was performed with 10 mM $MgCl_2$ (N0) or *Pst* DC3000 (P0) five times with 3-d intervals. The first dipping was carried out when plants were 5-w-old. (B) Representative leaves of N1 and P1 plants at 0 and 3 dpi. (C) Assays for bacterial cell growth using N1 and P1 plants at 0 and 3 dpi. Values are the means ± SE of three biological replicates. 'ns' means a statistically no significant difference in a Student's *t*-test. (D, E) RT-qPCR analyses of *PR1* transcript levels in N1 and P1 plants after INA treatment (D) or *Pst* DC3000 infection (E). Values were normalized to *UBQ10*. Means ± SE of three biological replicates are shown. (F) H3Ac levels within *PR1* chromatin in N1 and P1 plants before (−) and after (+) INA treatment. Values were normalized to input and *UBQ10*. Means ± SE of three biological replicates are shown. Asterisks indicate statistically significant differences between −INA and +INA samples ($P < 0.05$ in a Student's *t*-test).

next generation through increased H3Ac within the chromatin of defense genes including *PR1*. Therefore, we examined H3Ac levels within *PR1* chromatin in the N1 and P1 plants by chromatin immunoprecipitation (ChIP) assay (Fig. 4F). In consistence with the transcriptional induction of *PR1* (Fig. 4D) and previous reports (12, 13), INA treatments increased H3Ac levels in several regions of the *PR1* locus (Fig. 4F). However, H3Ac levels between the N1 and P1 plants were not significantly different at basal as well as induced states in all *PR1* regions tested.

Thus, all our results from the pressure-infiltration (Fig. 2) and dip-infection studies before (Fig. 3) or after flowering (Fig. 4) consistently demonstrate that repeated pathogen stress on plants did not result in transgenerational defense priming in the subsequent generation.

DISCUSSION

Non-DNA sequence-based transgenerational inheritances have been reported in various organisms (14). Especially in plants, transgenerational inheritance of disease resistance is an attractive subject because of its great application value. Recently, several studies reported that plant defense systems are transcriptionally primed, enabling faster and stronger immune responses in the progeny generation of parental plants suffered from repeated biotic stresses (8-10). Although DNA methylation has previously been proposed as a candidate epigenetic mark that might be responsible for the defense priming (8-10), there has been no direct demonstration of the role of DNA methylation. Therefore, we intended to understand an epigenetic mechanism and mark responsible for the transgenerational defense priming. However, we found, in contrast to the previous reports, no evidence of inheritance of acquired disease resistance in the next-generation plants in this study. In our study, the descendants of parental plants which experienced repeated pathogen attacks regardless of the infection methods (pressure-infiltration or dipping) and developmental statuses (vegetative or reproductive) did not show any evidence of acquired resistance against bacterial pathogens.

One possibility for the conflicting results might be in the differences in experimental conditions such as plant growth conditions, plant developmental states when the pathogen infection occurred, pathogen infection methods, etc. Such differences might affect the extent of host responses to pathogens. For example, photoperiods, critical environmental cues for plant growth and development, can affect biochemical and physiological features of plant cells which in turn may influence the level of susceptibility to pathogens. Although severely stressed parental phenotypes were commonly observed under two different photoperiodic regimes (8L/16D photoperiod (7) and 16L/8D photoperiod (8)), the same phenotypes were not observed under our 12L/12L photoperiod. Thus, photoperiods might not be the sole factor affecting parental susceptibility. Among other environmental factors, humidity is especially considered important for the virulence of pathogens (15). Given that surrounding humidity could substantially affect plant-pathogen interaction, the conflicting results from other groups might also have be caused by different humidity conditions given during pathogen inoculation. Under the assumption that the extent of stress given to parental plants is likely one of the factors affecting the transgenerational inheritance, the infection stresses used by the groups reported the transgenerational resistance of defense priming might be strong enough to generate defense priming that is transmittable to the next generation, whereas those used in our experiments might not be strong enough.

We also noticed that the approaches used to assess the disease resistance of progeny generation were different between groups. Luna *et al.* (8) used less bacterial cells to infect the progenies in comparison to our study. In addition, Luna *et al.* (8) and Slaughter *et al.* (7) quantified *Pst* DC3000 in the in-

fected leaves of progenies by measuring bioluminescence from luxCDABE-tagged *Pst* strain and the transcript level of a *Pst* gene, respectively, instead of counting the number of bacterial cells as we did. Thus, the differences in the infection methods and measuring the infection status of progenies might be other reasons for the discrepancies between the groups. At this point, we are not sure which methods would allow more accurate assays on the status of defense priming of the progeny generations.

