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Hidetoshi Saze
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Plant Epigenetics Unit (Hidetoshi Saze)  
FY2023 Annual Report

Plant Epigenetics Unit  
Professor Hidetoshi Saze



Epigenetic Regulation of Genes and Transposable Elements in Plant Genomes

Our research focuses on investigating the epigenetic regulation of genes and transposable elements (TEs) within plant genomes. In plants, genome defense mechanisms employ various epigenetic modifications, such as DNA cytosine methylation, small RNAs, and histone protein modifications, to repress TEs. Notably, these repressive epigenetic modifications are typically absent from actively transcribed genes. Our primary objective is to unravel the mechanisms by which epigenetic processes differentiate between gene and TE sequences, how they precisely deposit specific chromatin modifications at their targets, and to comprehend their biological significance in terms of adaptation and genome evolution. Additionally, we aim to explore the influence of environmental signals on epigenome dynamics and phenotypic diversity in plants. Furthermore, we are actively engaged in collaborative research projects with the local Okinawan government, institutes, and industries, utilizing plant genomics approaches to address pressing issues in the Okinawan society.

1. Staff

- Hidetoshi Saze, Professor
- Leonardo Furci, Researcher (JSPS PD)
- Jeremy Berthelier, Researcher
- Atsushi Shimada, Staff scientist
- Hiroki Tsutsui, Researcher
- Kenji Osabe, Senior staff scientist/Group leader
- Oscar Juez Neira, Ph.D. Student
- Munissa Sadykova, Ph.D. Student
- Anna Magdalena Klarkowska, Ph.D. Student
- Peter James Reynolds, Ph.D. Student
- Yoshiko Harukawa, Technical Staff
- Yoko Fujitomi, Research Unit Administrator

2. Collaborations

2.1 Epigenetic regulation of immune response in *Arabidopsis*

- Type of collaboration: Joint research
- Researchers: Drs. Ken Shirasu and Shuta Asai, RIKEN Center for Sustainable Resource Science, Japan.

2.2 Regulation of smallRNA and DNA methylation in *Arabidopsis*

- Type of collaboration: Joint research
- Researcher: Dr. Takahiro Hamada, Faculty of Life Science, Okayama University of Science, Okayama, Japan

