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Toward a biophysical understanding of the salt stress response of individual plant cells

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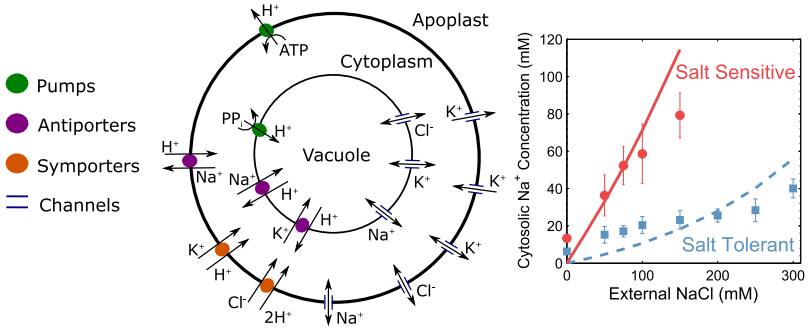
HIGHLIGHTS

- We model the ionic and osmotic response of an individual plant cell to salt stress.
- Primary active, secondary active and passive ion transport are modelled.
- The model includes osmotically driven water transport across the cell membranes.
- The model results are quantitatively comparable with available experimental data.
- We use this model to explore the effects of modifying transporter genes.

GRAPHICAL ABSTRACT



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ABSTRACT

We present and explore a kinetic model of ion transport across and between the membranes of an isolated plant cell with an emphasis on the cell's response to salt (Na^+) stress. The vacuole, cytoplasm and apoplast are treated as concentric regions separated by tonoplast and plasma membranes. The model includes the transport of Na^+, K^+, Cl^- and H^+ across both membranes via primary active proton pumps, secondary active antiporters and symporters, as well as passive ion channels. In addition, water transport is included, allowing us to investigate both the osmotic and ionic components of salt stress. The model's predictions of steady state and transient cytosolic pH and Na^+ concentrations were found to be quantitatively comparable to measured experimental values. Through an extensive simulation study we have identified and characterized scenarios in which individual transport processes (H^+ pumps, Na^+/H^+ antiporters and channels involved in the transport of Na^+) and their combinations have major effects on the level of Na^+ in each of the cell compartments. This systematic study emulates the effects of overexpressing and inhibiting transporter genes by genetic modification and hence we have compared our simulations with observations from experiments conducted on transgenic plants. The simulations suggest that overexpressing tonoplast Na^+/H^+ antiporter genes and tonoplast H^+ pump genes lead to an increase in the storage of Na^+ in the vacuole (helping the cell to maintain water uptake under salt stress), with only a transient influence on the cytoplasmic Na^+ concentration. The model predicts effects of varying the expression of transporter genes (individually or in combination) which have yet to be investigated in experiments. For example, our findings indicate that simultaneously overexpressing

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plasma membrane and tonoplast Na^+/H^+ antiporter genes would lead to improvements in both ionic and osmotic stress tolerance. The results demonstrate the importance of simultaneously modelling the transport of Na^+ across both the tonoplast and plasma membrane, a task not undertaken previously.

1. Introduction

Under low salt conditions Na^+ can be a beneficial nutrient in some plant species, with external concentrations as high as several millimolar being reported as beneficial even in some glycophytes (Kronzucker et al., 2013). However, under high salt stress plants experience significant problems. High salinity inhibits plant growth and does so in two phases: a rapidly occurring osmotic stress phase in which the plant's ability to take up water (and hence nutrients) is disrupted by the increase in osmotic pressure in the soil; and a slower ionic stress phase in which the accumulation of salt ions (usually Na^+) leads to concentrations that are toxic (Munns and Tester, 2008). Exposure to Na^+ can also disrupt the homeostasis of K^+ which is the most abundant cation in plant cells and is essential for many cell processes. For instance, the depolarization of the plasma membrane resulting from Na^+ influx can lead to an efflux of K^+ and hence a reduced cytosolic K^+ concentration (Shabala and Cuin, 2008). As a result, salinity can lead to significantly reduced crop growth rates, early leaf senescence and premature death. In this paper, we investigate the physical processes responsible for these events using a mathematical model of ion and water transport applied to a model plant cell. Moreover, we utilize the transport model to identify advantageous cell conditions that could be explored genetically to improve plant cell response to salt stress.