For the transgenerational inheritance of defense priming, the primed state should be transmitted through the germline to progenies and thus maintained through cell divisions (i.e., mitosis and meiosis). Although DNA methylation has been considered as an epigenetic mark implicated in transgenerational inheritance of acquired resistance, there is still a lack of direct evidence showing that environmental stress-induced changes in DNA methylation are stably inherited to successive generations in the absence of the stress and linked to changes in stress-specific and phenotype-related gene expressions (8-10). Furthermore, inheritance of the altered cytosine methylation and gene-expression patterns after pathogen attack were not evident, especially at disease-related loci (16).

The magnitude of given pathogen stresses appears to determine how long the acquired resistance and DNA-methylation change will last over subsequent generations (7, 8, 16). Luna *et al*. (8) reported that the disease resistance was inherited through one pathogen stress-free generation contrary to Slaughter *et al*. (7). It is noteworthy that in the report of Luna *et al*. (8), parental plants were given repeated pathogen stresses until they reached the reproductive phase. This raises a question on whether the reported defense priming in the progenies can be considered as true transgenerational inheritance, excluding the effects from direct germline-cell infection or germline cells received SAR signal. Therefore, to evaluate whether the acquired resistance is inherited across generations, it would be critical to avoid the possibility of germline cells (intergenerational inheritance), zygotes, and/or developing embryos being directly affected by pathogen stress. On the other hand, it is difficult to tell between intergenerational inheritances from transgenerational inheritances in plants considering the characteristics of plant development: Unlike animals that possess differentiated germline cells from early embryogenesis stages, plants keep producing new organs including reproductive organs from the shoot apical meristem (SAM). Therefore, if the SAM is affected by environmental stresses, it might be possible that the information recorded in the SAM passes onto gametes generated later from the SAM. For this reason, and to eliminate the confusion and question on the transgenerational inheritance of defense priming in plants, researchers should study descendants after at least one stress-free generation (if the stress is given only during the vegetative phase) or on progenies produced from the crosses using male gametes from stress-free plants of the stressed-lineage and female gametes from plants that were never exposed to the stress.

MATERIALS AND METHODS

Plant materials, growth conditions, and pathogen infection

Arabidopsis thaliana Columbia-0 ecotype (Col-0) was used in all experiments. Parental plants were grown under 12-hr light/12-hr dark (12L/12D) photoperiod at 22°C. For syringe-infiltrations, 3-week (w)-old plants were infiltrated with 10^8 colony-forming units (CFU)/ml of *Pst* DC3000 or *Pst* DC3000 *avrRpt2* (OD_{600} = 0.2). 10 mM $MgCl_2$ was used for mock treatment. This was repeated a total of five times with 3-day (d) intervals. For infection by dipping, 3-week (w)- or 5-w-old plants were dipped with *Pst* DC3000 suspension containing 10 mM $MgCl_2$ and 0.01% silwet L-77 (LEHLE SEEDS VIS-02). This was repeated a total of five times with 3-d intervals: The first three dippings were performed with *Pst* DC3000 suspensions at OD_{600} = 0.2 (10^8 CFU/ml), whereas the following two with *Pst* DC3000 at OD_{600} = 2.0 (10^9 CFU/ml). For mock treatment, 10 mM $MgCl_2$ containing 0.01% silwet L-77 was used. N1 and P1 plants in the next generation were grown for 4 w under 12L/12D at 22°C for pathogen-resistance test and RT-qPCR analyses after pathogen infection. For 2,6-dichloroisonicotinic acid (INA) treatment, 0.3 mM INA was sprayed onto 2-w-old plants grown under 16L/8D on Murashige & Skoog (MS) media or onto 4-w-old plants grown under 12L/12D in soil and used for RT-qPCR analyses or chromatin immunoprecipitation (ChIP) assays. All plants were grown under 100 μE m^{-2} sec^{-1} cool white fluorescence light.

