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Analysis of *Arabidopsis thaliana* mutants defective in the oligopeptide transporter *OPT3*

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The transport of peptides across membranes is a phenomenon found in both prokaryotes and eukaryotes as a method of obtaining amino acids, nitrogen, and carbon. Peptides can be transported by ATP-dependent transporters, as well as proton-coupled transporters. Among the latter are members of the oligopeptide transport (OPT) family, which transport tetra- and pentapeptides. Sequence comparisons led to the identification of nine OPT genes in Arabidopsis and our laboratory is investigating the role of these transporters in plant growth and development. Previous studies showed that mutations in the *OPT3* gene resulted in embryo lethality. More recently, *OPT3* expression was shown to increase under conditions of iron limitation, suggesting a possible role for *OPT3* in transporting iron-chelates. The lethal nature of *OPT3* T-DNA insertion mutation makes them difficult to study in a homozygous condition. Therefore, we sought non-lethal mutations within the *OPT3* gene sequence, which can be maintained as homozygous plants. To create such mutations, we used the process of Targeted Induced Local Lesions IN Genomes (TILLING) to identify non-lethal, point mutations in the *OPT3* gene. Eight mutant alleles, *opt3*-1 to *opt3*-8, were identified by TILLING. These mutants were sequenced and aligned with the other members of the OPT family to determine whether the mutations occurred within conserved regions of the protein. The mutations *opt3*-5 (P628S) and *opt3*-8 (P547L) were the first homozygous mutants identified which occurred within a highly conserved region and, therefore, were the likely candidates to disturb *OPT3* function. These mutations were followed in segregating populations by CAPS (Cleaved Amplified Polymorphic Sequence) markers. Homozygous mutant lines and wild-type controls were grown on medium containing limited, moderate, or excess iron. The iron effects on the plant were determined by assaying the chlorophyll content in whole plants. These assays revealed no measurable effect of the *OPT3* mutations on chlorophyll content under the conditions tested. We are now examining other *opt3* alleles for a role in iron transport and other possible phenotypes displayed during plant growth and development.