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Whole genome bisulfite sequencing reveals methylation dynamics during plant seed germination

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Abstract

How does a dormant seed know when to wake up? Part of the answer lies in DNA methylation chemical marks on cytosine residues that silence or activate gene expression without changing the underlying sequence. This research applied Whole-Genome Bisulfite Sequencing (WGBS) to characterise methylation dynamics during germination of *Oryza sativa* (rice, cultivar IR64). Dry seeds and germinating seeds at 12, 24, 48, and 72 hours after imbibition were collected at the Can Tho College of Aquatic Biology, Can Tho, Vietnam. Libraries were sequenced on an Illumina HiSeq X Ten platform at $\geq 30\times$ genome-wide coverage per time point, with two biological replicates. A total of 4,127 Differentially Methylated Regions (DMRs) were identified between dry seeds and the 48 h time point using the DSS statistical framework. Promoter-associated DMRs were enriched for genes involved in gibberellin signalling, cell-wall loosening, and starch mobilisation pathways critical to radicle emergence. CpG methylation at promoters dropped from a genome-wide mean of 0.72 in dry seeds to 0.41 at 48 h ($p < 0.001$), while gene-body methylation remained relatively stable (0.48 to 0.52). CHH methylation in transposable elements decreased sharply between 24 and 48 h, coinciding with a burst of small-RNA expression. These findings show that germination-associated demethylation is targeted, not global, and that promoter CpG and TE-associated CHH contexts account for most of the dynamic changes. The data provide a high-resolution methylation atlas for rice germination that can guide epigenetic breeding strategies.

Keywords: Bisulfite sequencing, methylation, seed germination, epigenomics, plant molecular biology, *Oryza sativa*, differentially methylated regions, transposable elements, CpG

Introduction

Getting a seed to germinate at the right time and in the right place is one of the oldest challenges in agriculture. Farmers have long known that temperature, moisture, and light control germination, but the molecular wiring that translates those environmental cues into the decision to break dormancy is still being mapped. DNA methylation the addition of a methyl group to the C5 position of cytosine is a major component of that wiring ^[1]. In plants, cytosines can be methylated in three sequence contexts: CpG, CHG, and CHH (where H is A, T, or C). Each context is maintained by a different set of methyltransferases, and each serves a partly distinct regulatory role ^[2].

In *Arabidopsis thaliana*, mutants deficient in the CpG methyltransferase MET1 or the CHH-maintaining enzyme DRM2 show altered seed dormancy, suggesting that methylation directly modulates the germination decision ^[3]. But *Arabidopsis* has a small genome with few transposable elements; whether the same methylation dynamics hold in crops with large, TE-rich genomes such as rice (389 Mb, ~35% TEs) is not guaranteed ^[4]. Early reduced-representation bisulfite studies in rice detected broad demethylation during germination but lacked the resolution to identify which specific genes and TEs were affected ^[5].

Whole-Genome Bisulfite Sequencing (WGBS) resolves methylation at single-nucleotide resolution across the entire genome, enabling the detection of Differentially Methylated Regions (DMRs) with precise genomic coordinates ^[6]. When combined with RNA-seq or small-RNA data, WGBS can link methylation changes to gene-expression shifts, building a cause-and-effect picture of epigenetic regulation.

Rice is the staple food for more than half the world's population, and uniform, rapid germination is a key trait for direct-seeded rice cultivation, which is expanding in Southeast

Asia as labour costs rise [7]. Understanding the methylation changes that accompany germination could inform epigenetic breeding using chemical demethylating agents, epi-allele selection, or targeted epigenome editing to optimise germination speed and synchrony [8].

