

Exogenous application of histone demethylase inhibitor trans-2-phenylcyclopropylamine mimics *FLD* loss-of-function phenotype in terms of systemic acquired resistance in *Arabidopsis thaliana*

Vijayata Singh, Zeeshan Zahoor Bandy, and Ashis Kumar Nandi*

School of Life Sciences; Jawaharlal Nehru University; New Delhi, India

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Abbreviations: RSI1, Reduced Systemic Immunity 1; FLD, Flowering Locus D; H3K4me2, Dimethylated Histone3 at Lysine4; ChIP, Chromatin immuno-precipitation; LSD1, Lysine Specific Demethylase 1; SAR, Systemic Acquired Resistance; 2-PCPA, Trans-2-phenylcyclopropylamine

Plants often learn from previous infections to mount higher level of resistance during subsequent infections, a phenomenon referred to as systemic acquired resistance (SAR). During primary infection, mobile signals generated at the infection site subsequently move to the rest of plant to activate SAR. SAR activation is associated with alteration in the nucleosomal composition at the promoters of several defense-related genes. However, genetic regulations of such epigenetic modifications are largely obscure. Recently, we have demonstrated that *Reduced Systemic Immunity1/FLORING LOCUS D (RSI1; alias FLD)* a homolog of human histone demethylase, is required for SAR development in *Arabidopsis*. Here, we report that exogenous application of a histone demethylase inhibitor trans-2-phenylcyclopropylamine (2-PCPA) mimics *rsi1/fld* loss-of-function phenotypes in terms of SAR and associated histone demethylation at the promoters of *PR1*, *WRKY 29*, and *WRKY6* genes, and as well as flowering phenotypes. Our results suggest histone demethylase activity of FLD is important for controlling SAR activation.

Plants, when challenged with pathogens, induce strong resistance at the site of infection to control the spread of the pathogen. In addition, plants often retain the infection memory to activate faster defense responses that provide SAR against subsequent infections.¹ The effect of SAR is long lasting and protects against a wide range of pathogens. At the primary infection site, plants synthesize mobile signals, such as salicylic acid and its methylated derivative, azelaic acid, glycerol-3-phosphate, dehydroabietinalditerpenoid, and pipercolic acid that move to the distal tissues to induce SAR.²⁻⁷ The distal tissues upon receiving the SAR inducing signals achieve preparedness for mounting faster defense response during secondary infections, a phenomenon referred to as priming.⁸ There is substantial evidence that priming is associated with epigenetic modifications at the promoters of defense related genes.^{9,10} Epigenetic modifications can be long lasting and inherited through meiosis over successive generations.⁹ Despite knowing the large numbers of mobile signals and their interactions, it is not

known how these mobile signals link to alteration chromosomal compositions associated with SAR development.

Earlier we had shown that the FLD gene is required for SAR activation in *Arabidopsis*.¹¹ Through mutagenesis of wild-type (WT) *Arabidopsis* and screening for SAR deficient mutants, we identified *reduced systemic immunity 1 (rsi1)* that is defective in SAR but not in local resistance. Through further map-based positional cloning we identified *rsi1* as allelic to FLD. FLD functions at the downstream of mobile signals in the distal tissues to activate SAR. Similar to *fld* loss-of-function plants, the *rsi1* mutant shows delayed flowering phenotype.^{11,12} FLD codes for a homolog of human *lysine specific demethylase 1 (LSD1)*. In accordance, the *fld* mutants contain higher levels of H3K4me2.¹³ Further we reported that *rsi1/fld* mutation affects H3K4me2 accumulation at the promoters of several WRKY genes.¹⁴ However, it was not known whether histone demethylase activity of FLD is required for SAR activation.

