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THE PIN AUXIN EFFLUX CARRIERS FAMILY IN *ZEA MAYS*: PHYLOGENETIC ANALYSIS, EXPRESSION PATTERNS AND SUBCELLULAR LOCALIZATION

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Crop plants with desirable architectures are able to produce much higher grain yields and understanding the molecular mechanisms that underlie plant architecture will facilitate the breeding of more productive crop varieties. The plant hormone auxin regulates and coordinates the whole plant growth, playing a crucial role in plant architecture determination and seed production. It is indeed involved in many developmental processes, including embryogenesis, meristems maintenance, organogenesis, lateral root initiation, vascular tissue differentiation and tropisms. Auxin is synthesized primarily in meristematic regions at the SAM and is then intercellularly transported in a polar fashion to the whole plant. Specific trans-membrane carriers (AUX1/LAX, PGP and PIN protein families) mediate the cell to cell auxin transport, creating auxin gradients that, in turn, control gene expression. Eight PIN proteins (AtPIN1-AtPIN8) were found in Arabidopsis and have been shown to play a rate-limiting role in the catalysis of auxin efflux from cells determining the direction of cell-to-cell auxin flow and, as a consequence, creating the auxin gradients that regulate plant development. 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 rice revealed that the monocot PIN family is wider and divergent than dicots one, with three or four genes homologous to one Arabidopsis *PIN* gene. Rice proteins that do not clusterize with any dicot sequence has been identified as well, suggesting the presence of monocot-specific PIN proteins.

In the last years we characterized three *PIN1* orthologs in maize. Despite some differences in protein localization patterns between Arabidopsis and maize, our results confirmed the fundamental role of PIN1-driven auxin accumulation for proper plant development also in monocots.

Moreover, to better understand role of PIN-mediated auxin transport in controlling maize development, we are now isolating all the members of maize *PIN* family and studying the regulation of plasma-membrane insertion of three previously characterized ZmPIN1 proteins. We identified nine new *ZmPIN* genes confirming the widening of monocots *PIN* family compared to dicots one. In addition to the three previously identified *PIN1* orthologous, an additional *PIN1* gene (*ZmPIN1d*), a gene closely related to *AtPIN2* (*ZmPIN2*), three putative orthologs of *AtPIN5* (*ZmPIN5a*, *ZmPIN5b* and *ZmPIN5c*) and the ortholog of *AtPIN8* (*ZmPIN8*) have been isolated. As previously demonstrated in rice, we identified also in maize three monocot-specific proteins (ZmPIN9, ZmPIN10a and ZmPIN10b). RT-PCR expression analysis revealed that these genes are differentially expressed during maize development and the analysis of their expression patterns at cell and tissue level is in progress. Our preliminary results suggest that the more elaborated pattern

of maize development compared to Arabidopsis is accompanied by a more complex *PIN* gene family.

We are also analyzing plasma-membrane insertion abilities of ZmPIN1 proteins by tobacco protoplasts transformation using ZmPIN1::GFP fusion constructs. Preliminary experiments revealed that the ZmPIN1 proteins may have different plasma-membrane insertion abilities or, more likely, may be subjected to different regulation mechanisms.