Expression and role of plasma membrane aquaporins in Zea mays leaves

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Expression and role of plasma membrane aquaporins in *Zea mays* leaves

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INTRODUCTION

Plant growth and development are dependent on essential physiological processes taking place in the leaf, such as photosynthesis and transpiration. In order to function properly, the leaf needs to maintain a balanced water content. As transcellular water flow is facilitated and regulated by membrane water channels, named aquaporins (AQPs), studies on their mRNA and protein expression are essential to better understand their involvement in physiological processes. We quantified and localized the expression of *Zea mays* plasma membrane AQPs (ZmPIPs, plasma membrane intrinsic proteins) in the leaf using quantitative RT-PCR and immunodetection approaches. Specific primer pairs selected in the non-conserved 3' untranslated regions allowed specific and efficient (Efficiency: 95-105%) cDNA amplification of the 13 highly homologue *ZmPIPs*.

RESULTS

➢ *ZmPIP* genes display a developmental expression pattern

Quantification of ZmPIP mRNA and protein expression was performed on the 7-day-old third leaf. All *ZmPIP* genes except *ZmPIP2;7* were expressed although to different extents (Fig. 1). mRNA and protein expression was found to be dependent on the developmental stage of the leaf tissue, with, in general, an increase in expression at the end of the elongation zone where the leaf emerges from the sheath (Fig. 1 and 2).

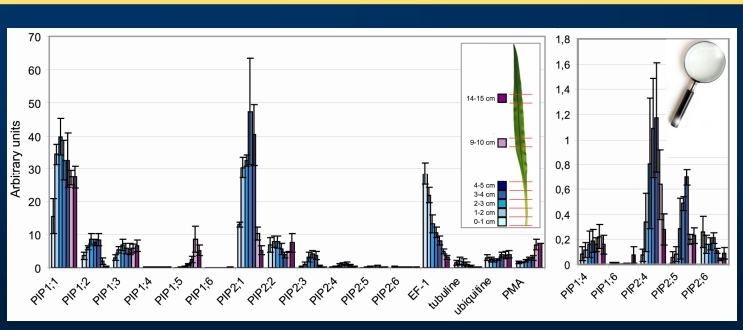

Fig. 1: Levels of *ZmPIP* transcripts in different sections of 7-day-old maize leaf 3. cDNA was synthesized from total RNA from each section and RT-PCR was carried out using SYBR Green. The geometric mean of the expression level of three control genes (*α-tubulin*, *ubiquitin* and *H⁺-ATPase* (*PMA*)) was used to normalize the data. The expanded scale shows the expression level of weakly expressed *ZmPIPs*.

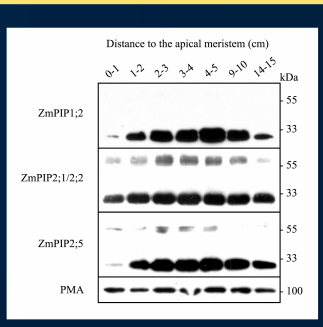

Fig. 2: Levels of ZmPIP proteins in different sections of the third leaf analyzed by Western blot using specific antibodies.

➢ *ZmPIP* expression analysis in stomatal complexes collected by Laser Capture Microdissection (LCM)

Stomata play a crucial role in balancing the essential CO_2 uptake with the unavoidable loss of water. In *Z. mays*, the stomatal complex is composed of two guard cells (GC) responsible for the opening/closing mechanism and two adjacent subsidiary cells (Fig. 3A). ZmPIP1;2 and ZmPIP2;1/2;2 were shown to be expressed in both cell types by *in situ* immunolocalization (Fig. 3B-E). Lacking specific antibodies against all 13 ZmPIPs, we are currently optimizing a Real-Time PCR expression analysis starting from 2000 microdissected stomatal complexes (Fig. 4).

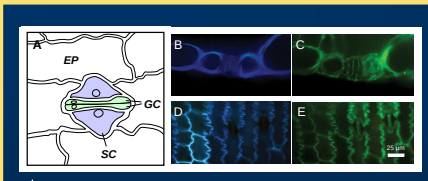

Fig. 3: (A) Schematic view of a closed stomata. Maize stomata are composed of two guard cells (GC) surrounded by two subsidiary cells (SC). (B) *In situ* immunolocalization of ZmPIP1;2 (C) and ZmPIP2;1/2;2 (E) proteins in the stomatal complex. (B, D) Autofluorescence showing the tissue anatomy.