Pathogen resistance test

Pst DC3000 was grown at 28°C in King's B medium supplemented with 60 mg/L rifampicin and 50 mg/L kanamycin. Pathogen inoculation was performed as described previously (12, 17). Briefly, leaves of 4-week (w)-old plants were pressure-infiltrated with 5×10^5 CFU/ml (OD_{600} = 0.001) of *Pst* DC3000 using a needless syringe. Three inoculated leaf discs per plant were collected and homogenized in sterile H_2O at 0 or 3 days post-infection (dpi). Leaf extracts were serially diluted and plated on King's B medium. Bacteria were cultured at 28°C, and CFU was counted after 2 d.

RNA extraction and RT-qPCR analysis

Total RNA was extracted using TRI reagent (Molecular Research Center TR118) from 2-w-old seedlings excluding roots or from leaves of 4-w-old plants. Reverse transcription (RT) was performed as previously described (18) with RevertAid reverse transcriptase (Thermo Scientific EP0442) using 3 μg of total RNA. Real-time quantitative PCR (qPCR) was performed using SYBR Green PreMIX (Enzynomics RT500) on the Rotor-Gene Q (QIAGEN). The sequences of primers used for RT-qPCR assays are listed in Supplementary Table 1.

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as previously described (12). The antibody used for ChIP was α-H3Ac (Millipore 06-599). The

amount of immunoprecipitated chromatin was measured by qPCR with primers listed in Supplementary Table 2. The $2^{-\Delta\Delta CT}$ method (19) was used to determine the relative amounts of amplified products in samples. The value of each fragment was normalized to the respective input DNA (DNA isolated from chromatin that was cross-linked and fragmented under the same conditions as the immunoprecipitated DNA) and to *Ubiquitin 10* (*UBQ10*) to obtain H3Ac enrichment.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. D'Urso A and Brickner JH (2014) Mechanisms of epigenetic memory. Trends Genet 30, 230-236
2. Heard E and Martienssen RA (2014) Transgenerational epigenetic inheritance: myths and mechanisms. Cell 157, 95-109
3. Macho AP and Zipfel C (2014) Plant PRRs and the activation of innate immune signaling. Mol Cell 54, 263-272
4. Pieterse CM, Leon-Reyes A, Van der Ent S and Van Wees SC (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5, 308-316
5. Kourelis J and van der Hoorn RAL (2018) Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. Plant Cell 30, 285-299
6. Singh P, Yekondi S, Chen PW et al (2014) Environmental history modulates Arabidopsis pattern-triggered immunity in a histone acetyl transferase1-dependent manner. Plant Cell 26, 2676-2688
7. Slaughter A, Daniel X, Flors V, Luna E, Hohn B and Mauch-Mani B (2012) Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. Plant Physiol 158, 835-843
8. Luna E, Bruce TJ, Roberts MR, Flors V and Ton J (2012) Next-generation systemic acquired resistance. Plant Physiol 158, 844-853
9. Rasmann S, Vos MD, Casteel CL et al (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiol 158, 854-863
10. Lopez Sanchez A, Stassen JH, Furci L, Smith LM and Ton J (2016) The role of DNA (de)methylation in immune responsiveness of Arabidopsis. Plant J 88, 361-374
11. Conrath U, Beckers GJ, Langenbach CJ and Jaskiewicz MR (2015) Priming for enhanced defense. Annu Rev Phytopathol 53, 97-119
12. Choi SM, Song HR, Han SK et al (2012) HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis. Plant J 71, 125-146
13. Jin H, Choi SM, Kang MJ et al (2018) Salicylic acid-induced transcriptional reprogramming by the HAC-NPR1-TGA histone acetyltransferase complex in Arabidopsis. Nucleic Acids Res 46, 11712-11725
14. Perez MF and Lehner B (2019) Intergenerational and transgenerational epigenetic inheritance in animals. Nat Cell Biol 21, 143-151
15. Xin XF, Nomura K, Aung K et al (2016) Bacteria establish an aqueous living space in plants crucial for virulence. Nature 539, 524-529
16. Stassen JHM, Lopez A, Jain R et al (2018) The relationship between transgenerational acquired resistance and global DNA methylation in Arabidopsis. Sci Rep 8, 14761
17. Katagiri F, Thilmony R, He SY (2002) The Arabidopsis thaliana-pseudomonas syringae interaction. Arabidopsis Book 1, e0039
18. Song SK, Jang HU, Kim YH et al (2020) Overexpression of three related root-cap outermost-cell-specific C2H2-type zinc-finger protein genes suppresses the growth of Arabidopsis in an EAR-motif-dependent manner. BMB Rep 53, 160-165
19. Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402-408