Genetic analysis of Ca^{2+}-signaling in *Arabidopsis* in response to drought and salt stress

Aaron Stephan, Division of Biological Sciences, University of California San Diego
Department of Energy, Office of Science, Basic Energy Sciences Fellow of the Life Sciences Research Foundation (2011-2014)

Overview and Significance- A primary objective of modern agriculture and biofuel production is to utilize arable land to its fullest potential. However, sub-optimal growing conditions—arising from abiotic stresses such as drought, soil salinity, low humidity, cold, and heat—reduce crop yield and quality. Optimal yield under both stressed and non-stressed conditions requires the plant to activate coping mechanisms at a level commensurate with the severity of the drought stress. The osmotic sensors and associated regulatory mechanisms that initiate drought- and salt-tolerance responses in plants are largely unknown (Figure 1). My fellowship research aimed to identify and characterize these initial sensory components.

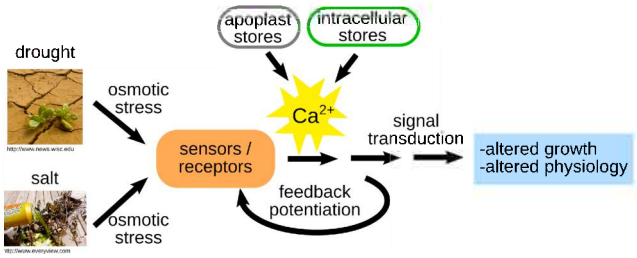

Figure 1. Role of osmotic sensors in initiating drought and salt responses in plants. A rise in intracellular calcium is the fastest-known response to osmotic stress and is the most direct readout of osmotic sensor activity.

Research Milestones- Rapid osmotic-induced calcium responses in *Arabidopsis thaliana*

Plants develop many drought responses within hours to days after osmotic stress, but they exhibit a rapid calcium influx within seconds of osmotic stress (Knight et al., 1997) (Figures 1 and 2). Genetic interrogation of this rapid osmotic-induced calcium response in plants is key to identify sensors of osmotic stress as well as to understand upstream regulation of these sensory mechanisms. At the onset of my fellowship, I established a system for measuring osmotic-induced calcium responses in the model plant *Arabidopsis thaliana* expressing the calcium indicator aequorin (Figure 2A). Data obtained from these experiments are fed into a detailed R-script analysis that quantifies over 40 different aspects of the response profiles, including peak amplitudes, rise times, decay constants, integrated response magnitudes, and principal components.

1) *Osmo-sensory potentiation-* I found that prior exposure of *Arabidopsis* seedlings to hyper-osmotic stress potentiates subsequent osmotic-induced calcium responses— amplitudes are larger, and the responses are more reproducible (Figure 2B). This result points toward a mechanism whereby positive feedback on the sensory machinery results in more robust activation of downstream responses to subsequent stress (Figure 1). Interestingly, I found that the abiotic stress hormone abscisic acid (ABA) also potentiates the osmotic-induced calcium responses. Potentiation by ABA is partially distinct from osmotic-induced potentiation. Through a collaboration I initiated with Dr. Carlos Guerrero in the Department of Chemistry and Biochemistry at the University of California, San Diego, we have synthesized the ABA antagonist hexa-sulfanyl-ABA (Takeuchi et al., 2014) to investigate the ABA-independent potentiation pathway. Other reported "drought-priming" agents are being evaluated for possible roles in osmo-sensory potentiation, including NO, H_2S, H_2O_2, polyamines, and acetate (Filippou et al., 2013).

2) *Genetics of osmo-sensation-* Candidate mutant *Arabidopsis* lines for genes with the potential to influence osmo-sensing were tested for osmotic-induced Ca^{2+} responses. While many proposed mechano-sensitive channel mutants showed no phenotype (Fig. 2C-D), I identified three mutant genes that result in a reduction in osmotic-induced calcium responses. The first gene, *Cyp86a1*, is necessary for suberin biosynthesis (Hofer et al., 2008; Li et al., 2007) (Fig. 2E). I hypothesize that the site of osmotic stress perception is located within the root at- or outside-of the endodermal cell layer, and an increase in hydraulic conductivity at the limits the magnitude of the perceived water potential difference (Ψ_w). I have established a collaboration with Professor Niko Geldner

at The University of Lausanne to test this hypothesis using mutants in genes necessary for biosynthesis of the Casparian Strip, which provides an independent means to increase root hydraulic conductance (Naseer et al., 2012). I also identified a mutation in the plasma membrane-localized, mechanosensitive, Tandem-Pore Potassium Channel *tpk4* gene that results in a reduced osmotic-induced calcium response (Maathuis, 2011). Lastly, through a collaboration with Dr. Hans-Henning Kunz (now at Washington State University), I found an involvement of the plastidial potassium/proton antiporters KEA1 and KEA2 (Kunz et al., 2014) in regulating osmotic-induced calcium responses. Preliminary observations indicate that this phenotype may be due to impaired sensory potentiation rather than a difference in initial sensitivity. Tissue-specific expression of these genes will be used to determine the site of perception. Work is currently underway to determine the link between altered intracellular and cell-surface potassium gradients and the osmotic-induced calcium response.