*Correspondence to: Ashis Kumar Nandi; Email: ashis_nandi@mail.jnu.ac.in

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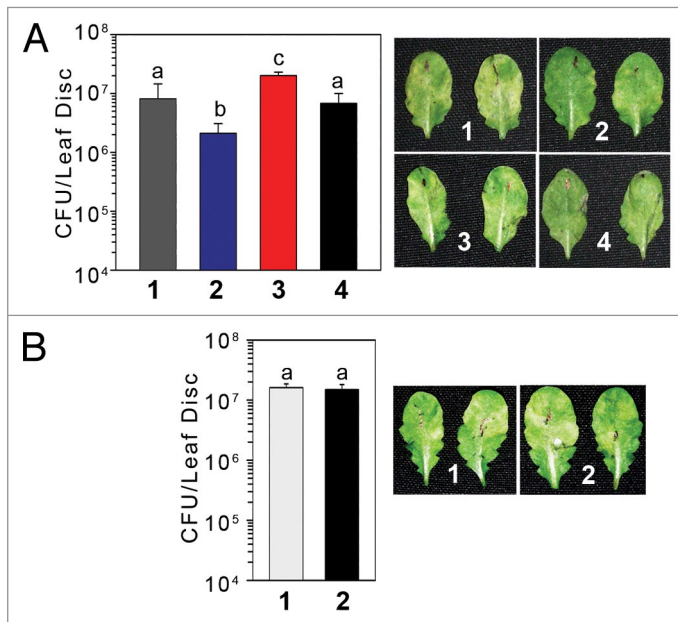


Figure 1. Effect of 2-PCPA on SAR and local resistance. **(A)** Bacterial numbers and disease symptoms after 3 d of challenge inoculation with *Psm* (2°). The plants were either pre-treated with 10 mM MgCl₂ or Avr-Pst (1°) at 1 X 10⁷cfu/ml with or without 2-PCPA 3 d before the challenge inoculation. Secondary challenge inoculation in all plants were performed with *Psm* at 5 X 10⁵ CFU/ml (2°). 1- 1° treatment with 10 mM MgCl₂ and 2° treatment with *Psm*. 2- 1° treatment with Avr-Pst and 2° treatment with *Psm*, 3 - 1° treatment with Avr-Pst + 2-PCPA and 2° treatment with *Psm*, 4 - 1° treatment with Avr-Pst, and 2° treatment with *Psm*+ 2-PCPA. **(B)** *Psm* counts from locally inoculated leaves and disease symptom at 3 dpi with or without 2-PCPA. Inoculation was done at 5X10⁵ CFU/ml. Each bar represents the mean ± standard deviation (SD) of 4 samples each carrying 5 leaf discs of 5 mm diameters randomly taken from different plants. Letters above the bars indicate values that are significantly different ($P < 0.05$) from each other as analyzed by one-way ANOVA (post hoc Holm Sidak method).

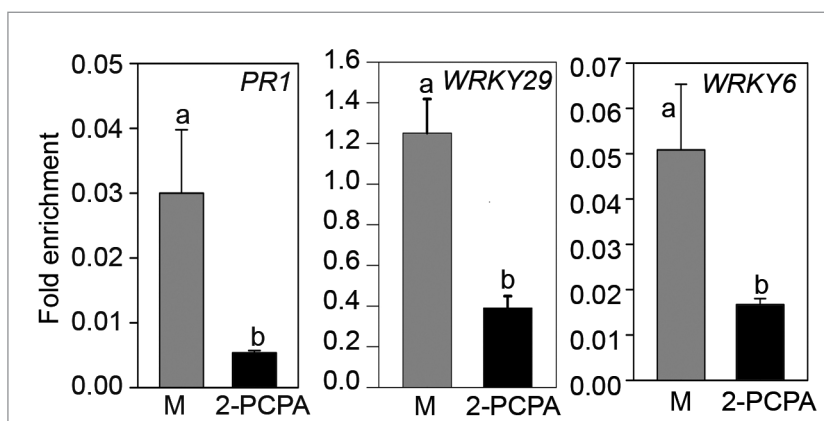


Figure 2. Effect of 2-PCPA on H3K4me2 occupancy in promoters of defense related genes. Five-week-old wild type plants were infiltrated with either 10mM MgCl₂ (M) or 10 mM of 2-PCPA. Three days later upper untreated leaves were harvested, and chromatin isolated from these leaves was precipitated with anti-H3K4me2 antibody. Abundance of individual target loci (relative to *ACTIN 2*) that were associated with H3K4me2 was determined by real-time PCR. Each bar represents the mean ± SD ($n = 3$). Different letters above the bars indicate values that are significantly different ($P < 0.05$) from each other as determined by one-way ANOVA (Holm-Sidak method).

