

Genome-wide bisulfite sequencing analysis of cultivated and wild rice species reveals epigenome variation in response to aluminum stress

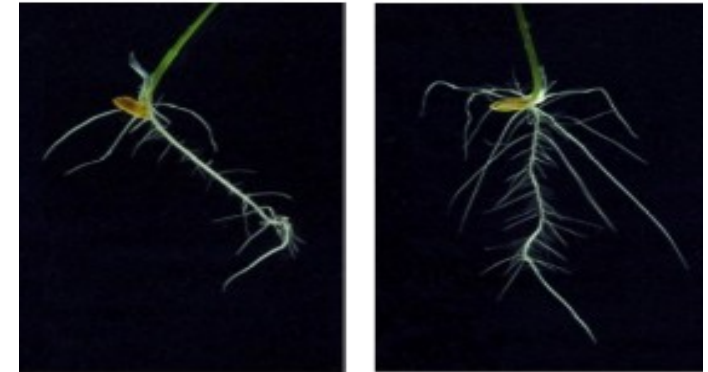
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Why study aluminum toxicity ?

- Aluminum (Al) is the third **most common** element in the world (~7%)
- When the **pH of the soil** is below 5, it becomes its most toxic form Al^{3+}
- Most of the **cereals** around the world such as corn, wheat and rice, grow in acid soils and are exposed to the Al^{3+} toxicity, which **inhibits** the **plants development**.



+Al

-Al



[Al^{3+}]



Why study the methylome of rice crops?

Rice is an important crop that represents the **food security** of more than half of the world's population.

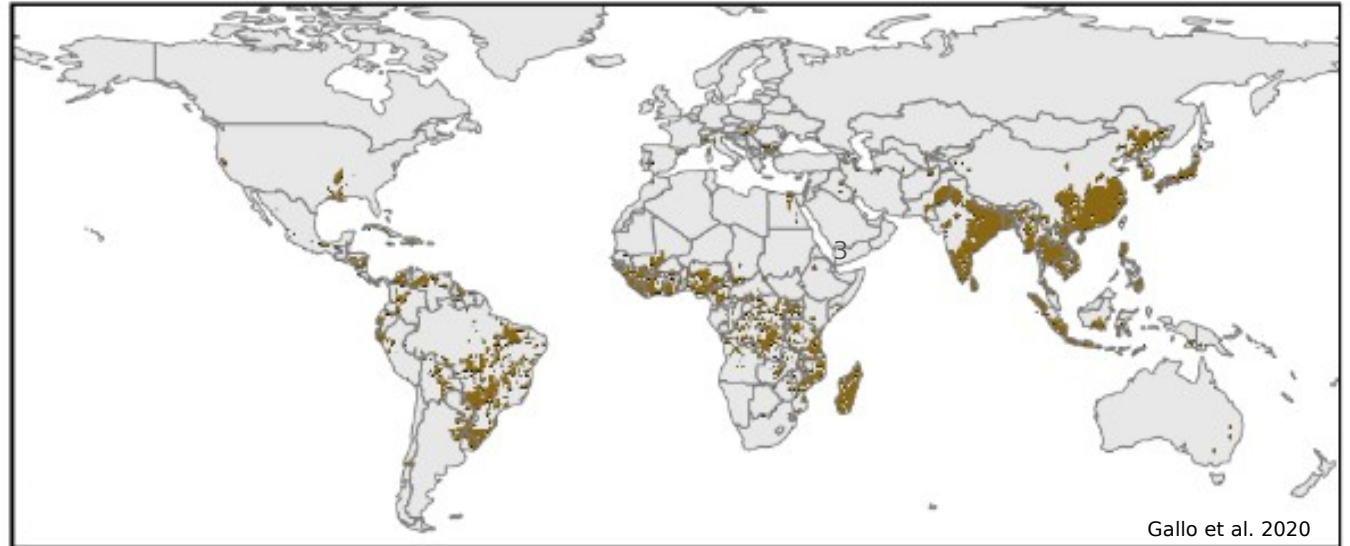
Class: Monocotyledoneae

Order: Poales

Family: Poaceae

Tribe: Oryzeae

Gender: Oryza



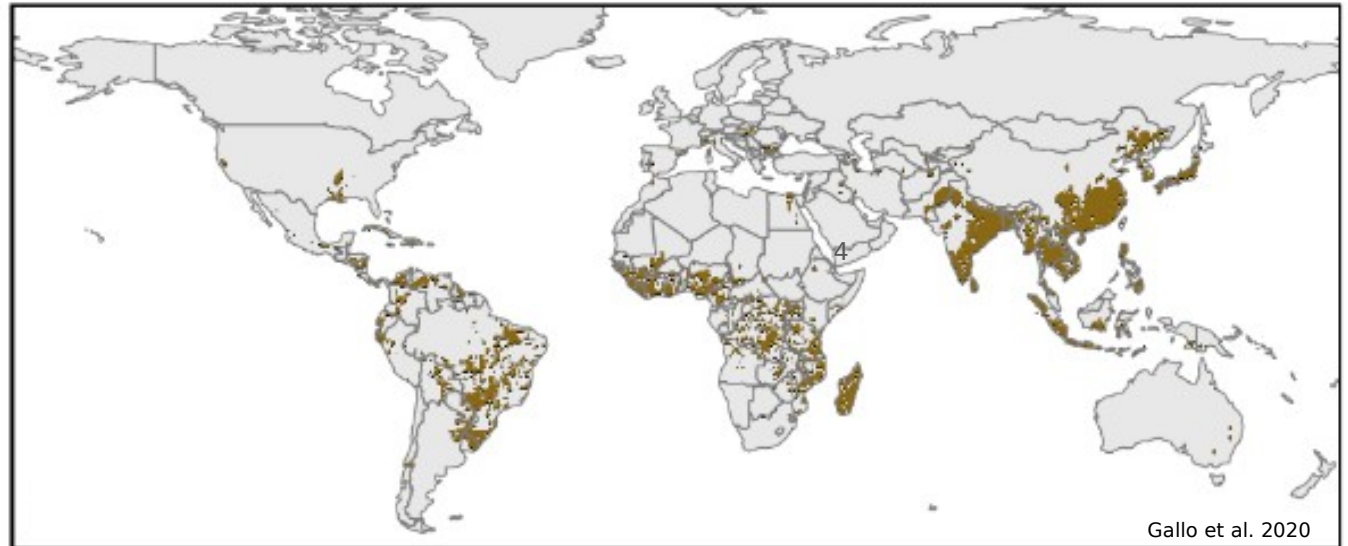


Why study the methylome of rice crops?

