Whole genome methylation patterns of *Oryza sativa* L. and *Oryza glumaepatula* Steud. genotypes with contrast response to aluminum stress

Gallo-Franco J.J., Ghneim-Herrera, T., Tobar-Tosse, F. & Quimbaya, M.

Virtual Symposium in Plant Omics Sciences
November 23-27
2020

Pontificia Universidad
JAVERIANA
Cali

ómicas

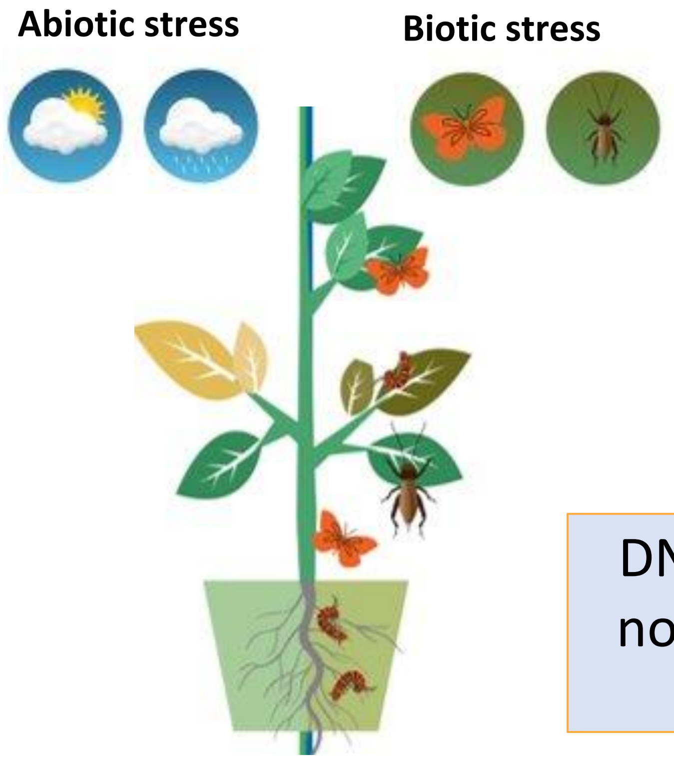
Introduction

Plants can respond and adapt to environmental conditions

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

(Thiebaut et al. 2019)

Plants can respond and adapt to environmental conditions

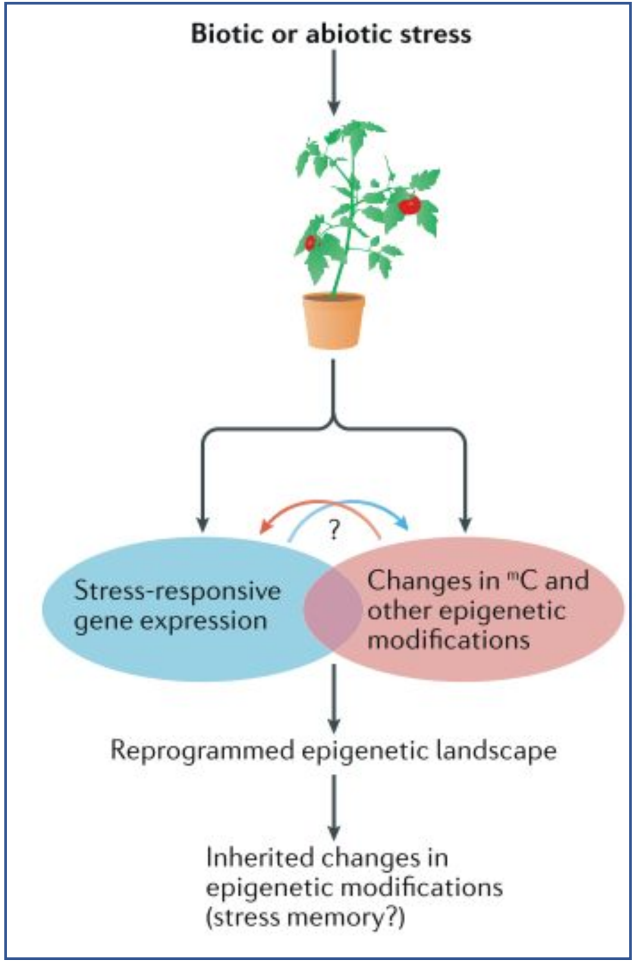


- Genetic information
- Signal transduction
- Modulation of transcription
- **Epigenetics**

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

(Thiebaut et al. 2019)

Epigenetic changes in response to stress conditions



Biotic or abiotic stress

Stress-responsive gene expression

?

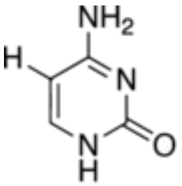
Changes in ^{m}C and other epigenetic modifications

Reprogrammed epigenetic landscape

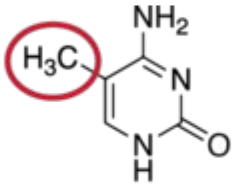
Inherited changes in epigenetic modifications (stress memory?)

