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

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 a opt3 T-DNA insertion mutation makes it difficult to study in a homozygous condition. Therefore, we sought non-lethal mutations within the oopt3 gene sequence, which can be maintained as homozygous plants. To obtain 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. 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 likely candidates to disturb OPT3 function. These mutations were followed in segregating populations by CAPS (Cleaved Amplified Polymorphic Sequence) markers. OPT3 over-expression lines were also obtained by linking opt3 to a CaMV35s promoter and inserting this within the genome. Homozygous mutant lines and wild-type controls were grown on medium containing limited or moderate iron. The iron effects on the plant were determined by assaying the reductase activity and the iron levels, via Perl's staining and Turnbull's blue stain, in whole plants. The reductase assays revealed no measurable effect of the TILLed oopt3 mutations under the conditions tested. Perl's staining and Turnbull's blue stain revealed a measurable effect of the CaMV35s-linked OPT3 over-expression.

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