Model organism for evolutionary and molecular studies in cereals and monocotyledonous plants.

Oryza sativa is the most AI tolerant crop.

Class: Monocotyledoneae
Order: Poales
Family: Poaceae
Tribe: Oryzeae
Gender: *Oryza*



Why *Oryza glumaepatula*?

Potential source of Al^{3+} tolerant varieties

- Wild type rice species
- Endemic from Center and South America
- Unknown tolerance mechanisms to Al^{3+}
- Biological collection in the Icesi University (Cali-Colombia)
- 65 accessions of tolerant and susceptible *O. glumaepatula* genotypes



Plants can respond and adapt to environmental conditions

- Genetic information
- Signal transduction
- Modulation of transcription
- **Epigenetics**



DNA and histone modifications which do not affect the DNA sequence, that are stable and heritable

Abiotic stress



Biotic stress



Plants can respond and adapt to environmental conditions

How are DNA methylation patterns affected by **aluminum stress** in rice?

Are there differences between **cultivated and rice wild species**?

Are there differences between **tolerant and susceptible species**?

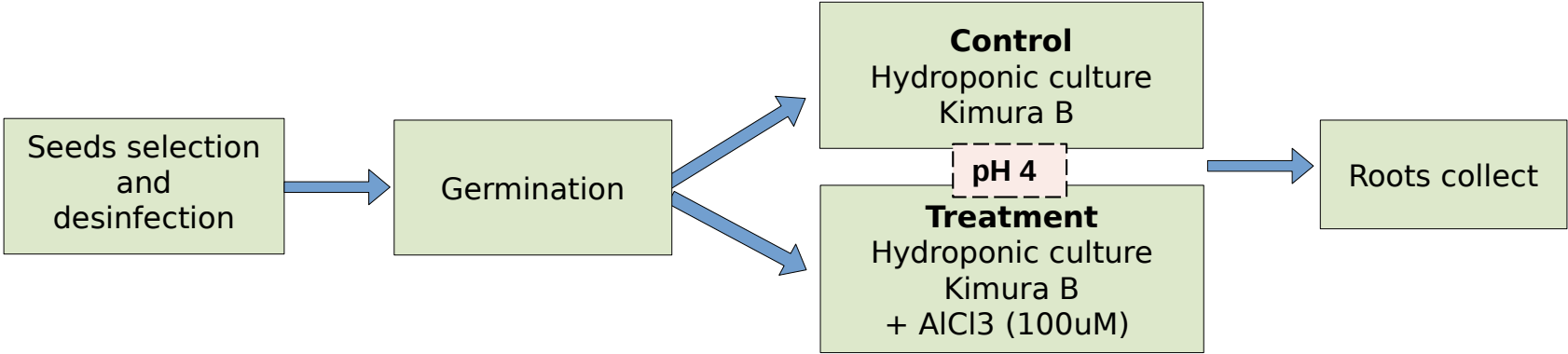
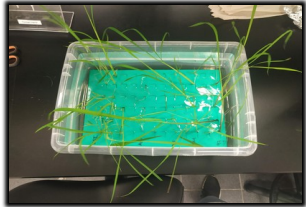
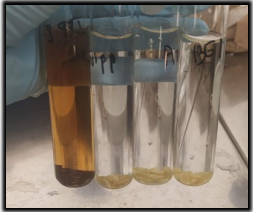
Abiotic stress



Biotic stress

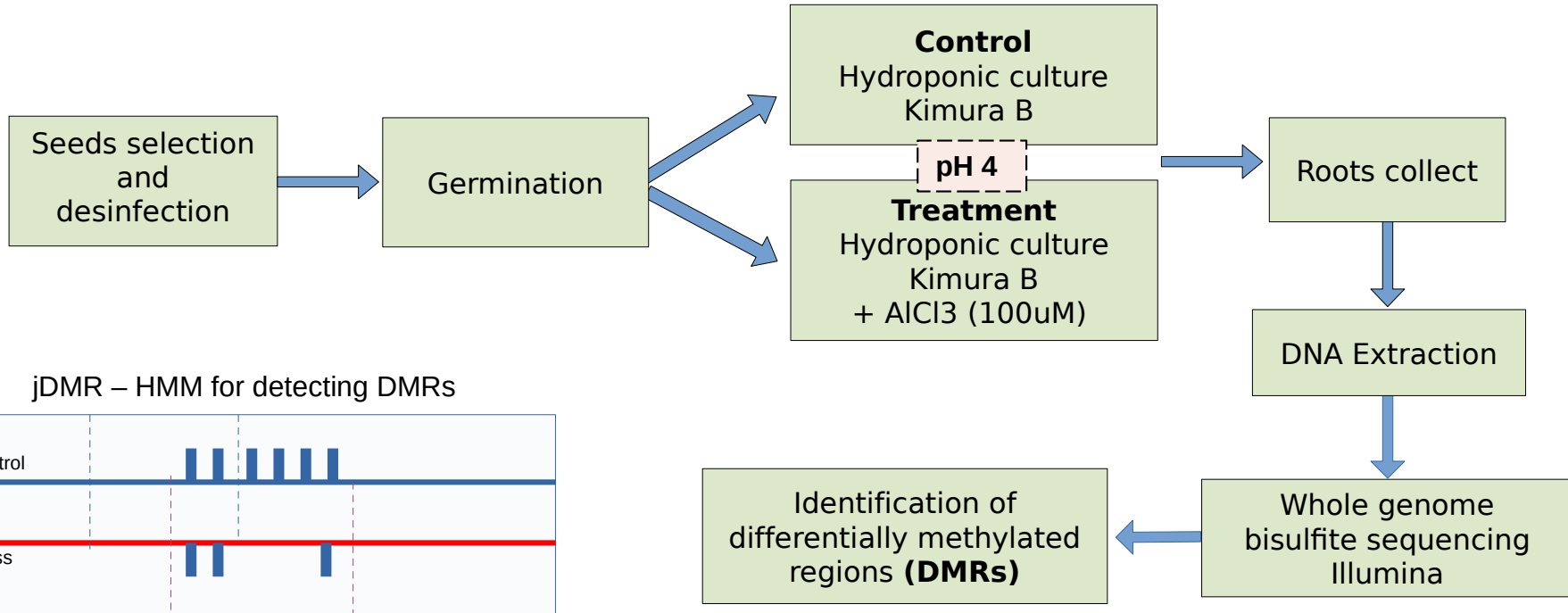
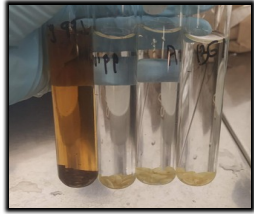


