EXPLORING ION MOVEMENT IN PLANT AQUAPORINS

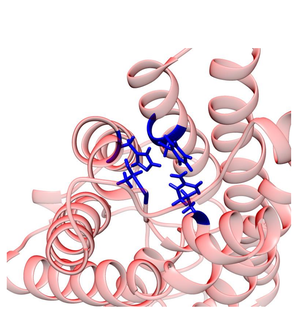
González Altieri, Lucía[1,2]; Beltrán González, Andrea[3]; Calvo, Daniel[3]; Milesi, Verónica [2]; Alleva, Karina, [1,4]; Martín, Pedro [2]

[1] *Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini", Universidad de Buenos Aires - CONICET, Capital Federal, Argentina.*
[2] *Instituto de Estudios Inmunológicos y Fisiopatológicos "Dr. Carlos Alberto Fossati", Universidad Nacional de La Plata - CICPBA - CONICET, La Plata, Argentina.*
[3] *Instituto de Fisiología, Biología Molecular y Neurociencias, Universidad de Buenos Aires - CONICET, Capital Federal, Argentina.*
[4] *Departamento de Fisicomatemática, Cátedra de Física, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Capital Federal, Argentina.*

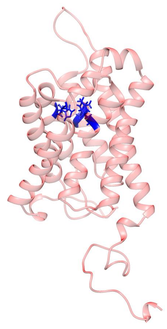
CONTACT ME
gonzalezaltierilucia@gmail.com

INTRODUCTION

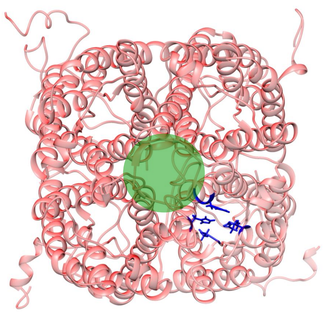
Plasma Membrane Intrinsic Proteins (PIP) are plant aquaporins from the Major Intrinsic Protein (MIP) family, known for transport water (H_2O) and hydrogen peroxide (H_2O_2)[1]. PIP assemble as tetramers, where each protomer contains a highly selective pore that enables the passage of H_2O and H_2O_2 while effectively excluding charged solutes through the **arR filter**. Furthermore, H_2O and H_2O_2 permeation through these protomers can be blocked by intracellular acidification or phosporylation. Recent studies suggest that some PIP are also ion channels, classified as icAQPs. The ion permeation pathway is unclear but is speculated to differ from the water one. One possible via of ion permeation is the **tetrameric pore**.