In order to investigate the requirement of histone-demethylase activity for SAR, we exogenously applied trans-2-phenylcyclopropylamine (2-PCPA), which is widely used for inhibiting LSD1 activity^{15,16} and followed SAR activation in *Arabidopsis*. Five-week-old *Arabidopsis* plants were SAR induced with *Pseudomonas syringae* pv *tomato* DC3000 carrying *AvrRpt2* gene (Avr-Pst) at 10⁷ CFU/ml concentration, suspended in 10 mM MgCl₂, or mock induced with 10 mM MgCl₂ by infiltrating 3 lower leaves.^{11,14,17} After 3 days of primary inoculation, both SAR and mock induced plants were treated with same dose of *Pseudomonas syringae* pv *maculicola* ES4326 (*Psm*) (5 X 10⁵ CFU/ml) and bacterial numbers were determined after 3 days of secondary inoculation. To study the effect of inhibitor, in parallel sets, 2-PCPA was co-applied along with either primary or secondary inoculations at 10mM final concentrations. We observed that the exogenous 2-PCPA application, either during primary or secondary inoculations, effectively blocks SAR induction in *Arabidopsis* (Fig. 1A). However, 2-PCPA does not affect growth of bacteria in plants (Fig. 1B), suggesting that the effect of 2-PCPA is SAR specific, similar to *fld1rsi1* mutant.

Though putative function of FLD is to remove methylations from histones, the *fld* mutants accumulate reduced levels of H3K4me2 at the promoters of several *WRKY* genes including *WRKY29* and *WRKY6*.¹⁴ It was predicted to be an indirect effect of FLD on defense-related promoters.¹⁴ Nevertheless, in order to examine whether 2-PCPA application exerts similar effects on these promoters, we performed Chromatin-immuno-precipitation (ChIP) using anti-H3K4me2 antibody, as described earlier.¹⁴ *Arabidopsis* leaves were infiltrated with 10 mM 2-PCPA and samples were harvested after 48 hours for the ChIP experiment. Chromatins were precipitated using anti-H3K4me2 antibody (Cat#16–157, Millipore, USA). Relative occupancy of H3K4me2 was determined by quantitative real-time PCR (qRT-PCR) and plotted as fold difference with *ACTIN2* (*At3g18780*).¹⁴ As shown in Figure 2, 2-PCPA application effectively reduces H3K4me2 accumulation at the promoter of *PR1*, *WRKY29*, and *WRKY6* genes. This effect of 2-PCPA is also highly similar to *fld* loss-of-function phenotypes.

FLD negatively regulates floral repressor FLC and thus promotes flowering. Therefore, the *fld* mutants are delayed in flowering.^{11,12} However, the flowering phenotype of FLD is not associated with SAR development. The flowering phenotype is rescued in *fld flc* double mutant but not the SAR phenotype.¹¹ However, since FLD affects both the phenotypes, we anticipated that 2-PCPA would also affect flowering phenotype. We grew *Arabidopsis* under normal growth conditions for 3 weeks, after which we applied 10 μl of 2-PCPA (10 mM dissolved in water) per leaf, in 3 leaves of each plant. The control plants received only water. The treatment was repeated once a week until plants showed transition. We found significant delay in flowering in the 2-PCPA treated plants,

compared with water treated plants. In our growth conditions, at 12 h-12 h light-dark cycle, *Arabidopsis* plants flower at the end of 5 weeks. The 2-PCPA treated plants took 7 to 9 weeks to flower, and also produced more rosette leaves (Fig. 3).

