

A MULTIPLE APPROACH TO STUDY ENVIRONMENTAL STRESS-INDUCED EPIALLELE FORMATION AND INHERITANCE IN *ZEA MAYS*

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In the last years it has become evident that epigenetic marks such as DNA methylation, histone modifications, histone variants and small RNA populations play a primary role in creating alternative states of gene expression. Some marks define different epigenetic reversible states in responses to environmental or/and developmental inputs and thus will not have any evolutionary impact. On the contrary, the formation of epialleles that can be propagated mitotically and meiotically transmitted to the progeny, remaining stable for several generations, could play an important role in plant adaptation and evolution. In particular, environmental cues activate specific epigenetic mechanisms, which add epigenetic marks, altering patterns of gene expression, destabilizing the plant genome and causing phenotypic changes. Thus, environmentally triggered formation of epialleles and their transmission represent an important, yet unexplored, source of variation and adaptive power that can contribute to improvement of crop plants.

The FP7 European project entitled AENEAS (Acquired Environmental Epigenetics Advances: from Arabidopsis to maize) aims to “explore” environmentally-induced epigenetic changes as the “new frontier” of natural and artificial variability. In this framework we are investigating the mechanisms of environment-induced epiallele formation and their heritable maintenance in maize. More in details, we are analyzing at genome-wide level the effects of cold stress on DNA methylation profiles coupling bisulfite conversion of unmethylated cytosines with Illumina sequencing (BIS-Seq). Preliminary results from this analysis will be presented and integrated with the data on epigenetic gene expression regulation in response to cold stress obtained *via* mRNA-, miRNA- and CHIP-Seq by other groups participating in AENEAS project. These results obtained by different approaches will allow to identify a robust list of sequences target of epigenetic regulation (epitargets) belonging to three main epigenetic pathways (autonomous, small RNA and CpG methylation). In this context we are also characterizing several epiregulators belonging to three pathways by gene expression analysis and mutant production. In this first step we focused our attention on *NFC102*, the maize ortholog of the Arabidopsis *FVE* gene, which encodes a MSI-like WD-repeat protein belonging to autonomous flowering pathway. Expression analysis revealed a complex expression pattern in young and actively dividing tissues and an antisense transcript has been detected as well.

Finally, reproducible protocols for temperature shift treatments, salinity and drought stresses have been optimized to induce and investigate epigenetic changes in maize, validating the previously identified epitargets and analyzing their trans-generational inheritance in stressed wt and

mutant plants. Since the induction of alternative epigenetic states not only triggers the formation of novel epialleles but also promotes the movement of DNA transposons and retroelements that are very abundant in plant genomes, we are investigating the stress effect on transposons mobility.