

EXPRESSION ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF TWO *NF-Y* GENES IN *ARABIDOPSIS THALIANA*

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In a statistical analysis of over 500 eukaryotic promoters, including many from plant species, the CCAAT box was one of the most ubiquitous elements, being present in 30% of them. In yeast and mammals, the CCAAT box is recognized by NF-Y, a trimer composed of distinct subunits: NF-YA, NF-YB and NF-YC, all required for DNA-binding. The NF-YB and NF-YC subunits form a tight dimer, via protein structures similar to the Histone Fold Motif -HFM- a conserved protein-protein and DNA-binding interaction module. Heterodimerization results in a surface for NF-YA association and the trimer can then bind to DNA with high specificity and affinity.

In *Arabidopsis thaliana*, the complete NF-Y family is composed of 29 genes: 10 *NF-YAs*, 10 *NF-YBs* and 9 *NF-YCs*. We studied two *NF-Y* genes of the *AtNF-YA* sub-family, which are very close in the phylogenetic tree and both expressed in vegetative and reproductive tissues. Our aim was the characterization of their functional role.

Detailed expression analysis revealed that they are expressed during ovule and pollen development and have a similar expression pattern. In one single mutant, pollen development is damaged, in since some pollen grains at the mature stage are defective; instead the other single mutant does not show any phenotypic defect.

In order to verify the functional relationship between these genes, we decided to analyze the double mutants. These are lethal and moreover the segregation analysis of plants homozygous for one gene and heterozygous for the other gene revealed that the ratio of normal and defective seeds (aborted seeds and arrested embryos) is 1:1, suggesting that there is a gametophytic lethality.

Morphological characterization of these plants showed that there is male lethality: in fact the ovule development is normal and the pollen development at early stages is the same to wild-type, but a lot of mature pollen grains are defective.

These data indicate that these *AtNF-YA* genes have a role during pollen development and that probably they are partially redundant. Reciprocal crosses and segregation analysis will confirm the male lethality and further analysis will show what determines the pollen development arrest.