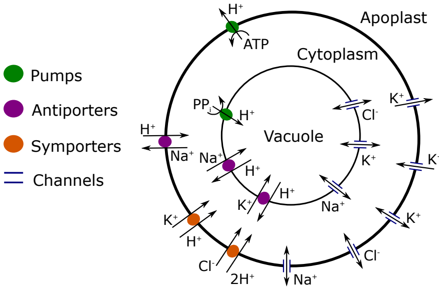
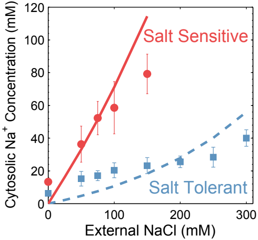
Mathematically modelling the salt stress response of individual plant cells

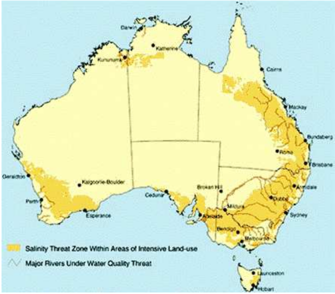
Kylie Foster
Supervisor: Prof. Stan Miklavcic
Phenomics and Bioinformatics Research Centre
University of South Australia





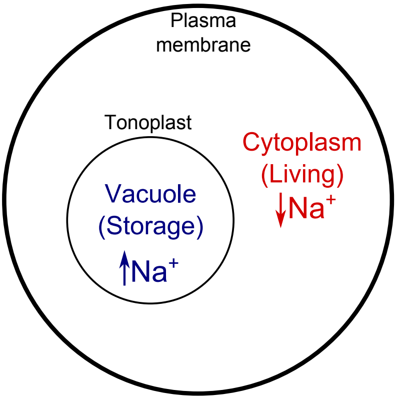
Motivation: Salinity

- Salinity is a problem globally
- In Australia high salinity affects **two-thirds** of cereal crops
- Impacts of salinity are expected to become worse
- Need to breed salt tolerant plants:
  - Requires identifying and understanding important transport processes
  - Need to identify the functions of relevant genes
  - Mathematical modelling can help!



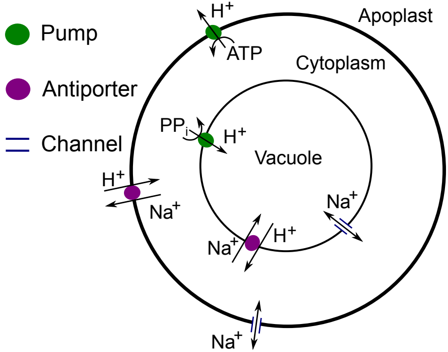
Plant cell biology: Salt stress stages

- Two main stages of salt stress:
  - **Osmotic** stress
    - Disrupts the cell's ability to take up water
  - **Ionic** stress
    - Na^+ in the living part of the cell disrupts important cell processes
- The effects of these stresses can be minimised by:
  - Storing Na^+ in the vacuole (to avoid osmotic stress)
  - Maintaining low levels of Na^+ in the cytoplasm (to avoid ionic stress)



Plant cell biology: Salinity tolerance mechanisms

- Membrane transporters control the movement of ions across cell membranes
- These include
  - H^+ pumps which power Na^+/H^+ antiporters
  - Channels that allow Na^+ to leak back into the cytoplasm

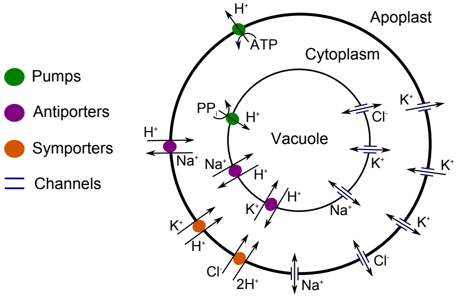


Existing cell models

- There has been extensive modelling of animal cells
  - But: Na^+ is transported by different mechanisms (Na^+/K^+ pump)
- Plant cell models:
  - Often do not include Na^+ (e.g. Hills et al., 2012)
  - Do not include transport across both membranes (Melkikh and Seleznev, 2005, 2012)

