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## **Adventures in Arabidopsis: Two projects investigating iron homeostasis in plants**

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In order to identify genes involved in iron homeostasis, a novel genetic screen was developed. Seed from a line transgenic for Green Fluorescent Protein (GFP) driven by the Fer1 promoter, a gene whose mRNA expression levels are increased during iron sufficiency and thus reflects iron status in plants were mutagenized with EMS and screened for high or low GFP fluorescence relative to non-mutagenized transgenic controls. From this screen, approximately 300 putative mutants for iron homeostasis were identified based on either high or low fluorescence relative to the transgenic control on iron-sufficient media. In order to more accurately determine the GFP protein level in the plants, total protein from each line grown on iron-sufficient media for 14 days then transferred to iron-deficient or fresh iron-sufficient media is being extracted, and will be separated by SDS-PAGE, and probed for GFP using a two-antibody detection system. One mutant, OAF102, had been previously identified for its constitutive fluorescence during both iron-sufficient and –deficient conditions three days after transfer, and had been shown to have uncoupled GFP and Fer1 mRNA levels between iron-sufficient and –deficient media at this point. OAF 102 and the non-mutagenized transgenic control were grown for 14 days on iron-sufficient media, then transferred to iron-sufficient or –deficient media. GFP and Fer1 mRNA levels were determined by northern blotting at 2, 3, 4, and 5 days after transfer. Two repetitions of the experiment were performed. Neither one showed the uncoupling seen in previous experiments at three days after transfer. OAF 102 may have constitutively elevated GFP mRNA, but normal regulation of Fer1 mRNA levels. Results have not been consistent and more experiments are being done. Four closely related transcription factors, bHLH's 038, 039, 100, and 101, had previously been shown to be upregulated in Arabidopsis during iron deficiency, but T-DNA insertion lines for each transcription factor did not show any changes in ferric-chelate reductase activity, chlorophyll content, or iron content, all of which are indicators of iron status in the plant. In order to further evaluate the potential role of these transcription factors in iron homeostasis, crosses were performed to create five double mutants with T-DNA insertions in two of these transcription factors in different combinations. Seed from these lines were germinated and grown on iron-sufficient media for 14 days and transferred to iron-deficient media or fresh iron-sufficient media. After three days, ferric-chelate reductase activity in each line was determined. None of the lines' reductase activities differed consistently from that of wild-type controls. Further characterization, including measurement of mRNA levels of these transcription factors and other iron-regulated genes, of the double and single T-DNA insertion lines is being performed.