

UNRAVELLING THE ROLE OF CHROMATIN-MEDIATED MEMORY OF STRESS ADAPTATION IN RESPONSE TO EXTENDED DROUGHT AND RECOVERY IN SOLANUM LYCOPERSICUM

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ChIP-Seq, Solanum lycopersicum, drought stress, histone modifications, epigenetic memory

Drought is a major environmental stress that exerts adverse effects on plant growth, development and productivity by altering biological functions and metabolic activity. However, plants have evolved molecular mechanisms to cope with water scarcity by regulating gene expression and their metabolisms through epigenetic switches. In eukaryotic cell, the structure/function of chromatin depends upon several regulatory epigenetic mechanisms, including DNA methylation and histone modifications and noncoding RNAs.

These mechanisms may provide a basis for epigenetic memory, integrating environmental conditions and developmental programs, and enabling plants to respond more efficiently to recurring stress or to prepare their offspring for potential future assaults.

In plants, cytosine methylation, an epigenetic mark critical for transposon silencing, is maintained over generations by key enzymes that directly methylate DNA and is facilitated by chromatin remodelers, like DECREASE IN DNA METHYLATION1 (DDM1). More in detail, the DDM1 enzyme is a SWI2/SNF2

chromatin remodeling protein that allows DNA methyltransferases to access heterochromatin thereby facilitating DNA methylation. The *ddm1* knockout mutants, generated by CRISPR/Cas9 technology, are lacking the two DDM1 genes identified in tomato. While each single *ddm1* mutant, *Slddm1a* and *Slddm1b*, was indistinguishable from wild-type plants, the double mutant *Slddm1a Slddm1b* displayed pleiotropic developmental phenotypes, associated with severe hypomethylation of the heterochromatic transposons in both the CG and CHG methylation contexts.

To gain insights into chromatin-mediated memory of drought stress in tomato, we are investigating the genome-wide distribution of histone modifications (H3K4me3, H3K27me3, H3K36me3 and H2A.Z histone variant) and the transcriptome in wild-type (cv M82) plants and *ddm1* mutants, *Slddm1a*, *Slddm1b* and *Slddm1aSlddm1b*, subjected to a mild and prolonged drought stress in the early development stage.

Additionally, to better address our aim we are analyzing also amiARP6 (herein *arp6-11* and *arp6-14*) lines and cv. Ailsa Craig as wild-type (herein WT). These tomato mutants lacking the ACTIN-RELATED PROTEIN 6 (ARP6), one of the subunits of the SWR1c, and could be very informative since has been widely used to study the effects of H2A.Z depletion from chromatin.

In our stress application, drought stress cycle is followed by re-watering for the time needed to allow a complete recovery from the stress, to identify genomic loci that show transcriptional/chromatin memory and elucidate different categories of stress-memory genes. By integrating RNA-seq, ChIP-seq and genomic data, we will shed light on the correlation between transcriptome variation and variation in epigenetic states at specific genomic regions.