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Characterization of a putative mutant for iron homeostasis

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Little is known about the genetics of iron homeostasis in plants. A novel genetic screen was used to identify mutants with alterations in iron homeostasis. Because Ferritin (Fer1) mRNA expression is upregulated by intracellular iron concentration in leaves, this gene can be used to predict intercellular iron concentrations in leaves. To identify mutants that overor under-accumulate leaf iron, Arabidopsis was transformed with the reporter gene Green Fluorescent Protein (GFP) driven by the Ferl promoter. Seed from this transgenic plant were mutagenized with EMS. The resulting M2 seed were screened for high or low GFP fluorescence relative to transgenic controls grown on iron-sufficient medium. A putative Over-Accumulator of Fe, pOAF40, was identified that expressed high levels of GFP fluorescence. Our objective was to characterize this mutant for alterations in iron homeostasis. Seed of pOAF40 and the non-mutagenized transgenic control were germinated and plants grown on iron-sufficient medium for 14 days before transferring to ironsufficient or -deficient media for four days. Fer1 mRNA levels, chlorophyll content, and ferric-chelate reductase activity (an enzyme whose activity increases during iron deficiency) were determined at the point of transfer and again four days after transfer. Fer1 mRNA expression was the same at the time of transfer, but greater relative to transgenic controls regardless of iron concentration 4 days later. The average concentration of chlorophyll in pOAF40 was less than the control regardless of sampling time or iron concentration. pOAF40 exhibited lower reductase activity than control on the day of transfer, however this difference in activity was not detected four days after transfer within iron-sufficient or -deficient treatments. Furthermore, ferric-chelatate reductase activity was greater in irondeficient than -sufficient media for both mutant and control suggesting normal response to iron-deficiency by pOAF40. Further characterization of this mutant is being performed to determine whether the mutation deregulates ferritin expression or leads to over-accumulation of iron in leaves.