In addition to a reverse-genetic approach, I performed a forward-genetic screen of ~2,000 EMS-mutagenized *Arabidopsis* lines and isolated 20 mutant lines that revealed reproducible, heritable, altered osmotic-induced Ca^{2+} responses (e.g., Fig. 2F-H). The causative mutations in the strongest of these mutants, line "9.3C09K", has been rough-mapped. Whole-genome Illumina re-sequencing revealed that this interval contains just a few EMS mutations that are predicted to affect protein function. One gene is of utmost interest and is the subject of intense investigation with regard to osmosensory functions.

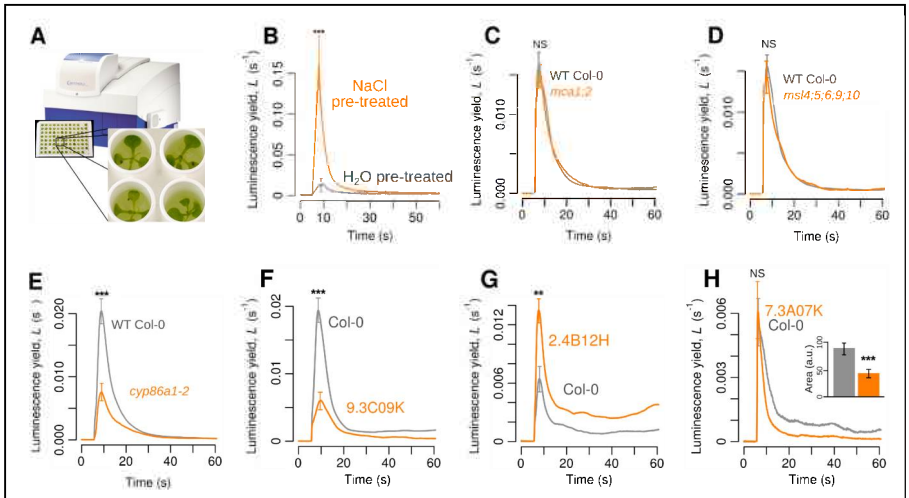

Figure 2. Potentiation of osmotic-induced calcium responses and genetic analyses.

3) *Physiological consequences of altered osmo-sensation*- I tested many of the mutants that displayed altered osmotic-induced calcium responses for downstream effects on drought and osmotic responses. I found that mutant line "9.3C09K" (Fig. 1F), for instance, displayed faster transpiration rates than wildtype in response to water stress (Fig. 3A). Furthermore, this mutant and others exhibit an impaired hydrotropic response, a growth phenotype whereby roots grow toward regions of highest water potential (Cassab et al., 2013) (Figs. 3B-C). Interestingly, while none of the mutants displayed impaired root growth on hyper-osmotic plates, I made the observation that the left-ward root skew tendency that is present in wildtype plants was absent in several calcium-signaling mutant lines (Fig. 3D). This finding is exceptional for a number of reasons. First, it provides a novel screen-able trait never before associated with osmotic stress. Second, understanding the underlying mechanisms may shed light into previously unknown effects of osmotic stress on molecular interactions and developmental processes. Third, these mechanisms may provide new insights into the origins of biological chirality and how the environment can

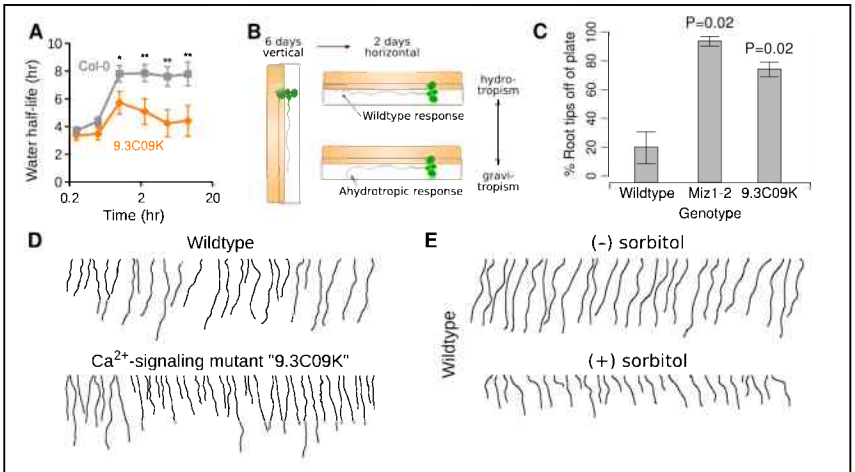

Figure 3. Physiological consequences of impaired osmotic sensing. (A), increased water loss. (B-C), reduced hydrotropism. (D-E), inverted root chirality.

influence the direction of chirality (Riehl, 2010).

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Publications resulting from the grant:

Stephan, A.B., and Schroeder, J.I. Sensory potentiation of rapid osmotic-induced Ca^{2+} responses in *Arabidposis thaliana*. (in preparation).

Stephan, A.B., Yang, E., Zhang, T., and Schroeder, J.I. Genetic identification of regulators of osmo-sensation in plants. (in preparation).

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Anticipated unexpended funds at the end of the budget period

There are no unexpended funds at the end of this budget period.