

# New insights into genome-wide change of DNA methylation and its association with gene expression in cadmium-exposed rice (*Oryza sativa*)

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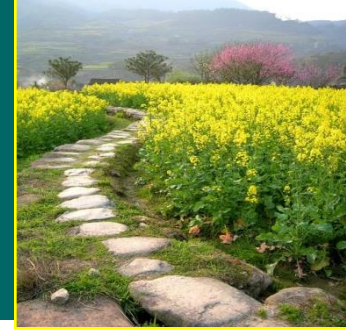
**College of Life Science**

**Nanjing Agricultural University**

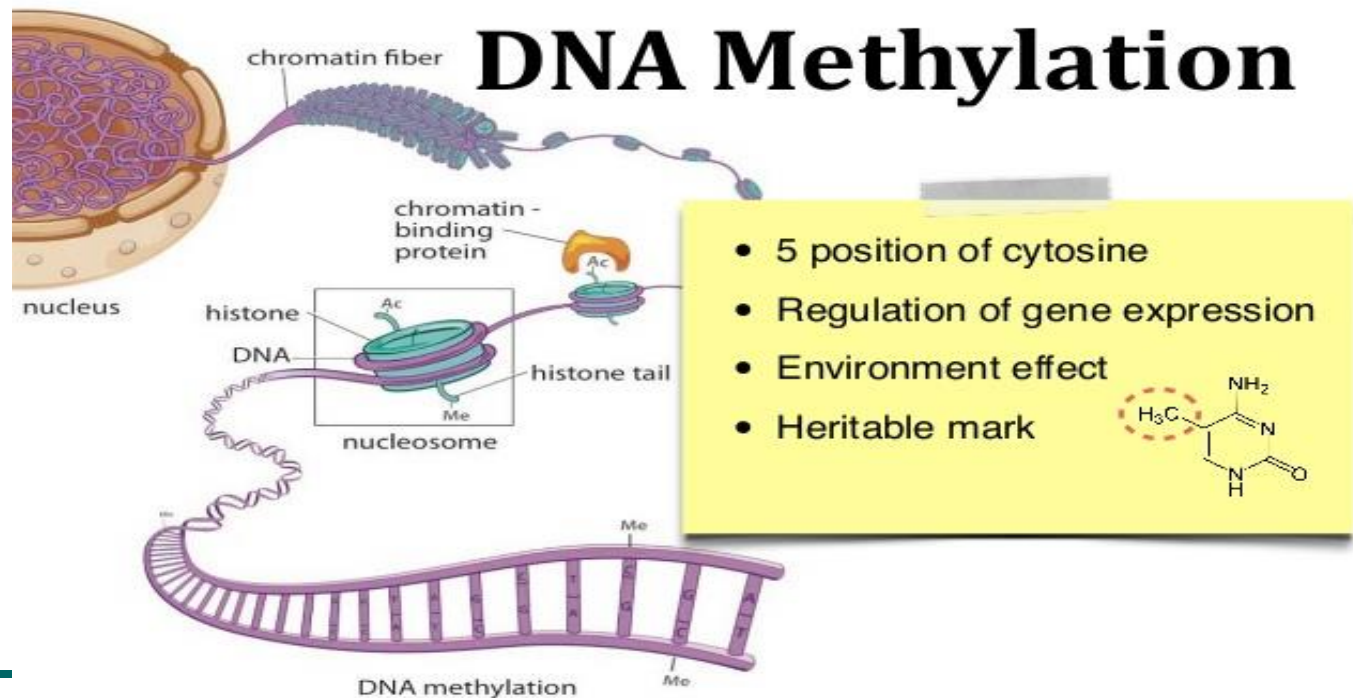
**Nanjing 210095, China**

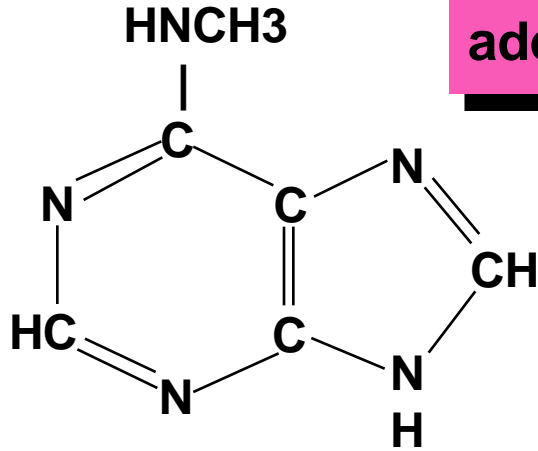


# ➤ Introduction-DNA methylation

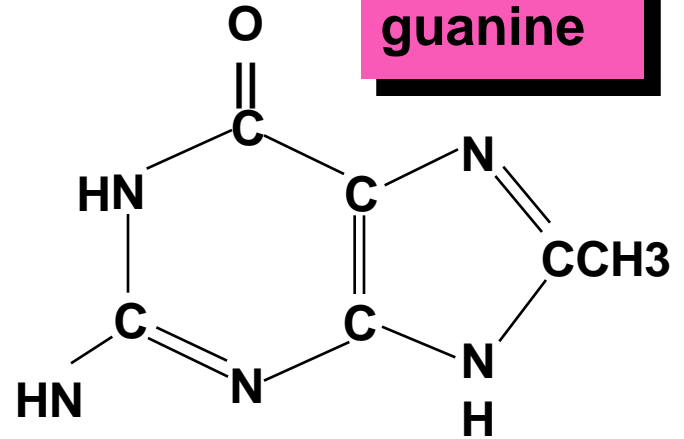


DNA methylation is epigenetic marks related to genomic stability, transposon silencing, and gene expression in plant development and response to environmental stresses.

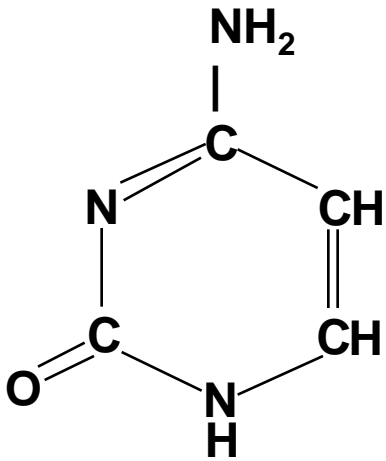




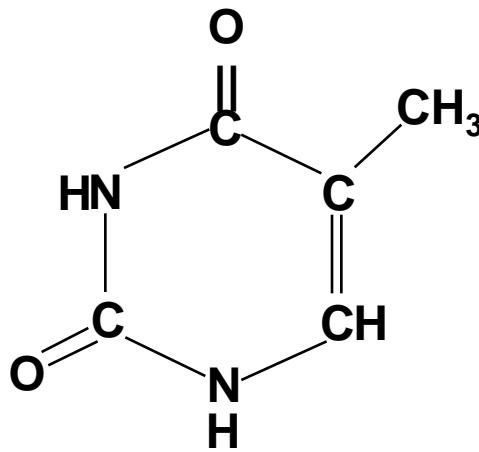
adenine



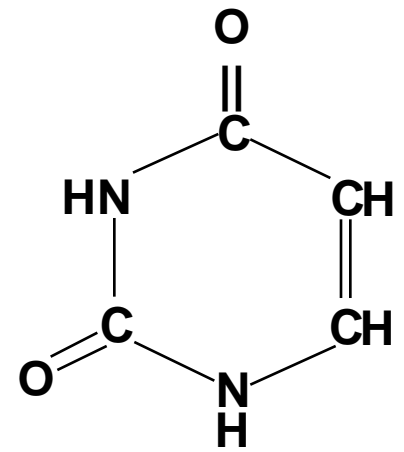
guanine



cytosine

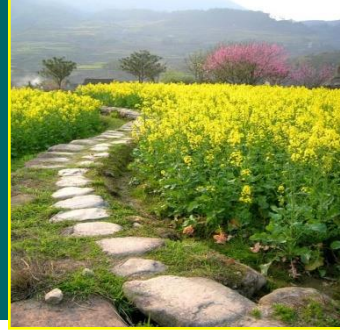


thymine

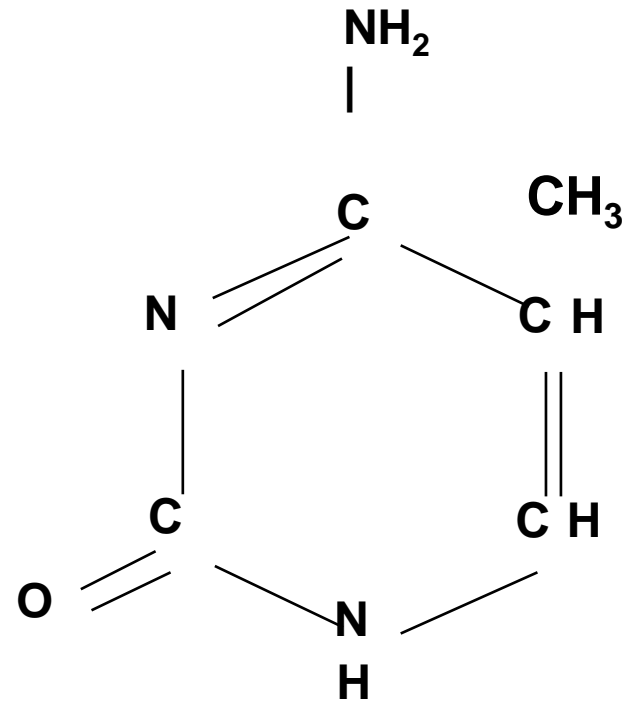


uracil

## ➤ Introduction-DNA methylation

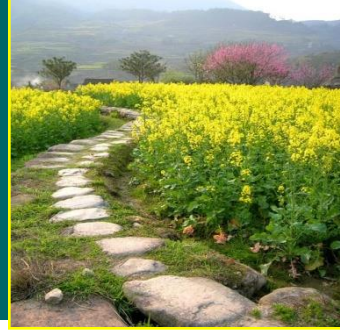


In higher plant genomes, DNA methylation usually occurs in CG, CHG and CHH (H=A, T, C) that are mainly located at the 5-position of cytosine, yielding 5-methylcytosine.