Zhang et al. 2018

DNA methylation



Cytosine



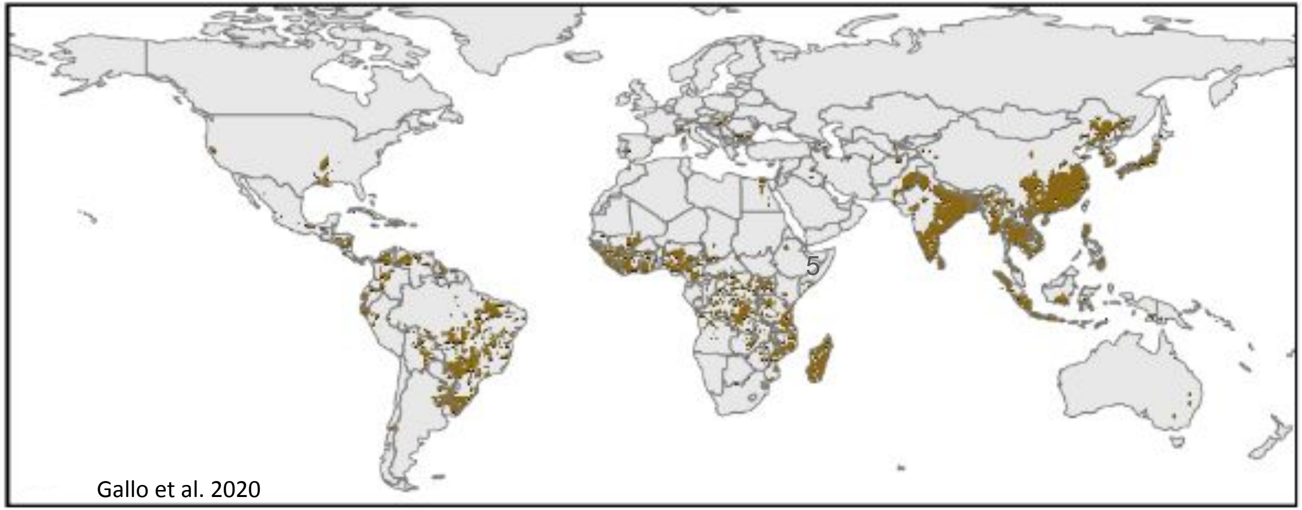
methylated Cytosine

¿ 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*

***Oryza sativa* y**
Oryza glumaepatula



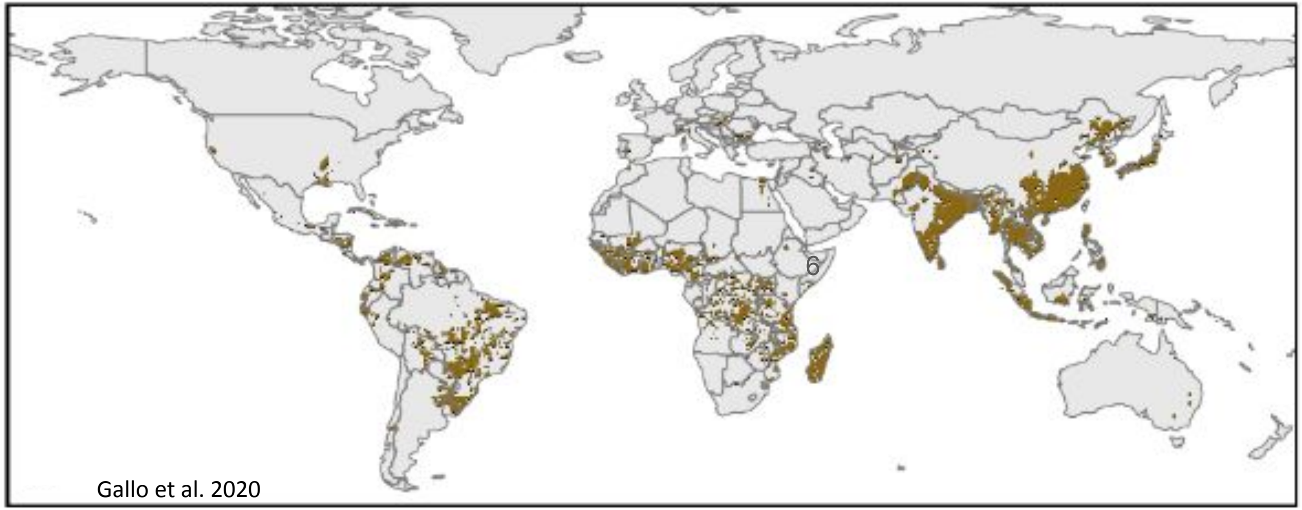
Gallo et al. 2020

¿ Why study the methylome of rice crops?

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

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

***Oryza sativa* y**
Oryza glumaepatula



Gallo et al. 2020

¿ Why study aluminum toxicity ?

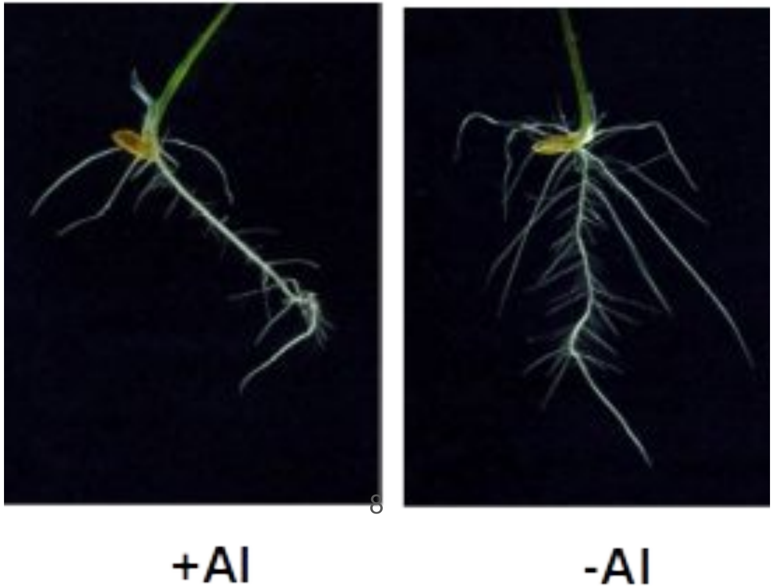
- Aluminum (Al) is the third **most common** element in the world (~ 7%).

- 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.**

(Famoso et al. 2010)

Effects of aluminum on cereal physiology



(Famoso et al. 2010)

Epigenetics play a role in regulating the response of rice plants to stress conditions

In rice plants, does **epigenetics** participate as a **regulatory factor** in the response to **aluminum stress?**

Are there differential methylation patterns?

Are they different **between species** of rice?

Are the **changes in methylation pre-established** in the tolerant varieties with respect to the susceptible ones or do they occur in response to the stress condition?

Control Al^{3+} stress

Materials and methods

Cultivo de plantas control

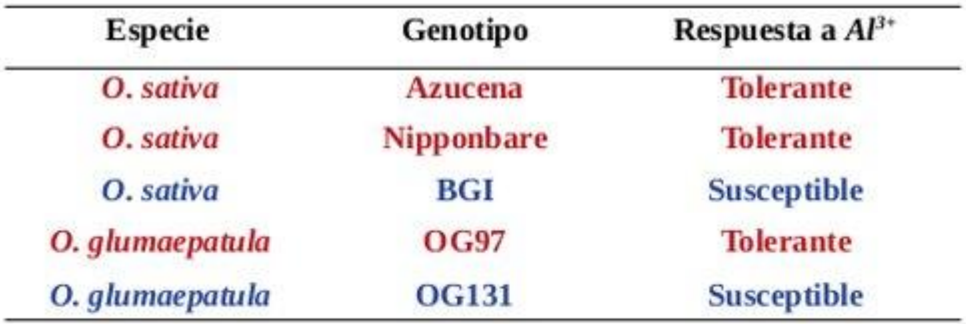
Extracción de ADN

Análisis de calidad del ADN

Secuenciación por bisulfito

Análisis de datos

Rice genotypes

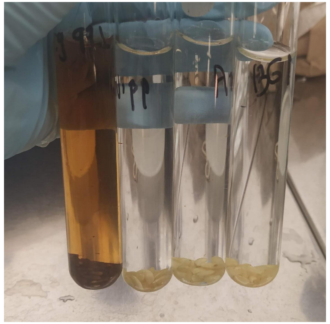


Especie	Genotipo	Respuesta a Al^{3+}
O. sativa	Azucena	Tolerante
O. sativa	Nipponbare	Tolerante
O. sativa	BGI	Susceptible
O. glumaepatula	OG97	Tolerante
O. glumaepatula	OG131	Susceptible

