

Chapter 8

Ca^{2+} - Sensitive and Non-selective Na^+/K^+ Channel Activity of a Barley Aquaporin HvPIP2;8 under Saline Conditions

Thi Huong Sen TRAN

Summary: HvPIP2;8 transcript abundance increased in barley shoot tissues following salt treatments in a salt-tolerant cultivar, Haruna-Nijo, but not in salt-sensitive I743. Here we report that one of the 12 barley PIPs (PIP1 and PIP2) tested, HvPIP2;8, facilitated cation transport when expressed in *Xenopus laevis* oocytes. HvPIP2;8-associated ion currents were detected with Na^+ and K^+ , but not Cs^+ , Rb^+ , or Li^+ , and were inhibited by Ba^{2+} , Ca^{2+} , Cd^{2+} and, to a lesser extent, Mg^{2+} , which also interacted with Ca^{2+} . Currents were reduced in the presence of K^+ , Cs^+ , Rb^+ , or Li^+ relative to Na^+ alone. Co-expression of HvPIP2;8 with HvPIP1s, barley aquaporins in the PIP1 subfamily, significantly abolished the ions permeability of HvPIP2;8, but not water permeability. There is potential for HvPIP2;8 to be involved in barley salt-stress responses, and HvPIP2;8 could facilitate both water and Na^+/K^+ transport activity, depending on the phosphorylation status. Future research will uncover the molecular and structural mechanisms that control the dual permeability of aquaporins for ion and water, and more testing of the physiological role of HvPIP2;8 in planta.

1. Introduction

Salt stress encompasses two primary components that affect

plant physiology and growth: ionic stress, which results from the accumulation of toxic ions within plant tissues, and osmotic stress, which is caused by the reduction in water potential in the soil solution that limits water uptake by plant roots (Figure 8.1).

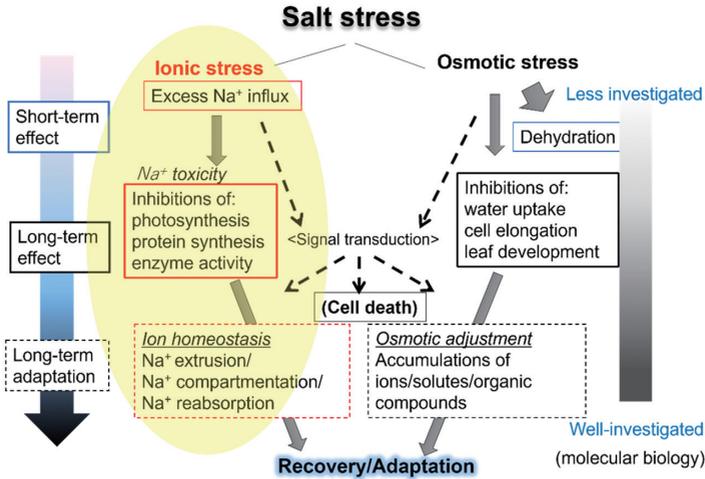


Figure 8.1. Mechanism of Salt Stress

Source: (Horie et al. 2012)

Plant aquaporins are specialized membrane proteins that function primarily as channels responsible for the transportation of water molecules and various neutral solutes across cellular membranes. These integral membrane proteins play a crucial role in maintaining cellular water homeostasis and facilitating the movement of specific molecules. In recent scientific investigations, researchers have discovered that certain aquaporins possess dual functionality, exhibiting the capability to transport both water and ions simultaneously. This remarkable dual permeability has significant implications for understanding plant physiology and cellular transport mechanisms. Despite these

advancements in knowledge, there remains a substantial gap in our understanding regarding the precise mechanisms by which plasma membrane intrinsic proteins (PIPs), a subfamily of aquaporins (Katsuhara and Shibasaka 2007), facilitate ion influxes across the plasma membrane in various plant species. This area of research requires further investigation to fully elucidate the functional diversity and physiological significance of these transport proteins in different plant contexts.