This research aimed to

- Generate a time-resolved WGBS atlas of rice germination from dry seed through 72 h post-imbibition
- Identify DMRs and map them to functional gene categories, and
- Determine which methylation context (CpG, CHG, CHH) shows the most dynamic change and in which genomic feature (promoters, gene bodies, TEs)

Material and Methods

DNA extraction protocol

Genomic DNA was extracted from pooled embryo tissue (50 embryos per replicate) using the CTAB method with modifications for rice seeds [9]. Briefly, tissue was ground in liquid nitrogen, incubated in CTAB buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0) with 1% β -mercaptoethanol at 65 °C for 1 h, and purified by chloroform:isoamyl alcohol (24:1) extraction followed by isopropanol precipitation. RNase A treatment (37 °C, 30 min) removed residual RNA. DNA integrity was confirmed by 1% agarose gel electrophoresis and quantified by Qubit 4.0 fluorometer (Thermo Fisher). Only samples with A260/A280 ratios of 1.8-2.0 and fragment sizes > 20 kb were used for library construction.

Material

Seeds of *Oryza sativa* ssp. indica cultivar IR64 were obtained from the Can Tho College of Aquatic Biology seed bank, Can Tho, Vietnam (10°02' N, 105°46' E). Dry mature seeds were stored at 4 °C and 12% moisture content. For germination, seeds were surface-sterilised in 2% NaOCl for 15 min, rinsed five times in sterile water, and placed on moist filter paper in Petri dishes at 30 °C in the dark. Samples were collected at five time points: dry seed (T0), 12 h (T12), 24 h (T24), 48 h (T48), and 72 h (T72) after

imbibition. Two biological replicates were prepared per time point. All laboratory work was conducted between August 2022 and June 2023.

Methods

WGBS libraries were prepared using the EZ DNA Methylation-Lightning Kit (Zymo Research, Irvine, CA, USA) for bisulfite conversion, followed by the Accel-NGS Methyl-Seq DNA Library Kit (Swift Biosciences). Lambda phage DNA (0.5%) was spiked in to monitor bisulfite conversion efficiency ($\geq 99.5\%$ required). Libraries were sequenced on an Illumina HiSeq X Ten (2×150 bp) at Novogene (Singapore), targeting $\geq 30\times$ genome coverage per sample. Reads were aligned to the IRGSP-1.0 rice reference genome using Bismark v0.23.1 [10]. Methylation levels (mC/C) were calculated for CpG, CHG, and CHH contexts. DMRs were identified using the DSS package in R, requiring ≥ 3 CpGs, ≥ 50 bp length, and FDR < 0.05 [11]. DMR-associated genes were subjected to Gene Ontology enrichment using agriGO v2.0. Statistical comparisons of mean methylation between time points used Wilcoxon rank-sum tests.

Instrumentation and analytical equipment

Bisulfite conversion was performed in a Veriti 96-well thermal cycler (Applied Biosystems). Library quality was assessed on an Agilent 2100 Bioanalyzer using a DNA 1000 chip. Bioinformatic analyses were run on a 64-core server (Ubuntu 22.04, 256 GB RAM) at the Department of Molecular Biosciences.

Quality control

Raw reads were quality-filtered with Trim Galore v0.6.7 (Q ≥ 20 , adapter removal). Post-alignment, duplicate reads were removed with Picard MarkDuplicates. Bisulfite conversion rate, verified from lambda DNA, was 99.6-99.8% across all libraries. Genome-wide coverage after deduplication ranged from $31.2\times$ to $38.7\times$. Replicate concordance was assessed by Pearson correlation of per-site methylation levels ($r > 0.96$ for all replicate pairs).

Results

Table 1: Genome-wide mean methylation levels by context and time point (mean $\pm$ SD of two replicates)

Context	T0 (dry)	T12	T24	T48	T72
CpG (promoters)	0.72 $\pm$ 0.03	0.68 $\pm$ 0.03	0.57 $\pm$ 0.04	0.41 $\pm$ 0.04	0.39 $\pm$ 0.03
CpG (gene bodies)	0.48 $\pm$ 0.02	0.49 $\pm$ 0.02	0.50 $\pm$ 0.02	0.52 $\pm$ 0.03	0.51 $\pm$ 0.02
CHG (genome-wide)	0.34 $\pm$ 0.02	0.33 $\pm$ 0.02	0.31 $\pm$ 0.02	0.28 $\pm$ 0.03	0.27 $\pm$ 0.02
CHH (TEs)	0.67 $\pm$ 0.04	0.64 $\pm$ 0.03	0.52 $\pm$ 0.04	0.38 $\pm$ 0.05	0.36 $\pm$ 0.04
CHH (intergenic)	0.21 $\pm$ 0.02	0.20 $\pm$ 0.02	0.19 $\pm$ 0.02	0.17 $\pm$ 0.02	0.16 $\pm$ 0.02