cytosine

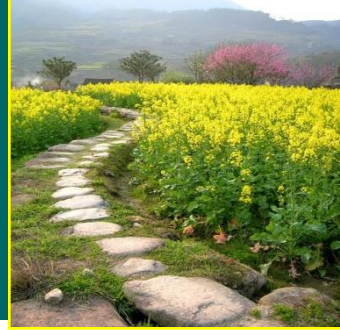
## ➤ Introduction-DNA methylation



**DNA methylation is catalyzed by a group of specific enzymes:**

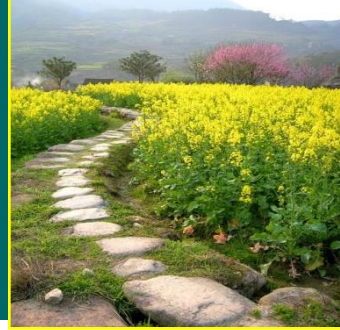
**(1): DMT1 or MET1 (DNA METHYLTRANSFERASE 1) and its members act as maintenance methyltransferases responsible for introducing methyl groups specifically into CG sequences**

## ➤ Introduction-DNA methylation



(2): The methyltransferase DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE 2) catalyzes asymmetric CHH methylation through persistent DNA methylation. siRNAs guide the RdDM pathway to regulate de novo DNA methylation.

## ➤ Introduction-DNA methylation



**(3):** The third type of methyltransferases CMTs (CHROMOMETHYLASES) is plant specific DNA methyltransferases involved primarily in the maintenance of symmetrical CHG methylation.

# Active DNA demethylation

- methylation in cytosine residues can be reversed by active demethylation, which is catalyzed by a group of demethylating enzymes such as **ROS1** (REPRESSOR OF SILENCING), **DME** (DEMETER), **DML2** (DEMETER-LIKE) and DML3. **ROS1 encodes a DNA glycosylase/lyase.**

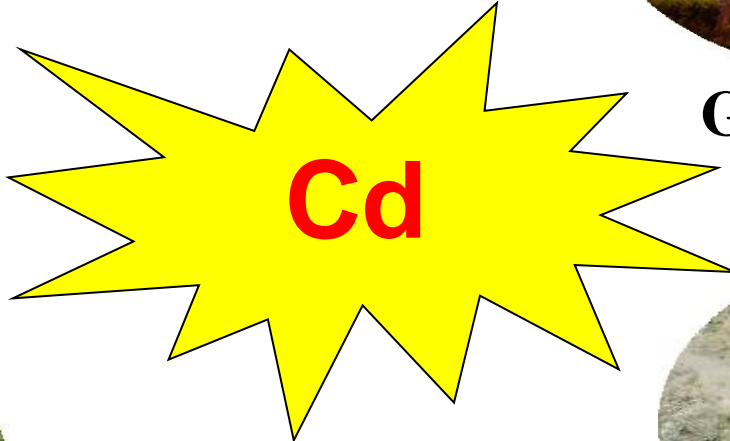


# ➤ Cd is toxic heavy metal released to environments

**Mining**



**Gaseous emission**

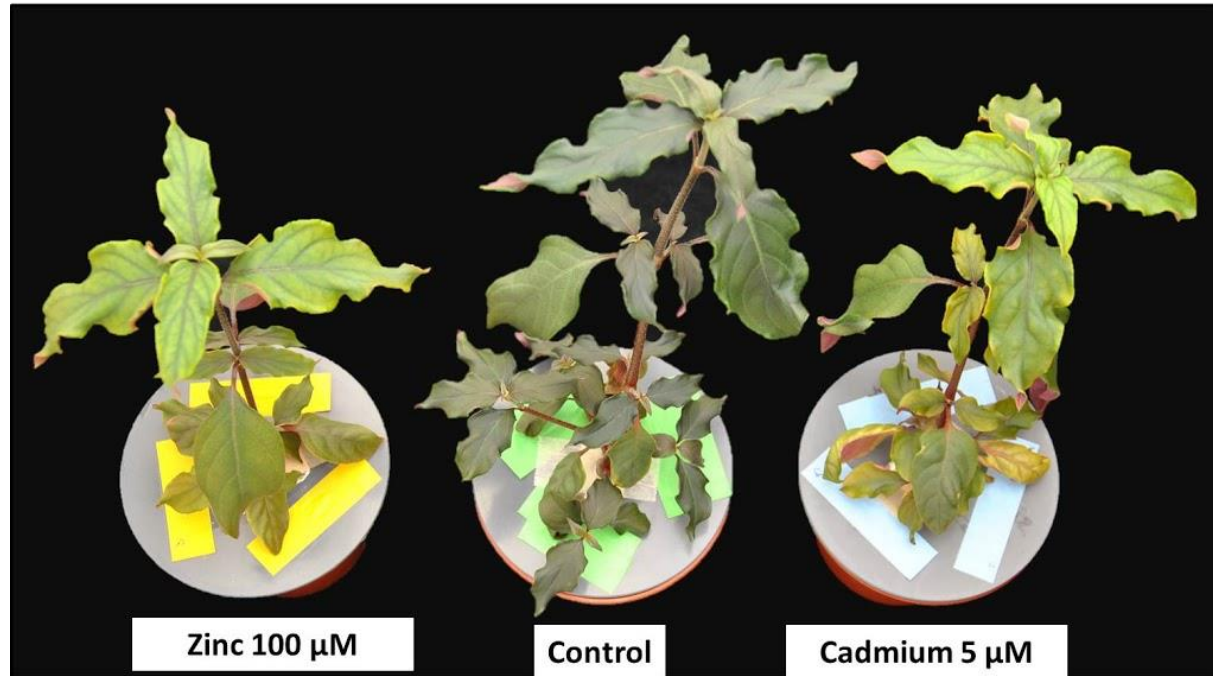
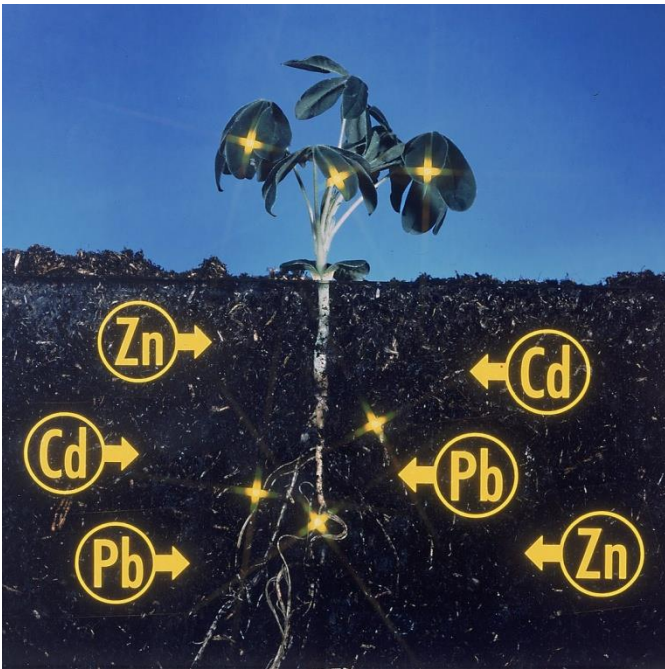


**Fertilizers & pesticides**



**Irrigation**

# Cd toxicity to plants as well as human beings



## ➤ Plant overload of Cd induced gene expression

1. Transcriptome of rice could be changed by Cd
2. Expression of many genes responsible for Cd uptake, transport, tolerance or detoxification are altered.
3. Affecting transposable elements, TEs (transposons and retrotransposons)

## ➤ Hypothesis for Cd-induced change in DNA methylation in plants

We assumed that Cd affects plant growth and development and its uptake/accumulation possibly through DNA methylation/demethylation of functional genes and transposable elements on a genome-wide scale.

## ➤ Hypothesis for Cd-induced change in DNA methylation in plants

In this study, we adopted the recent advances in high-throughput single-base-resolution bisulfite-sequencing (BS-Seq) and RNA-sequencing (RNA-Seq) to identify the pattern and degree of cytosine methylation in Cd-exposed rice seedlings (with 80  $\mu\text{M}$  Cd for 4 days ).