Single cell model: Active transport

- Time dependent model of the membrane transport of **water** and four mobile **ions**: Na^+, K^+, Cl^- and H^+
- Includes three compartments: Vacuole, Cytoplasm and Apoplast
- Constant conditions in the apoplast
- Simulates membrane transporters operating across **both** the tonoplast and plasma membrane:
  - **H^+ pumps** (primary active)
  - **Antiporters** (secondary active)
  - **Symporters** (secondary active)
  - **Channels** (passive)
- The model captures the key processes involved in salt stress response at the cell-level



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Model equations: Ion fluxes

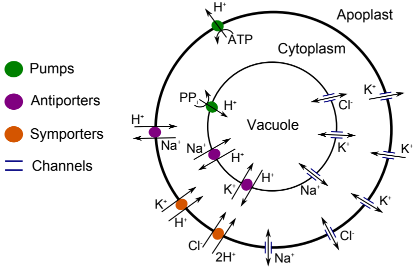
- Conservation equations for mobile ions in the vacuole:

$$\frac{d}{dt}\left(V_v[H^+]_v\right) = J_H^{A_v} + J_H^{XNa_v} + J_H^{XK_v}$$

$$\frac{d}{dt}\left(V_v[Na^+]_v\right) = J_{Na}^{C_v} + J_{Na}^{XNa_v}$$

$$\frac{d}{dt}\left(V_v[K^+]_v\right) = J_K^{C_v} + J_K^{XNa_v}$$

$$\frac{d}{dt}\left(V_v[Cl^-]_v\right) = J_{Cl}^{C_v}$$



- Fluxes (J) depend on
  - Concentrations in the different cell compartments ($[]_a$, $[]_c$, $[]_v$)
  - Electric potential differences across the cell membranes
  - Various transport specific parameters (channel permeabilities, antiporter reaction rates, pump rates)

Model equations: Volume and electric potential

- Cell compartment volumes change over time (due to water transport):

$$\frac{dV_c}{dt} = -Q_v - Q_a$$
$$\frac{dV_v}{dt} = Q_v$$

  - Water flow rates across membranes (Q's) driven by osmotic pressure differences

- Transmembrane potential, $\Delta\psi$:

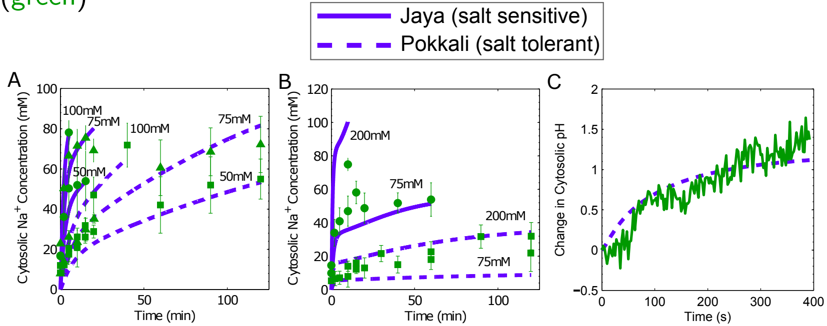
$$\frac{d\Delta\psi_{a/v}}{dt} = -\frac{I_{a/v}}{C_m^{a/v}}$$

  - I = the total current flowing across the membrane (determined by ion fluxes, J's)
  - C_m = membrane capacitance

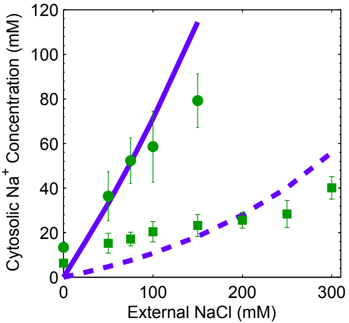
- Nonlinear system of ordinary differential equations.