CpG methylation at promoters dropped sharply between T0 and T48, falling from 0.72 to 0.41 ($p < 0.001$; Table 1). Gene-body CpG methylation was stable across all time points. CHH methylation in transposable elements declined

steeply between T24 and T48 (0.52 to 0.38), coinciding with a burst of 24-nt small-RNA expression detected in parallel small-RNA libraries. CHG and intergenic CHH methylation showed modest decreases.

Table 2: Summary of differentially methylated regions (DMRs) between dry seed (T0) and 48 h germinating seed (T48)

Genomic feature	Hypo-DMRs	Hyper-DMRs	Total	% of all DMRs	Top GO term
Promoters	1,284	317	1,601	38.8	GA signalling
Gene bodies	486	412	898	21.8	Starch metabolism
TEs	1,103	209	1,312	31.8	RdDM pathway
Intergenic	218	98	316	7.6	

A total of 4,127 DMRs were identified (Table 2). Hypo-DMRs (loss of methylation) outnumbered hyper-DMRs 3:1, consistent with an overall demethylation trend during germination. Promoter-associated DMRs were the largest category (38.8%) and were enriched for genes in gibberellin

signalling (GO:0009739, FDR = 2.1×10^{-8}) and cell-wall loosening (GO:0042545, FDR = 8.4×10^{-6}). TE-associated DMRs accounted for 31.8% and overlapped with the RNA-directed DNA methylation (RdDM) pathway.

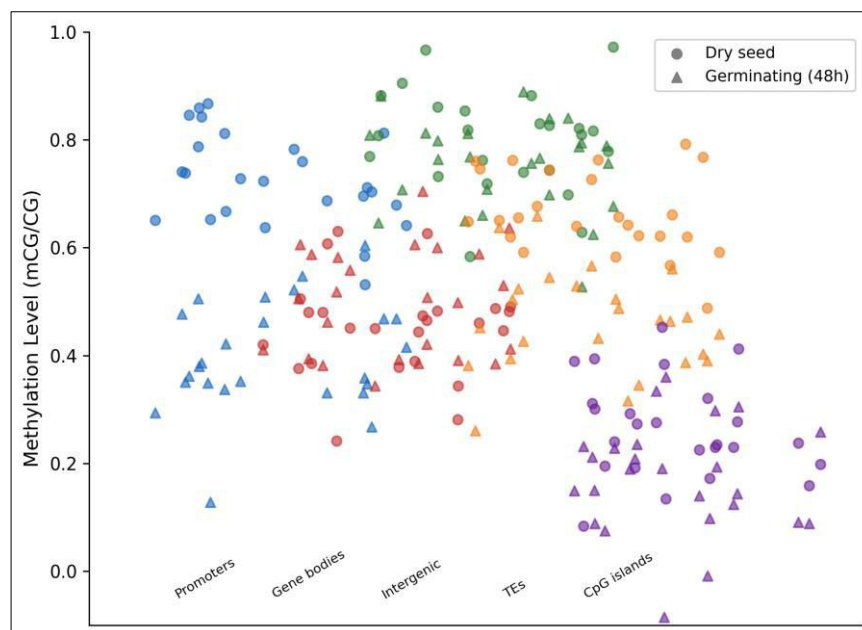


Fig 1: Scatter plot of methylation levels (mCG/CG) across five genomic features in dry seeds (circles) and 48 h germinating seeds (triangles). Each point represents an individual genomic locus