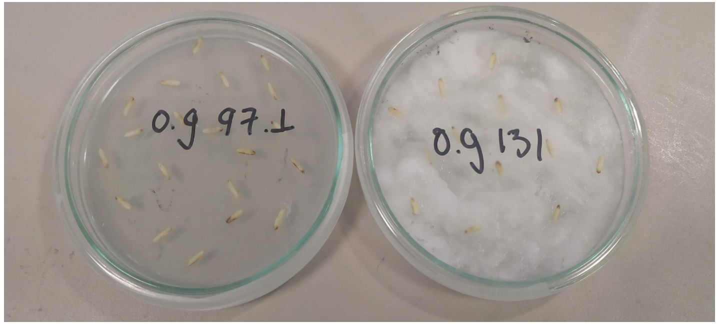
Materials y methods

Plants culture

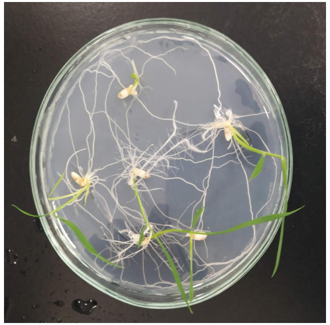
Seed disinfection

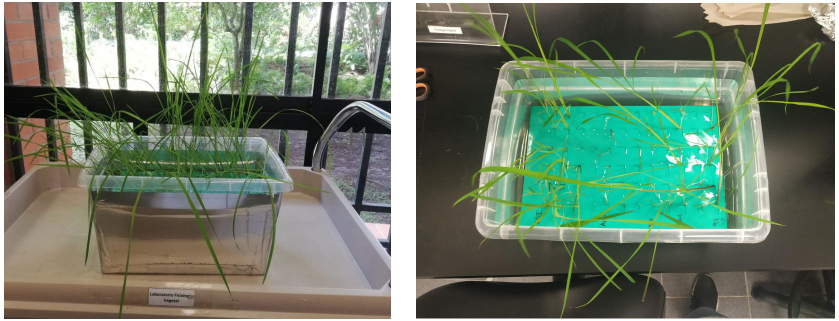


3 days at 56°C - Darkness

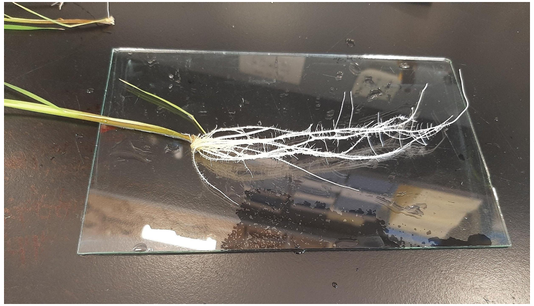


7 days- Light 12:12 h

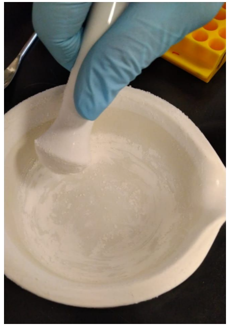




Hydroponic culture
Kimura B – Arnon micronutrients



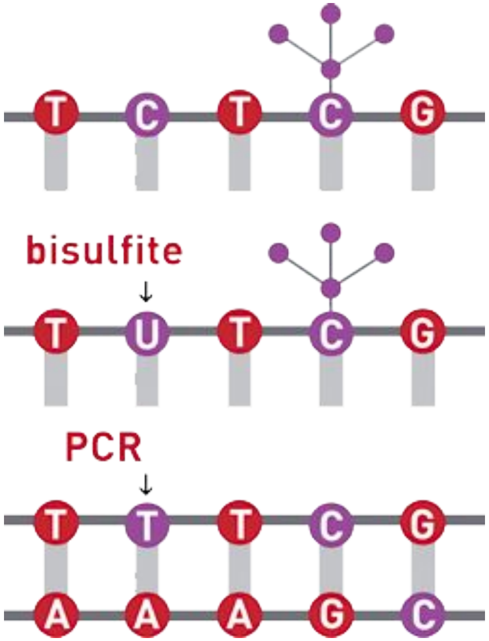
Root collection
Storage at –80°C



DNA extraction
CTAB method

Materials y methods

Whole genome bisulfite sequencing (WGBS)

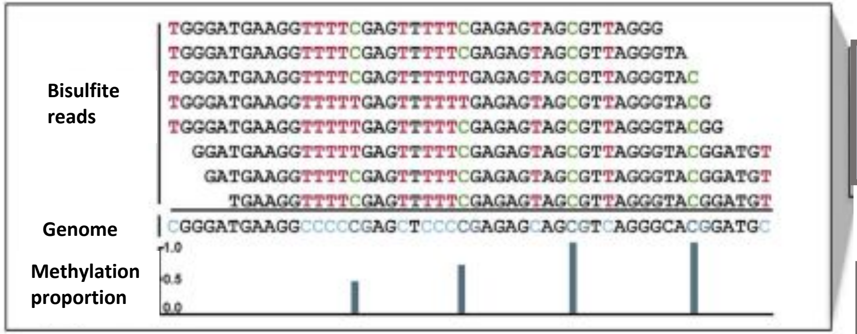


- **Unmethylated cytosine** nucleotides are converted to uracil, which are identified as **thymines (T)** when sequenced.

- **Methylated cytosines** are protected from conversion, thus they are still identified as **cytosine (C).**

Bisulfite sequencing data analysis

Sequencing, quality check and remove adapters → **Align to the genome Bowtie2** → **Remove PCR bias (Deduplication)** → **Methylation calling** → **Coverage filtering** → **Visualization and Clustering** → **Diferential methylation** → **Results as tables, summary statistics, BED files**



Bismark v0.22.3

Babraham Bioinformatics

(Krueger & Andrews, 2011)

Methylkit v.1.15

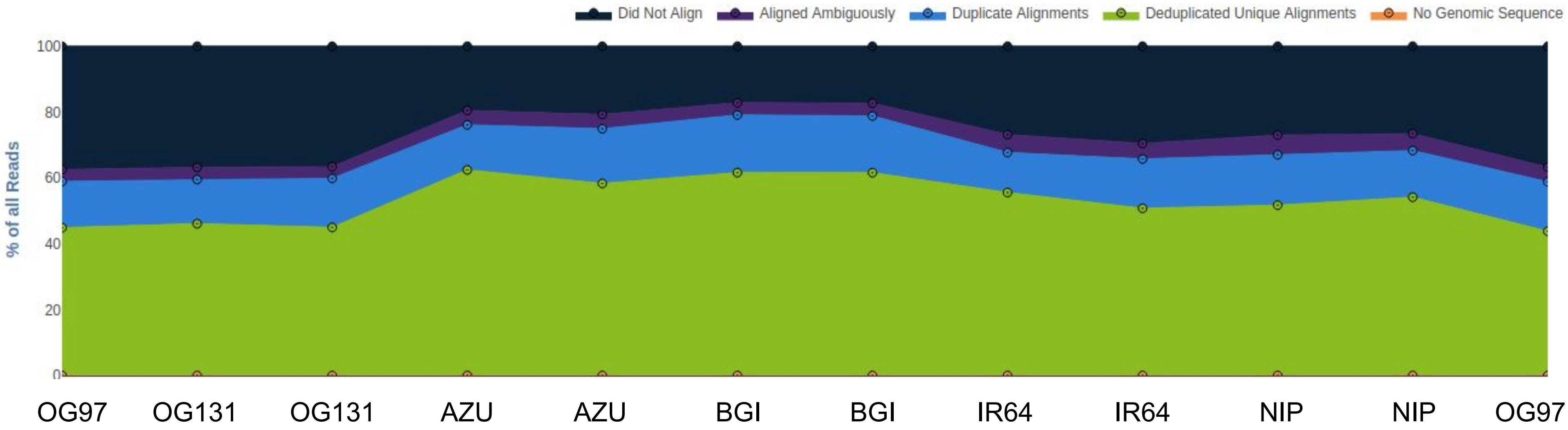
methylKit

(Akalin et al., 2012)

