

Molecular mobility and concentration of plant cells intracellular compartments, as derived from ice crystal size measured on cryo-SEM micrographs

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Cryo-SEM (scanning electron) micrographs present black imprints, corresponding to the ice crystals formed during the cooling process of aqueous solutions within cells and tissues. The etching process (partial sublimation of water from ice crystals at vacuum, inside the microscope) is the responsible of the observed black ice crystal imprints. A first sight of a plant tissue micrograph with subcellular resolution shows how the different subcellular compartments contain crystals of different size. Ice crystal size can be related (for similar cooling rate conditions) to the molecular mobility in the solution before freezing: low mobility allows a larger number of nuclei to form, before all available water is frozen (and, hence, forms smaller crystals), while with higher mobilities, a reduced number of initial nuclei grow to include all water molecules. Molecular mobility (and its opposite, viscosity) are depending on solute type and their concentration. For example, for the same solute composition, mobility will decrease with water content reduction. Intracellular compartments are characterized by having different solute/water ratios, which may change with physiological state.

We propose that the different molecular mobilities/viscosities (as derived from different concentrations/water contents) in subcellular compartments of plant tissues, can be derived or estimated from the corresponding ice crystal sizes, as observed in cryo-SEM images.

Examples for mint tissue equilibrated to different global water contents and incubated with different solutions are presented. The observation of cryo-SEM micrographs allows easy measurement of ice crystal size and its distribution, with the help of image analysis software. Global water content data and composition for incubation solutions allow estimations of concentrations and motilities, and in spite of the lack of independent data of mobility within each compartment, available information allows at least comparative estimations. The possibilities and limitation of this method, as well as its utility for cryopreservation studies are discussed.