Changes in flower protein and metabolite profiles in an *Arabidopsis* ferritin null mutant

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Ferritins are plastid Fe storage proteins directly involved in plant Fe homeostasis. The loss of ferritin causes sensitivity to excess Fe and strong defects in flower development. The triple null ferritin mutant *atfer1,3,4* is particularly well adapted to study Fe regulatory processes in the different plant organs (leaves and flowers) in case of Fe excess. The aim of the work was to study changes in the flower protein and metabolite profiles in this mutant as compared to the Col-0 wild type (WT) in two Fe nutrition regimes, control and excess Fe (++Fe). A gel-based technique (2-D IEF-SDS-PAGE) and GC-MS were used to study the protein and metabolite profiles, respectively, of flower extracts. The four flower classes were compared using 5 and 6 biological replicates in the cases of proteomic and metabolomic analysis, respectively. The 2-DE gels resolved 362±35, 328±39, 341±25 and 350±40 protein spots in WT control, WT++Fe, *atfer1,3,4* control and *atfer1,3,4*++Fe, respectively, with 377 spots being consistently detected. Only consistent spots (those occurring in 4 out of 5 gels), showing changes statistically significant (t-test, $p<0.1$) and having increases >2-fold or decreases >50% were considered for the differential analysis: in total, 58 proteins were found to change in relative abundance in the different classes. When compared to the WT control, the number of increases/decreases were 0/11, 2/18 and 9/12 in WT++Fe, *atfer1,3,4* control and *atfer1,3,4*++Fe, respectively. In the *atfer1,3,4* mutant, the excess Fe treatment caused increases/decreases *in* 20/2 protein spots. More than 90% of the proteins changing in abundance were identified using nLC-MS/MS. Metabolites consistently present in flower extracts (in 5 out of 6 biological replicates in at least one class) were 252, including 104 identified ones and 148 unknowns. Metabolites showing changes in response ratios (over the WT values) above 1.5-fold were 10 known ones (5 increasing and 5 decreasing) and 25 unknowns (15 increasing and 10 decreasing). The combined metabolomics/proteomics analysis provides a framework to understand the changes brought about by the absolute lack of ferritin in *Arabidopsis thaliana* flowers.

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