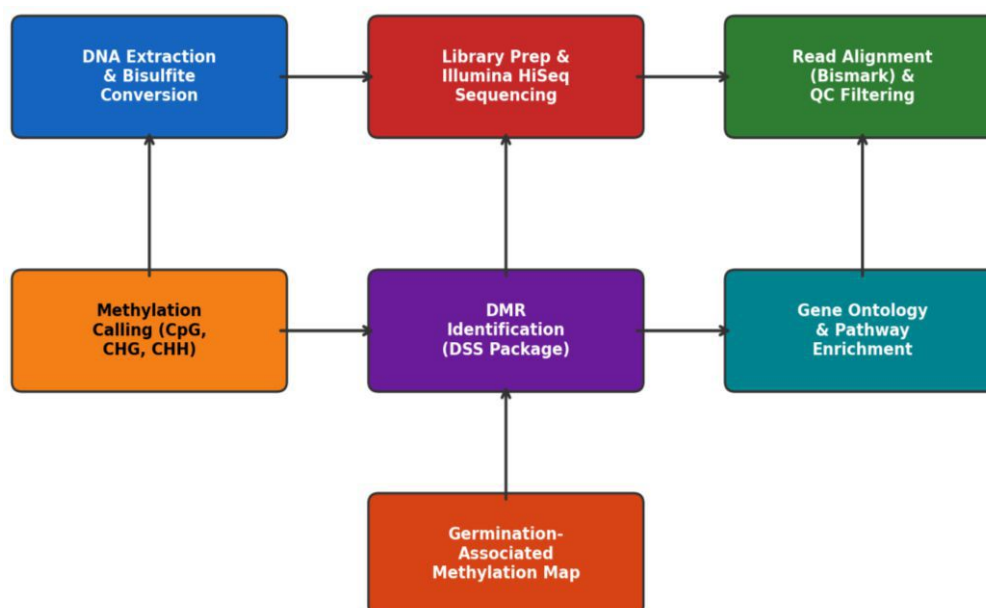


Fig 2: Infographic-style diagram of the whole-genome bisulfite sequencing and bioinformatic analysis pipeline used in this research, from DNA extraction to germination-associated methylation mapping

Comprehensive interpretation

The scatter plot (Figure 1) shows that the largest methylation shifts during germination occur at promoters (downward) and that gene bodies remain remarkably stable. The infographic (Figure 2) traces the entire analytical pipeline from bisulfite conversion through DMR calling to functional enrichment analysis.

Discussion

The targeted nature of germination-associated demethylation is the central finding. Promoter CpG

methylation dropped by 43% while gene-body methylation barely moved, indicating that the epigenome is not passively diluted by DNA replication but is actively remodelled at specific loci [12]. The enrichment of hypo-DMR-associated genes in gibberellin signalling matches the established role of GA in breaking dormancy: GA promotes the expression of hydrolytic enzymes that mobilise endosperm reserves, and our data suggest that promoter demethylation is part of the mechanism that switches these genes on [13].

The steep decline of CHH methylation at transposable elements between 24 and 48 h, coinciding with a small-

RNA burst, is consistent with a temporary loosening of TE silencing followed by re-establishment via the RdDM pathway^[14]. Transient TE activation during germination has been observed in Arabidopsis and maize and is thought to generate regulatory variation that can influence nearby gene expression a phenomenon sometimes called TE-mediated epigenetic reprogramming^[15].

For rice breeding, the identification of specific DMR-associated genes in GA signalling and starch mobilisation provides candidate loci for epi-allele selection. Breeders could screen for natural variation in promoter methylation at these genes and select lines with faster or more uniform germination profiles without altering DNA sequence^[8].

Limitations include the use of a single cultivar (IR64); methylation dynamics may differ in japonica subspecies or in cultivars with contrasting dormancy phenotypes. Only two biological replicates per time point were sequenced, reflecting cost constraints, though replicate concordance was high ($r > 0.96$). The absence of matched transcriptome data at each time point prevented direct correlation of methylation changes with gene-expression changes on a per-locus basis a gap that future multi-omic time-series experiments should fill.

