Subcellular Characterization of Salinity Tolerance in Almonds with Novel Tools

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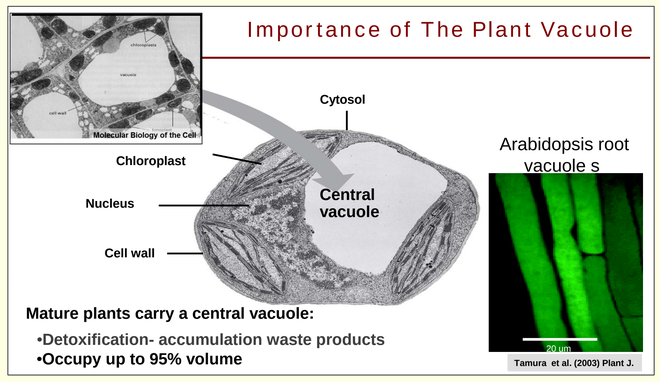
California is experiencing increasing soil salinization, which is projected to accelerate in the current drought conditions due to the increased use of saline ground water. Almond plants, one of the most economically important crops in California and one with the highest expansion rate, are strongly sensitive to salt stress. Selection of elite rootstocks with improved salinity tolerance affords a way to ensure high yield production in this long term trend. However, currently there exist no robust screening tools for the large scale evaluation of salinity tolerance for rootstocks at the seedling level. Our proposed work is aiming developing and applying these, driven by the hypothesis that sodium sequestration and compartmentalization in almond tree cells is a quantitative predictor for salinity tolerance.
The foundation for the development of universal cellular and molecular methodologies towards identifying sodium uptake, ion sequestration and its effect on cellular morphology and viability for various rootstocks and rootstock/scion combinations is a hitherto unexplored approach. Live cell fluorescent microscopy affords localizing and evaluating saline induced structural and morphological changes in the cell and cell wall as a robust criterion for halotolerance across various rootstocks.

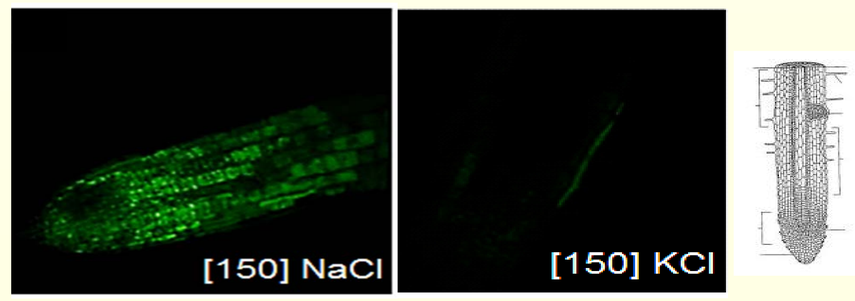
Hypothesis and Rationale

Roots play a key role in the salt tolerance of plants, for they represent the first organs to control the uptake and translocation of nutrients and salts throughout the plant. Accumulation of Na^+ in the roots is an adaptive response used by several woody species to minimize its toxicological effects on shoots. Accordingly, the control of the root-to-shoot transport of salt can serve as a criterion for tolerance.
Our working hypothesis is that sodium and chloride sequestration in the vacuole of almond cells is important for salinity tolerance. Thus assessing and understanding the subcellular sequestration of Na^+ and Cl^- into the almond root cells, in a quantitative manner, can provide an effective and economical way for the identification of the most suitable genotypes.



Results

Selectivity of CoroNa-Green for Sodium



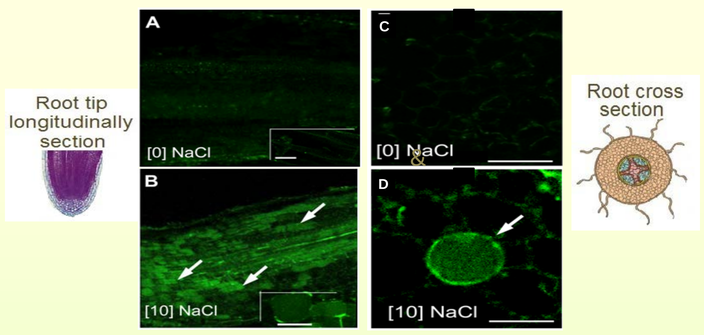
The specificity of sodium and potassium dyes in live cells was tested. Figure shows selectivity of CoroNa-Green for Na^+ versus K^+. CoroNa-Green intensity increases in response to 150mM NaCl treatment, whereas no signal is observed under 150mM KCl.

Results

Towards the selection of elite material, we develop confocal based assays for sodium and potassium detection. We established a methodology and protocols to specifically image sodium and potassium in the context of referential cellular markers, such as vacuoles, in woody plant cells. This allows us to quantitate sodium transport in roots, stems and leaves toward assessing the subcellular sodium sequestration.

