# Cellular, Subcellular and Molecular Characterization of Salinity Tolerance in Pistachio with Novel Tools

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## INTRODUCTION

Soil salinization in California is increasing. Pistachio plants with relative high saline tolerance afford the possibility to use marginal land and improve yield on current acreage. So far, little is known about the cellular uptake and translocation of nutrients and salts throughout the pistachio plant under salt stress, and more importantly how to systematically and economically select for elite cultivars. Pistachio trees are strongly contributing to California's economy. Their relative saline tolerance provides great potential for future expansion into marginal land with elevated salt levels.

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. 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. We focus on the phenotypical, genetic and molecular characterization of this hitherto ill-understood mechanism. Understanding the mechanism of sodium uptake, transport and sequestration at the cellular and molecular levels is valuable and can provide a convenient and economical way of identifying desired plant "characteristics" to be selected in rootstocks, scions or their combinations in order to achieve optimal plant performance and composition. Insights gained will assist in the development of better agricultural practices under saline conditions.

Our work aims at the establishment of cellular and molecular methodologies to identify sodium, potassium and chloride uptake; and ion sequestration and its effect on cellular morphology and viability for various rootstock scion combinations. Our working hypothesis is that sodium and chloride transport and sequestration in pistachio cells is an important and identifiable trait for salinity tolerance, and that it is mediated by the activity of specific transporters. By observing sodium, potassium and chloride localization in live plants, at the subcellular level with non-invasive fluorescence microscopy and saline-induced structural/morphological cell and cell wall changes, we are aiming at the in-depth characterization of salinity tolerance. Thus, understanding the cellular ion sequestration, in combination of root structural characterization, can provide evaluation criteria for the identification of most suitable rootstocks.

## RESULTS

We developed and adopted methodologies for the precise tissue staining of sodium, potassium and chloride in root and leaves of pistachio seedlings. The developed approaches allow *live, in vivo* imaging at the cellular and subcellular levels. In order for the indicator dyes to be effective, a number of custom steps in the staining process had to be developed. Toward establishment of the subcellular distribution, co-staining methods were developed with markers for subcellular compartments and membrane structures, while circumventing signal form the auto fluorescence

from plant tissue, in particular chlorophyll. This was achieved by taking advantage of the difference in the time that a fluorophore spends in the exited state before it emits light, which can differ by 0.5- 4 nanoseconds depending on the fluorophore. The Leica SP8 microscope used in our studies allows a signal selection via gating for collecting time-dependent fluorescence.

**Phenotypic analysis of salt treatment:** The salinity treatment led to an accumulation of pigments in leaves, manifesting itself in a “red-brown” leaf color. This effect was observed after one week of 100 mM of sodium application. Differences in leaf browning was observed between UCB-1*, P. atlantica* and *P. integerrima,* with *P. integerrima* being the most severely affected. Further, the overall “robustness” and root architecture was considerably different between the various genotypes, suggesting that this characteristic of the rootstocks may play a role in mechanisms of salinity tolerance. Notably *P. integerrima showed* significantly different root development and overall root system architecture compared to UCB-1 and *P. atlantica*. It is likely that observed differences in the overall root system architecture contribute to differences in the response of these genotypes to salt stress.

**Cellular imaging of sodium and potassium:** Localization of sodium under salinity treatment was observed in root cells of UCB-1, *P atlantica* and *P. integerrima*. Sequestration of sodium under salinity treatment was observed in root parenchyma cells of UCB-1 and was also present in *P. integerrima*, while this effect was not pronounced in *P. atlantica*. Characteristic cell damage was observed during prolonged salt treatment, manifesting itself in sodium dispersing throughout the cytoplasm. Overall, these data showed sodium uptake under salinity treatment, which is enhanced in *P. integerrima.* Further, examination of leaf tissue in UCB-1 using an SP8 confocal microscope with gating imaging, revealed a specialized cell type accumulation of sodium compared to nontreated plants. These specialized cells, in UCB-1, may contribute in the overall salinity tolerance.

On the basis of these identified ion-localization patterns, we hypothesize that both sodium uptake via the roots and its subsequent specific distribution, determines cytotoxic effects during NaCl stress. This is most likely effected by a mechanism related to ion balances that is not yet fully explored and understood. The mechanism appears to control transport of sodium to the leaf tissue with an observable reduction in plants exhibiting less cytotoxic effects compared to the plants with “red-brown rendered leaves.” In agreement with our subcellular data, ion content analysis showed increased sodium and chloride in roots and leaves of *P. integerrima* compared to UCB-1 and *P. atlantica*. Altogether, these data suggest that increased uptake and transport of sodium and chloride takes place in *P. integerrima,* compared to the other two genotypes tested that lead to an increased cytotoxicity and leaf damage.

Further, root cellular structures were analyzed by staining root sections with aniline-berberine, a vital stain for suberin, lignin, and callose, showing a characteristic staining of the root endodermis. With the methodology developed, the role of endodermis under stress conditions will be determined in continuing studies to examine its role in salt stress response. The root structural differences observable in tissues within the different genotypes can be related to halo-tolerance.

## CONCLUSION AND APPLICATIONS

The overall results suggest that both ions uptake and sequestration are mechanisms contributing to salinity tolerance. Further, structural differences observed in tissues within the different genotypes can be related to halo-tolerance. With our established methodologies, we

will conclude the evaluation of different rootstock genotypes and contribute to the understanding of mechanisms contributing to salinity tolerance. Application of these methods in genotype characterization efforts affords a unique opportunity to assess rootstocks and elite cultivars in an efficient and cost-effective way. Genotypes, with these phenotypic characteristics, will be examined for altered gene expression of specific transporters, allowing the identification of molecular markers for salinity tolerance