# ➤ Results-BS-seq data

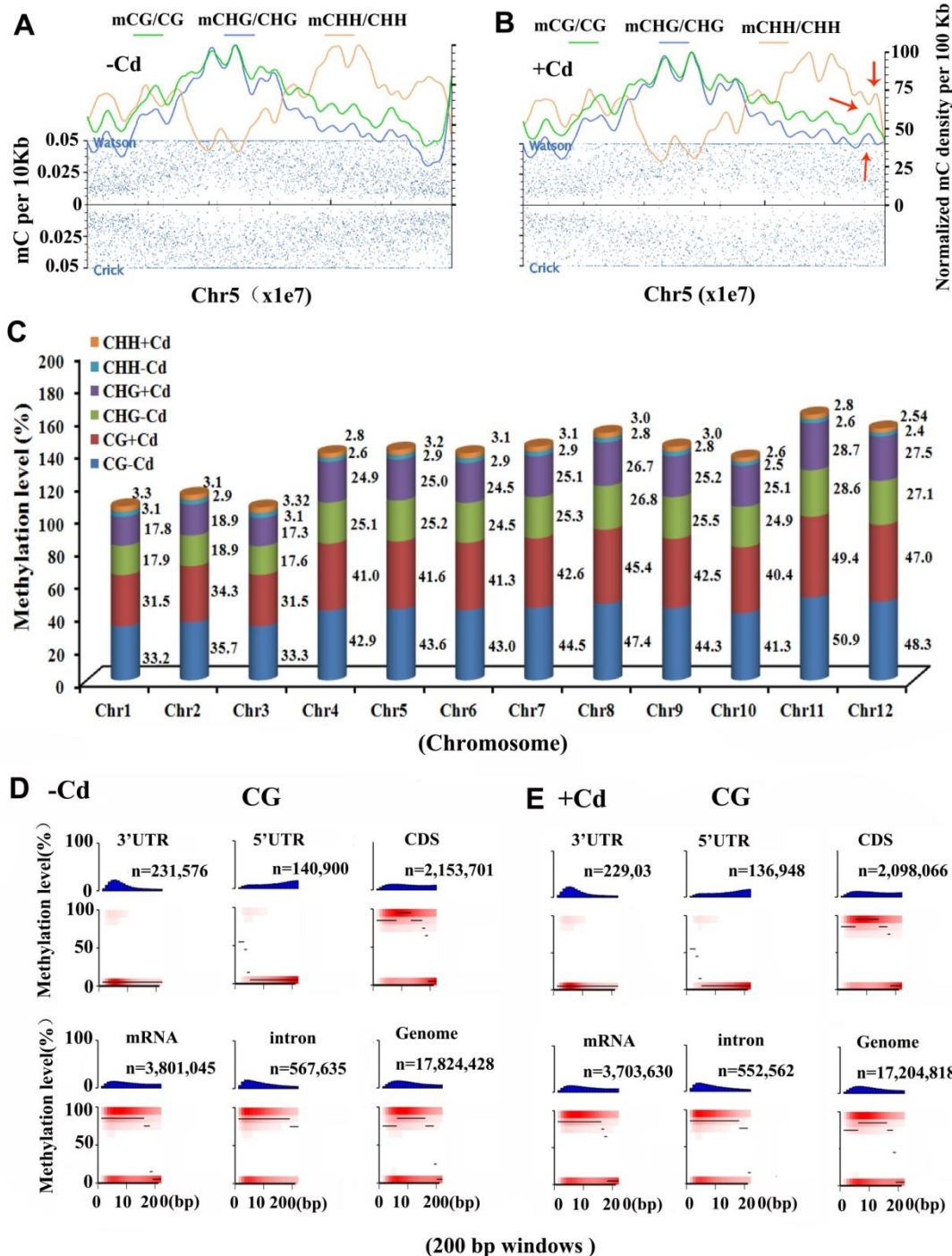
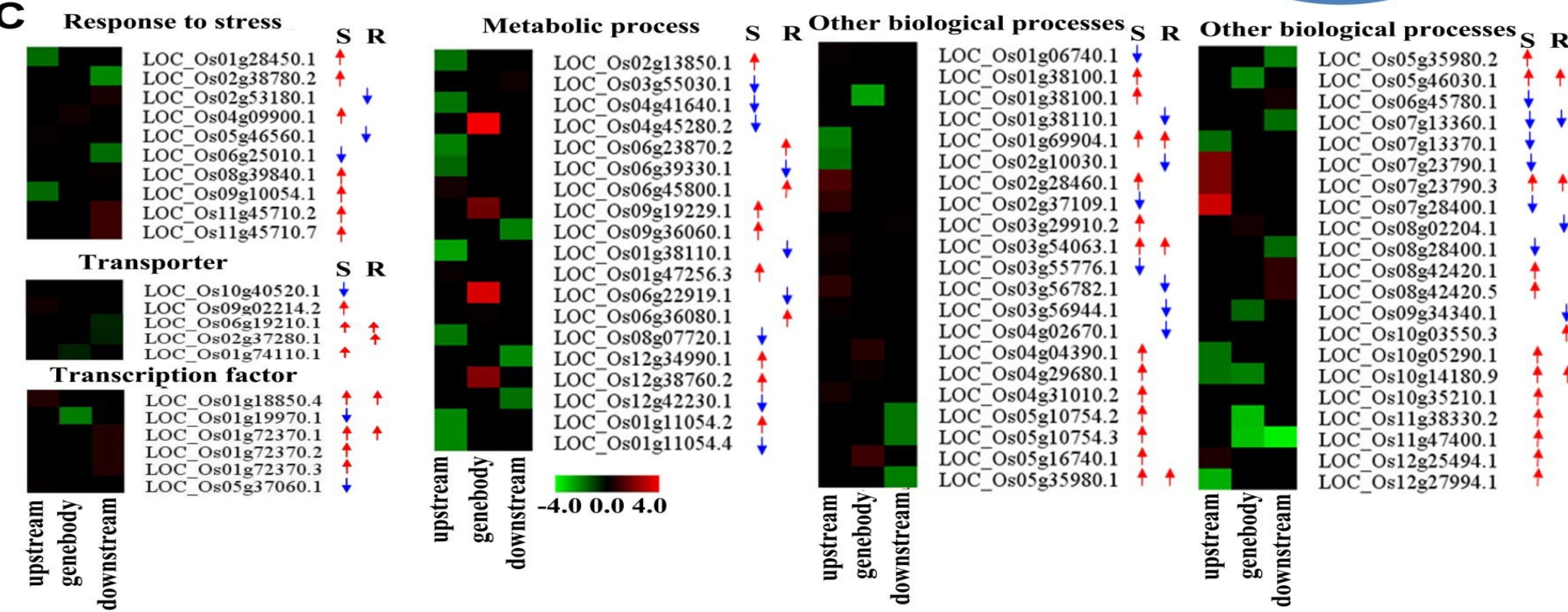
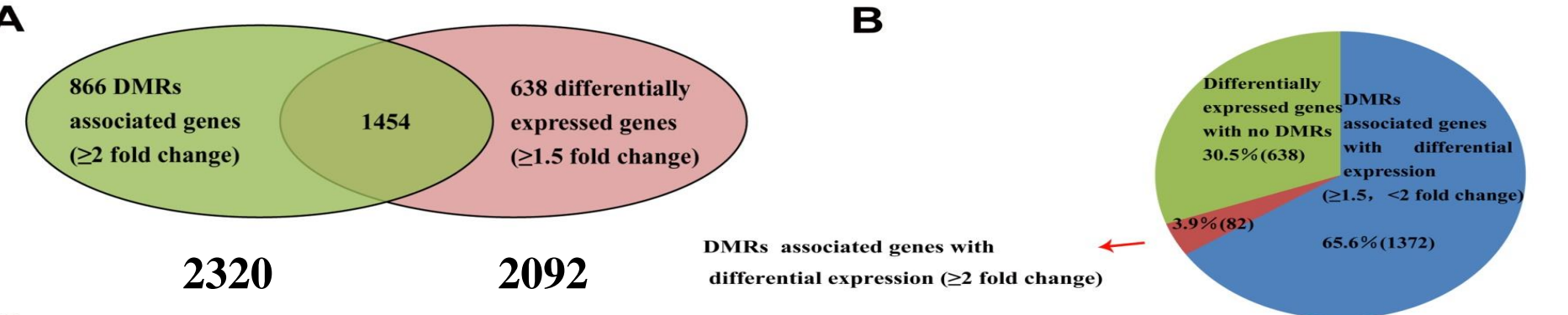
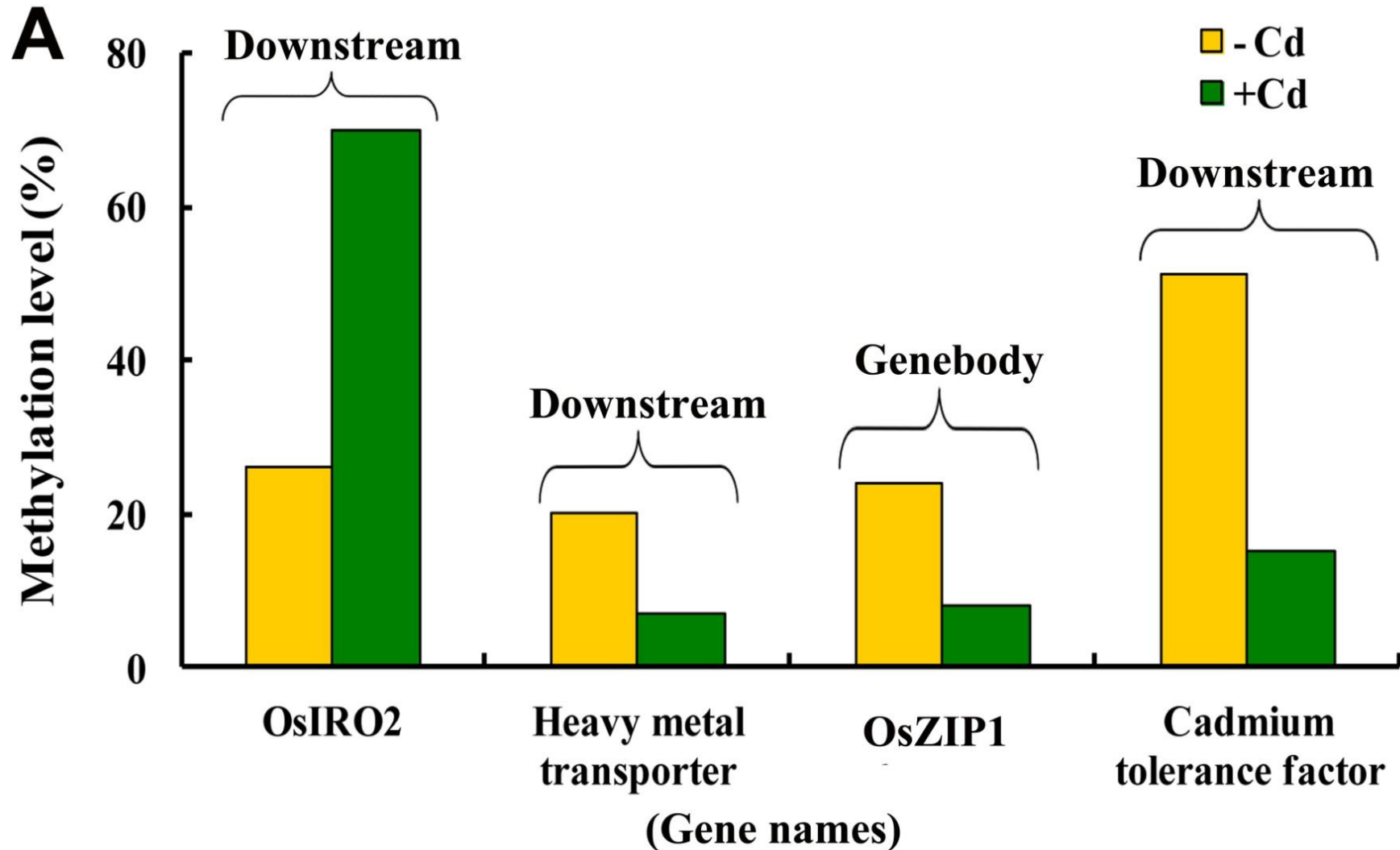