Experimental design

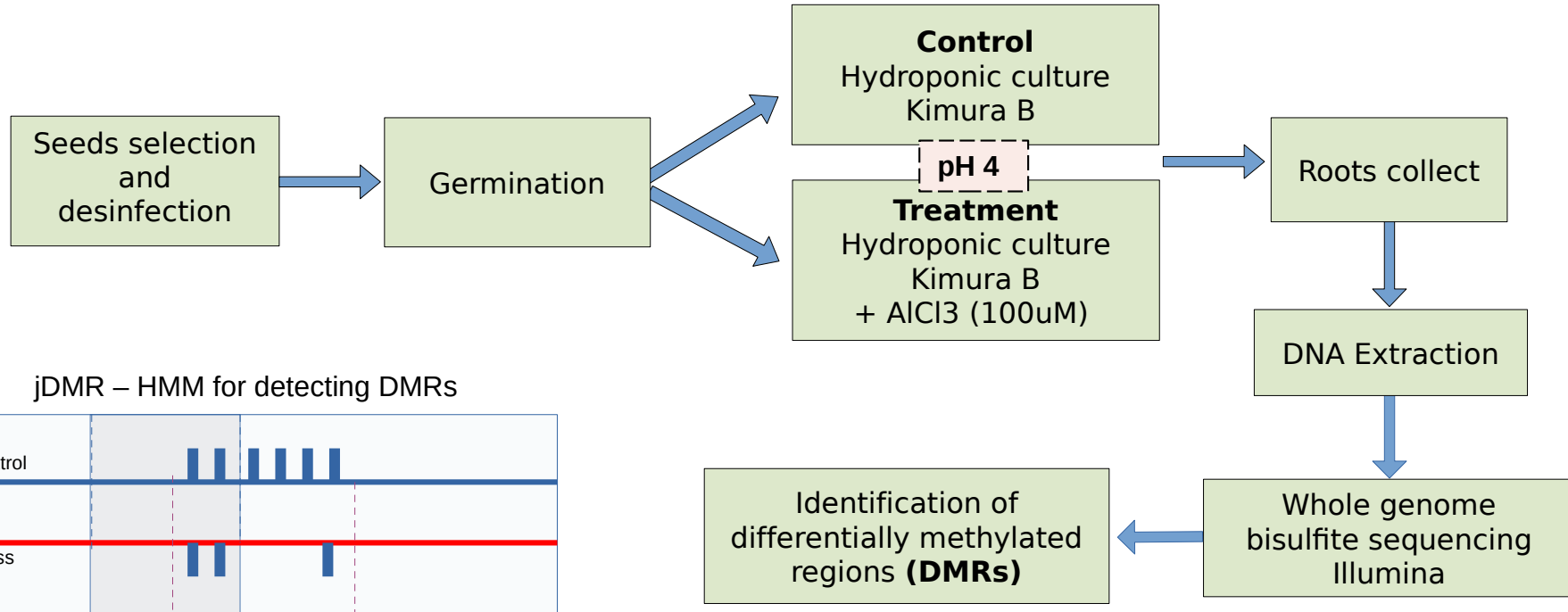
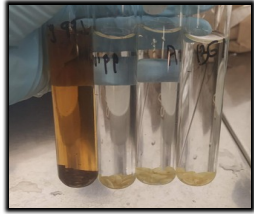


Species	Genotype	Response to Al ³⁺
<i>O. sativa</i>	Azucena	Tolerant
<i>O. sativa</i>	BGI	Susceptible
<i>O. glumaepatula</i>	Og97	Tolerant
<i>O. glumaepatula</i>	Og131	Susceptible

