FUNCTIONAL CHARACTERIZATION OF THE NEWLY DISCOVERED FAMILY OF MUT9 KINASES IN ARABIDOPSIS THALIANA AND SUGARCANE

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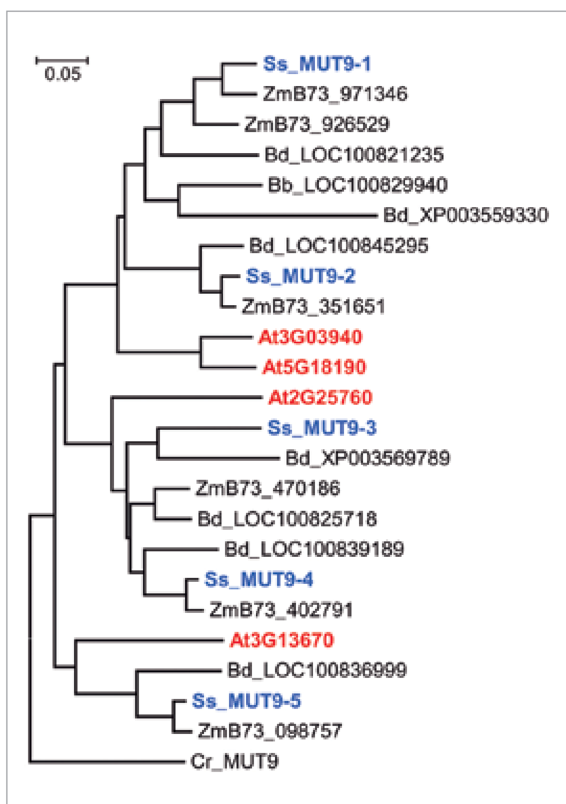

Figure 1. Phylogenetic tree showing the relationship among MUT9 proteins from different plants. Amino acids sequences were aligned by using the ClustalX program, and the tree was drawn with the MEGA 7.0 program. The MUT9 proteins from Arabidopsis and sugarcane are indicated in the tree by red and blue, respectively. MUT9 proteins used to draw the tree are from Arabidopsis thaliana *(At),* Brachypodium distachyon *(Bd),* Chlamydomonas reinhardtii *(Cr),* Saccharum sp *sugarcane cultivar SP80-3280 (Ss) and* Zea mays *B73(Zm)*

The aim of this project is to gain understanding of the role that MUT9 kinases play in the organization and maintenance of gene expression patterns during development, the response to environmental stresses and gene silencing in higher plants. The MUT9 protein was first described as a novel kinase involved in maintaining gene silencing in the green alga *Chlamydomonas reinhardtii*. In this alga the MUT9 kinase is responsible for the phosphorylation of histone H3 at threonine 3 (H3T3ph), which is a covalent modification associated with silent genes and transposons. True orthologs of *MUT9* are conserved in the plant kingdom and have undergone multiple duplications, giving origin to a small gene family with members present in both, mono- and dicotyledonous plants. However, information about the function of these genes in higher plants is almost non-existent. Thus, in order to learn more about *MUT9* genes we are taking advantage of the reverse genetic tools available in the model plant *Arabidopsis thaliana* and the genomic and transcriptomic resources recently developed in sugarcane. In these species, we are studying the spatial and temporal patterns of gene expression of the different *MUT9* homologs. The pathways and genes affected on mutants or lines with disrupted expression of *MUT9* are being investigated by phenotypic and transcriptomic analyses. These studies will be complemented by *in vitro* kinase assays of MUT9 proteins, and analysis of histone phosphorylation and other covalent modifications. With this project, we expect to contribute to the understanding of the molecular mechanisms that eukaryotes use to coordinate their development or to cope with environmental stresses. Preliminary evidence indicates that MUT9 kinases may be involved in regulation development and responses to environmental stresses in higher plants. Thus, the study of the MUT9 kinases could open avenues for the development of novel strategies for manipulation of plant growth and the engineering of stress tolerance in sugarcane and other crop plants.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Searches in genome databases and phylogenetic analysis indicate that the Arabidopsis genome encodes four MUT9 proteins whereas at least five MUT9 orthologs could be identified in sugarcane (Figure 1). These proteins contain a serine/threonine kinase domain and a conserved C-terminal region present exclusively in MUT9 kinases. In Arabidopsis, reverse transcription-PCR (RT-PCR) and promoter fusions with the gene reporter GUS indicate that the four MUT9 genes are expressed in the majority of plant organs. Analysis of translation fusions with the fluorescent protein GFP showed that MUT9 proteins localize into the nuclei, sometimes distributed as a discrete foci. We also isolated homozygous T-DNA mutants in the four Arabidopsis MUT9 genes. Analysis of these mutants did not reveal any obvious phenotypes or developmental defects. However, crossing the T-DNA lines in all possible ways revealed a double mutant combination displaying a drastic phenotype. Plants of this double mutant were dwarf in appearance, often produced floral buds with narrow sepals resulting in exposed floral organs. RNA-seq and a candidate gene approach showed that this mutant is defective in the silencing of developmentally regulated genes.

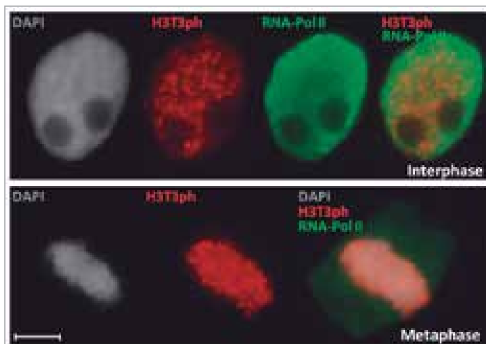

Figure 2. Distribution of H3T3ph and RNA polymerase II (RNA-pol II) in sugarcane root nuclei. H3T3ph (red signals) does not co-localize with actively transcribed regions rich in RNA Polymerase II (green signals). Instead, it appears to be associated with silent chromatin, DAPI densely stained regions (grey nucleus) coincide with H3T3ph brighter foci (red nucleus). Bars = 5 μm. Adapted from Moraes et al. 2015

In sugarcane, RT-PCR analysis of *MUT9* genes in different organs and tissues indicates they are downregulated in mature tissues and also during internode development. RT-PCR analysis also showed that the five *MUT9* genes are drastically downregulated during drought stress in sugarcane roots, and to a lesser extent in leaves. Since H3T3 phosphorylation is dependent on MUT9 activity, we also analyzed the distribution of this modification in nuclei isolated from root cells. In sugarcane interphase nuclei, H3T3ph was distributed as small foci that appear to co-localize with condensed chromatin. Strong H3T3ph signals were also found associated with metaphase chromosomes, indicating an association with chromosome condensation during cell division in sugarcane (*Figure 2*). Our initial observations suggest that MUT9 kinases may play a role during cell differentiation and in responses to drought in sugarcane.

MAIN PUBLICATIONS

Moraes I, Yuan Z-F, Liu S, Souza GM, Garcia BA, Casas-Mollano JA. 2015. Analysis of histones H3 and H4 reveals novel and conserved post-translational modifications in sugarcane. *PLoS ONE.* **10(7)**: e0134586.

Moraes I, Casas Mollano JA. 2014. Histone phosphorylation in plants and other organisms. In Alvarez-Venegas R, De la Pena C, Casas-Mollano JA (Eds) Epigenetics in Plants of Agronomic Importance: Fundaments and Applications. *Springer SBM*. ISBN 978-3-319-07971-4.

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