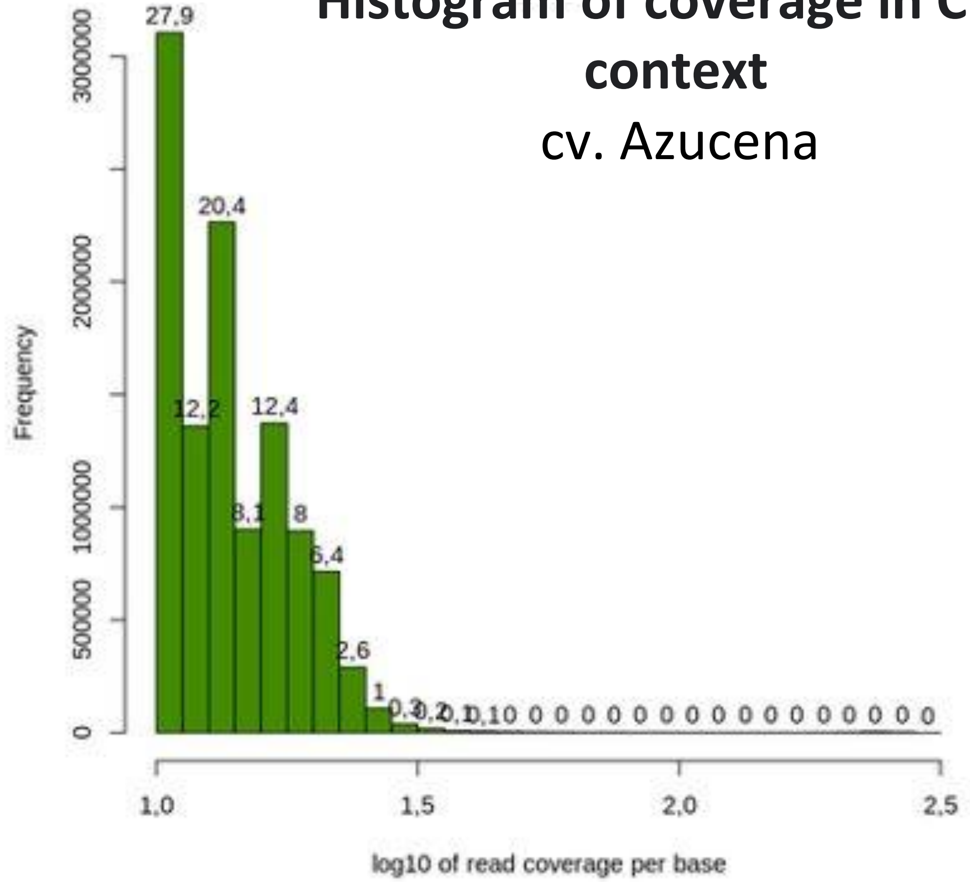
Results and Discussion

Bismark alignment statistics

Results obtained after removing duplicate sequences



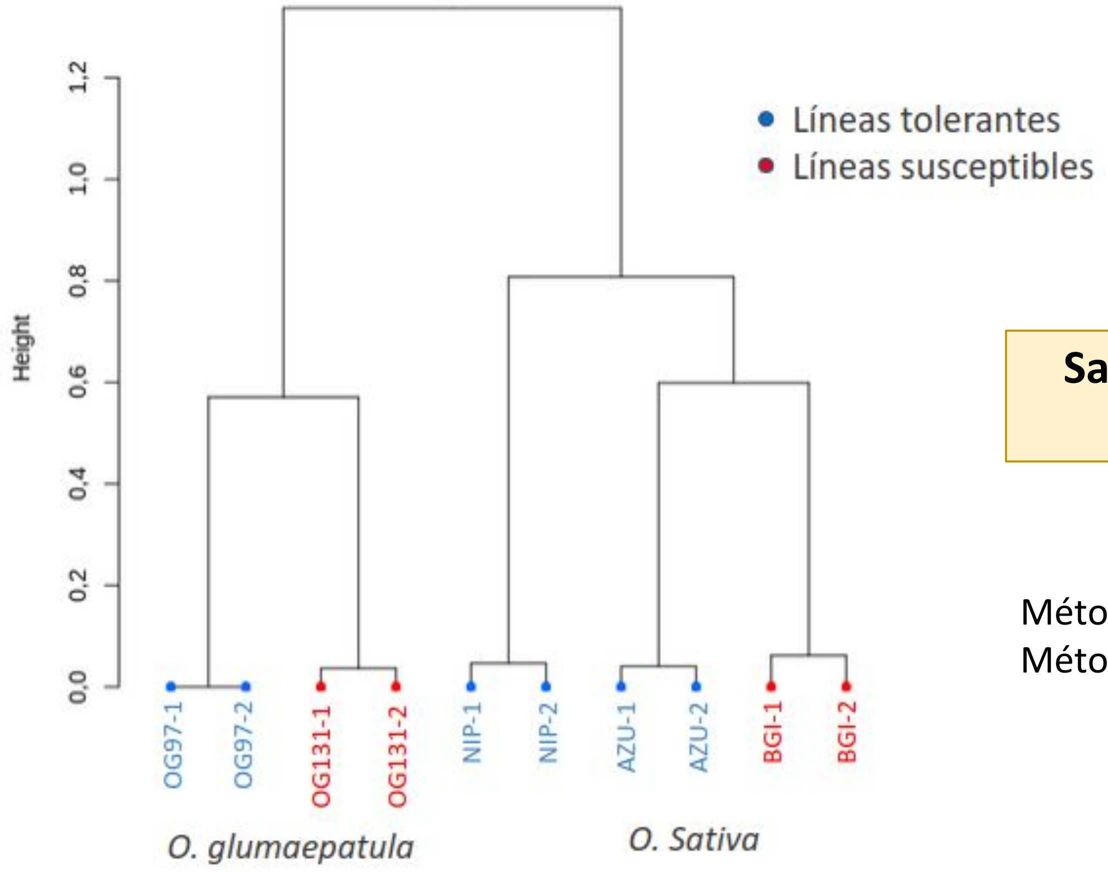
Histogram of coverage in CpG context
cv. Azucena



Methylation statistics in the CpG context

Experiments with high PCR duplication bias will have a secondary peak towards the right side of the histogram.