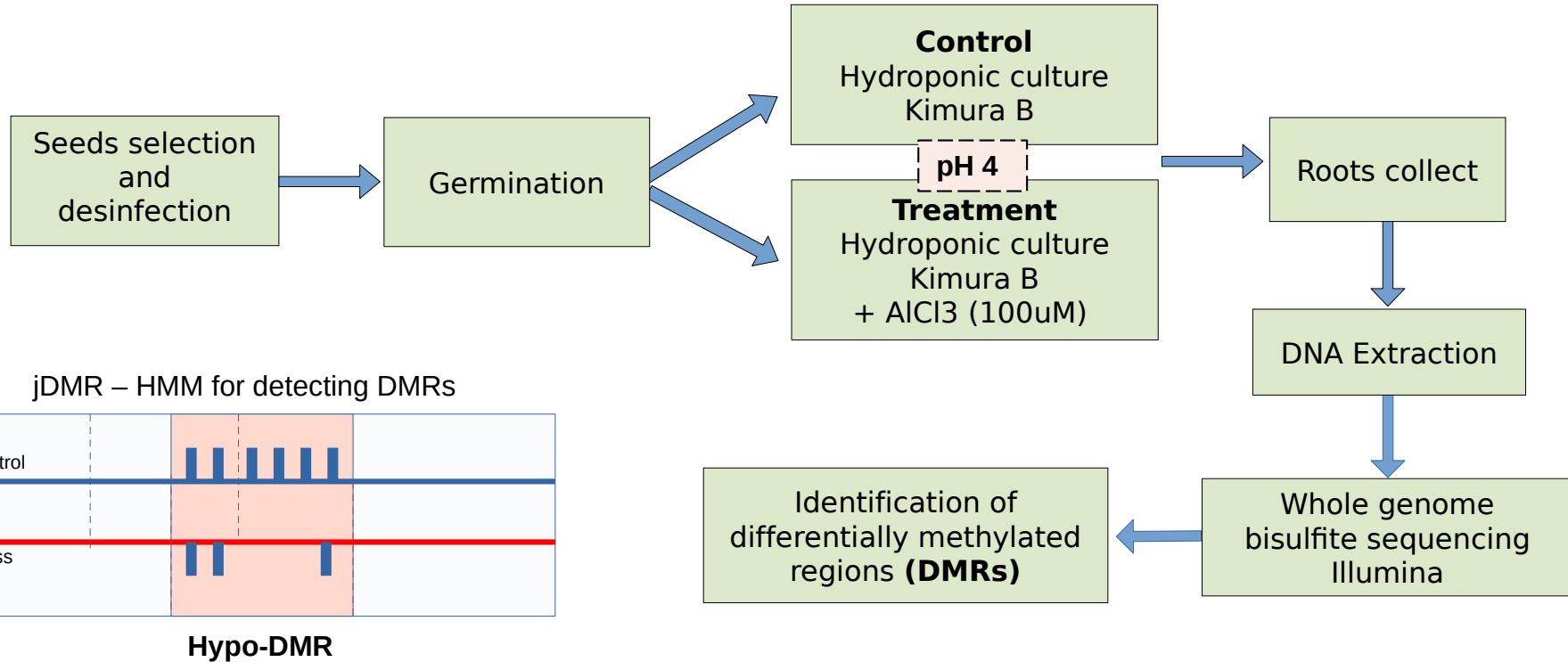
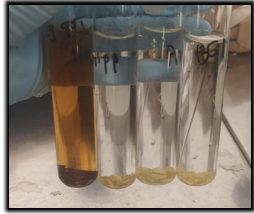
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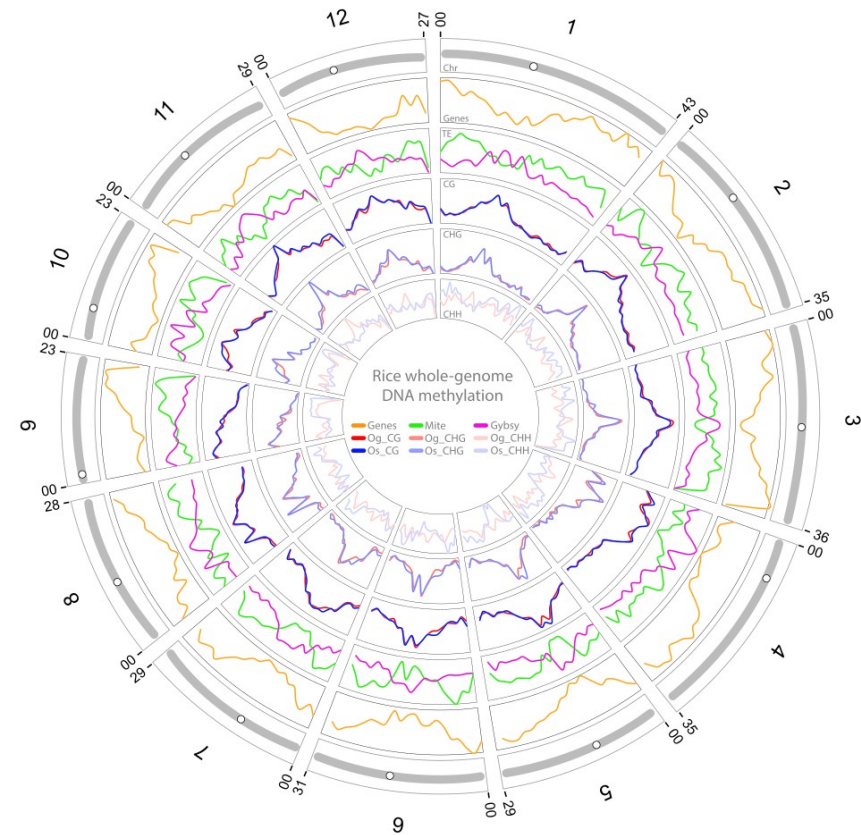
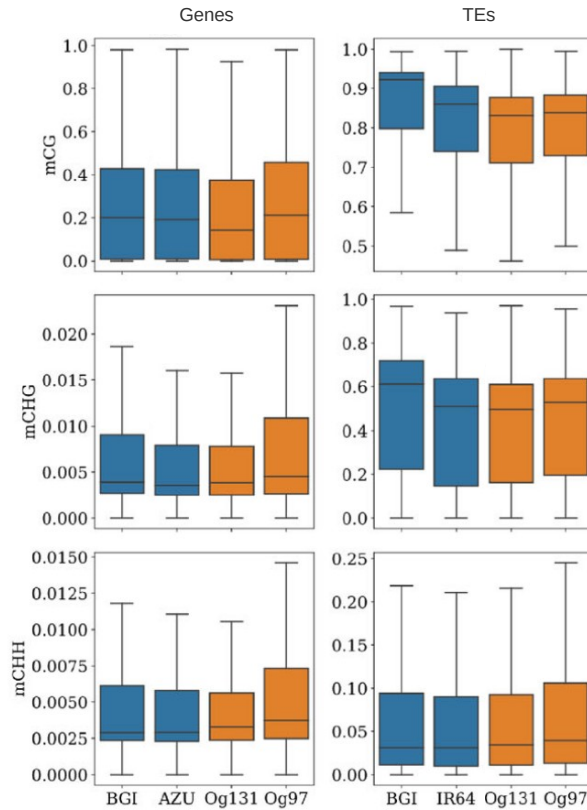
Experimental design



