

## SEEKING FOR THE *PIN* GENE FAMILY OF AUXIN EFFLUX CARRIERS IN *ZEA MAYS*: A MULTIPLE APPROACH

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After the formulation of the chemiosmotic hypothesis for auxin transport, genetic approaches allowed the identification of genes involved in auxin polar transport in *Arabidopsis thaliana*. Because the chemical properties of IAA suggested that auxin efflux is the limiting step, the isolation of auxin efflux carriers became the main objective of scientists. Molecular characterization of the *pin1* mutant let the identification of the first member of PIN-FORMED gene family that encode transmembrane proteins with a similarity to a group of bacterial transporters. Subsequently, seven other genes similar to *PIN1* were found in Arabidopsis genome and PIN proteins have been shown to play a rate-limiting role in the catalysis of efflux of auxin from cells. PIN proteins asymmetrical cellular localization determines the direction of cell-to-cell auxin flow, creating auxin gradients that regulate a wide variety of processes, including embryogenesis, all type of organogenesis, vascular tissue differentiation, root meristem maintenance, root elongation, apical dominance and tropic growth responses to environmental stimuli.

Genes homologous to the Arabidopsis *PIN* are present in genomes throughout the plant kingdom, from the model moss *Physcomitrella patens* to all vascular plants and the relatively high amino acid identity between PIN proteins suggests that all the *PIN* genes diverged from a single ancestral sequences. Phylogenetic analysis of PIN sequences from *Oryza sativa* and *Triticum aestivum* revealed that the monocot *PIN* family is wider and divergent than dicots one, with two or three genes homologous to one Arabidopsis *PIN* gene. Wheat and rice present respectively three and two closely related *PIN1* genes. On the other hand TaPIN9 and OsPIN9 do not clusterize with any dicot sequence, suggesting the presence of at least one monocot-specific PIN protein.

*Zea mays* presents an even wider and more divergent *PIN* family if compared with the wheat and rice ones. We identified three orthologs of *AtPIN1*, called *ZmPIN1a*, *ZmPIN1b* and *ZmPIN1c* and we mapped them respectively on the chromosomes 9, 5 and 4. Our aim is to identify maize *pin1* mutations by the genetic and molecular characterization of spontaneous mutants mapped in proximity of the *ZmPIN1* gene positions. At the same time, we are complementing the Arabidopsis *pin1* mutant with *ZmPIN1* cDNA full length sequences to asses if they really act as auxin efflux carrier.

Moreover, the widening of maize *PIN* family was confirmed by the cloning of two genes closely related to *AtPIN2*, and by the identification of the putative orthologs to *AtPIN3*, *AtPIN4* and *AtPIN6*. Currently, we are analyzing their expression patterns by semiquantitative RT-PCR and by *in situ* hybridization.

In addition RT-PCR experiments allow us to identify splicing variants for *ZmPIN1b*, *ZmPIN1c*, *ZmPIN2* and *ZmPIN3*. Their role in maize development is still under analysis.