Figure 1. Differential CG and non-CG methylation levels in genomic regions (A and B): The density profiling of methylcytosines in chromosome 5. Blue dots indicate methylcytosine density. Smoothed lines represent the methylcytosine (CG、CHG and CHH) density in each context. (C): Levels of CG methylation and non-CG methylation in rice chromosomes. (D, E): The heatmap of genomic regional distribution (3-UTR, 5-UTR, CDS, intron and mRNA) characteristics.

# Combined analyses of genes changed in DNA methylation and genes changed in transcriptional expression

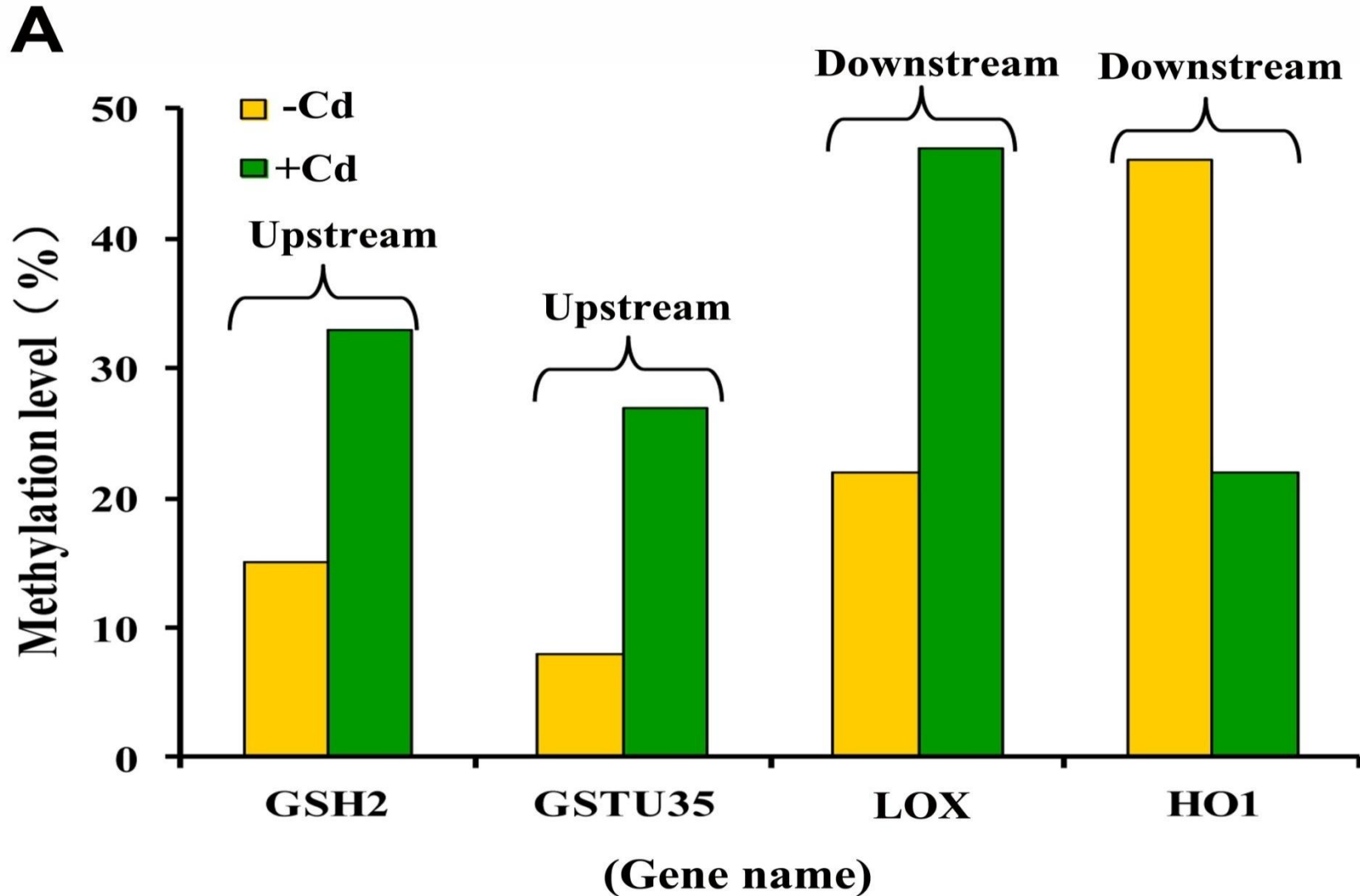


# Profiling of DNA methylation of selected genes for metal transporters

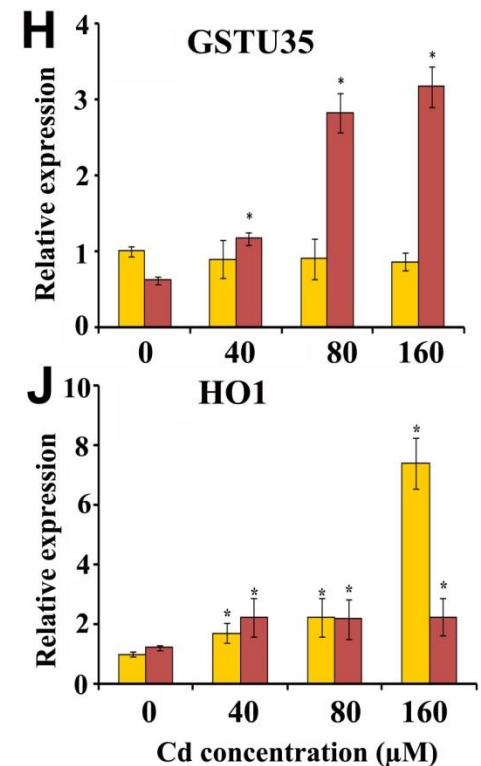
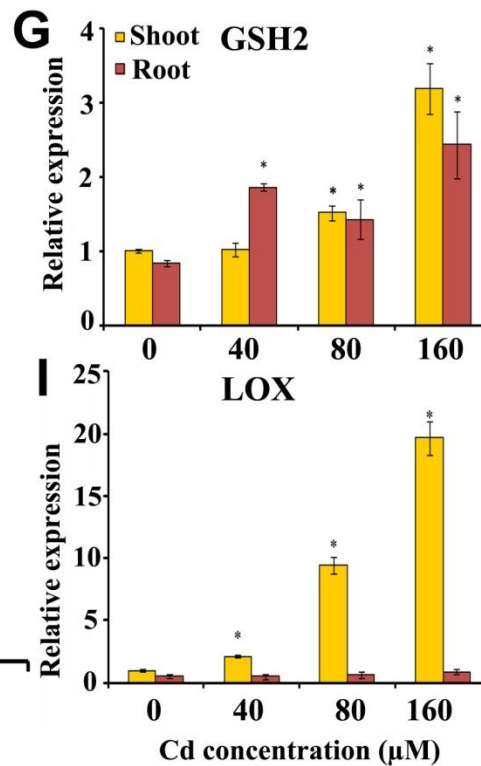
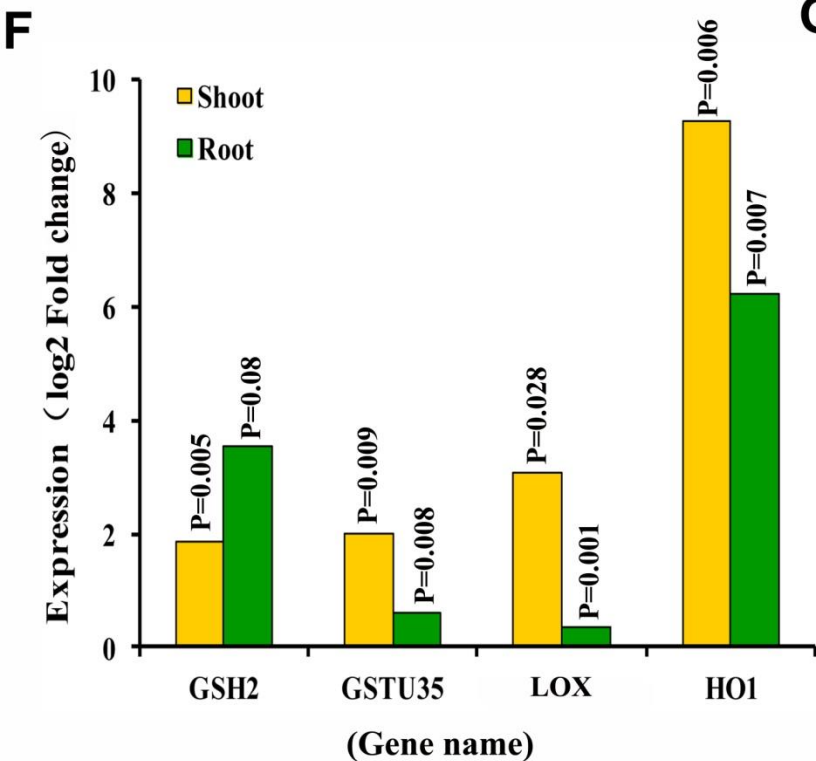
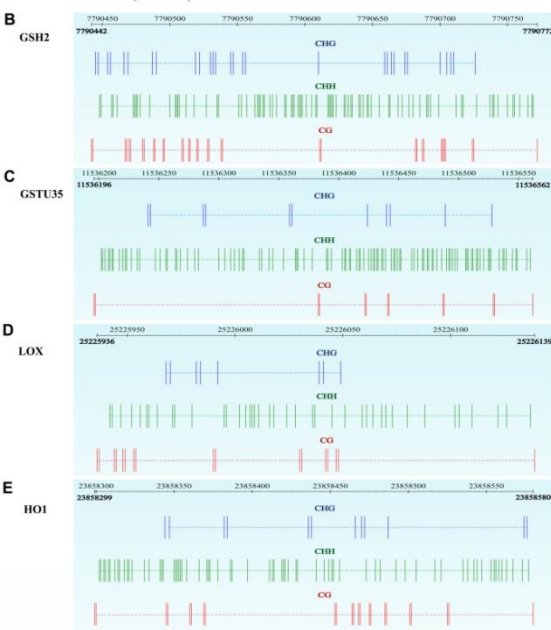




