Universitat Autònoma de Barcelona
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Data analysis

Epigenetic regulation in response to environmental changes in plants: The role of Polycomb activity

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INTRODUCTION

The Polycomb proteins (PcG) are important **epigenetic regulators** involved in gene repression. They form **Polycomb Repressive Complexes (PRC)** with various subunits.

PRC complexes **regulate stable or flexible gene repression in response to internal and environmental signals**.

PRC2 adds methyl groups to histone H3 at the lysine 27 position, **generating the H3K27me3 mark**. **LHP1 binds to this mark** on chromatin and helps recruit additional genetic repression complexes, **leading to the repression of gene expression in these regions**.

OBJECTIVES

There has been evidence of a **close relationship between LHP1 and H3K27me3**, which is catalyzed by PRC2. Additionally, **Polycomb** is **associated** with **homeotic and developmental changes**. In recent years, **climate change** has been affecting the planet more than ever, and **plants need to adapt to these changes**.

In this study, we will analyze whether there is **a relationship between Polycomb activity and the response to environmental stress in *Arabidopsis thaliana* using RNA-seq data**.

MATERIAL AND METHODS

Chip-seq and RNA-seq data from *A. thaliana*, Columbia ecotype, were used in this study. The data were sourced from various articles from **Veluchamy** and **Zhou** and the laboratory of **Dr. Jordi Moreno Romero**.

The **RNA-seq data** for **stress conditions** were obtained from the Arabidopsis RNA-seq Database. These data were processed using the **WebMeV web tool**. Based on their log2FC values, the lists of upregulated genes (UG) and downregulated genes (DG) were generated, which were used in this study.

In this analysis were also used ShinnyGO 0.77 to study **Gene Ontology (GO) categories**, the web Venny 2.1 to examine **gene overlapping** and several online bioinformatics tools to generate **metagene plots**, **heatmaps** and **boxplots**, in addition to Excel.

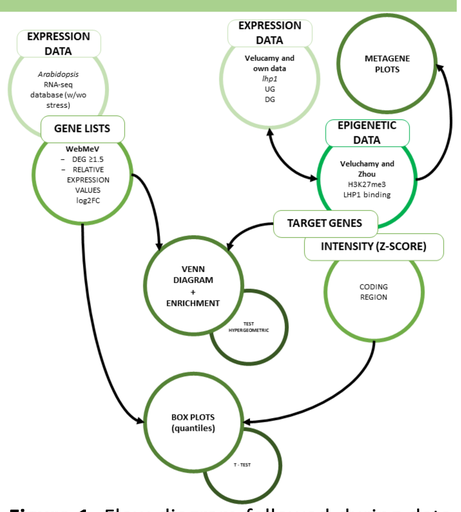


Figure 1. Flow diagram followed during data analysis.