Under normal plant cell conditions of low cytoplasm concentration of Na^+ and negative electric potential difference across the plasma membrane, the influx of Na^+ into plant cells occurs passively (for instance, via channels) driven readily by electrochemical potential differences. Therefore, under high external salt conditions, active processes, occurring at the cell level, are required to maintain low levels of cytosolic Na^+. The important mechanisms include those that maintain low Na^+ ion concentrations in the cytoplasm either by sequestering Na^+ ions in the vacuole, by restricting the amount of Na^+ entering the cell initially or by the active efflux of Na^+ from the cell into the apoplast (Kronzucker and Britto, 2011). Both active extrusion and active compartmentalization of Na^+ are achieved by secondary active transport via Na^+/H^+ antiporters on the plasma membrane and tonoplast, respectively. These are driven by the primary active membrane transport of H^+ by proton pumps (H^+-ATPases on the plasma membrane and tonoplast, and the H^+-PPase on the tonoplast).

Numerous experimental studies seeking to identify and assess the relative and absolute importance of active processes in the plant salt response have been reported. For example, the overexpression of a tonoplast Na^+/H^+ antiporter gene (Apse et al., 1999) and overexpression of a tonoplast H^+ pump gene (Gaxiola et al., 2001) in *Arabidopsis thaliana* have been shown to improve salt tolerance. Nevertheless, many questions remain unanswered. For instance, what precise physical mechanisms are triggered by the overexpression of these transporter genes that lead to enhanced salinity tolerance (Kronzucker and Britto, 2011)? Focus on a single cell cannot lead to conclusions on the influence of transporters on long distance transport of salt ions in intact plants. However, an analysis of single cell behaviour is nevertheless relevant; enhanced salinity tolerance has been found in transgenic cell suspension cultures and protoplasts following the overexpression of various transporter genes (see for example, Fukuda et al., 2004). This indicates that improved salinity tolerance on the whole plant level is due in part to the combined action of individual mechanisms occurring at the single cell level.

There has been extensive modelling of ion transport in animal cells (see for example, Mori, 2012). However, in animal cells the active transport of Na^+ is achieved via Na^+/K^+ pumps, whereas in plant cells the active transport of Na^+ is achieved by the combined action of Na^+/H^+ antiporters and H^+ pumps. Hence, in parallel with transport modelling in animal cells, several mathematical models of ion transport in plant cells have been developed. These include primary active membrane transport mechanisms (such as H^+ and Ca^{2+} pumps), secondary active transport mechanisms (such as antiporters and symporters) and passive movement via channels. In particular, Hills et al. (2012) developed a detailed model of ion transport to investigate the behaviour of specialized leaf cells, guard cells. Other time dependent models have been developed to model the oscillatory behaviour of such quantities as membrane action potentials (for example, Sukhov and Vodeneev, 2009; Gradmann, 2001). However, Na^+ transport was not explicitly studied by these models. In contrast, Melkikh and Seleznev developed cell level models of ion, particularly Na^+, transport across the plant plasma membrane (Melkikh and Seleznev, 2005) as well as across the tonoplast (Melkikh and Seleznev, 2012). However, their analyses were limited to steady state behaviour (*e.g.*, comparing theoretically predicted membrane resting potentials to those measured in real plant cells).

Of the plant cell models developed thus far, only the model of Hills et al. (2012) included simultaneous ion transport across both the plasma and tonoplast membranes. Models considering Na^+ transport have either focused on transport across the plasma membrane (Melkikh and Seleznev, 2005) or across the tonoplast (Melkikh and Seleznev, 2012), but not both. However, it is clear that only by considering the simultaneous action of transport processes occurring across both membranes, resulting in ion redistribution between the cytoplasm and vacuole of plant cells and the apoplast, will a more holistic picture of the salt response of plants emerge.

As a step toward understanding the whole plant response to salt stress we have developed a kinetic model of a single, generic plant cell that incorporates primary active, secondary active and passive transport of Na^+, K^+, Cl^- and H^+ across both the tonoplast and the plasma membranes. We outline this physical and mathematical model of membrane ion transport in Section 2, stipulating our underlying assumptions. The detailed equations of the mathematical model are provided in the Supplementary Data file. The results of our simulations, including comparisons with experiments, are presented in Section 3 and discussed in Section 4 in the context of the plant cell response to salt stress. In particular, the discussion emphasizes the relevance of the model and its predictions to improved understanding of salinity tolerance and highlights suggestions of possible targets for future genetic studies seeking to enhance specific transport processes that contribute to salinity tolerance. The paper concludes with summary remarks and a note on a future research direction.