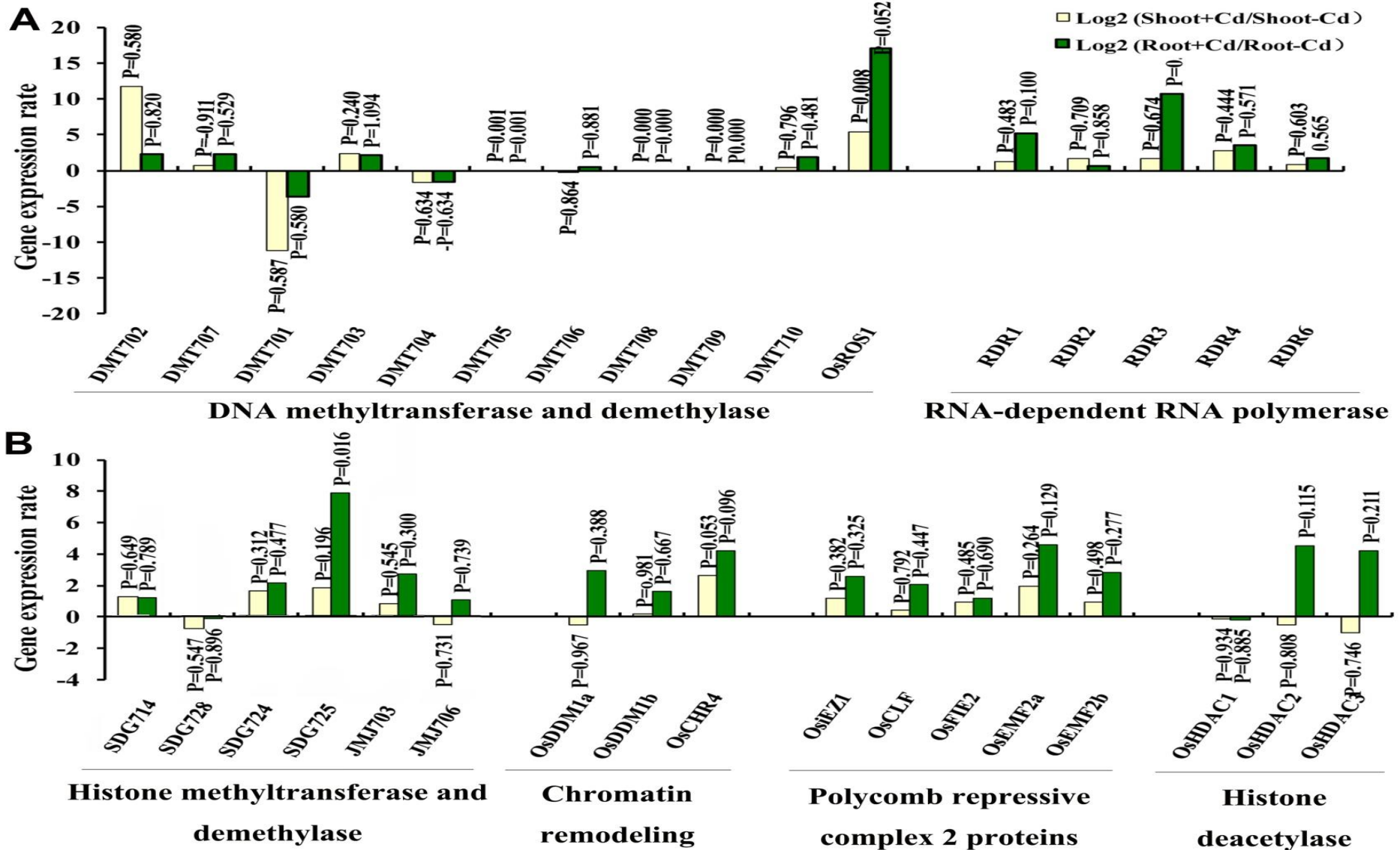
# Profiling of DNA methylation of selected genes for Cd detoxification



Profiling of DNA methylation and expression of representative genes coding for glutathione S-transferase 2 (GSH2), glutathione S-transferase U35 (GSHU35), lipoxygenase (LOX) and heme oxygenase-1 (HO1). Differences of DNA methylation levels ( $\geq 2$  fold) and patterns of genes under Cd stress



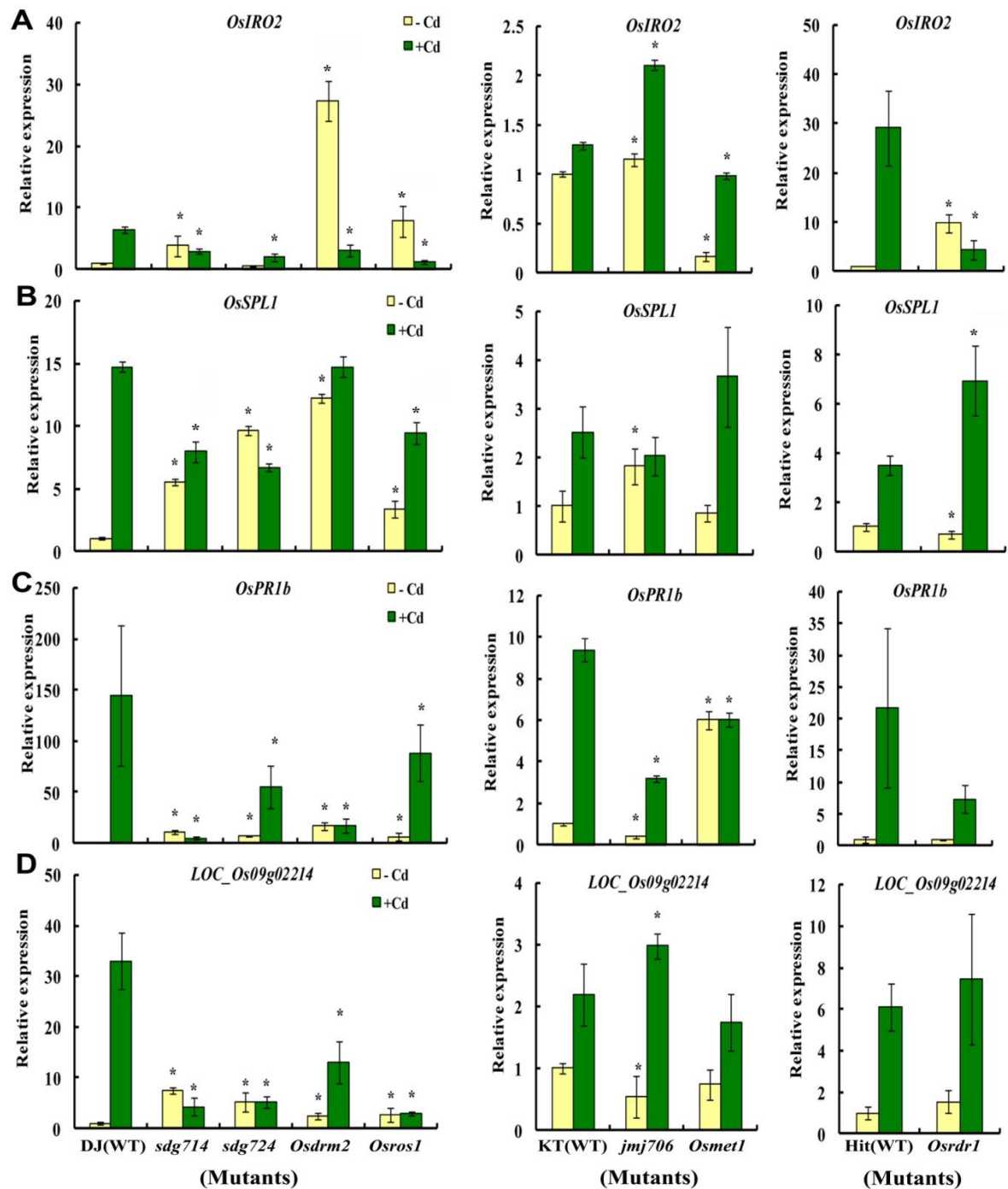
# Cd exposure alters expression of genes relevant to DNA methylation