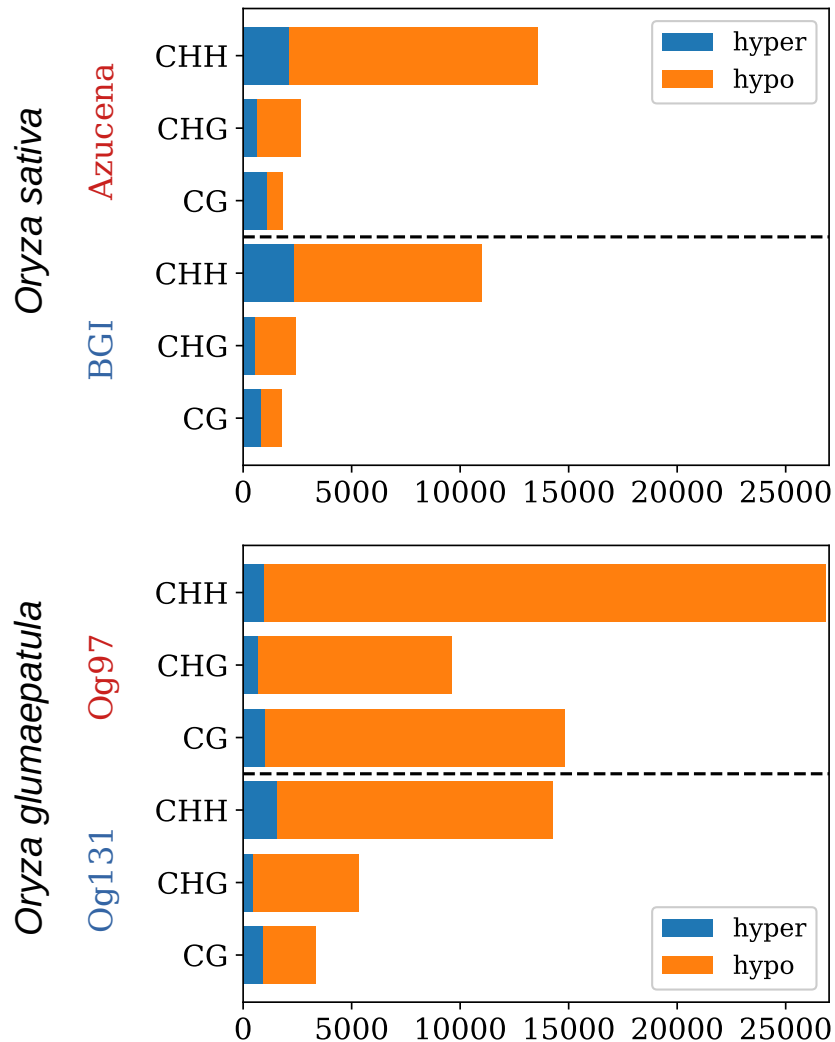
Experimental design



Genome-wide DNA methylation patterns in cultivated and wild rice



General methylation patterns for both rice species are conserved

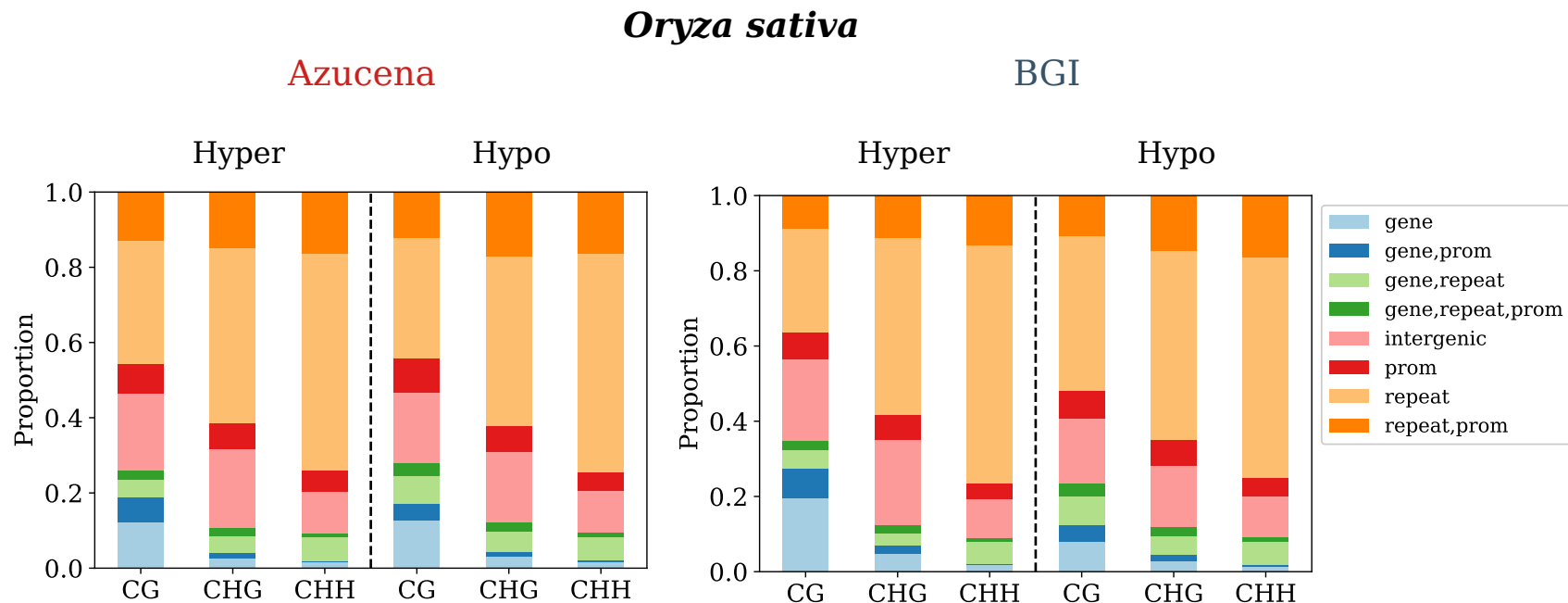


Number of differentially methylated regions under aluminum stress conditions

Hypomethylation under stress conditions

- Mainly Hypo-methylation for CHH sequence context
- Higher number of DMRs in the wild species
- Mainly Hypo-methylation for tolerant varieties

Where are located the DMRs along the genome for *O. sativa*?



Similar trend between hyper and hypo-methylation

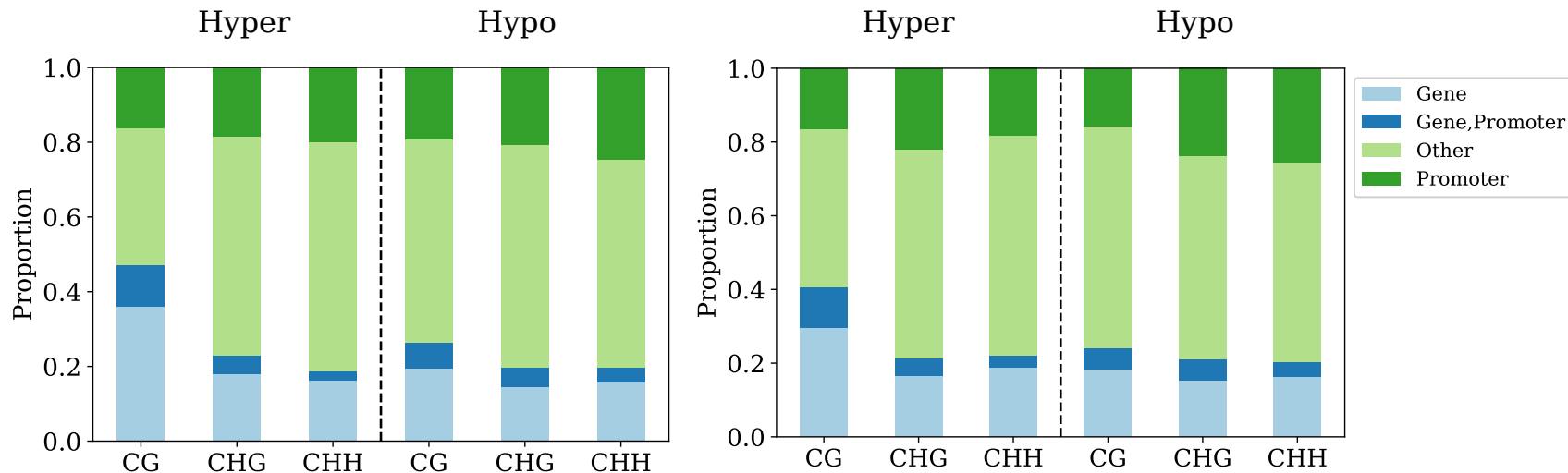
Similar trend between tolerant and susceptible varieties

Where are located the DMRs along the genome for *O. sativa*?