Our results altogether suggest that 2-PCPA application inhibits FLD function in *Arabidopsis*, and with the established role of histone demethylase inhibition activity of 2-PCPA, we may infer that histone-demethylase activity is required for SAR activation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Fu ZQ, Dong X. Systemic acquired resistance: turning local infection into global defense. *Annu Rev Plant Biol* 2013; 64:839-63; PMID:23373699; <http://dx.doi.org/10.1146/annurev-arplant-042811-105606>
- Návarová H, Bernsdorff F, Döring AC, Zeier J. Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell* 2012; 24:5123-41; PMID:23221596; <http://dx.doi.org/10.1105/tpc.112.103564>
- Chaturvedi R, Venables B, Petros RA, Nalam V, Li M, Wang X, Takemoto LJ, Shah J. An abietane diterpenoid is a potent activator of systemic acquired resistance. *Plant J* 2012; 71:161-72; PMID:22385469; <http://dx.doi.org/10.1111/j.1365-313X.2012.04981.x>
- Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* 2007; 318:113-6; PMID:17916738; <http://dx.doi.org/10.1126/science.1147113>
- Dempsey DA, Klessig DF. SOS - too many signals for systemic acquired resistance? *Trends Plant Sci* 2012; 17:538-45; PMID:22749315; <http://dx.doi.org/10.1016/j.tplants.2012.05.011>
- Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. Priming in systemic plant immunity. *Science* 2009; 324:89-91; PMID:19342588; <http://dx.doi.org/10.1126/science.1170025>
- Chanda B, Xia Y, Mandal MK, Yu K, Sekine KT, Gao QM, Selote D, Hu Y, Stromberg A, Navarre D, et al. Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. *Nat Genet* 2011; 43:421-7; PMID:21441932; <http://dx.doi.org/10.1038/ng.798>
- Conrath U, Pieterse CM, Mauch-Mani B. Priming in plant-pathogen interactions. *Trends Plant Sci* 2002; 7:210-6; PMID:11992826; [http://dx.doi.org/10.1016/S1360-1385\(02\)02244-6](http://dx.doi.org/10.1016/S1360-1385(02)02244-6)
- Luna E, Bruce TJ, Roberts MR, Flors V, Ton J. Next-generation systemic acquired resistance. *Plant Physiol* 2012; 158:844-53; PMID:22147520; <http://dx.doi.org/10.1104/pp.111.187468>
- Jaskiewicz M, Conrath U, Peterhansel C. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep* 2011; 12:50-5; PMID:21132017; <http://dx.doi.org/10.1038/embor.2010.186>
- Singh V, Roy S, Giri MK, Chaturvedi R, Chowdhury Z, Shah J, Nandi AK. *Arabidopsis thaliana* FLOWERING LOCUS D is required for systemic acquired resistance. *Mol Plant Microbe Interact* 2013; 26:1079-88; PMID:23745676; <http://dx.doi.org/10.1094/MPMI-04-13-0096-R>
- He Y, Michaels SD, Amasino RM. Regulation of flowering time by histone acetylation in *Arabidopsis*. *Science* 2003; 302:1751-4; PMID:14593187; <http://dx.doi.org/10.1126/science.1091109>
- Liu F, Quesada V, Crevillén P, Bäurle I, Swiezewski S, Dean C. The *Arabidopsis* RNA-binding protein FCA requires a lysine-specific demethylase 1 homolog to downregulate FLC. *Mol Cell* 2007; 28:398-407; PMID:17996704; <http://dx.doi.org/10.1016/j.molcel.2007.10.018>
- Singh V, Roy S, Singh D, Nandi AK. *Arabidopsis* flowering locus D influences systemic-acquired-resistance-induced expression and histone modifications of WRKY genes. *J Biosci* 2014; 39:119-26; PMID:24499796; <http://dx.doi.org/10.1007/s12038-013-9407-7>
- Schmidt DM, McCafferty DG. trans-2-Phenylcyclopropylamine is a mechanism-based inactivator of the histone demethylase LSD1. *Biochemistry* 2007; 46:4408-16; PMID:17367163; <http://dx.doi.org/10.1021/bi0618621>
- Neelamegam R, Ricq EL, Malvaez M, Patnaik D, Norton S, Carlin SM, Hill IT, Wood MA, Haggarty SJ, Hooker JM. Brain-penetrant LSD1 inhibitors can block memory consolidation. *ACS Chem Neurosci* 2012; 3:120-8; PMID:22754608; <http://dx.doi.org/10.1021/cn200104y>
- Nandi A, Welti R, Shah J. The *Arabidopsis thaliana* dihydroxyacetone phosphate reductase gene *SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY1* is required for glycerolipid metabolism and for the activation of systemic acquired resistance. *Plant Cell* 2004; 16:465-77; PMID:14729910; <http://dx.doi.org/10.1105/tpc.016907>

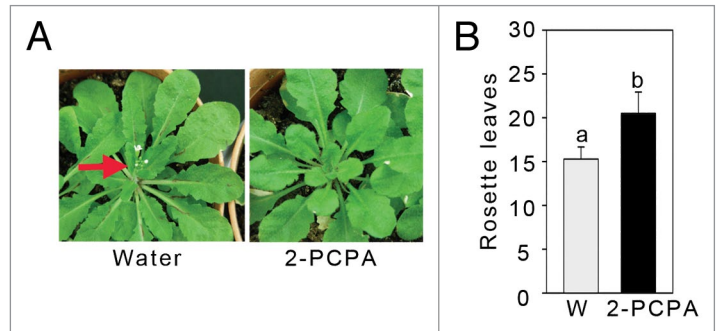


Figure 3. Effect of 2-PCPA on flowering. (A) Flowering phenotypes after treatment with water or 2-PCPA. Arrow indicates flowering in water treated plants. (B) Rosette leaves number at the time of transition. Leaves were counted just before the transition took place. Plants were kept in photoperiod condition 12 h-12 h, light-dark condition. Letters above the bars indicate values that are significantly different ($P < 0.05$) from each other as analyzed by one-way ANOVA (post hoc Holm Sidak method).