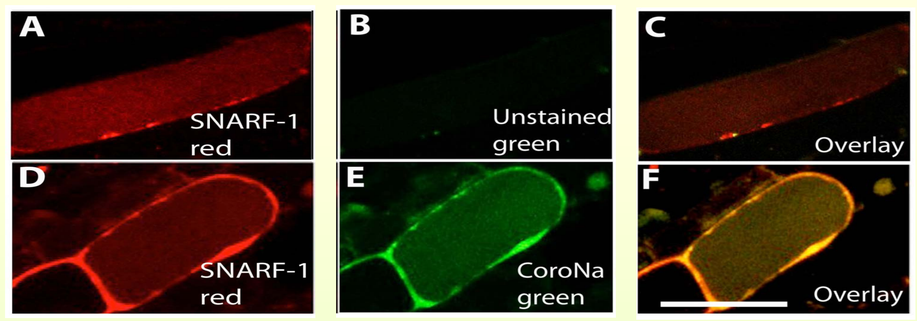
Sodium localization in Pistachio

Sodium Localization in Pistachio Roots after NaCl Treatment



Sodium localization in pistachio in roots after 3 weeks of NaCl treatment. Longitudinal sections (A-B) and transverse sections (C ,D) of UCB-1 roots are shown. Control roots (A, E) do not show significant signal. Intracellular sites of sodium accumulation were detected as indicated by arrows.
Scale bars = 20µm.

Sodium Localization in the Vacuole of Pistachio cells



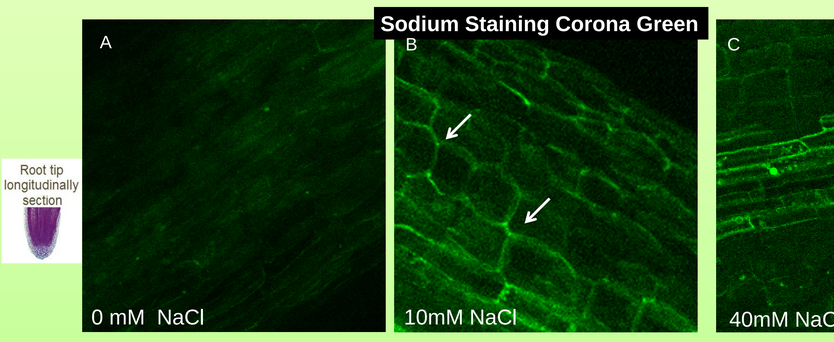
Sodium accumulates in the vacuole of pistachio cells.
Panel shows case for sections from plants treated with 50mM NaCl. Sodium localization is indicated in green. Vacuole localization is indicated in red. Scale bar = 20µm.

Salt Treatment of Almond Seedlings

Almond rootstock seedlings were treated under different NaCl concentrations. Root tissues were sectioned and stained for sodium and potassium.

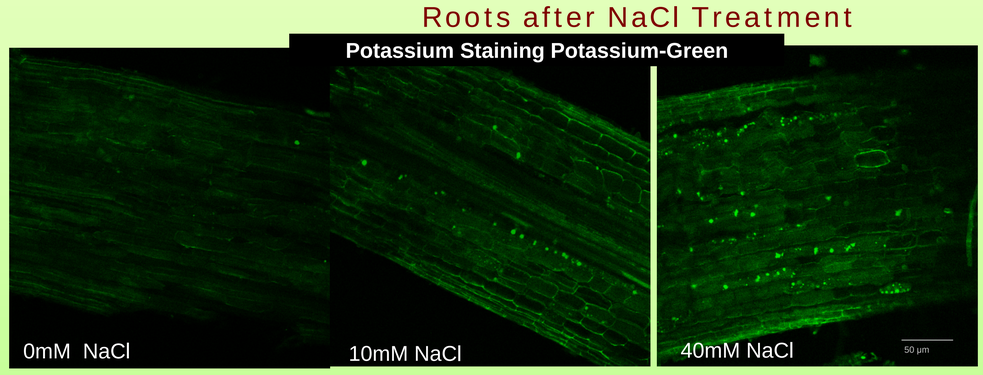
Sodium and Potassium localization in Almond

Sodium Localization in Almond Roots after NaCl Treatment



Sodium localization in almond roots after 2 weeks of NaCl treatment. Longitudinal sections of Hansen roots are shown. Longitudinal sections (A-C) and transverse sections (D ,E) of Hansen roots are shown. Control roots (A, D) do not show significant signal. Increased cellular accumulation of sodium is observed under salt treatment (B, C, E). Scale bars = 50µm.

Potassium Localization in Almond Roots after NaCl Treatment



Potassium localization in almond roots after 2 weeks of NaCl treatment. Longitudinal sections of Hansen roots are shown. Altered localization pattern of potassium is observed compared to the control. Scale bar= 50µm.

Future work

We will extend the fluorescence ion screening toward chloride localization in almond cells. The latter constituents a major step forward, since no approaches in plants have been reported so far. Structural root cell morphology changes and ion compartmentalization in selected rootstock genotypes will be examined throughout. Cellular morphologies under salinity will be characterized and comprehensively charted. Using specific fluorescent dyes for cell wall, key structural components in the root cell tissue will be analyzed, to provide comprehensive structural visualization. This will enable the correlation of salt tolerance with subcellular ion compartmentalization to efficiently characterize tolerant genotypes. The established methodology will provide the flexibility for future extensions beyond salinity stress to assess cellular structural modifications in response to biotic and abiotic stresses, including that of various pathogens. We expect that dissemination of the developed methodology will spur rapid adaptation and follow up studies, in tandem with a proliferation of advanced microscopy tools.

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