RESULTS AND DISCUSSION

UG and DG LHP1 target genes are enriched in GO categories related to stress

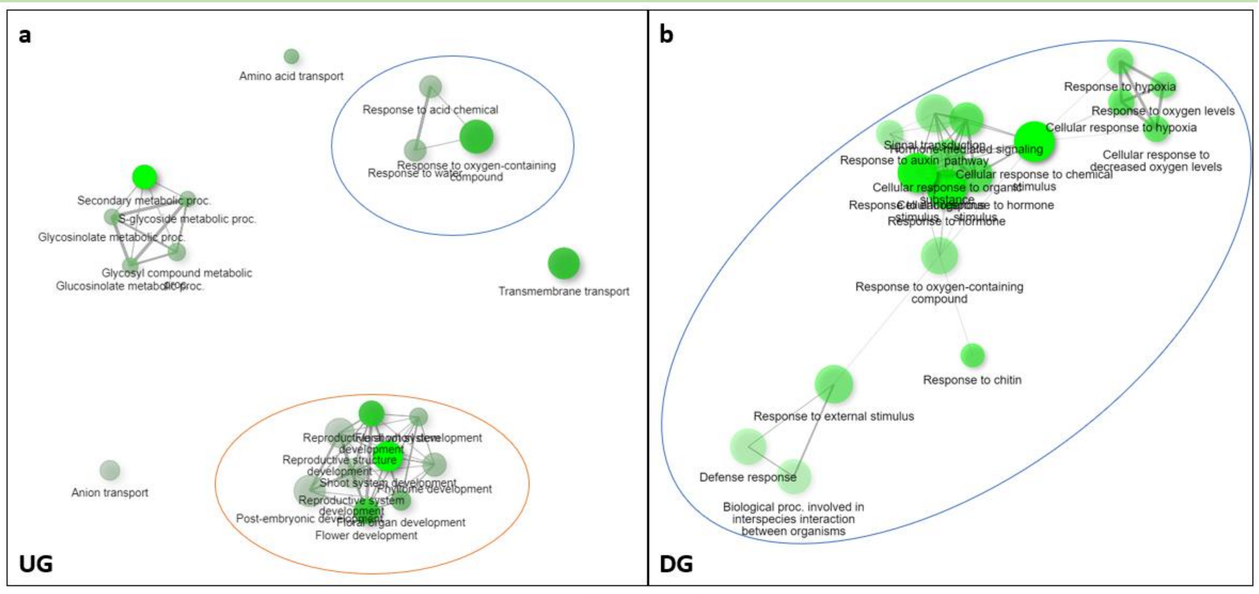


Figure 2. Graphic representation of the GO categories of **a)** UG and **b)** DG. Blue indicates stress-related GO categories, while orange represents development-related GO categories. The intensity of green color indicates the degree of enrichment.

LHP1 target genes overlap with stress-related genes

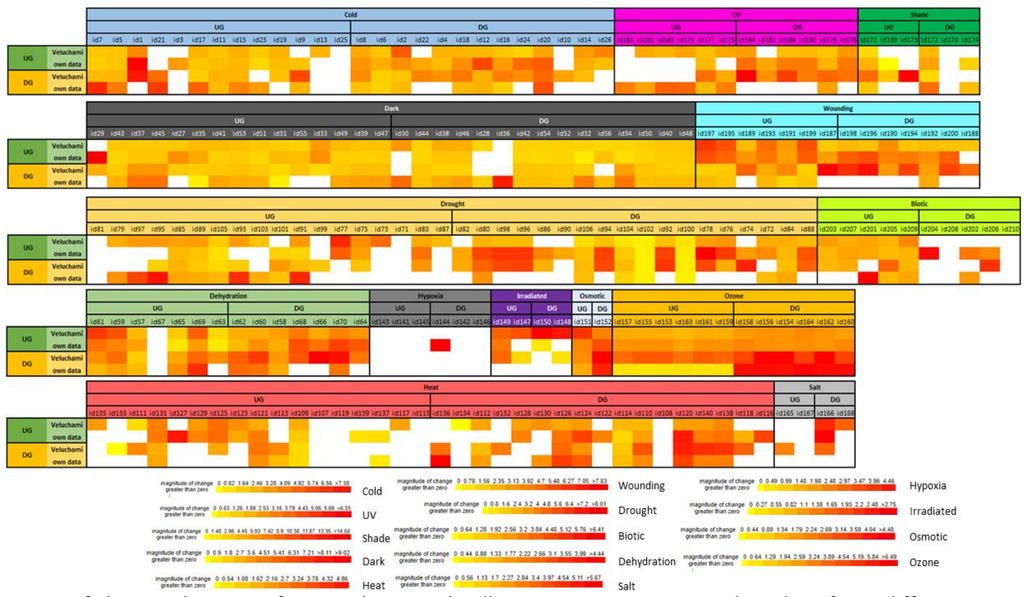


Figure 3. Heatmap of the overlapping of UG and DG in the *lhp1* mutant as compared to data from different stresses. White indicates non-significant data. Genes with a fold enrichment above 2 are marked in orange.

