ELUCIDATING THE ROLE OF ZmPIN1 GENES DURING MAIZE KERNEL DEVELOPMENT

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In Angiosperms the seed is the outcome of a double fertilization event, a process leading to the formation of the embryo and the endosperm. Proper seed development requires the co-ordinated expression of embryo and endosperm genes and relies on the interaction between the two seed compartments and between the seed and the maternal tissues. The large reservoir of auxin conjugates deposited into the developing maize endosperm, which provides a continuous environment for the developing embryos, has been suggested to be involved in these interactions, leading to polar embryo development. In Arabidopsis thaliana an apical-basal auxin gradient, established by PIN7, triggers the specification of apical embryo structures, whereas the subsequent PIN1 polar localization reorganizes the auxin gradient to specify the basal root pole. To verify whether this model also applies for monocotyledonous species, in which embryos develop with a more complex architecture and endosperm persists at later stages of seed development, we investigated on the behavior of ZmPIN1 genes and auxin accumulation patterns during Zea mays kernel development.

We identified ZmPIN1c, a novel putative ortholog of AtPIN1 in maize. This gene, encodes a putative protein of 597 amino acids that shows more than 80% of amino acid identity with ZmPIN1a and ZmPIN1b, the others two members of the ZmPIN1 family. Real time RT-PCR experiments demonstrated that ZmPIN1 genes show differential expression patterns during kernel formation. In situ hybridization assays with ZmPIN1 specific probes and immunolocalization assays using an anti-AtPIN1 antibody revealed different localization of PIN1 transcripts and proteins in developing kernels. During the differentiation of endosperm four different cellular domains (basal transfer layer, embryo surrounding region, starchy endosperm and aleurone), ZmPIN1a and ZmPIN1c localized in the basal transfer cells layer (BETL) and in the embryo-surrounding region (ESR), but ZmPIN1 proteins are not polarized both in BETL and ESR domains. During embryogenesis ZmPIN1 genes are expressed in the apical region of the proembryo, in the scutellum during the transition and the coleoptilar stage and in the shoot apex and root from L1 to L5 stages. The embryonic SAM is characterized by a central group of cells presenting a polarized PIN1 in a way that suggests auxin fluxes spreading at 360°. The protein also marks leaf primordia and L1 layer at the level of the incipient primordium. In the embryonic root the antibody suggests acropetal auxin fluxes directed towards the RAM. To better describe the auxin fluxes during kernel development we performed immunolocalization experiments using an anti-IAA antibody. Our data showed that auxin is present both in the maternal tissues of the seed and at higher level in specialized tissues of the endosperm: aleurone, transfer layer and embryo surrounding region.
defective endosperm-B18 maize mutant, that shows reduced levels of IAA in the endosperm leading to a reduced dry matter accumulation also showed altered expression of \textit{PIN1} genes and defects in differentiation of the transfer cell layer.