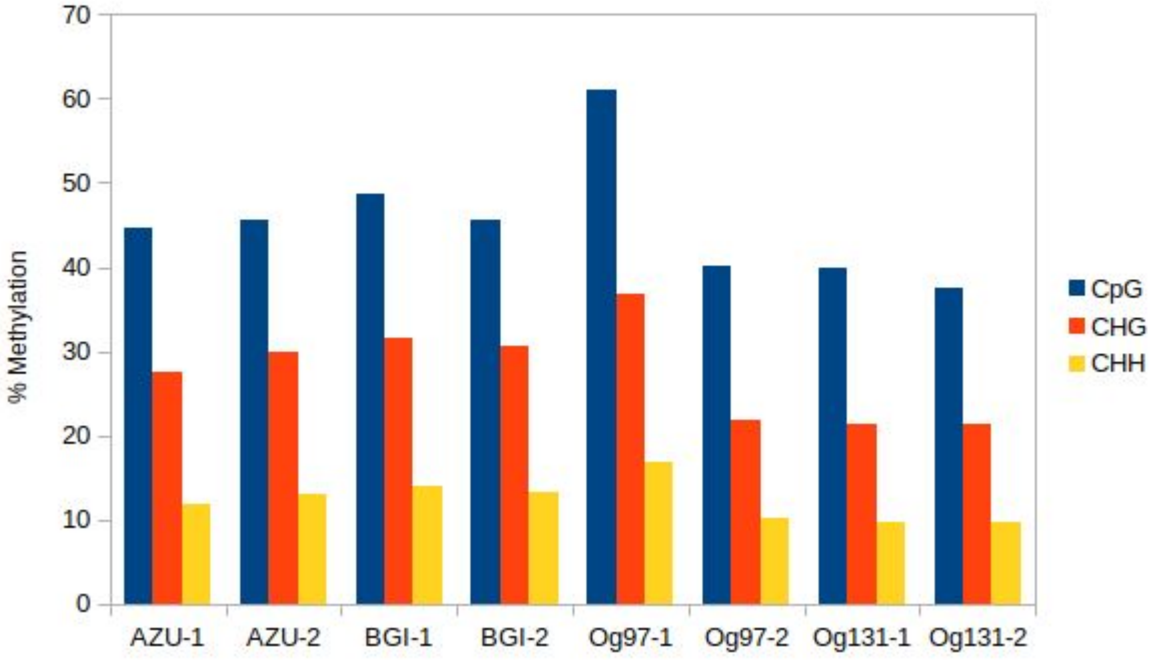
Sample correlation - CpG Context



Same pattern with CHG y CHH methylation

Método de distancia: 1 - Pearson
Método de agrupamiento: Ward

Relative frequency of methylcytosines (mCs) identified in each sequence context

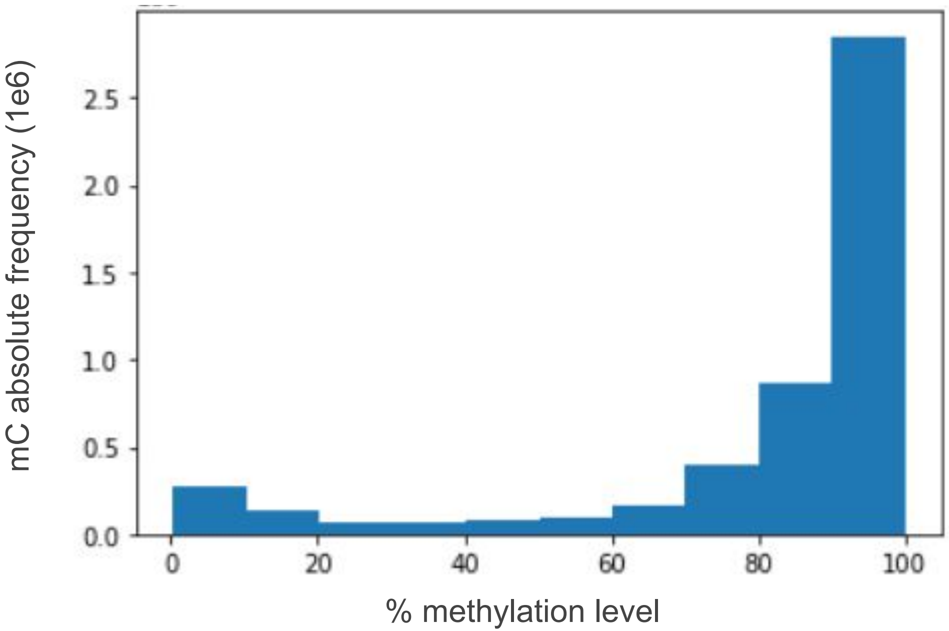


Greater mCs in CpG context

Histogram of methylation percentage in CpG context

cv. Azucena

Methylation statistics in the CpG context

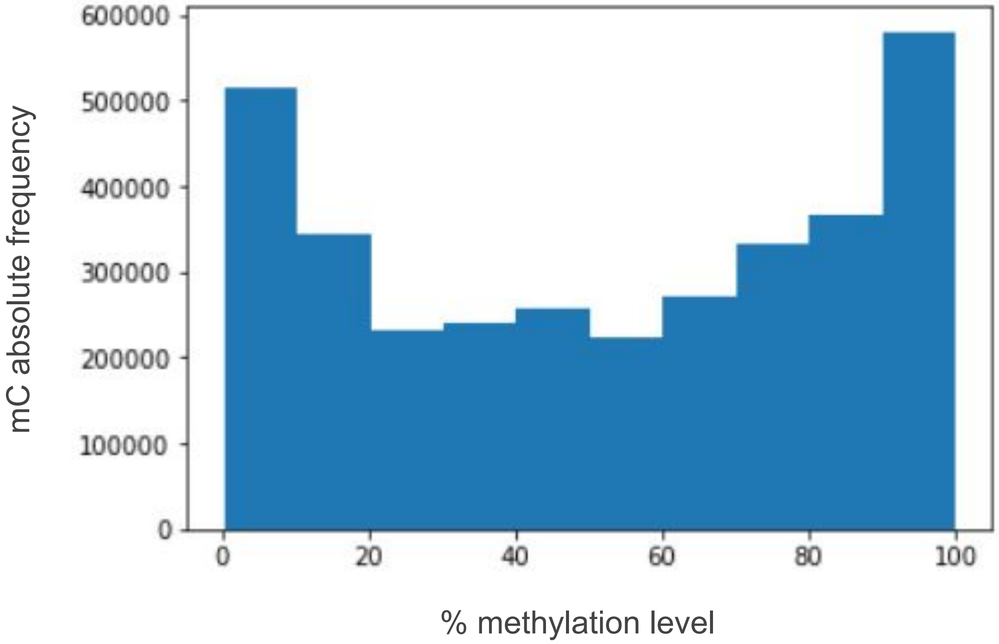


High levels of methylation in most mCs

Histogram of methylation percentage in CHG context

cv. Azucena

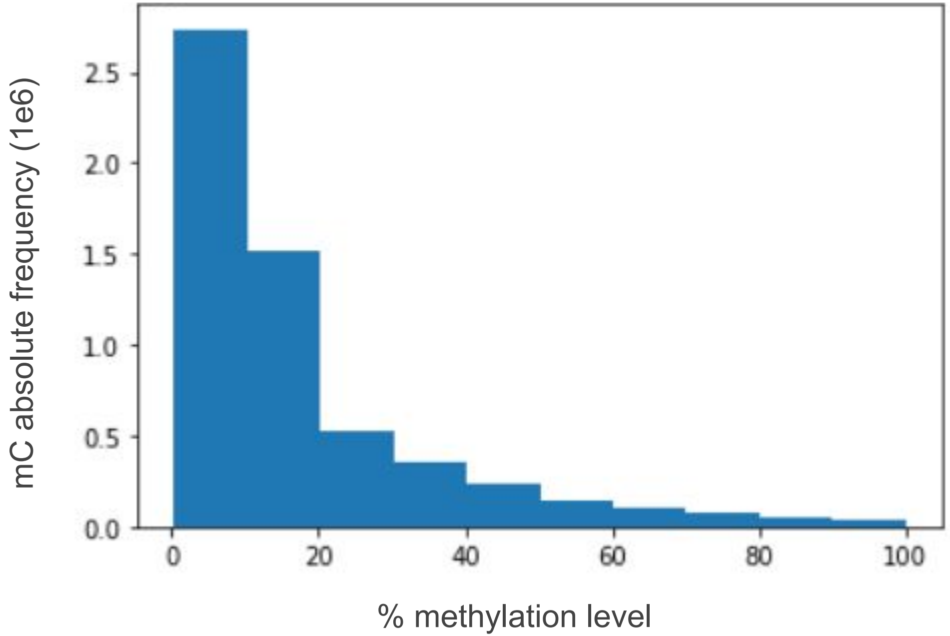
Methylation statistics in the CHG context