Oryza glumaepatula

Og97

Og131

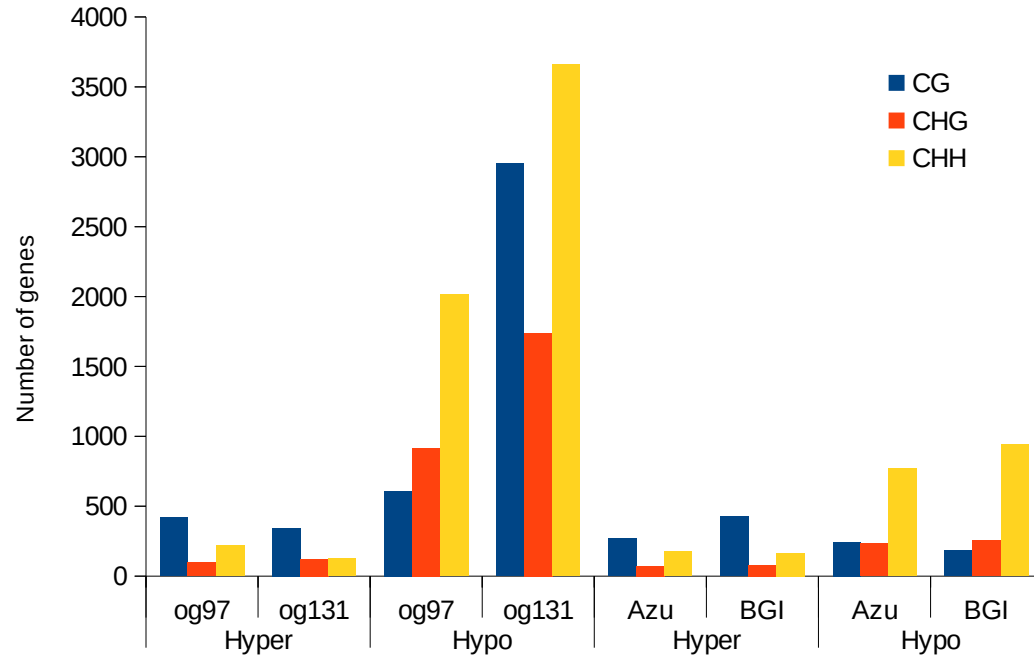


Similar trend between hyper and hypo-methylation

Similar trend between tolerant and susceptible varieties

There is a pattern of variation in the genome

DMR-associated genes for *O. sativa* and *O. glumaepatula*



15 hyper-methylated genes
for both *O. sativa* and *O. glumaepatula*

384 hypo-methylated genes
for both *O. sativa* and *O. glumaepatula*

Hyper-methylation in the CG context and hypo-methylation in the CHH context

DMR-associated genes previously reported as aluminum-responsive genes

- **OsFRDL4**

Hypo-methylated CG

Og131

- **OsALMT4**

Hypo-methylated CG

Og131

- **STAR1**

Hypo-methylated CHH

Azucena and Og131

DMR-associated genes previously reported as aluminum-responsive genes

- OsFRDL4

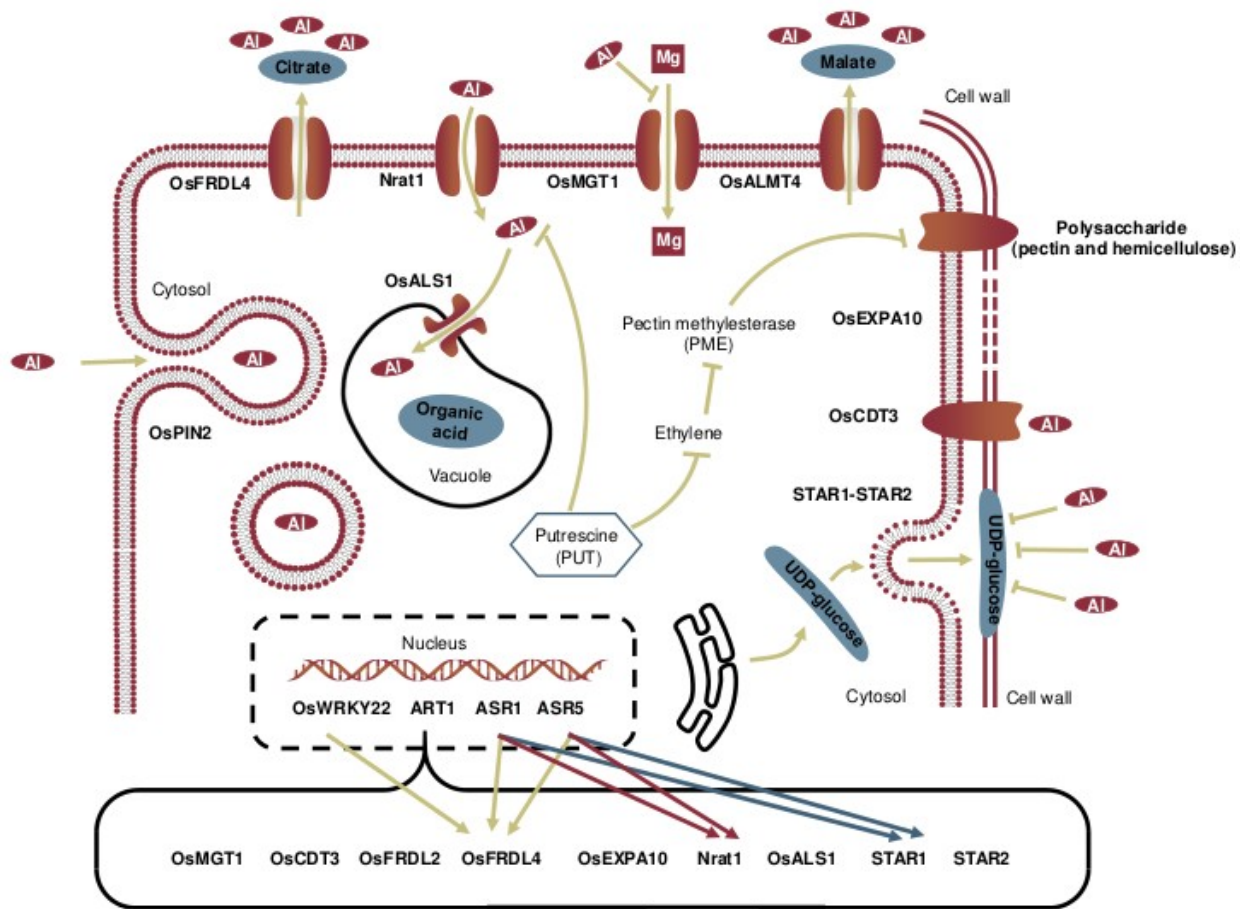
Hypo-methylated CG
Og131

- OsALMT4

Hypo-methylated CG
Og131

- STAR1

Hypo-methylated CHH
Azucena and Og131



DMR-associated genes previously reported as aluminum-responsive genes

- **OsFRDL4**

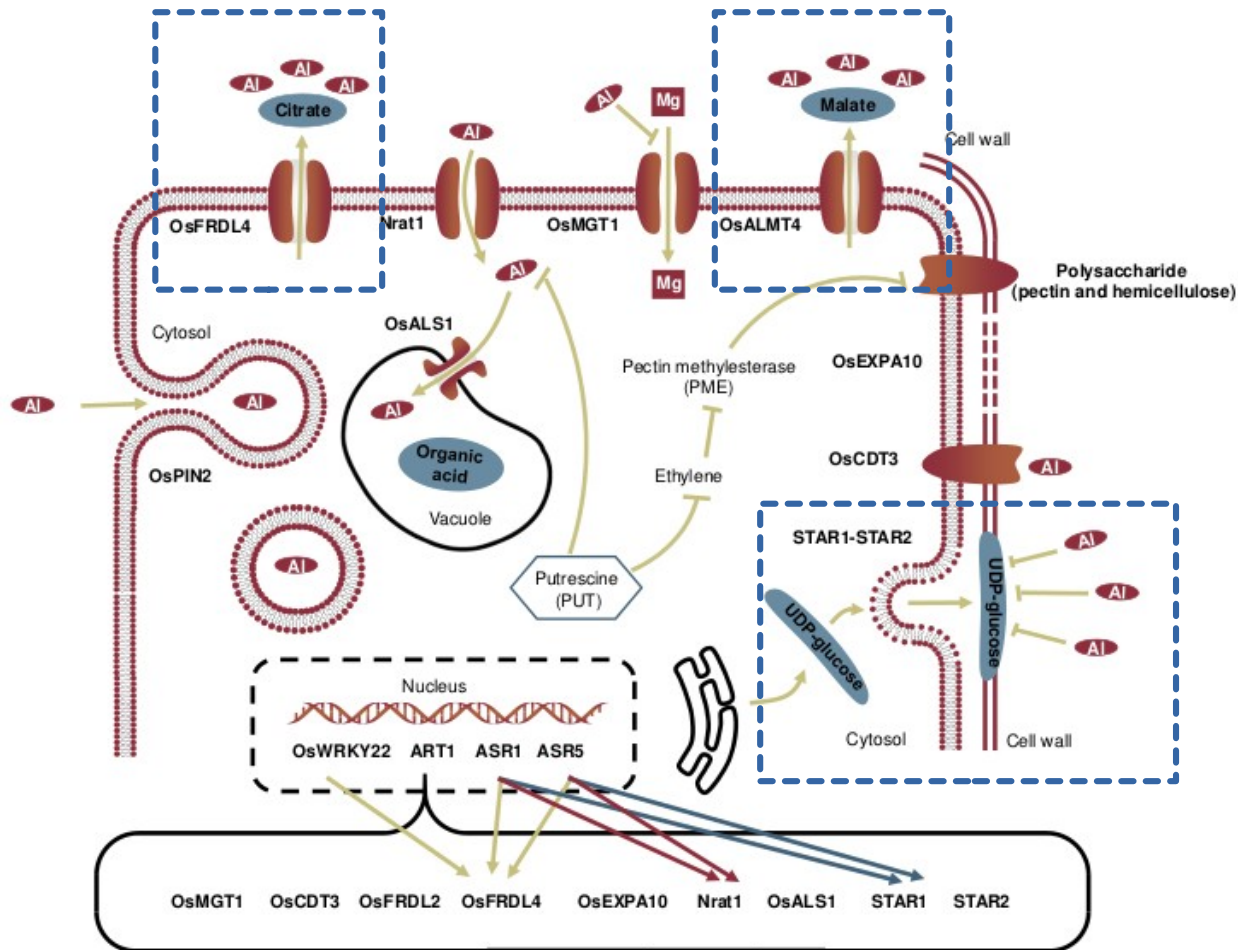
Hypo-methylated CG
Og131

- **OsALMT4**

Hypo-methylated CG
Og131

- **STAR1**

Hypo-methylated CHH
Azucena and Og131



DMR-associated genes previously reported as aluminum-responsive genes

-OsNRAMP6

Hypo-methylated
CHH for BGI and Og131

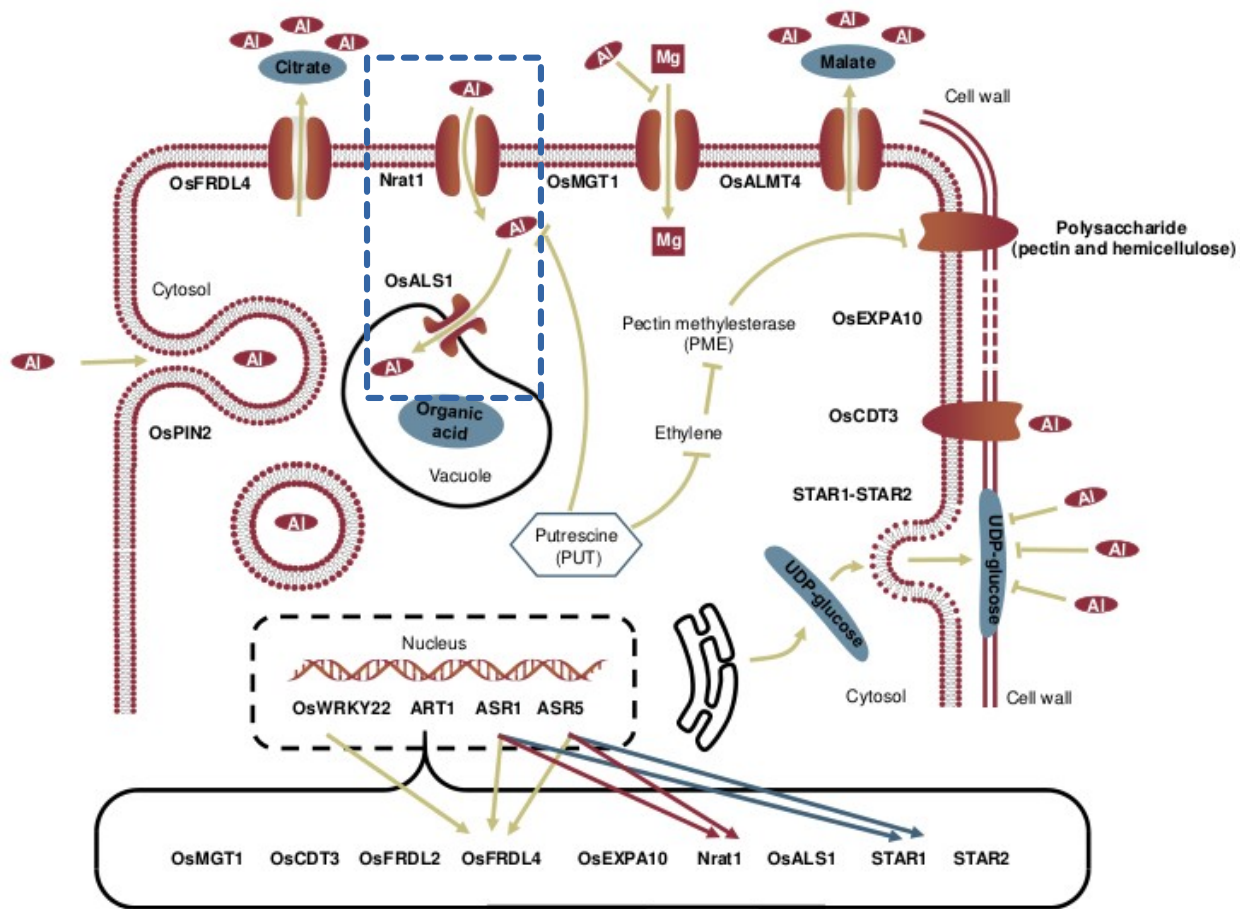
-OsNRAMP3

Hypo-methylated
CG, CHG, CHH Og131

Hyper-methylated
CG for BGI

-OsNRAMP4 - Nrat1

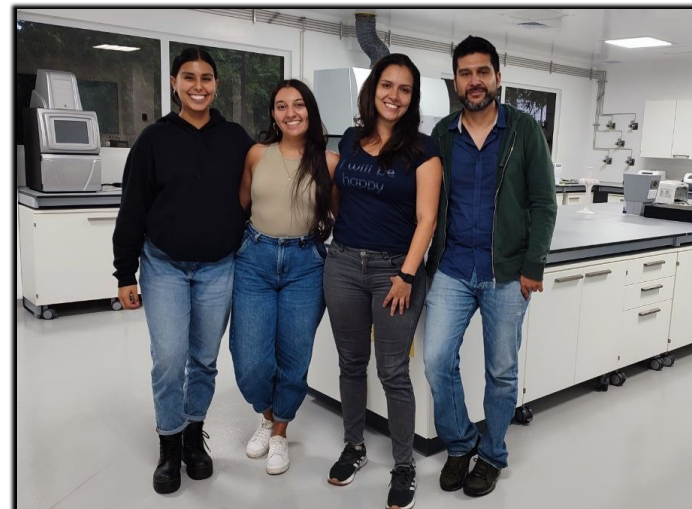
Hyper-methylated
CG og131



Conclusions and perspectives

- The methylation patterns associated with genes and TEs for both rice species are conserved.