Extracellular view of protomeric pore



Lateral view of a PIP2 protomer

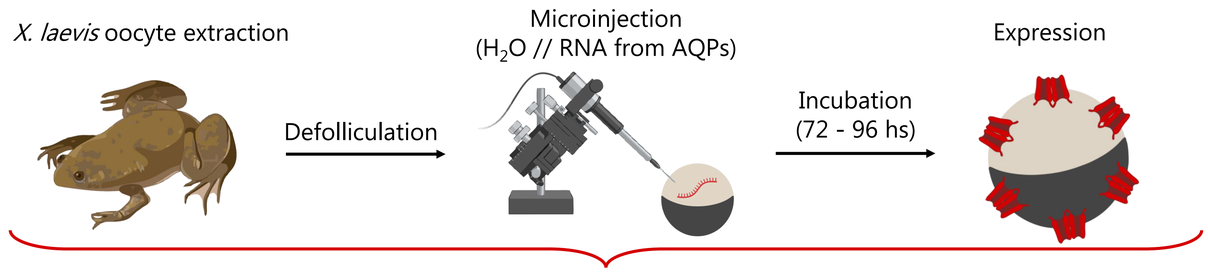


Extracellular view of PIP2 tetramer

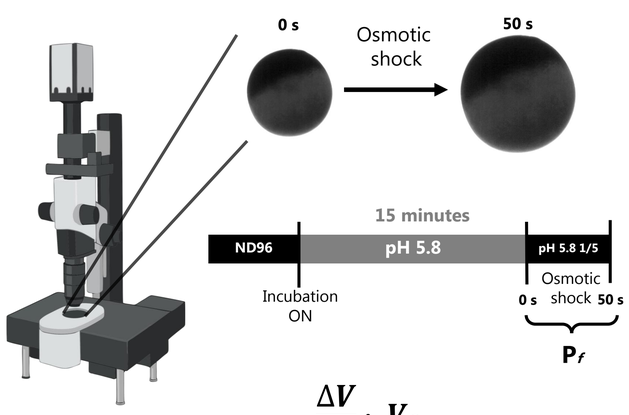
AIM

Our aim is to explore the ion permeation in plant aquaporins including the possibility that an inverse modulation between water permeability and ionic conductance may exist. In this case, we will use intracellular acidification as a trigger, which is a proved closing stimulus for protomeric pore of PIP2.

WORKFLOW

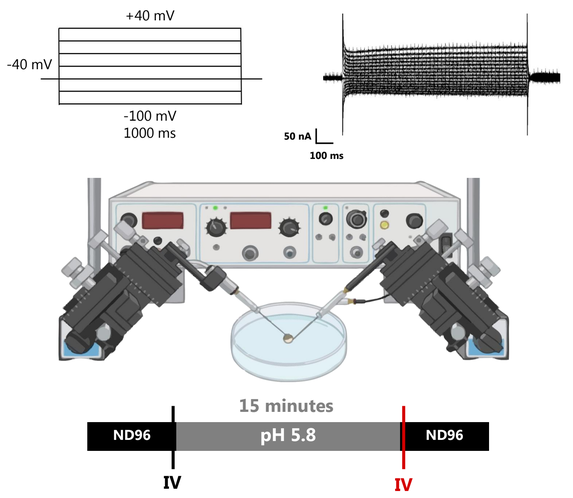


OSMOTIC PERMEABILITY ASSAY



$$P_f = \frac{\frac{\Delta V}{\Delta t} \cdot V_0}{V_w \cdot S \cdot \Delta \Pi}$$

TWO ELECTRODE VOLTAGE CLAMP



RESULTS

PIP2 ISOFORMS HAS NOT SHOWED CHANGES IN MACROSCOPIC CONDUCTANCE COMPARED TO H_2O-INJECTED OOCYTES IN ND96 BUFFER SOLUTION

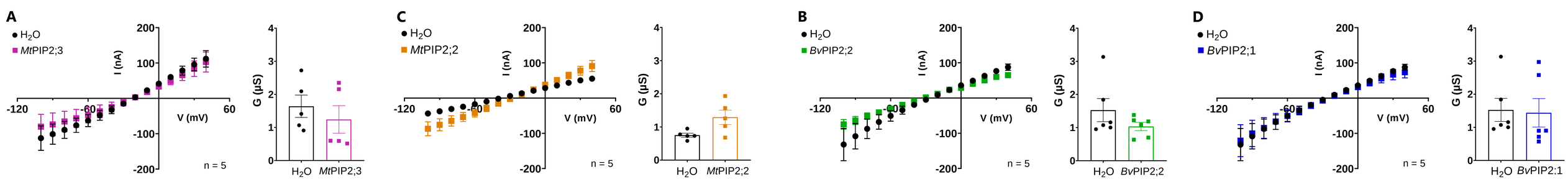


Fig 1. Current-voltaje (I-V) relationships and mean values of macroscopic conductance of *Xenopus laevis* oocytes injected with 4 ng of cRNA coding for *Mt*PIP2;3 (A), *Mt*PIP2;2 (B), 7.5 ng of cRNA coding for *Bv*PIP2;2 (C), 2 ng coding for *Bv*PIP2;1 and H_2O as a negative control. ND96 buffer solution has the following composition (in mM): NaCl 96, KCl 2, HEPES 5, $MgCl_2$ 1, $CaCl_2$ 1.8, pH 7.4 with NaOH. Data of macroscopic conductance is shown as mean ± SEM. PIP-injected oocytes did not show statistically significant differences using a Student *t* test or Mann Whitney test against H_2O-injected oocytes ($p > 0.05$).