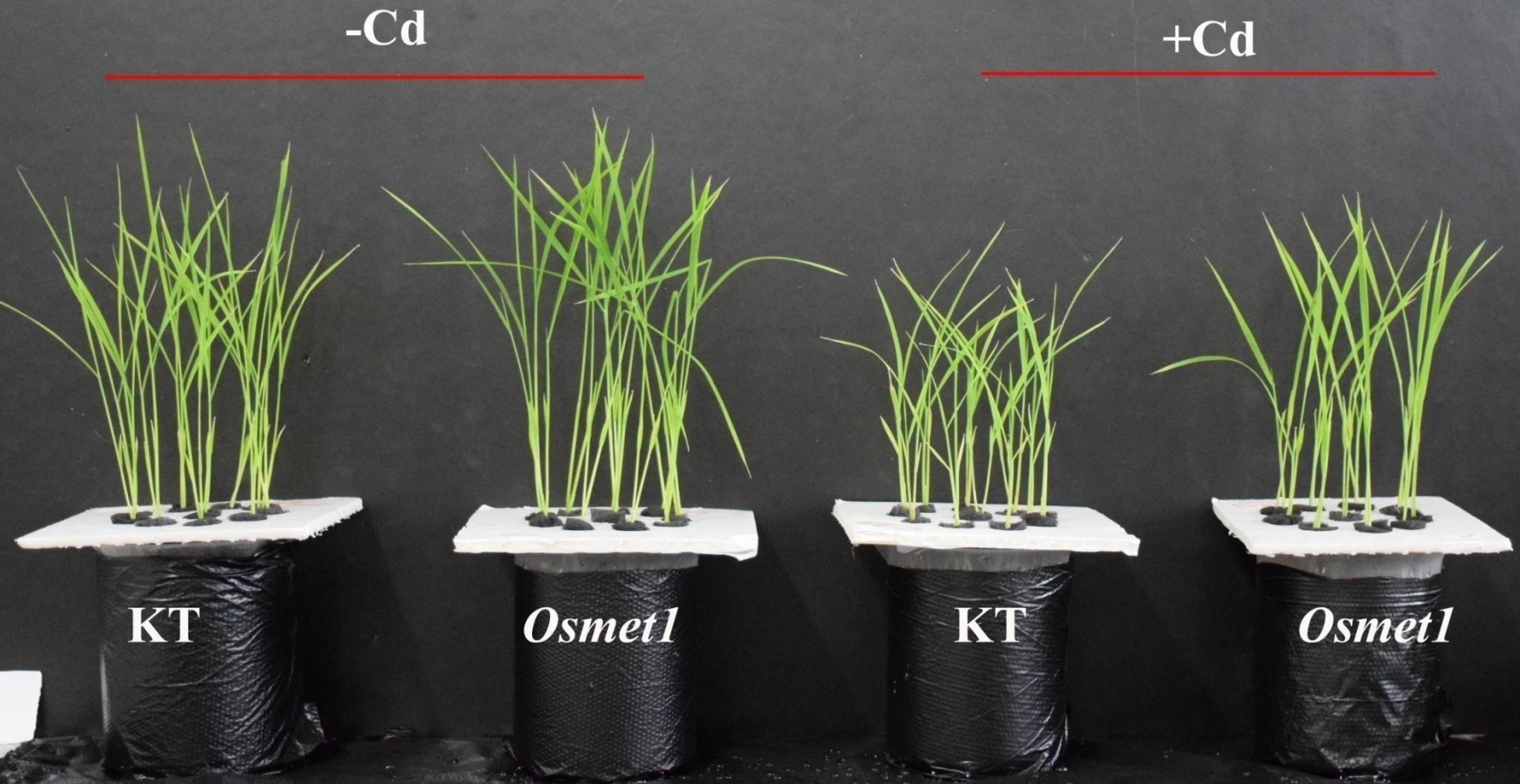
# ➤ Expression of several genes in mutants

*Osmet1*, *Osdrm2*, *sdg714*, *sdg724*, *jmj706*, *Osrd1*, and *Osros1*

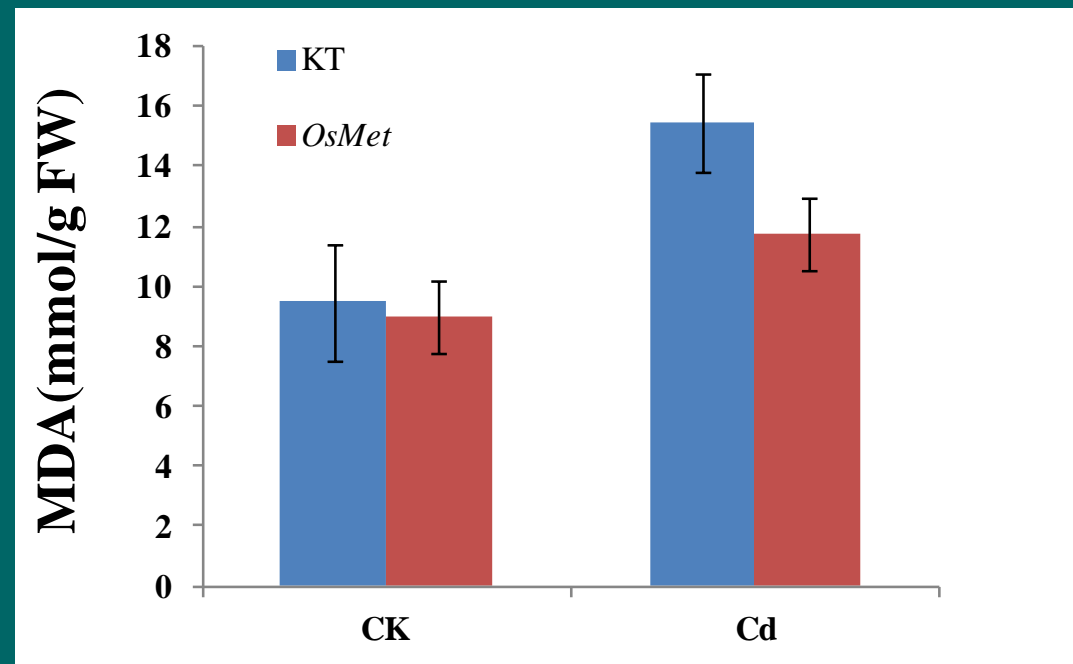
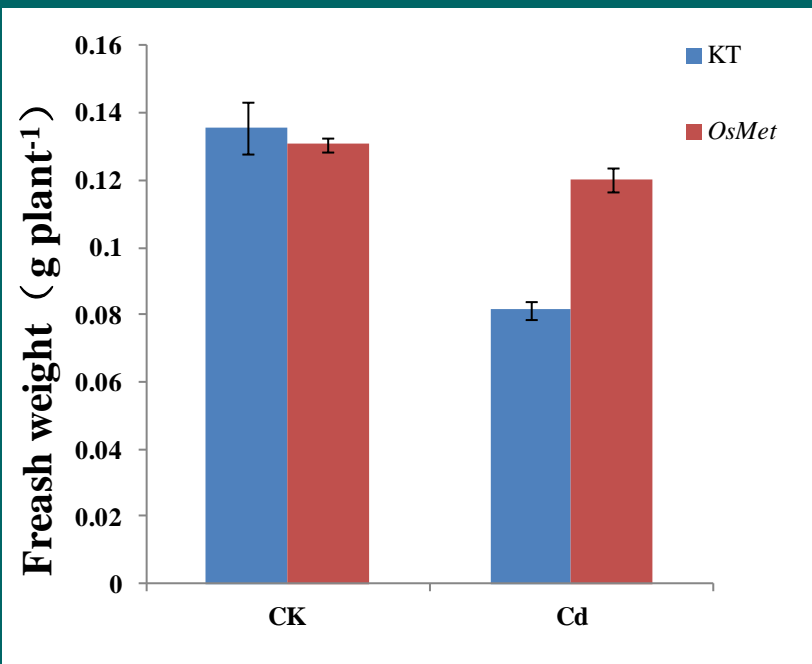
A: *OsIRO2*,  
B: *OsSPL1*, C:  
*OsPR1b*; D:  
*LOC\_Os09g02214*