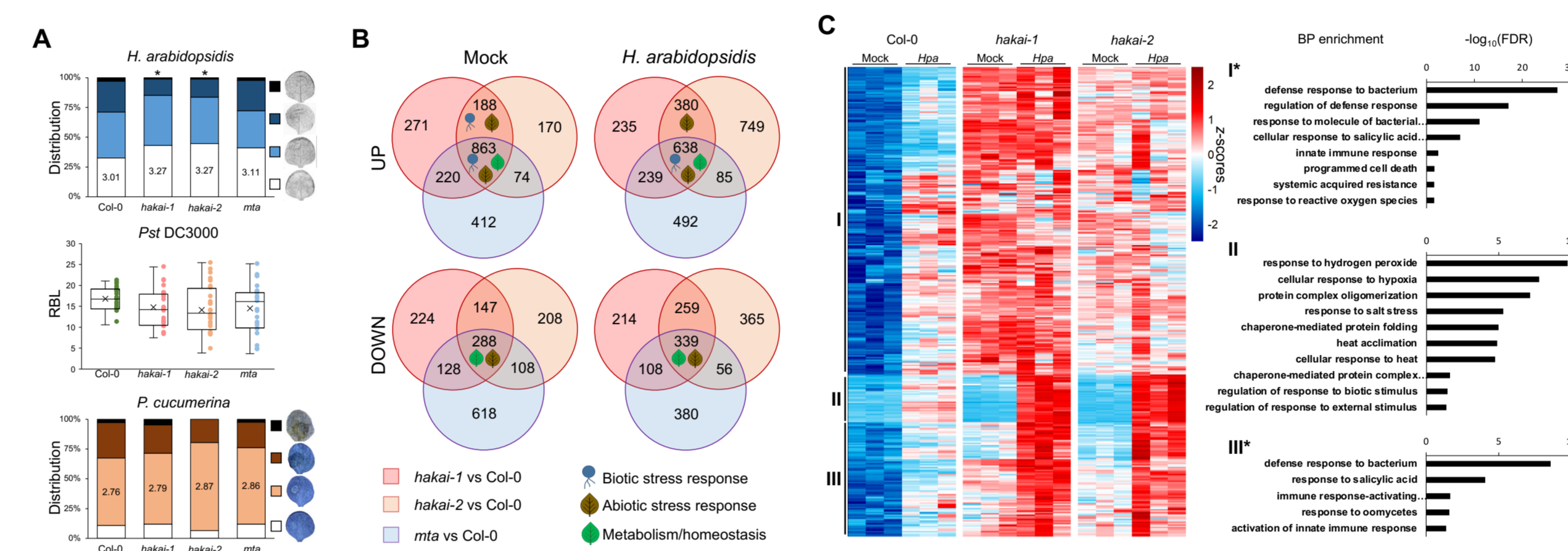
2.2 Okinawa Functional Rice project

- Type of collaboration: Joint research
- Researchers: Onna Village

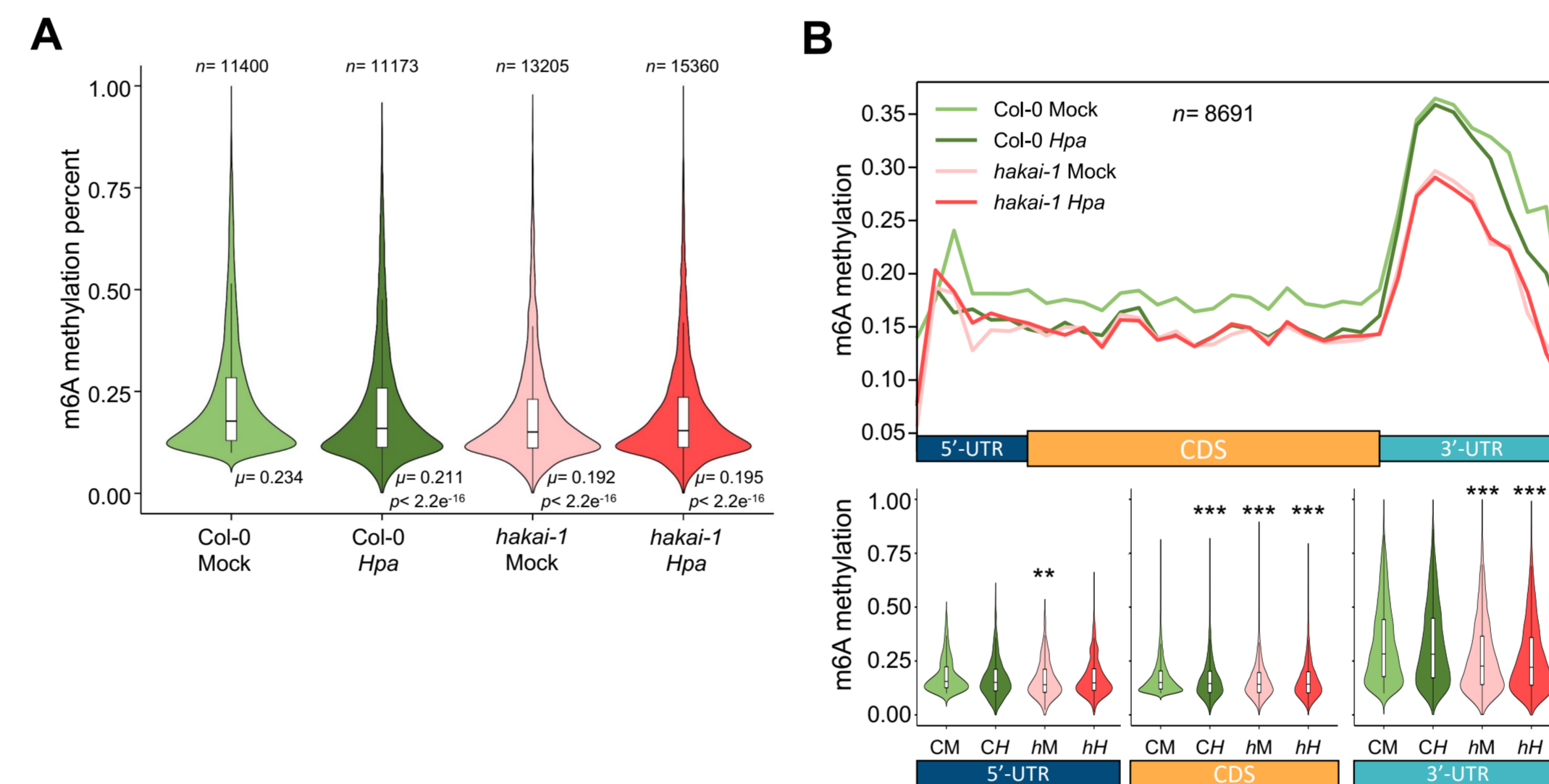
3. Activities and Findings

3.1 Epitranscriptome dynamics and plant immune responses

In plants, epitranscriptomic mark N6-adenine methylation (m6A) is dynamically regulated by (ab)iotic stressors. However, there is limited knowledge on m6A dynamics at single nucleotide resolution on specific RNA molecule under stresses, and their role in environmental adaptation. By using Oxford Nanopore Technology direct RNA sequencing (ONT-DRS), we investigated transcript-specific dynamics of m6A modification at single nucleotide resolution by biotic stress during *Hyaloperonospora arabidopsidis* (*Hpa*) infection in *Arabidopsis*. In wild-type seedlings, pathogen infection causes significant reduction of global m6A ratios, which correlates with activation of m6A-modified transcripts.



**Figure 1. Phenotypical and transcriptional characterization of m6A deposition mutants.** **A:** Basal resistance phenotype of *hskai-1*, *hskai-2* and *mta* mutants against different pathogens. Top graph shows distribution of infection classes for *Hpa*-treated leaves from each genotype, ranging from least infected (white), to most infected (black, examples shown on the right). Middle graph shows quantification of relative bioluminescence (RBL) from transgenic *Pst* colonization (x indicates mean RBL for each genotype). **B:** Distribution and overlaps of up- or down-regulated DEGs in m6A deposition mutants. Each circle represents DEGs in pairwise comparisons between the indicated mutant and WT at the appropriate condition (Mock or *Hpa*). **C:** Hierarchical clustering of *Hpa*-inducible genes in WT seedlings showing differential induction in both *hskai* mutants. For each cluster (I, II, III), GO terms enrichment for BP are shown on the right. Asterisks indicate clusters that contain additional GO terms not related to basal defences.



**Figure 2: Dynamics of m6A in WT and *hskai-1* *Arabidopsis* seedlings upon *Hpa* infection.