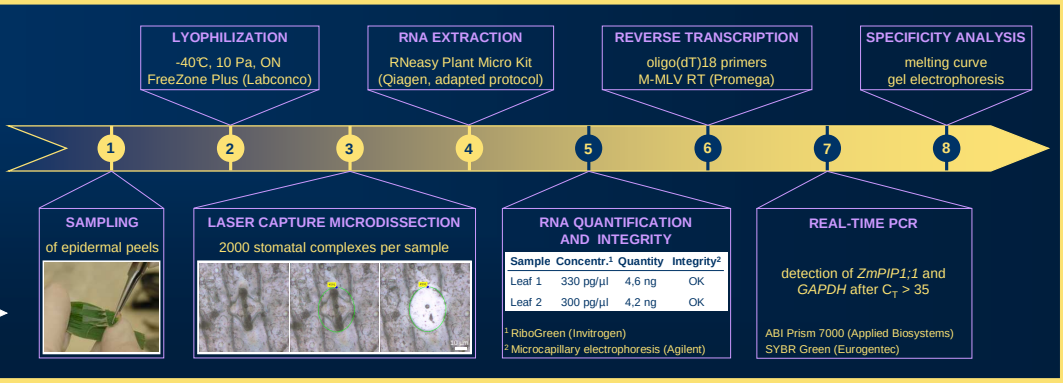

Fig. 4: Real-Time PCR experiment on microdissected stomatal complexes. Timeline showing the different steps which need to be optimized.

➢ *ZmPIP* expression and protoplast osmotic water permeability (P_f) display a diurnal pattern

ZmPIP expression was followed during 24 hours by Real-Time PCR and Western blot (Fig. 5 and 6). Almost all *ZmPIP* genes showed a diurnal expression pattern in the mature zone. In general, the highest transcript/protein level was observed during the morning. Protoplast swelling essays were carried out to assess their membrane permeability to water which is closely linked to the presence and activity of aquaporins. During the day, the protoplasts displayed higher P_f values (Fig. 7), which correlates with the higher *ZmPIP* expression (Fig. 5 and 6). Altogether, these data suggest a link between photosynthesis/transpiration, aquaporin expression and membrane permeability.

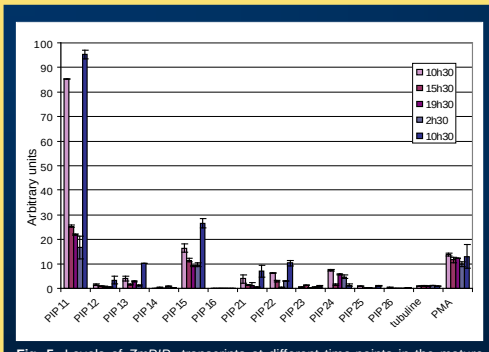

Fig. 5: Levels of *ZmPIP* transcripts at different time-points in the mature zone of leaf 3 analyzed by Real-Time PCR. The geometric mean of the expression level of *α-tubulin* and *H⁺-ATPase* (*PMA*) was used to normalize the data.

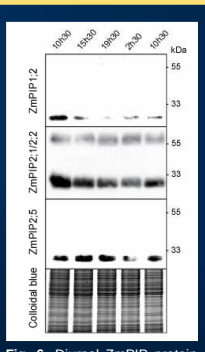

Fig. 6: Diurnal ZmPIP protein expression in the mature zone of leaf 3 analyzed by Western blot.

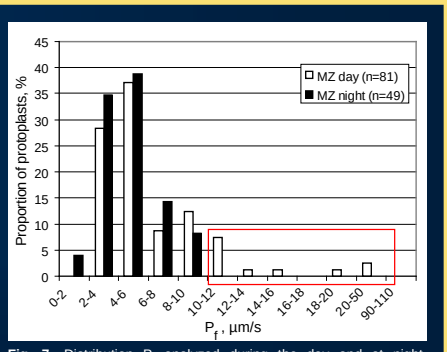

Fig. 7: Distribution P_f analyzed during the day and at night. Protoplasts were isolated from the mature zone (MZ) and their swelling induced by a hypotonic challenge was used to determine the P_f.

CONCLUSION

- **All *ZmPIP* genes except *ZmPIP2;7* are expressed in leaves although to different extents.**
- **ZmPIP expression is developmentally regulated.**
- **Stomatal complex RNA has to be pre-amplified before q RT-PCR**
- **ZmPIP expression is diurnally regulated and correlated to the osmotic water permeability of isolated protoplasts.**