# Phenotype of *Osmet1* mutants defective in DNA methylation activities in rice exposed to Cd



# Growth and physiological responses of *Osmet1* to Cd



# Phenotype of *Osrdr1* mutants defective in DNA methylation activities in rice exposed to Cd

-Cd

+Cd



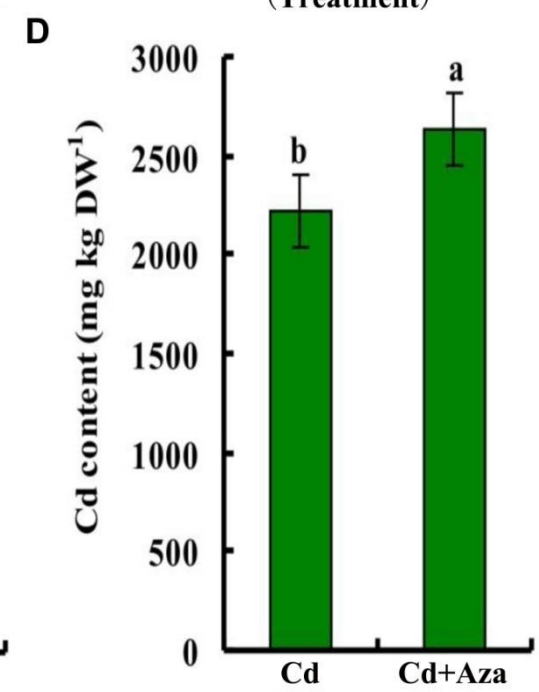
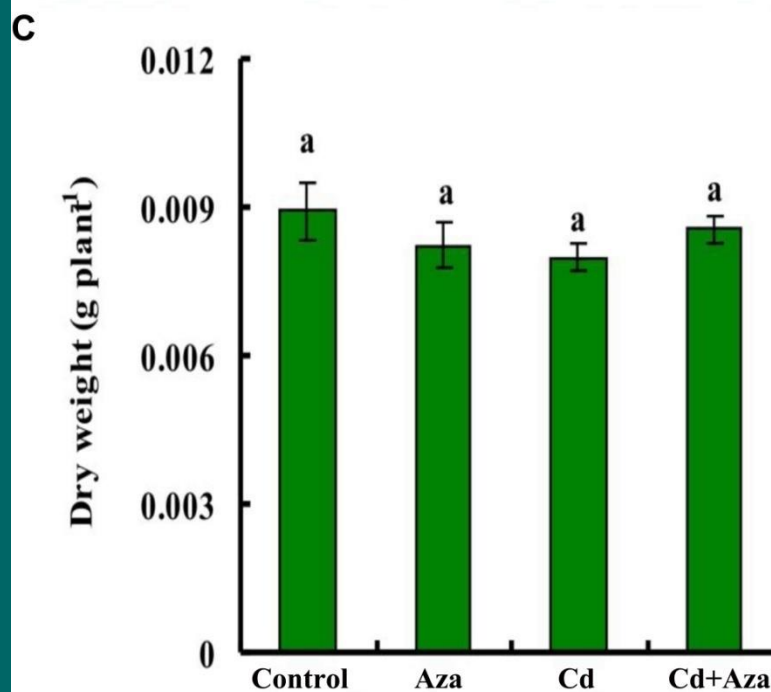
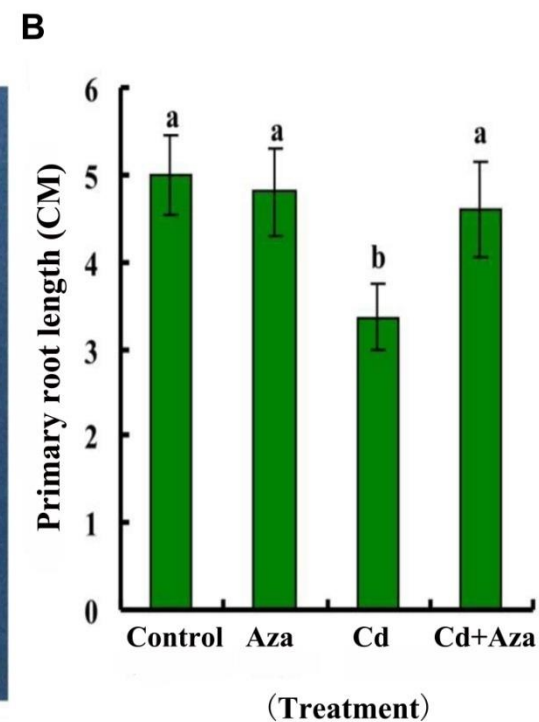
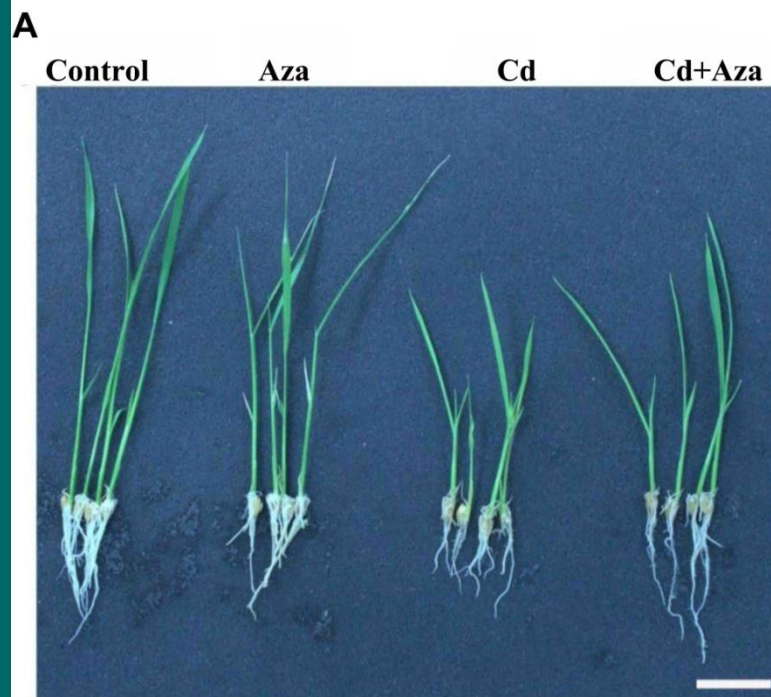
Hitomebore

*Osrdr1*

Hitomebore

*Osrdr1*

**Effect of DNA methylation inhibitor 5-azacytidine (Aza) on phenotypes and Cd accumulation in rice exposed to Cd**



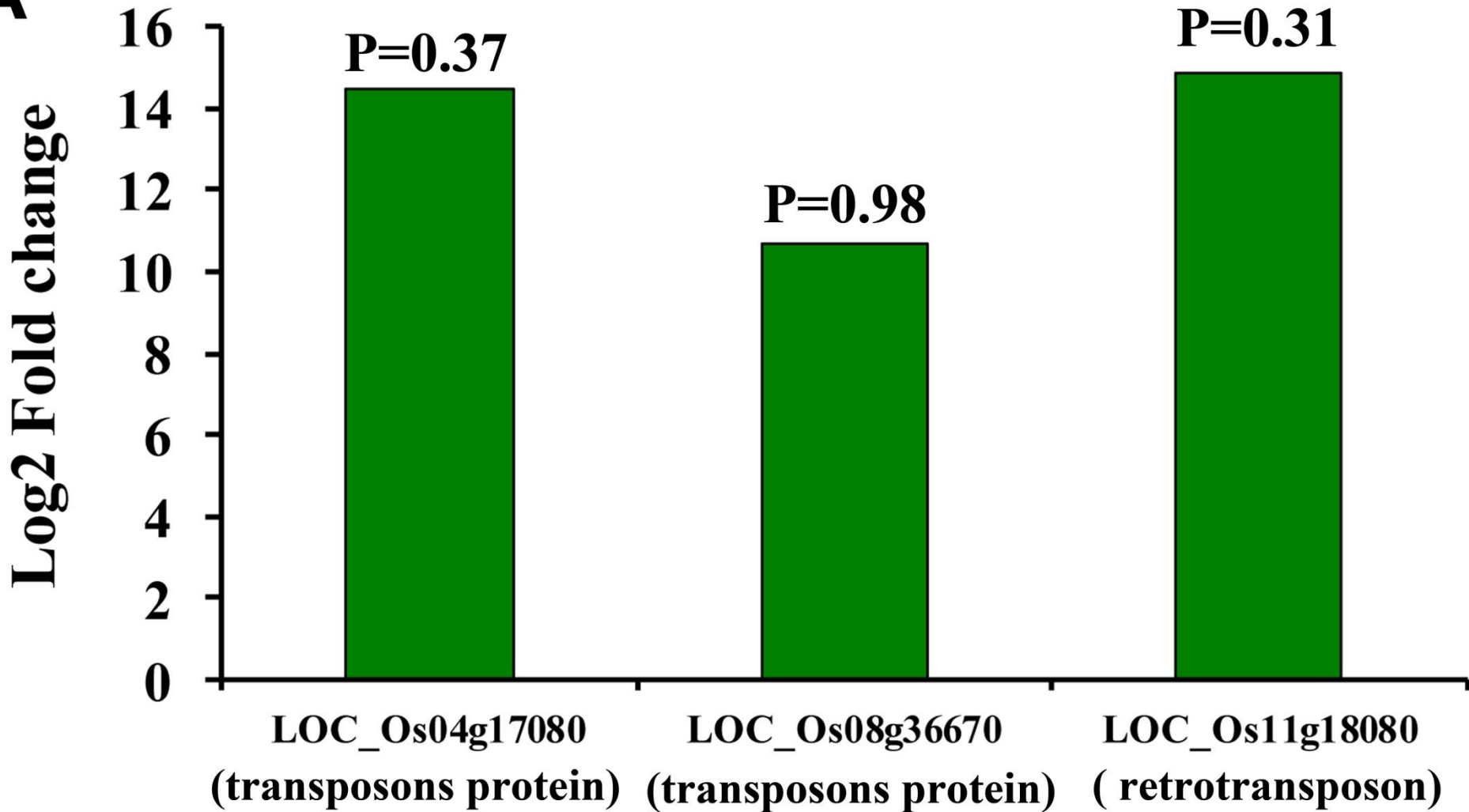


# Transposable elements: 362

108 transposons and 254 retrotransposons were differentially methylated ( $\geq 2$  fold change,  $p < 0.05$ ) under Cd stress