Low to high methylation levels in mCs

Histogram of methylation percentage in CHH context

cv. Azucena



Methylation statistics in the CHH context

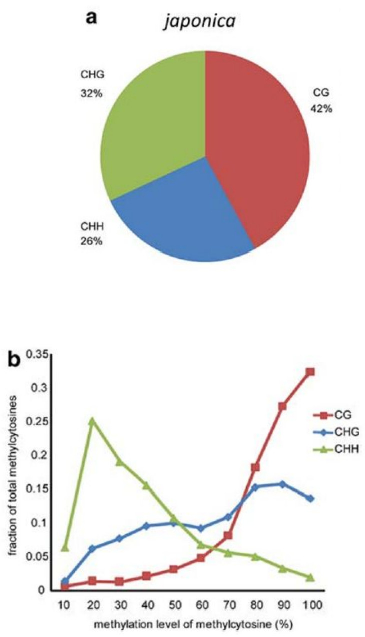
Low levels of methylation in most mCs

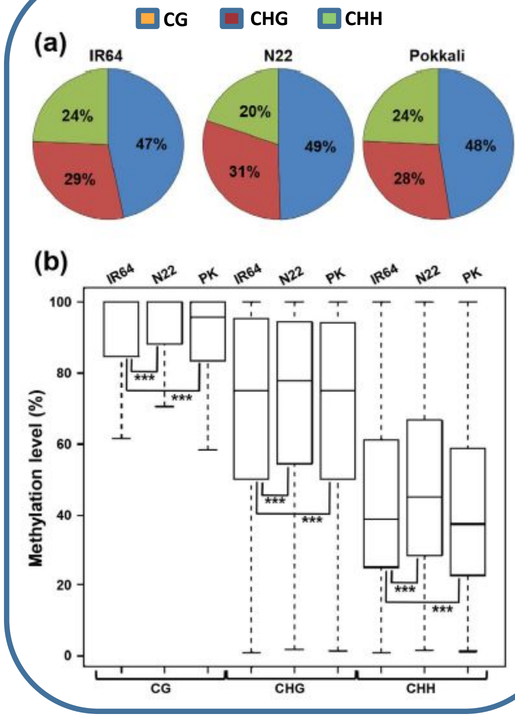
General methylome pattern in rice plants.

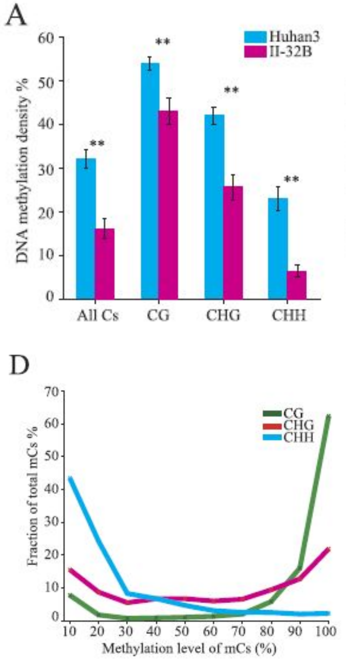
Li et al. 2012

Garg et al. 2015

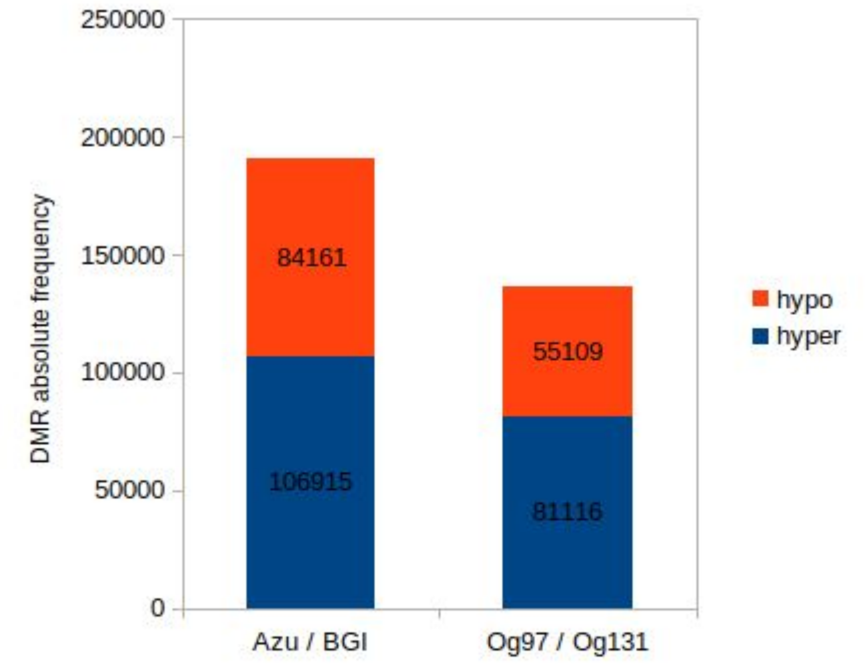
Zheng et al. 2017







Number of differentially methylated regions between tolerant and susceptible varieties



Mainly hypermethylation in tolerant genotypes

Greater number of DMRs in *o. glumaepatula*

Azucena (Tolerant) vs.
BGI (Susceptible)

Og97 (Tolerant) vs.
Og131 (Susceptible)

How do methylation patterns change in response to stress?

Garg et al. 2015

In **drought-tolerant** plants, **greater hypermethylation** occurs under normal conditions.

Feng et al. 2016

Higher number of **hypermethylated genes** when plants were **subjected to stress.**