** **A:** Global distribution and average m6A percentages in Mock- and *Hpa*-treated *Arabidopsis* seedlings. Statistically significant differences in distribution were assessed by Student's T-test in pairwise comparisons with Mock-treated WT ( $\mu = \text{mean}$ ;  $n = \text{number of m6A sites}$ ). **B:** Top panel: Mean m6A methylation ratio (50bp resolution) over transcript regions for shared m6A sites ( $n = 8691$ ) across conditions. Bottom panel: distribution of methylation ratios for shared m6A sites in each transcript region. Statistically significant differences in distribution were assessed by Student's T-test in pairwise comparisons with Mock-treated WT. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

Defect of m6A deposition in the m6A mutant mimics m6A reduction from *Hpa* infection at ~70% of sites, resulting in constitutive overexpression of basal defence genes and enhanced resistance against the pathogen. Our results demonstrate that m6A dynamics impact defence response against pathogen, providing a promising target for future crop improvement strategies.

4. Publications

4.1 Journals

- Berthelier J, Furci L, Asai S, Sadykova M, Shimazaki T, Shirasu K, Saze H. (2023) Long-read direct RNA sequencing reveals epigenetic regulation of chimeric gene-transposon transcripts in *Arabidopsis thaliana*. *Nat Commun.* Jun 5;14(1):3248. doi: 10.1038/s41467-023-38954-z. **Press release** : <https://www.oist.jp/ja/news-center/news/2023/6/15/jumping-genes-help-plants-adapt-extreme-temperature-and-pathogens>
- Chavan S, Saze H, Tanaka F. (2023) Chemical Modification of Peptides and Proteins Using Spirooxindole Oxirane Derivatives. *Adv Synth. Catal.* 2023. July 4; 363(13): 2171-2176. doi: doi.org/ 10.1002/adsc.202300578.
- Furci L, Berthelier J, Saze H (2024). RNA N6-adenine methylation dynamics impact *Hyaloperonospora arabidopsidis* resistance in *Arabidopsis*. *bioRxiv*, doi.org/10.1101/2024.01.30.577950

4.2 Books and other one-time publications

- Saze H, Springer N. (2023). Editorial overview: Delving into organizational principles of plant genomes. *Curr Opin Plant Biol.* Oct 11;76:102458. doi: 10.1016/j.pti.2023.102458.