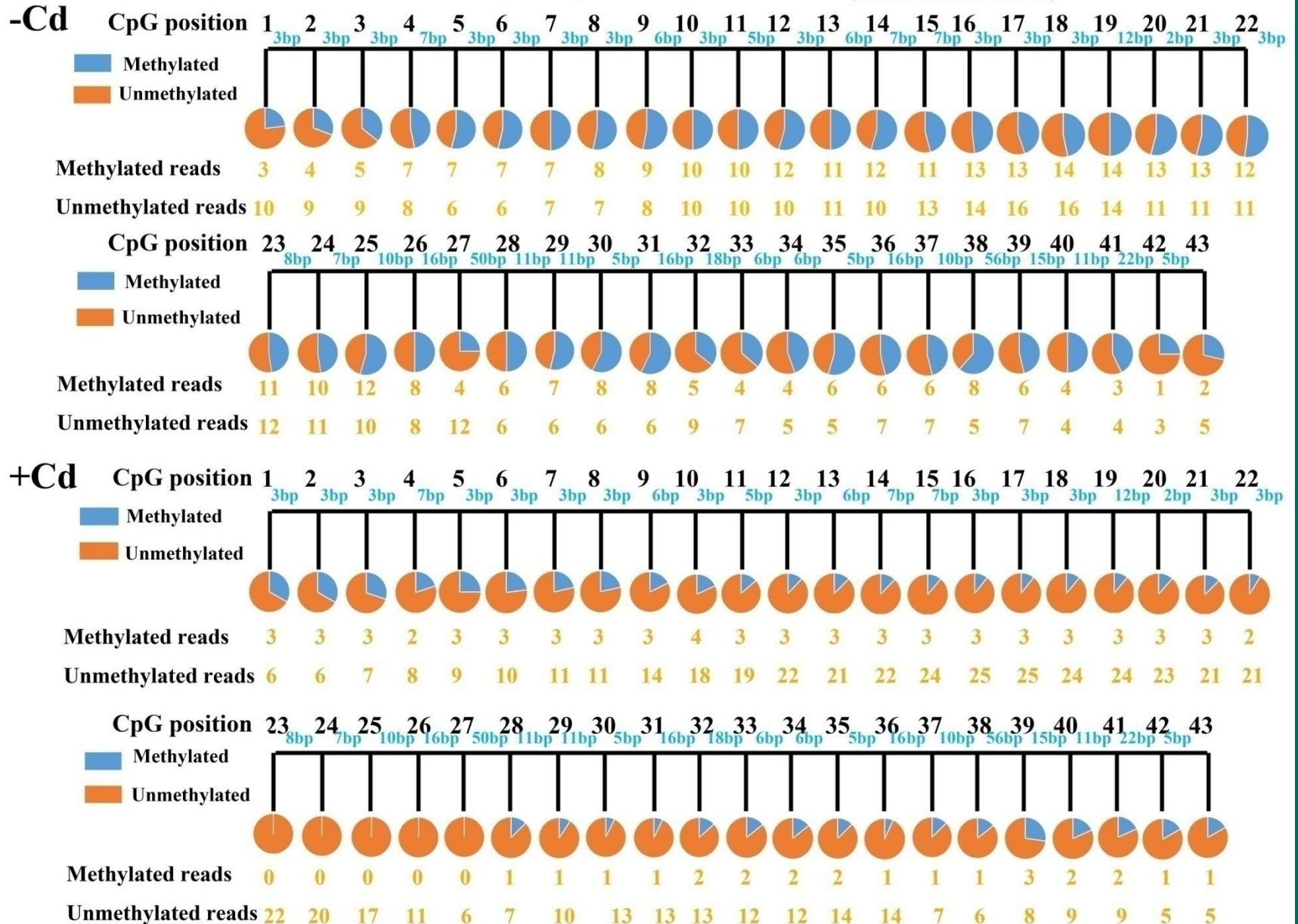
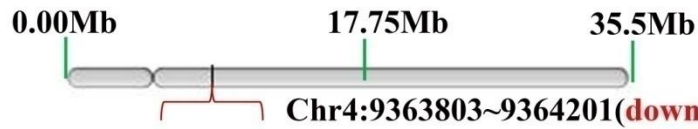
# Three TEs can be induced by Cd exposure

**A**



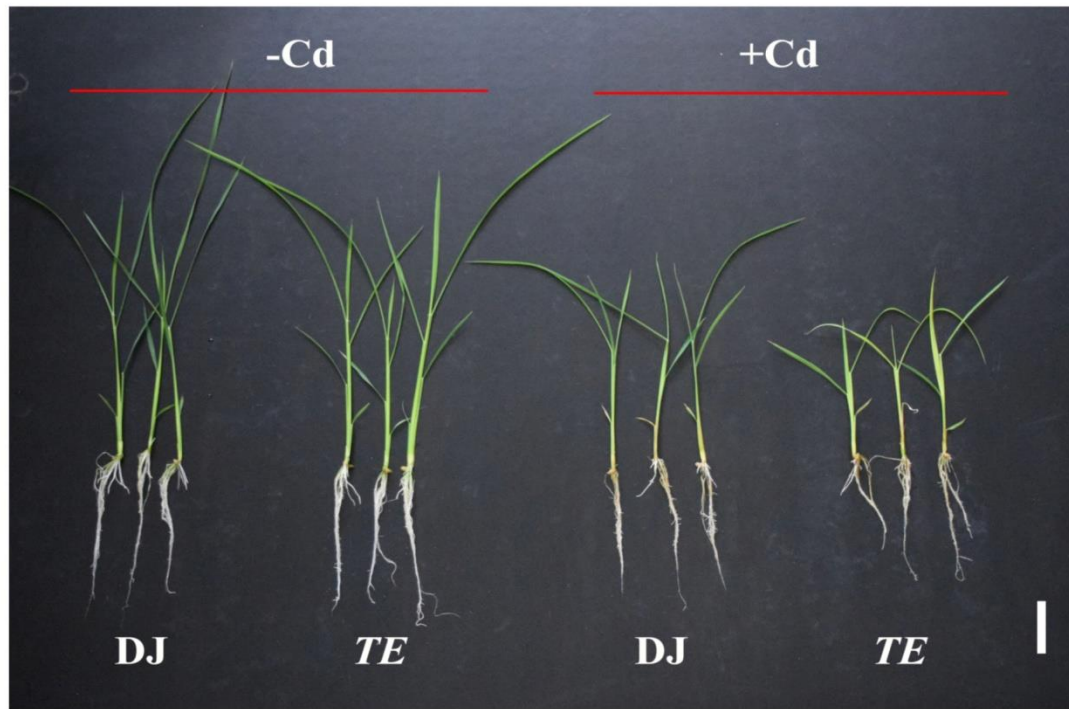
**B**

**Transposon**   
(LOC\_Os04g17080)

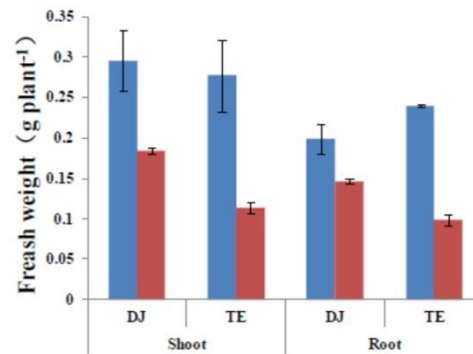


# Mutation of a TE LOC Os0417080 results in more sensitivity to Cd

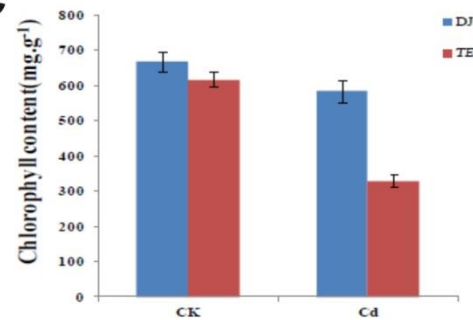
A



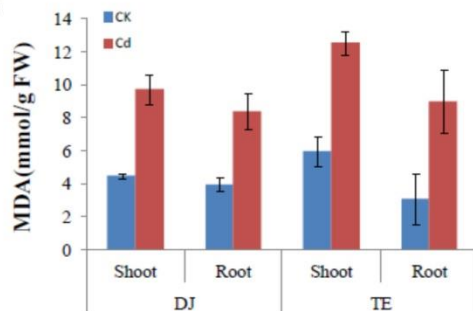
B



C



D



# Acknowledgements

## Lab members





Thank you  
for your attention