Comparison with experiments: Parameter fitting

- Anil et al. (2007) conducted two-photon microscopy experiments to derive cytosolic Na^+ concentrations of individual, cultured rice cells
- Kader et al. (2005) conducted experiments to estimate salt induced changes in cytosolic pH in salt tolerant rice cells
- Model simulations (purple) were performed by matching experimental conditions and fitting parameters to experimental data (green)



Comparison with experiments



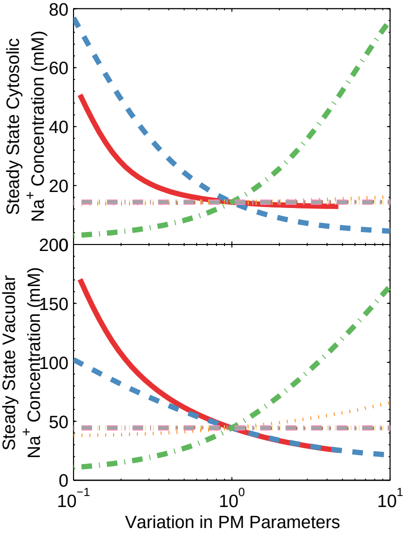
- *No further parameter fitting*
- Experimental cytosolic Na^+ concentrations (symbols) were measured after 1 hr of exposure to different external Na^+ concentrations
- Model simulations (lines) were carried out using the parameters found from the previous experimental results

- The model simulations show that the key differences between the cultivars are:
  - Plasma membrane Na^+ channel permeability
  - Ability to store Na^+ in the vacuole

Parameter sensitivity: Plasma membrane transporters

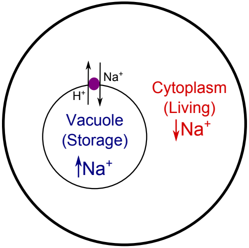
- Effects of varying **plasma membrane** parameters:
  - H^+ pump density
  - Na^+/H^+ antiporter strength
  - Na^+ channel permeability

- The changes in cytosolic Na^+ concentration lead to changes in vacuolar Na^+ concentration



Model applications

- Overexpression of **tonoplast** Na^+/H^+ **antiporter** genes has been shown to increase salinity tolerance in a wide variety of plant species
- The improved salinity tolerance could be due to
  1. Decreasing the level of Na^+ in the living cytoplasm (reduced ionic stress), and/or
  2. Increasing storage of Na^+ in the cell vacuole (reduced osmotic stress)



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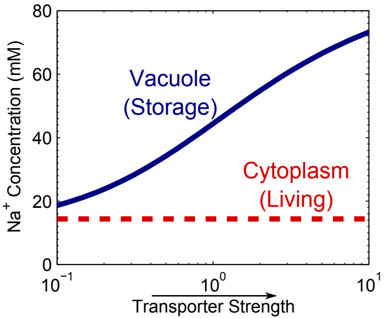
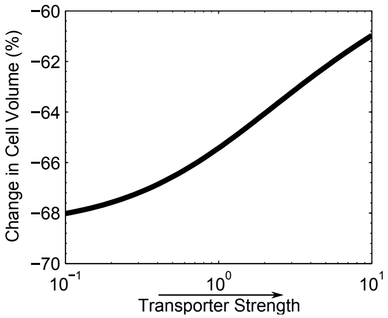
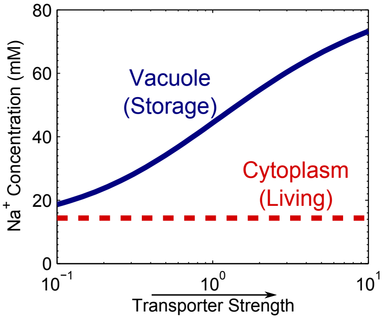
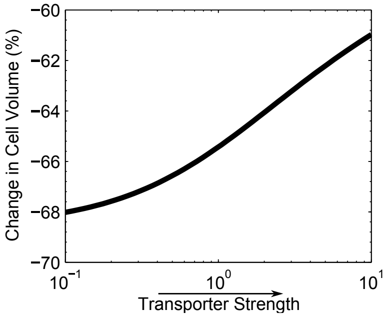