Specific DNA methylation sites can be modified by **exposure to Cd.**

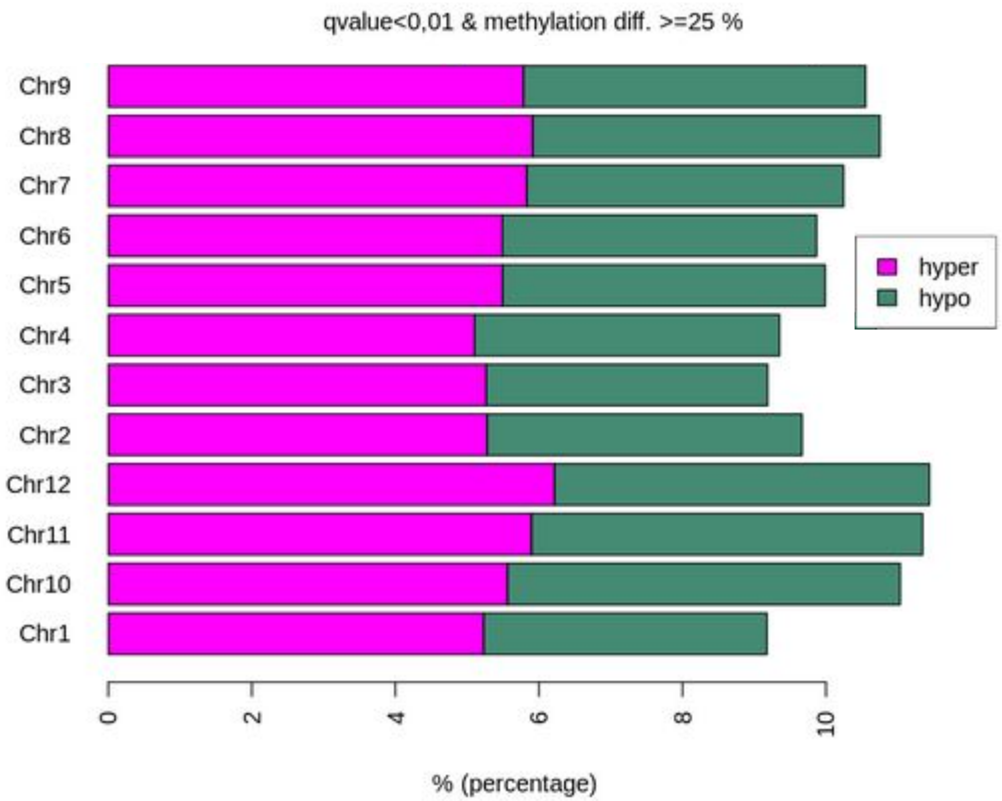
Wang et al. 2016

Most of the DMRs were observed in the **drought-susceptible** variety IR64. Mainly hypermethylation

Zheng et al. 2017

Increased number of **hypermethylated DMPs** in response to stress.

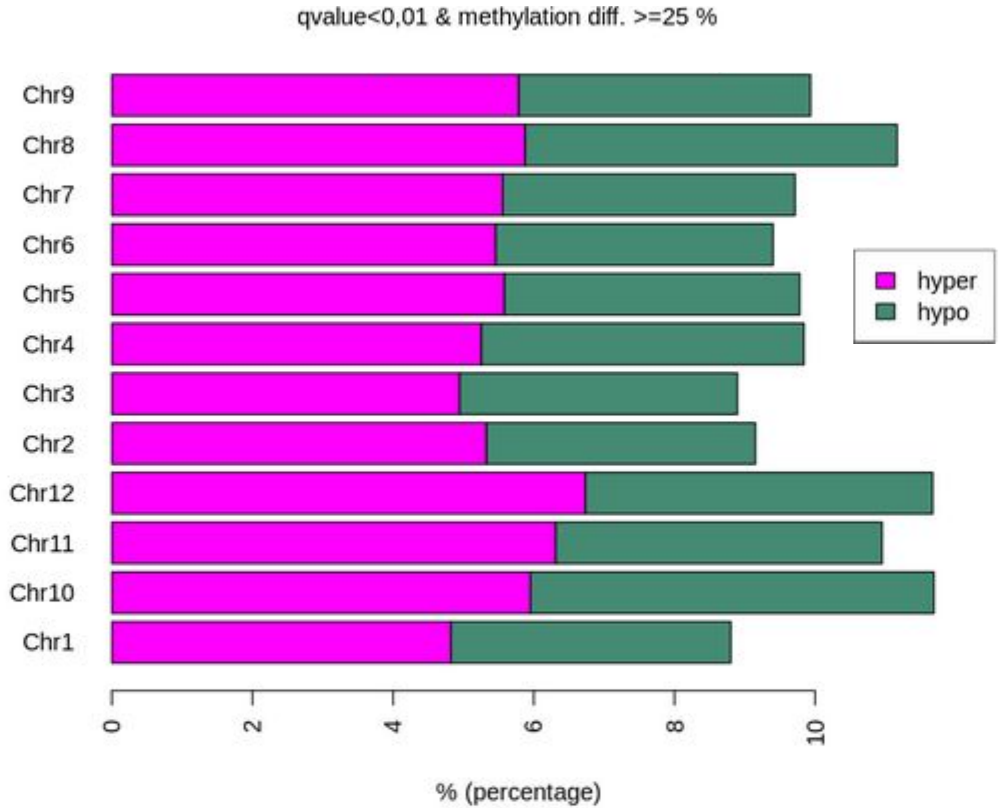
Percentage of hypo and hyper methylated regions per chromosome



Tolerant vs. susceptible genotypes

Both species included

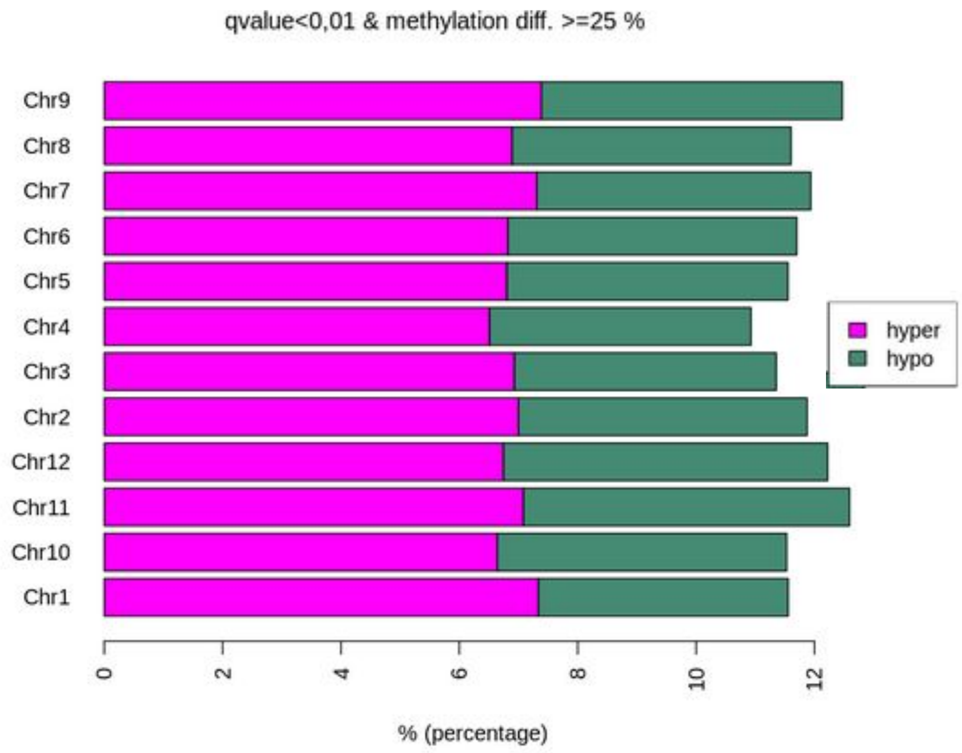
Percentage of hypo and hyper methylated regions per chromosome



Differentially methylated regions inside *O.sativa* genotypes

Azucena (Tolerant)
vs.
BGI (Susceptible)