LHP1 and H3K27me3 are involved in the response to stress-related genes

LHP1 and H3K27me3 are distributed within the gene body in *A. thaliana*

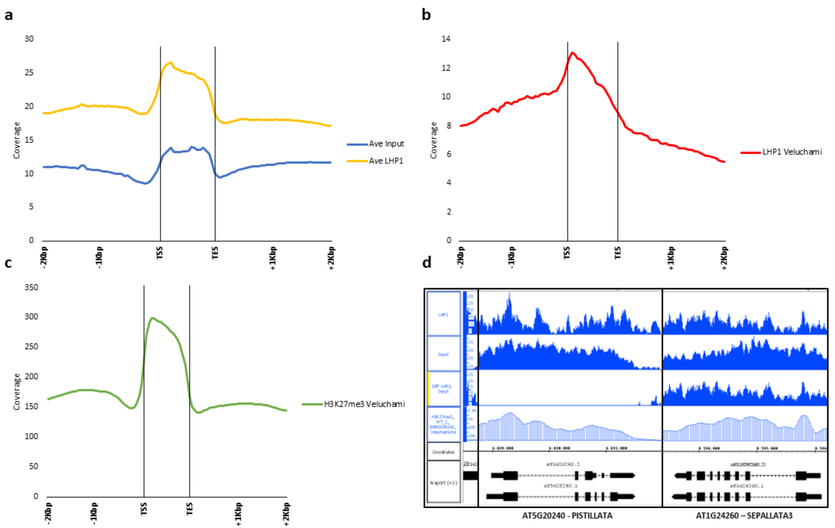


Figure 4. a) Distribution of LHP1 in the genome of *A. thaliana* and its input. **b)** Normalization of the distribution of LHP1 in the genome of *A. thaliana*, showing the difference between LHP1 and its input. **c)** Normalized distribution of the epigenetic mark H3K27me3 in the genome of *A. thaliana*. **d)** Distribution of the epigenetic mark LHP1, its input, the difference between LHP1 and its input, and H3K27me3 (from top to bottom) in two genes of *A. thaliana*: AT5G20240 (PISTILLATA) and AT1G24260 (SEPALLATA3).

LHP1 and H3K27me3 target genes overlap with stress-responsive genes

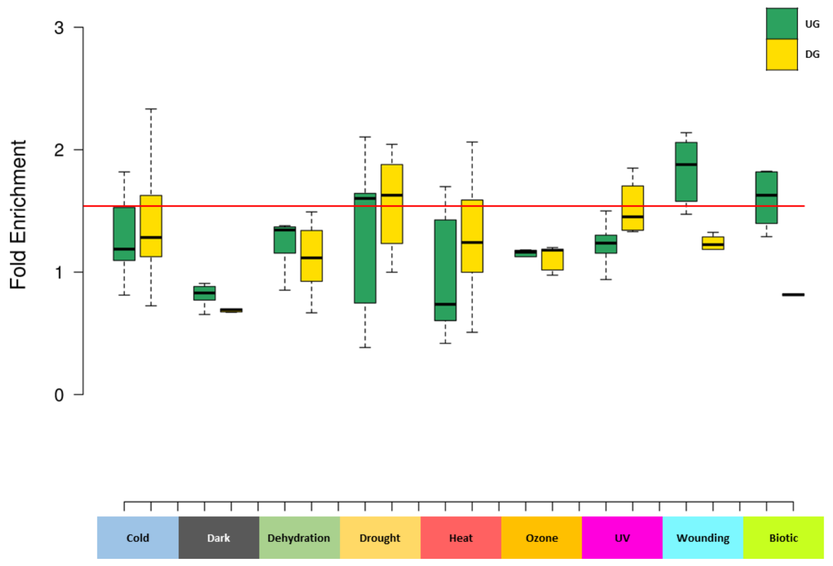


Figure 5. Fold enrichment of H3K27me3 in the different groups of UG and DG genes under different stresses. Genes above this 1,5 threshold are enriched in the mark, while those below it, are not.