Considering that none of the tested PIP2 isoforms exhibited changes in macroscopic conductance and knowing that H_2O and H_2O_2 permeability of *Mt*PIP2;3 is inhibited by intracellular acidification, we decided to perform recordings under this condition. We began this study with *Mt*PIP2;3 confirming that, under our experimental conditions, the aquaporin H_2O permeation is in fact inhibited by intracellular acidification. Subsequently, we measured ionic currents under analogous conditions.

*Mt*PIP2;3 INCREASES OSMOTIC PERMEABILITY OF PLASMATIC MEMBRANE OF *XENOPUS LAEVIS* OOCYTES AND IS INHIBITED BY INTRACELLULAR ACIDIFICATION

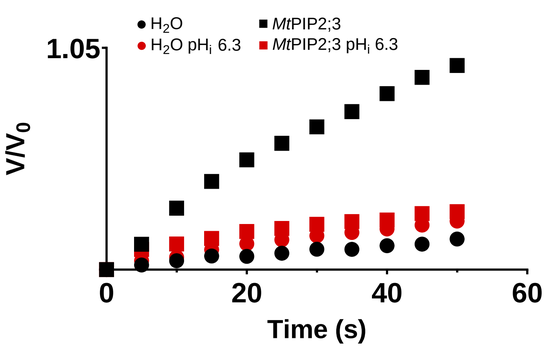


Fig 2. Time course evolution of the relative volume change in a single oocyte injected with 2 ng of cRNA coding for *Mt*PIP2;3 **with** and **without** preincubation at 5.8 pH sodium acetate solution for 15 minutes and then exposed to a hypo-osmotic gradient. H_2O corresponds to water-injected oocyte (negative control). The internal pH was calculated following the calibration performed in Ref. [2]. 5,8 pH solution contain (in mM): sodium acetate 50, MES 20 $CaCl_2$ 1,8 supplemented with 1M mannitol until osmolarity (200 ± 5 mOsm · kg^{-1}) was achieved.

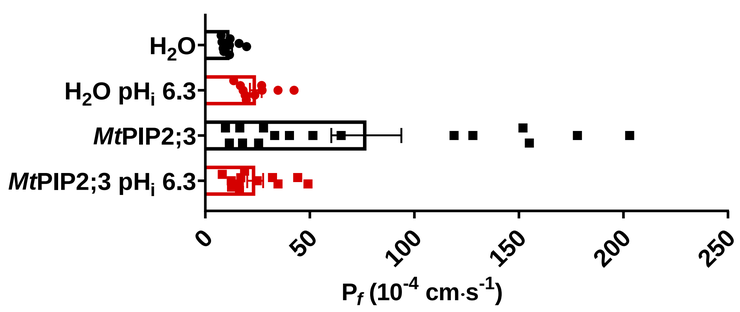


Fig 3. Mean ± SEM osmotic water permeability of *Mt*PIP2;3 **with** and **without** preincubation at 5.8 pH sodium acetate solution and exposed to a hypo-osmotic gradient. H_2O corresponds to water-injected oocyte (negative control). n = 9-16 oocytes. The internal pH was calculated following the calibration performed in Ref. [2].