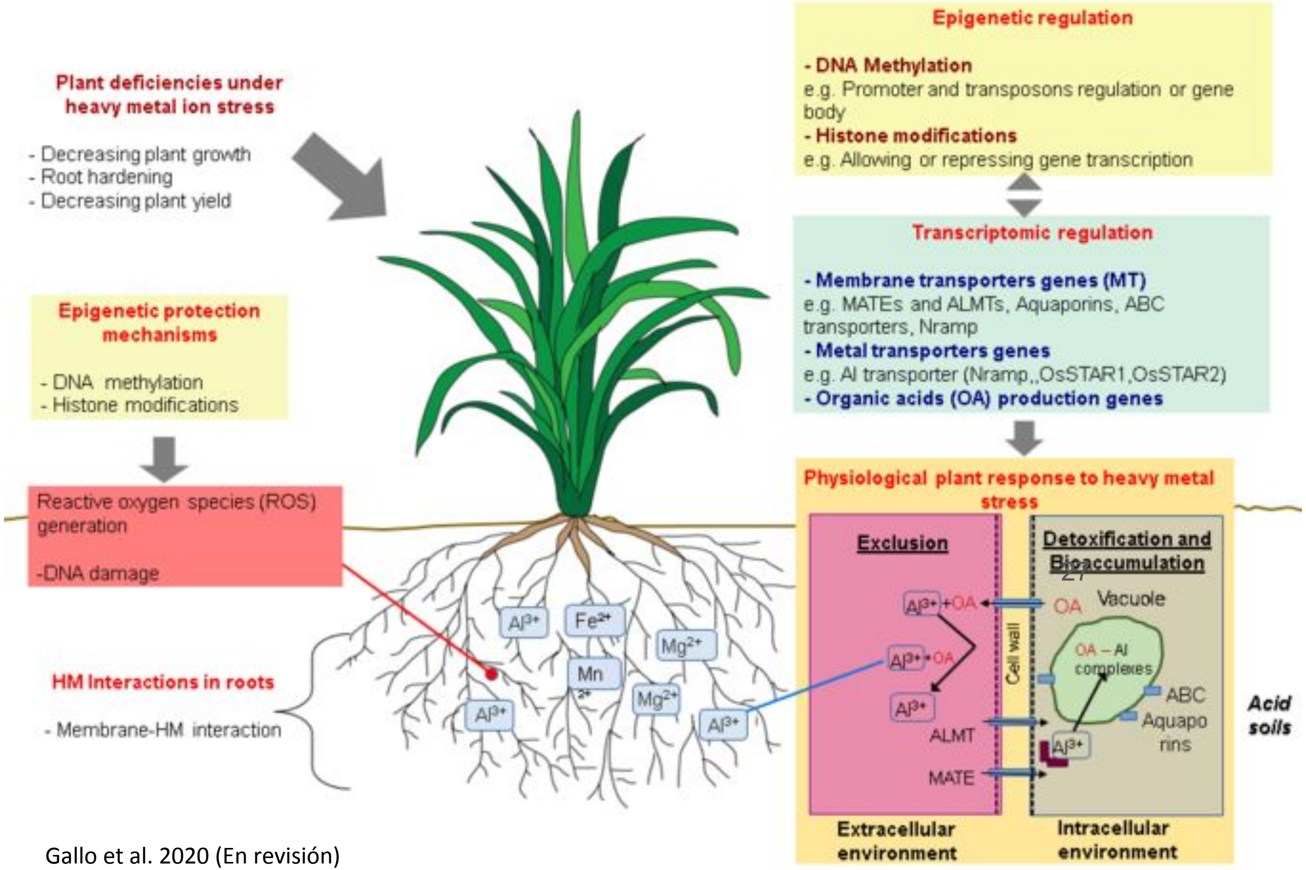
Percentage of hypo and hyper methylated regions per chromosome



Differentially methylated regions inside *O. glumaepatula* genotypes

Og97 (Tolerant)
vs.
Og131 (Susceptible)

Epigenetics as a key regulatory factor of the aluminum stress response in rice



Plant deficiencies under heavy metal ion stress

- Decreasing plant growth
- Root hardening
- Decreasing plant yield

Epigenetic protection mechanisms

- DNA methylation
- Histone modifications

Reactive oxygen species (ROS) generation

-DNA damage

HM Interactions in roots

- Membrane-HM interaction

Epigenetic regulation

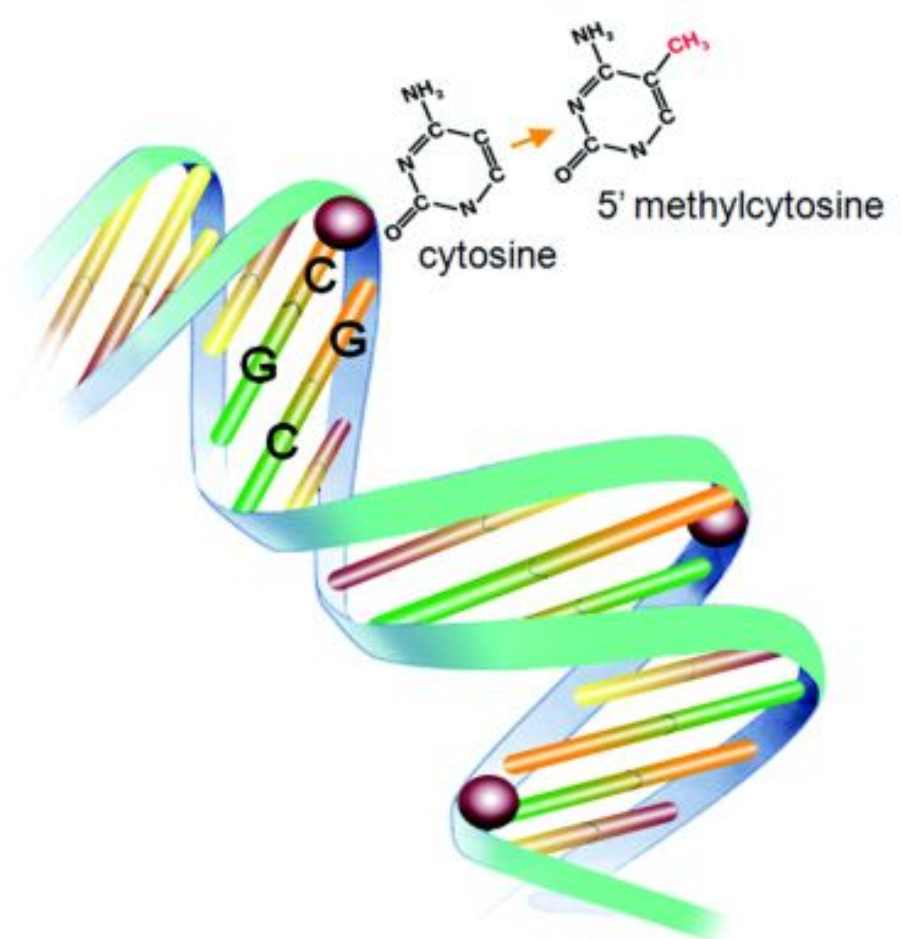
- **DNA Methylation**
  e.g. Promoter and transposons regulation or gene body
- **Histone modifications**
  e.g. Allowing or repressing gene transcription

Transcriptomic regulation

- **Membrane transporters genes (MT)**
  e.g. MATEs and ALMTs, Aquaporins, ABC transporters, Nramp
- **Metal transporters genes**
  e.g. Al transporter (Nramp,,OsSTAR1,OsSTAR2)
- **Organic acids (OA) production genes**

Physiological plant response to heavy metal stress

Gallo et al. 2020 (En revisión)



Gracias ...