- General hypo-methylation landscape under aluminum exposure in rice.
- The pattern of distribution of DMRs along the genome is similar for all the varieties analyzed
- There are genes potentially regulated through epigenetics that have been previously related to Al-tolerance in rice.
- Do these changes in methylation patterns affect gene expression?

Acknowledgments



Acknowledgments

Aliados



Apoyan



Thanks !

References

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- Nevo, Y., and Nelson, N. (2006). The NRAMP family of metal-ion transporters. Biochim. Biophys. Acta 1763: 609–620.
- Xia, J., Yamaji, N., Kasai, T., and Ma, J.F. (2010). Plasma membrane-localized transporter for aluminum in rice. Proc. Natl. Acad. Sci. USA 107: 18381–18385.

DMR-associated genes previously reported as aluminum-responsive genes

- **OsFRDL4**

Hypo-methylated CG
Og131



OsFRDL4 encodes a multidrug and toxin extrusion (MATE) transporter that mediates root citrate release (Yokosho et al., 2011)

- **OsALMT4**

Hypo-methylated CG
Og131

Al-activated release of organic acids from the root is a major physiological mechanism of plant Al resistance (Kochian et al., 2015)

- **STAR1**

Hypo-methylated CHH
Azucena and Og131

DMR-associated genes previously reported as aluminum-responsive genes

- **OsFRDL4**

Hypo-methylated CG
Og131

- **OsALMT4**

Hypo-methylated CG
Og131



Aluminum-activated malate transporter (ALMT)

family genes encode anion channels in plants

OsALMT4 protein localizes to the plasma membrane. Overexpression of OsALMT4 can increase the efflux of malate in rice roots and the Al tolerance