LHP1 and H3K27me3 intensity is higher in response to stress-responsive genes

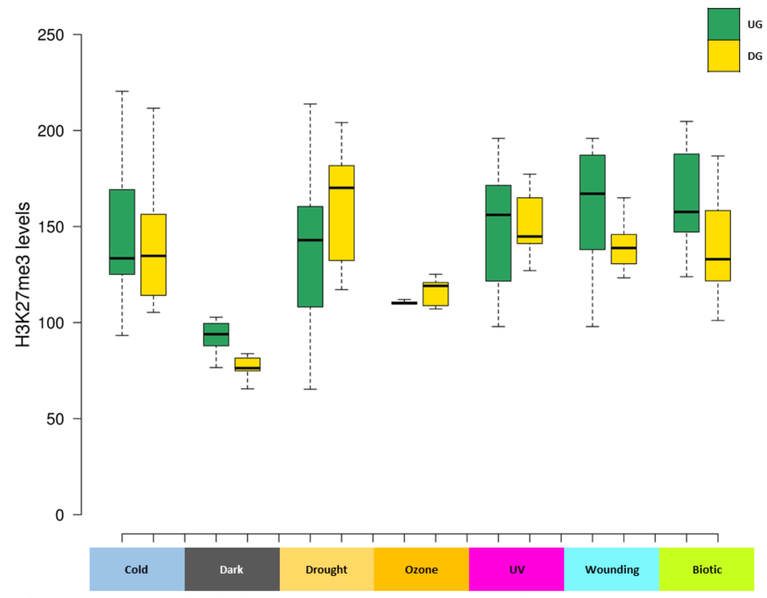
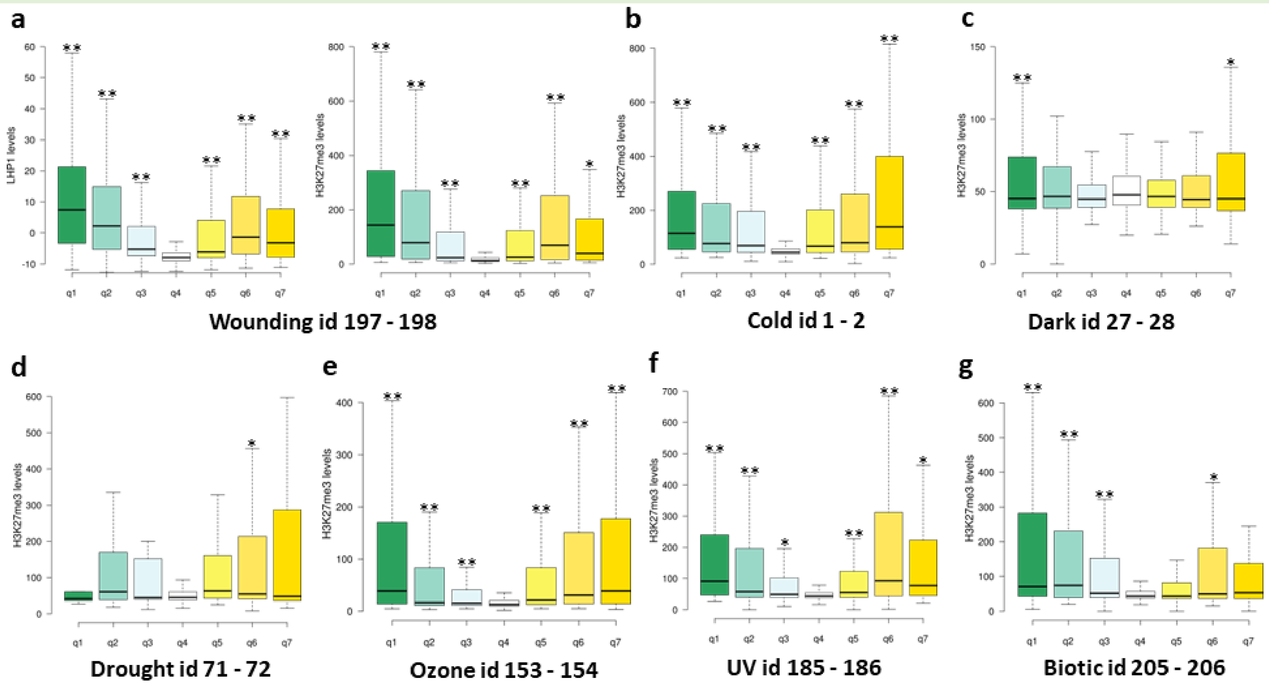


Figure 6. Levels of H3K27me3 in UG and DG genes (log2FC ≥ 1,5 and log2FC ≤ -1,5 with p-value ≤ 0,05) under different stresses.

LHP1 and H3K27me3 intensity is higher in UG and DG in response to stress-responsive genes



h

quantile	log2FC
q1	≥ 2
q2	[2, 1.5]
q3	(1.5, 1]
q4	(1, -1)
q5	[-1, -1.5)
q6	[-1.5, 2)
q7	≤ -2

Figure 7. a-g) Levels of H3K27me3 in genes which are divided into 7 quantiles based on their expression under different stresses. **h)** Reference table indicating the genes that belong to each quantile based on their log2FC value (p-value ≤ 0,05). The * symbol indicates significant values ≤ 0,01 and ** values ≤ 0,0001, according to the t-test.

CONCLUSIONS

The epigenetic mark H3K27me3, recognized and spread by LHP1, which is capable of interacting with PRC1 and PRC2, has been associated with gene repression.

In this study, we have analyzed **LHP1 target genes** and proved that they are **enriched in GO categories related to stress response**. These genes may be **subjected to coordinated regulation**, either positively or negatively. Within the genome of *A. thaliana*, **LHP1** and **H3K27me3** are **distributed within the gene body**, and their **presence decreases as we move towards the 3' end**.

There is evidence that the **mark is not only repressive but also activating**.

In future research, it could be valuable to investigate genome-wide methylation with post-stress data, since the methylation data used in this study are from wild type organisms without stress.

BIBLIOGRAPHY

Veluchamy, A., Jégu, T., Ariel, F., Latrasse, D., Mariappan, K. G., Kim, S.-K., Crespi, M., Hirt, H., Bergounioux, C., Raynaud, C., & Benhamed, M. (2016). LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. *PLOS ONE*, *11*(7), e0158936. https://doi.org/10.1371/journal.pone.0158936

Shen, Q., Lin, Y., Li, Y., & Wang, G. (2021b). Dynamics of H3K27me3 Modification on Plant Adaptation to Environmental Cues. *Plants*, *10*(6), 1165. https://doi.org/10.3390/plants10061165