Salinity (NaCl) affects PIP2 aquaporin expression in a time- and isoform-dependent manner. This regulation is noteworthy as studies show that the abundant AtPIP2;1 protein relocates from the plasma membrane to an internal position in *Arabidopsis* roots under saline conditions, potentially explaining reduced root hydraulic conductance (Katsuhara et al. 2003; McGaughey et al. 2018).

Barley (*Hordeum vulgare*) is an important grain crop worldwide and is relatively salt-tolerant compared to other crops (Ismail et al. 2017).

Therefore, this research aims to identify ion-conducting PIP aquaporins in barley and clarify the mechanisms of ion transport activity by PIP aquaporins (HvPIP2;8) and their regulation under salinity stress in barley.

2. Materials and Methods

This research used electrophysiological measurement techniques to investigate membrane transport phenomena at the cellular level. These sophisticated methodologies allowed for precise quantification of ion fluxes (i.e., two electrode voltage clamp) across plant cell membranes under controlled experimental conditions (Figure 8.2). Then, the data were analyzed using SPSS statistics software (version 20). All data are means \pm standard error (SE).

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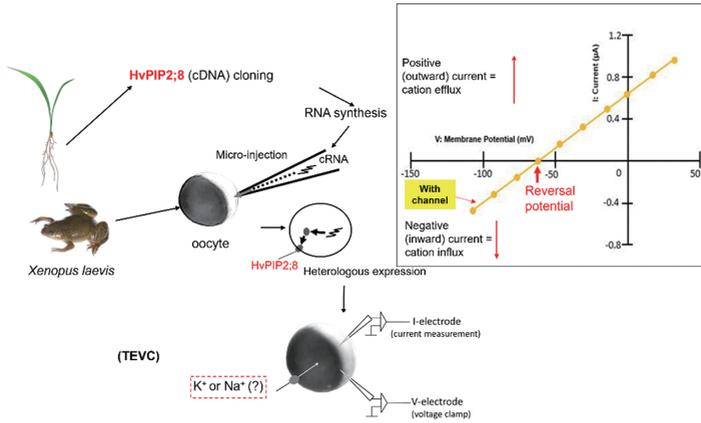


Figure 8.2. A Mechanism of Electrophysiological Measurement Techniques
Source: Author

3. Results

(1) Expression of HvPIP2;8 in Barley

The research found that only HvPIP2;8 could move ions in both directions across cell membranes under specific test conditions (Figures 8.3 and 8.4).