- **STAR1**

Hypo-methylated CHH
Azucena and Og131

DMR-associated genes previously reported as aluminum-responsive genes

- **OsFRDL4**

Hypo-methylated CG
Og131

- **OsALMT4**

Hypo-methylated CG
Og131

- **STAR1**

Hypo-methylated CHH
Azucena and Og131



STAR1 and **STAR2** encode an ATP-binding domain and a membrane-binding domain, respectively.

The STAR1-STAR2 complex localizes at the vesicles and transports UDP-glucose, which may be involved in cell wall modification, resulting in decreased Al accumulation in the cell wall (Huang et al, 2012).

DMR-associated genes previously reported as aluminum-responsive genes

-OsNRAMP6

Hypo-methylated
CHH for BGI and Og131

-OsNRAMP3

Hypo-methylated
CG, CHG, CHH Og131

Hyper-methylated
CG for BGI

-OsNRAMP4 (Nrat1)

Hyper-methylated
CG og131

Nramp family members function as proton-coupled metal ion transporters that can transport Mn^{2+} , Zn^{2+} , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Co^{2+} , and Al^{3+} (Nevo and Nelson, 2006; Xia et al., 2010)

DMR-associated genes previously reported as aluminum-responsive genes

-OsNRAMP6

Hypo-methylated
CHH for BGI and Og131

-OsNRAMP3

Hypo-methylated
CG, CHG, CHH Og131

Hyper-methylated
CG for BGI

-OsNRAMP4 (Nrat1)

Hyper-methylated
CG og131



Nramp family members function as proton-coupled metal ion transporters that can transport Mn^{2+} , Zn^{2+} , Cu^{2+} , Fe^{2+} , Cd^{2+} , Ni^{2+} , Co^{2+} , and Al^{3+} (Nevo and Nelson, 2006; Xia et al., 2010)

Nrat1 transports trivalent Al (Xia et al, 2010), which is required for sequestration of Al into the vacuoles for final detoxification.