4.3 Oral and Poster Presentations

- (Poster presentation) Atsushi Shimada, Hidetoshi Saze, RNA-directed DNA Methylation promotes genome integrity in *Arabidopsis* epIRILs, ICAR2023 The 33rd International Conference on Arabidopsis Research, Makuhari Messe, Chiba, Japan, June 6, 2023
- (Poster presentation) Leonardo Furci. A novel function for transcriptional regulators IBM2 and EDM2 as limiters of salicylic acid-dependent defence genes in *Arabidopsis*. ICAR2023 The 33rd International Conference on Arabidopsis Research, Makuhari Messe, Chiba, Japan, June 6, 2023
- (Poster presentation) Gene-transposon transcripts can be epigenetically regulated and impact gene response to stress conditions in *Arabidopsis thaliana*. ICAR2023 The 33rd International Conference on Arabidopsis Research, Makuhari Messe, Chiba, Japan, June 6, 2023
- (Poster presentation) Oscar Juez Neira, Hidetoshi Saze "Conserved (epi)genetic mechanisms of mammalian aging in whole-plant senescence", ICAR2023 The 33rd International Conference on Arabidopsis Research, Makuhari Messe, Chiba, Japan, June 6, 2023
- (Lecture) Long-read sequencing analysis to study chimeric gene-transposon sequences. TURIP/LabEx Graduate school, University of Perpignan Via Domitia (UPVD), Perpignan, France, Sep 15, 2023.
- (Seminar) Epigenetic regulation of intragenic transposons and gene transcription in plant genomes. Laboratoire des Interactions Plantes-Microbes-Environnement, INRAE/CNRS, Castanet-Tolosan Cedex, France, Sep 19, 2023.
- (Seminar) Epigenetic regulation of intragenic transposons and gene transcription in plant genomes. iGRÉD, INSTITUT OF GENETICS, REPRODUCTION & DEVELOPMENT, Clermont-Ferrand, France, Sep 22, 2023.
- (Seminar) Epigenetic regulation of intragenic transposons and gene transcription in plant genomes. Laboratoire Génome et Développement des Plantes, Perpignan, France, Sep 25, 2023.
- (Seminar) Epigenetic regulation of intragenic transposons and gene transcription in plant genomes. Institut de Biologie de l'École Normale Supérieure (IBENS), Paris, France, Sep 29, 2023.
- (Oral presentation) Atsushi Shimada, Hidetoshi Saze, The CCR4-NOT complex maintains centromere epigenome and function by promoting small interfering RNA synthesis, The 10th CCR4-NOT Meeting, THE LUIGANS Spa & Resort, Fukuoka, Japan, November 14, 2023
- (Poster presentation) Oscar Juez Neira, Hidetoshi Saze "Conserved (epi)genetic mechanisms of mammalian aging in whole-plant senescence", EMBO workshop on CRISPR-Cas, Seville, Spain, November 2023.
- (Poster presentation) Atsushi Shimada, Hidetoshi Saze, Poly(A) tail shortening by the CCR4-NOT complex promotes RNA interference in *Arabidopsis*, MBSJ2023 The 46th Annual Meeting of the Molecular Biology Society, Kobe Convention Center, Hyogo, Japan, December 6, 2023
- (Seminar) Epigenetic regulation of intragenic transposons and gene transcription in plant genomes. Faculty of science, Kyoto University, Kyoto, Japan, Dec 15, 2023.

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

(Invited Seminar) Dr. Aline V. Probst. GReD Institute, INSERM U1103, CNRS UMR6293, University Clermont Auvergne - Clermont Ferrand - France. "Controlling gene expression during development - from histone variants to GH1-domain proteins" Oct 20, 2023.

7. Other

Nothing to report.