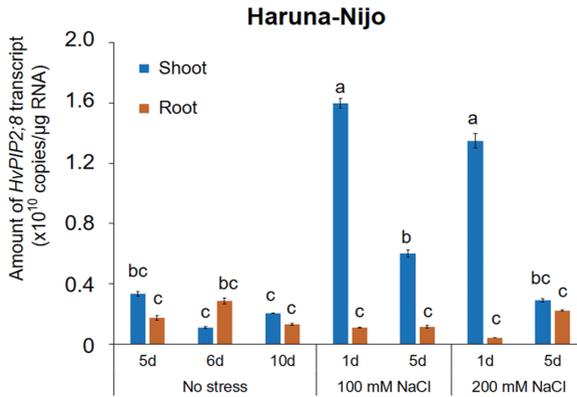


Figure 8.3. HvPIP2;8 Expression in Haruna-Nijo Barley

Note: d means day after exposed to salt. Source: Author

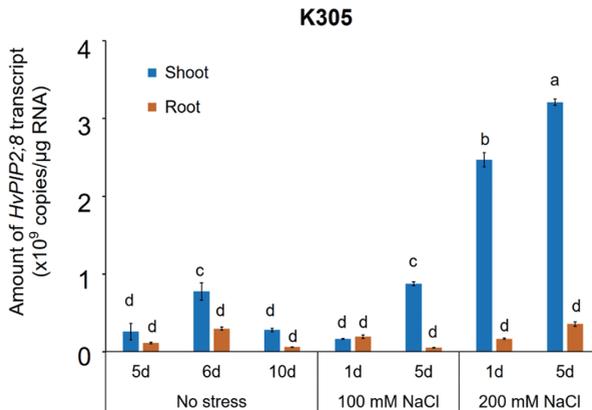


Figure 8.4. HvPIP2;8 Expression in K305 Barley

Note: d means day after exposed to salt. Source: Author

(2) HvPIP2;8 in Barley Can Transport Ions

Ca²⁺- Sensitive and Non-selective Na⁺/K⁺ Channel Activity of a Barley Aquaporin HvPIP2;8 under Saline Conditions

The study found that only HvPIP2;8 could move ions in both directions across cell membranes under specific test conditions (Figure 8.5).

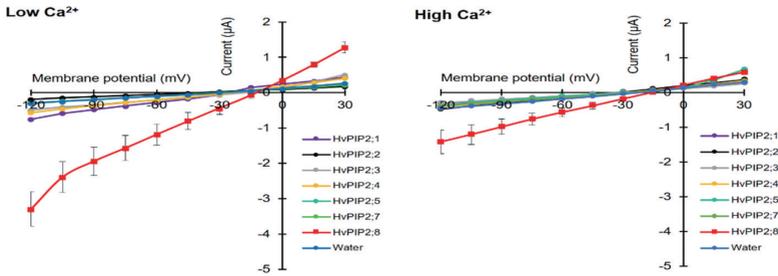


Figure 8.5. HvPIP Ion Transport Activity

Source: (Tran et al. 2020)

The ionic conductance was strongly inhibited in accordance with increases in the external free Ca²⁺ concentration (Figure 8.6). It reveals that its ionic conductance characteristics exhibit sophisticated patterns of differential sensitivity when exposed to various divalent cations in the external environment. Specifically, the presence of external calcium, cadmium, and barium ions results in pronounced inhibitory effects on the channel's conductance capabilities, whereas magnesium ions, despite their divalent nature, demonstrate a markedly reduced capacity to inhibit the channel's conductance properties.

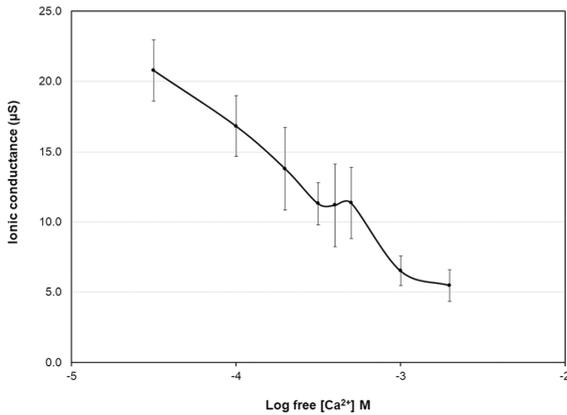


Figure 8.6. Relationships between the External Free Ca²⁺ Concentration and HvPIP2;8

Source: (Tran et al. 2020)

(3) Identification of HvPIP2;8 as a Na⁺/K⁺ Channel

HvPIP2;8 is a specialized protein channel in cell membranes that can transport both sodium (Na⁺) and potassium (K⁺) ions. When potassium ions are present in the environment, they block the channel's ability to transport sodium, but chloride ions don't have this blocking effect (Figure 8.7).