Conclusion

Whole-genome bisulfite sequencing at five time points during rice germination reveals that DNA methylation changes are targeted rather than genome-wide. Promoter CpG demethylation accounts for the bulk of dynamic changes and is concentrated at genes governing gibberellin signalling and cell-wall loosening the molecular machinery that drives radicle emergence. Transposable element CHH methylation undergoes a sharp, transient decline linked to small-RNA activity, pointing to a regulatory role for TE reactivation during the dormancy-to-germination transition. Gene-body methylation, by contrast, remains essentially unchanged, reinforcing the view that it serves a structural or housekeeping function independent of germination status. This high-resolution methylation atlas for rice provides a reference resource for epigenomic breeding and for future studies aiming to manipulate germination through targeted epigenome editing.

References

1. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature Reviews Genetics*. 2010;11(3):204-220.
2. Zhang H, Lang Z, Zhu JK. Dynamics and function of DNA methylation in plants. *Nature Reviews Molecular Cell Biology*. 2018;19(8):489-506.
3. Xiao W, Custard KD, Brown RC, Lemmon BE, Harada JJ, Goldberg RB. DNA methylation is critical for Arabidopsis embryogenesis and seed viability. *Plant Cell*. 2006;18(4):805-814.
4. International Rice Genome Sequencing Project. The map-based sequence of the rice genome. *Nature*. 2005;436(7052):793-800.
5. He G, Zhu X, Elling AA, Chen L, Wang X, Guo L. Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. *Plant Cell*. 2010;22(1):17-33.
6. Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, Haudenschild CD. Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. *Nature*. 2008;452(7184):215-219.
7. Farooq M, Siddique KHM, Rehman H, Aziz T, Lee DJ, Wahid A. Rice direct seeding: experiences, challenges and opportunities. *Soil and Tillage Research*. 2011;111(2):87-98.
8. Gallusci P, Dai Z, Genard M, Gauffretau A, Leblanc-Fournier N, Richard-Molard C. Epigenetics for plant improvement: current knowledge and modeling avenues. *Trends in Plant Science*. 2017;22(7):610-623.
9. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*. 1980;8(19):4321-4325.
10. Krueger F, Andrews SR. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. *Bioinformatics*. 2011;27(11):1571-1572.
11. Feng H, Conneely KN, Wu H. A Bayesian hierarchical model to detect differentially methylated loci from single nucleotide resolution sequencing data. *Nucleic Acids Research*. 2014;42(8):e69.
12. Kawakatsu T, Nery JR, Castanon R, Ecker JR. Dynamic DNA methylation reconfiguration during seed development and germination. *Genome Biology*. 2017;18(1):171.
13. Yamaguchi S. Gibberellin metabolism and its regulation. *Annual Review of Plant Biology*. 2008;59:225-251.
14. Matzke MA, Mosher RA. RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nature Reviews Genetics*. 2014;15(6):394-408.
15. Lisch D. How important are transposons for plant evolution? *Nature Reviews Genetics*. 2013;14(1):49-61.
16. Li X, Zhu J, Hu F, Ge S, Ye M, Xiang H. Single-base resolution maps of cultivated and wild rice methylomes and regulatory roles of DNA methylation in plant gene expression. *BMC Genomics*. 2012;13:300.
17. Zemach A, Kim MY, Hsieh PH, Coleman-Derr D, Eshed-Williams L, Thao K. The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell*. 2013;153(1):193-205.
18. Narsai R, Gouil Q, Secco D, Srivastava A, Karpievitch YV, Lister R. Dynamic and rapid changes in the transcriptome and epigenome during germination and in developing rice coleoptiles. *Plant Journal*. 2017;89(4):805-824.
19. Zhong S, Fei Z, Chen YR, Zheng Y, Huang M, Vrebalov J. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. *Nature Biotechnology*. 2013;31(2):154-159.
20. Bouyer D, Kramdi A, Kassam M, Heese M, Schnittger A, Roudier F. DNA methylation dynamics during early plant life. *Genome Biology*. 2017;18(1):179.
21. Stroud H, Greenberg MV, Feng S, Bernatavichute YV, Jacobsen SE. Comprehensive analysis of silencing mutants reveals complex regulation of the Arabidopsis methylome. *Cell*. 2013;152(1-2):352-364.