

Spatial and Temporal Expression Patterns of Genes in the Myo-Inositol Pathway to Ascorbate in Arabidopsis thaliana

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Also known as vitamin C, ascorbate (AsA) plays an important role in living organisms as a major antioxidant and cofactor of multiple vital enzymatic reactions; it is also the most abundant water-soluble vitamin and is widely distributed among species except for primates, and some fish and rodents that rely on dietary sources to cover their vitamin C requirements. The deficit in this vitamin causes a disease known as scurvy that can be deadly if remains untreated. There are four known biosynthetic routes by which plants produce vitamin C using the following intermediates: D-mannose/L-galactose, L-gulose, D-galacturonate, and *myo*-inositol. The *myo*-inositol route involves four enzymes: *myo*-inositol oxygenase (MIOX), glucuronate reductase (GlcUA), gulonolactonase (GNL), and gulono-1,4-lactone oxidase (GLOase). Our group has made significant progress on the characterization the first two gene families involved in the conversion of inositol to AsA in plants. The main objective of my PhD project is to study the spatial and temporal expression patterns of the genes involved in the inositol pathway to vitamin C in *A. thaliana* by using molecular biology, biochemical, and cell biology techniques. I will focus first on the enzymes already characterized in the Lorence Laboratory: MIOX and GlcUAR. In order to determine the subcellular localization of these enzymes, they will be tagged with fluorescent proteins and expressed as fusions in *A. thaliana* and or tobacco. In order to understand the tissue specificity and expression pattern of these enzymes, the corresponding gene promoters will be introduced in transformation cassettes that include the β -glucuronidase gene (GUS) reporter system. Fluorescence microscopy will provide a high resolution tool to track the fusion proteins of interest *in situ*. Histochemical analysis of plant expressing GUS will provide information about the pattern of expression in tissues at various developmental stages. Knowing how these genes regulate the synthesis of AsA may contribute to explain the relationship among AsA biosynthesis, and important cellular processes such as redox equilibrium, cell wall formation, protein synthesis, and photosynthesis.