*Mt*PIP2;3-INJECTED OOCYTES SHOWS A SIGNIFICANT INCREASE IN MACROSCOPIC CONDUCTANCE AFTER INTRACELLULAR ACIDIFICATION (pH_i 6.3)

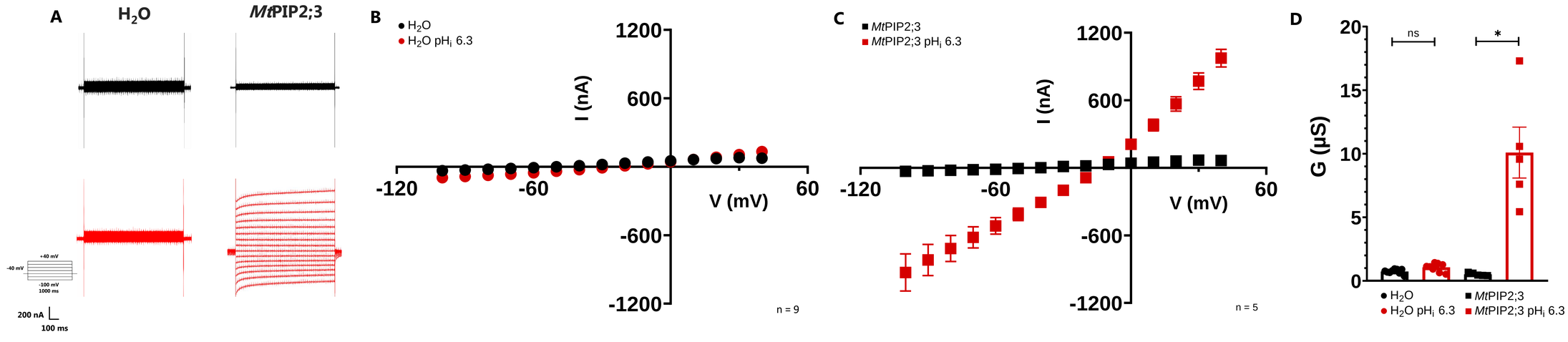


Fig 4. (A) Representative macroscopic current of H_2O and *Mt*PIP2;3-injected oocytes recorded in ND96 buffer solution **before** and **after** intracellular acidification by 15 minutes incubation with 5.8 pH sodium acetate solution. (B y C) Mean ± SEM I-V relationships of oocytes injected with H_2O as a negative control (B) or 4 ng of cRNA coding for *Mt*PIP2;3 (C). (D) Mean ± SEM values of macroscopic conductance measured between -80 and -50 mV in H_2O and *Mt*PIP2;3-injected oocytes **before** and **after** intracellular acidification. Differences between **before** vs **after** intracellular acidification for each condition were evaluated using a Student *t* test (* indicates $p < 0.05$, H_2O $n = 9$, *Mt*PIP2;3 n = 5).

	H_2O	*Mt*PIP2;3
Increased conductance	0/9	5/9
No change in conductance	9/9	4/9

Table 1. Frequency of oocytes that present an increased macroscopic conductance after intracellular acidification.

DISCUSSION AND CONCLUSIONS

Our results show that ion permeation is possible in plant aquaporins. These data showed that in more than half of the *Mt*PIP2;3-injected oocytes, intracellular acidification increases macroscopic ion conductance. Regarding the molecular mechanism of ion permeation, we hypothesize that the conformational change induced by cytosolic acidification, which closes the water-conducting pore, could open the tetrameric central pore facilitating the ion flux. Tyerman et al.,[3] have proposed this mechanism to explain their results[4] showing that a mutually exclusive gating among water and ions could be modulated by phosphorylation. Therefore, we want to extend this study to the other PIP2 isoforms and investigate in deep the mechanism of ionic permeation.

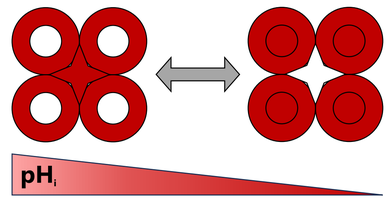


Fig 5. Scheme of hypothetical inverse modulation between water permeability and ionic conductance pH_i-mediated.

REFERENCES

1. Chevriau et al., *Biochem. J,* 2024.
2. Bellati et al., *Plant Mol. Biol,* 2010.
3. Tyerman et al., *Annu. Rev. Plant Biol,* 2021.
4. Qiu et al., *Plant Cell Environ.* 2020.