Model applications

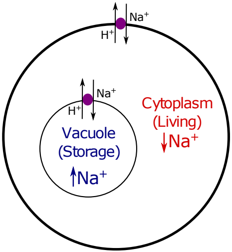
- The improved salinity tolerance could be due to:
  1. ~~Decreased Na$^+$ in the living cytoplasm (reduced ionic stress),~~ and/or
  2. **Increased storage of Na$^+$ in the cell vacuole (reduced osmotic stress)**

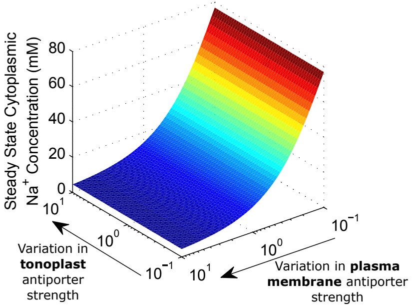


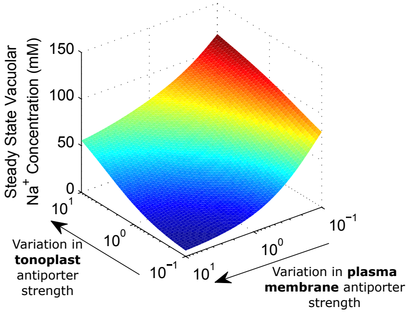


Model applications

- **Both** reduced ionic stress and reduced osmotic stress can be achieved by **simultaneously** overexpressing tonoplast and plasma membrane transporter genes
- To date this has not been achieved experimentally

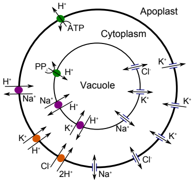


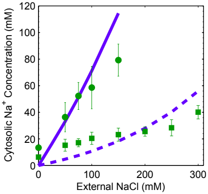


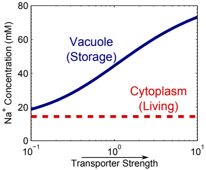


Single cell model: Summary remarks

- Our model captures the key processes responsible for salt stress response at the cell-level
- The model predictions are qualitatively and quantitatively comparable with experimental data
- The model simulations are particularly interesting in light of attempts to genetically alter Na^+ transport processes







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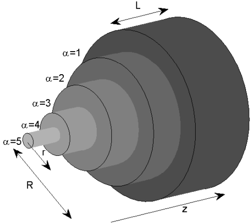
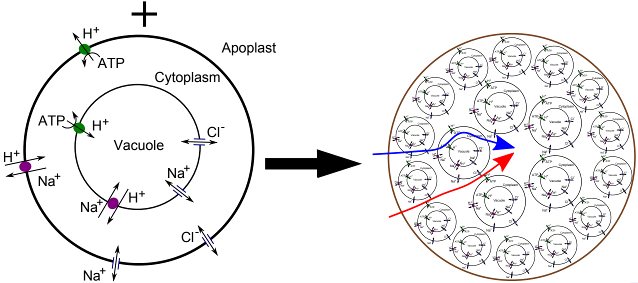
Future work: Multi-scale model

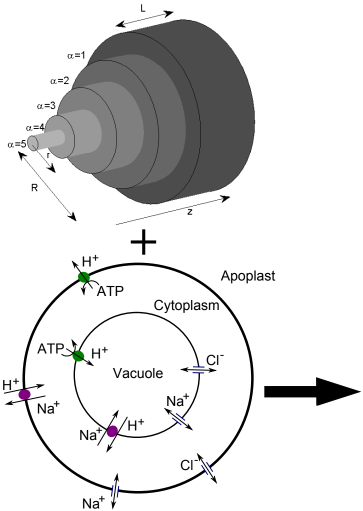
- Developing a multi-scale model of ion and water transport that combines features of:



- Organ-level model
  - Diffusion
  - Convection
- Cell-level model
  - Active extrusion of Na^+
  - Storage of Na+ in cell vacuoles



Acknowledgements



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- # Thank You!