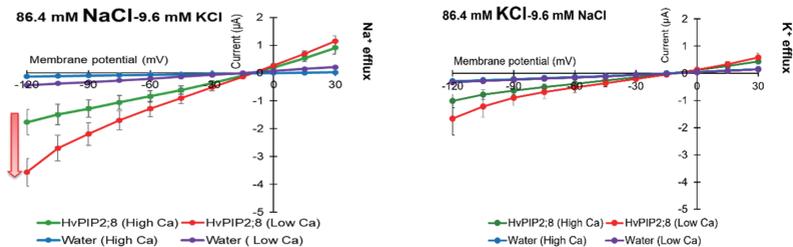


Figure 8.7. Identification of HvPIP2;8 as a Na⁺/K⁺ Channel

Source: Tran et al. 2020

(4) HvPIP2;8 Monovalent Alkaline Cation Selectivity

The investigation of HvPIP2;8's monovalent selectivity sequence has unveiled a particularly distinctive hierarchical pattern in ion permeability: the channel displays comparable levels of permeability for both sodium and potassium ions, with sodium showing marginally higher or equal permeability compared to potassium ($\text{Na}^+ \geq \text{K}^+$). Moving down the periodic table, rubidium ions demonstrate notably restricted permeability through the channel, while both cesium and lithium ions are completely excluded from passage through the HvPIP2;8 transport pathway.

The Na^+ permeability of HvPIP2;8 demonstrates a strong and significant dependence on the concentration of external K^+ ions, suggesting a complex interplay between these two ionic species in the transport mechanism. This relationship appears to be a key factor in determining the overall conductance properties of the protein channel.

(5) Co-expression of HvPIP2;8 with HvPIP1s

A particularly intriguing aspect of HvPIP2;8's behavior emerges during co-expression studies with HvPIP1s: this experimental scenario reveals a complex functional interaction whereby the HvPIP2;8-dependent ionic conductance undergoes substantial reduction, yet remarkably, the channel's capacity for water permeability remains completely unaltered by this co-expression arrangement, suggesting distinct regulatory mechanisms for ion and water transport pathways (Figure 8.8).

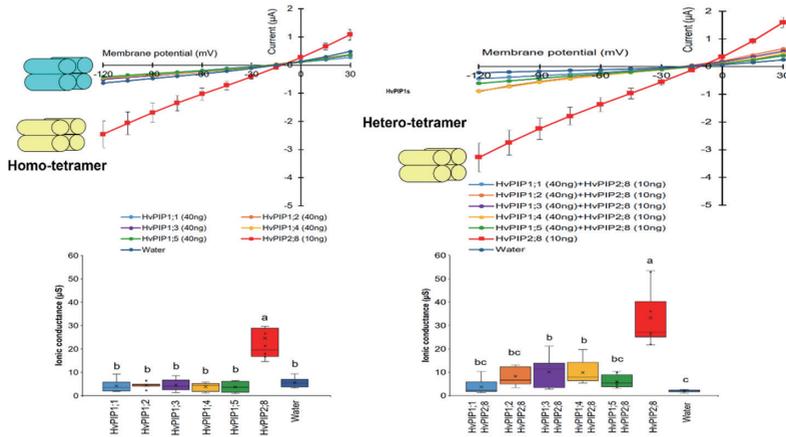


Figure 8.8. Co-expression of HvPIP2;8 with HvPIP1s Reduced HvPIP2;8 Ion Transport Activity

Source: Author

4. Conclusion

Our research on barley proteins shows that HvPIP2;8 can move specific ions (sodium and potassium) across cell membranes, but this ability depends on calcium levels outside the cell. When we combined HvPIP2;8 with related proteins (HvPIP1s), its ability to transport ions was significantly reduced. We also found that changes in protein phosphorylation suggest that HvPIP2;8 is controlled through a complex system involving both protein interactions and chemical modifications. These discoveries help us better understand how plants deal with salt stress. This research opens new paths for studying how these proteins can transport both ions and water, and for investigating the specific role of HvPIP2;8 in living barley plants.

References

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8. Dr. Thi Huong Sen TRAN



Chapter 8. Ca²⁺- Sensitive and Non-selective Na⁺/K⁺ Channel Activity of a Barley Aquaporin HvPIP2;8 under Saline Conditions

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