## Breeding for Flood tolerance: Experiences with time-series elemental imaging of fresh and live plants.

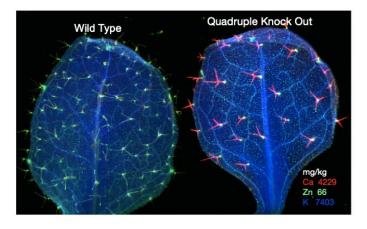
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Molecular genetics is a tool for understanding the basis for traits that make plants resistant or susceptible to environmental stress. This information feeds directly into plant breeding efforts. The impact of flooding on agricultural crops as a result of increased severe rainfall events is economically devastating, making flood stress tolerance a breeding priority for climate resilience. Flooding presents compound stressors to plants: combining oxygen deprivation (anoxia), temperature and light changes and osmotic stress. Calcium is a key element in the plant stress response; transporters regulate cytosolic Ca as a signal. Unexpected tolerance to anoxia was discovered when all four calcium exchanger genes (CAX 1-4) are silenced. Understanding why suppressed Ca transport causes anoxia tolerance requires fast elemental imaging of fresh (but preferably living) plants and a regimented experimental timeline after 8 hours of anoxia. Elemental imaging at both the NSLS2 4-BM beamline and using laser ablation inductively coupled plasma mass spectrometry found that under normal conditions, mutants without functional CAXs have a similar elemental distribution to wild type plants recovering from anoxia, with the hypothesis being that mutants have by default already made the metabolic adjustments that minimize anoxia-induced damage. Time series imaging showed that in wild type, Ca moves into the star-shaped leaf hairs (trichomes) after anoxia, whereas in mutants Ca cannot move but is already in trichomes at high concentrations. Elemental perturbations from anoxia also affect magnesium, chloride and zinc, the latter appearing in a punctate distribution that suggests guard cell and stomatal involvement. We present results from (1) time-series experiments from both X-ray and laser technques, (2) development of prototype custom sample environment for live plant imaging and (3) capture of the Ca signal using the new Maia detector at 4-BM.



Fresh *Arabidopsis* leaves from wild type (left) and mutants lacking all four Ca exchanger genes (right) grown under normal conditions. Leaves were analyzed together at the XFM beamline. Mutant plants have unusual tolerance to oxygen deprivation and a different elemental distribution profile.