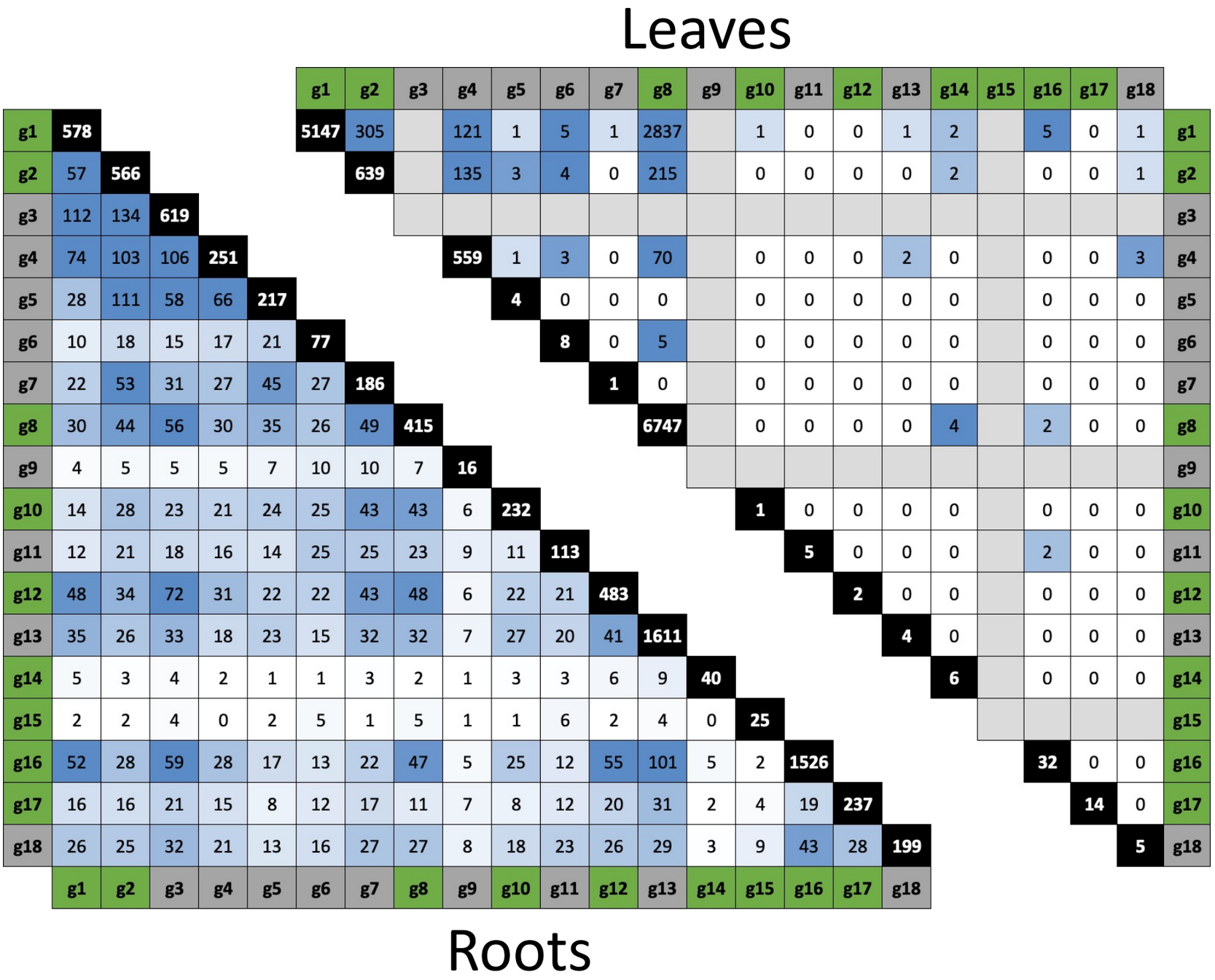
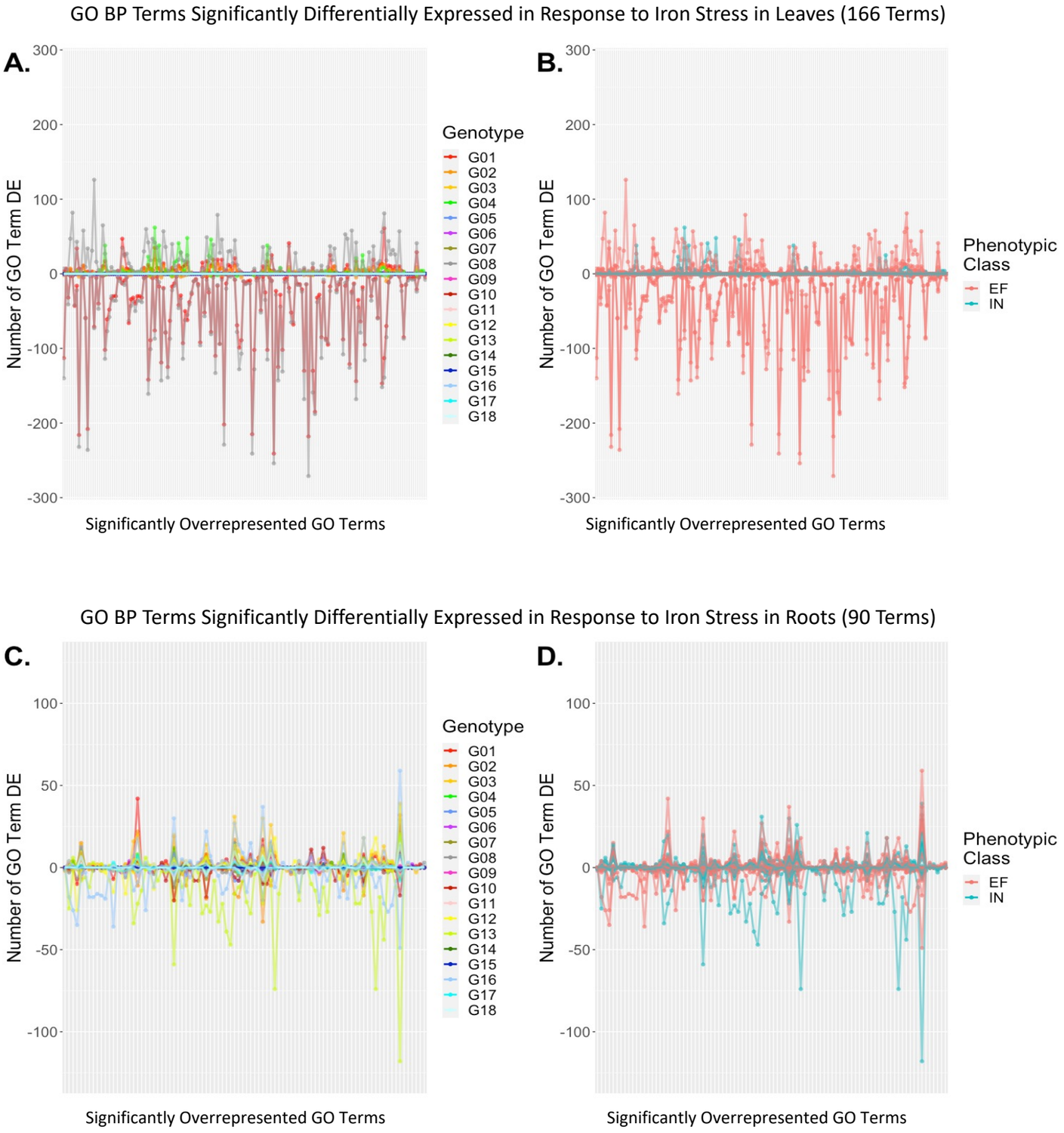
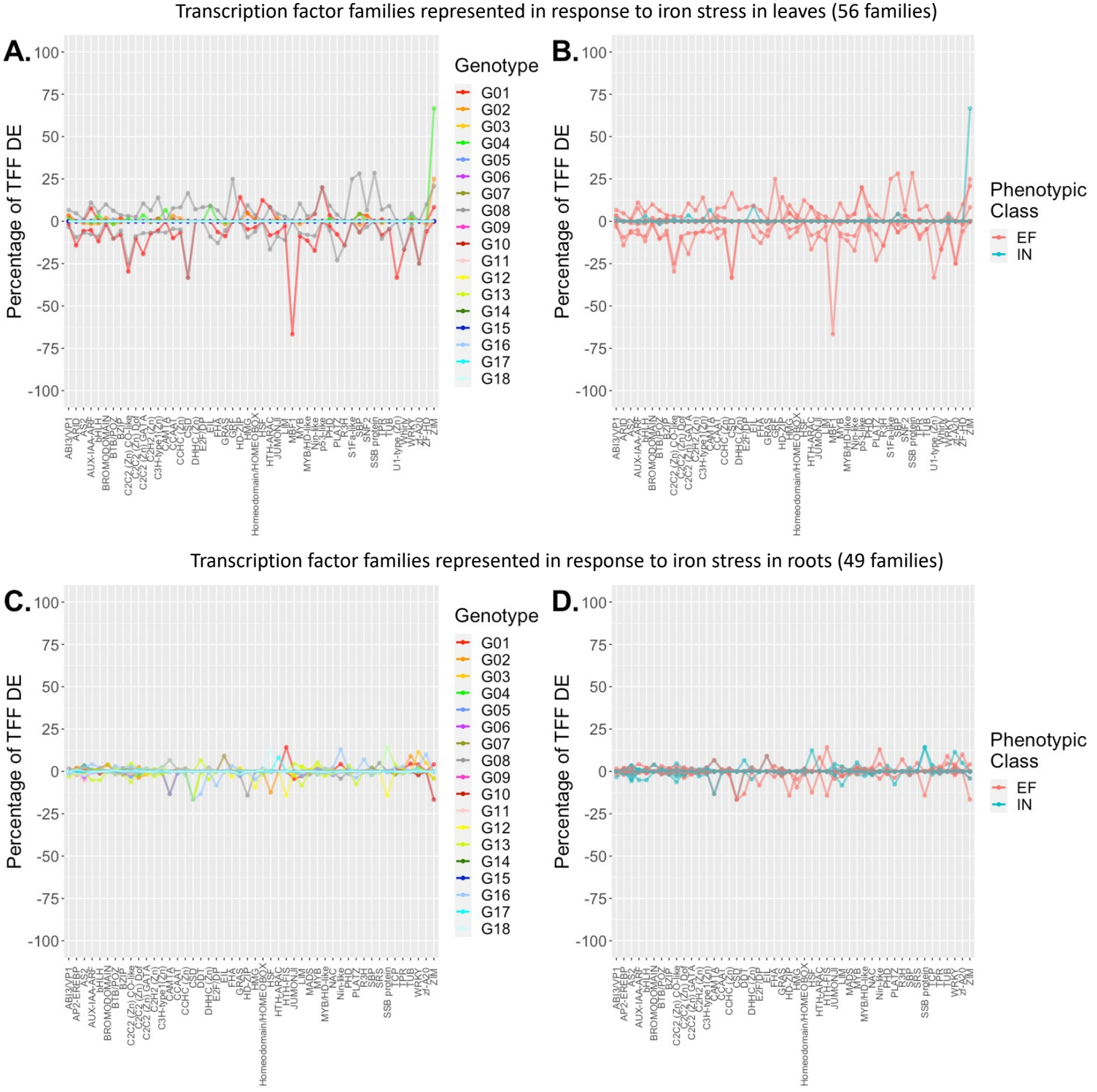


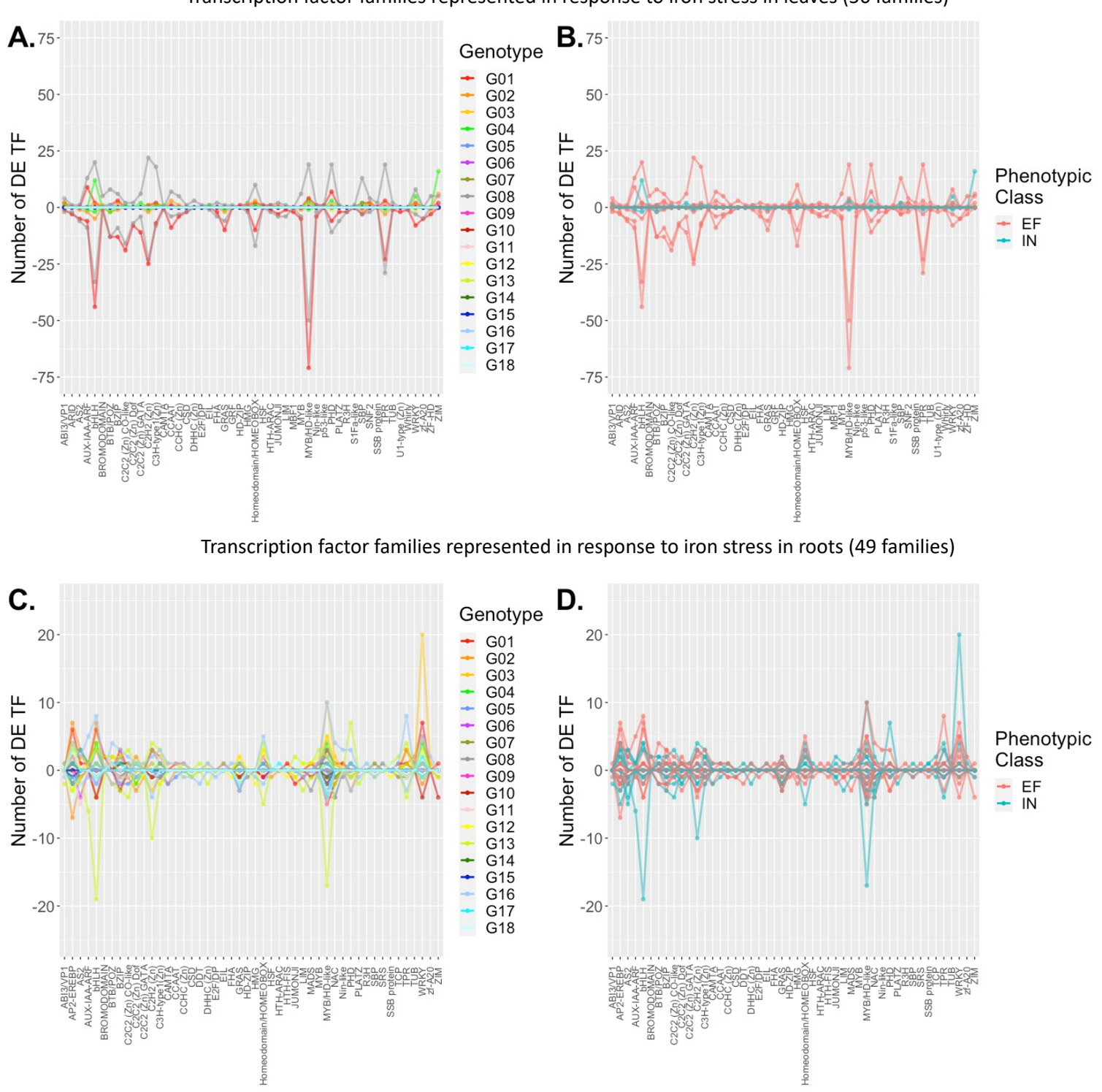
Supplemental figure S1. Pairwise comparison of overlapping differentially expressed genes (DEGs) between 18 soybean genotypes. Significant DEGs (FDR < 0.05) responding after 60 minutes of iron stress were identified in leaf and root tissue of 18 soybean genotypes shown in black squares on the diagonals. Within each tissue type all combinations of genotype pairs were evaluated for DEG overlap. The blue color scale within each genotype matrix indicates the number of DEGs in common with dark blue squares indicating higher numbers of common DEGs between the two genotypes. Hierarchical cluster analysis based on phenotypic symptoms of iron stress revealed two major clusters of soybean genotypes, iron efficient and iron inefficient, indicated by green and grey squares, respectively. Genotypes 3, 9, and 15 in the leaf matrix (shown on the right) contain missing values due to sample removal during sequence processing.



Supplemental figure S2. Number of differentially expressed genes (DEGs) associated with select gene ontology (GO) terms in leaf tissue of 18 soybean genotypes. GO term enrichment analysis was used on DEGs that were up-regulated or down-regulated in response to 60 minutes of iron stress in (A, B) leaf and (C, D) root tissue. GO terms that were significant in at least one genotype were compiled. The number of DEGs associated with each GO term were plotted for each genotype with up-regulated genes shown as positive values and down-regulated genes shown as negative values. (B, D) Previous hierarchical cluster analysis based on phenotypic measurements revealed two major clusters of soybean genotypes, iron efficient and iron inefficient, shown in red and blue, respectively.

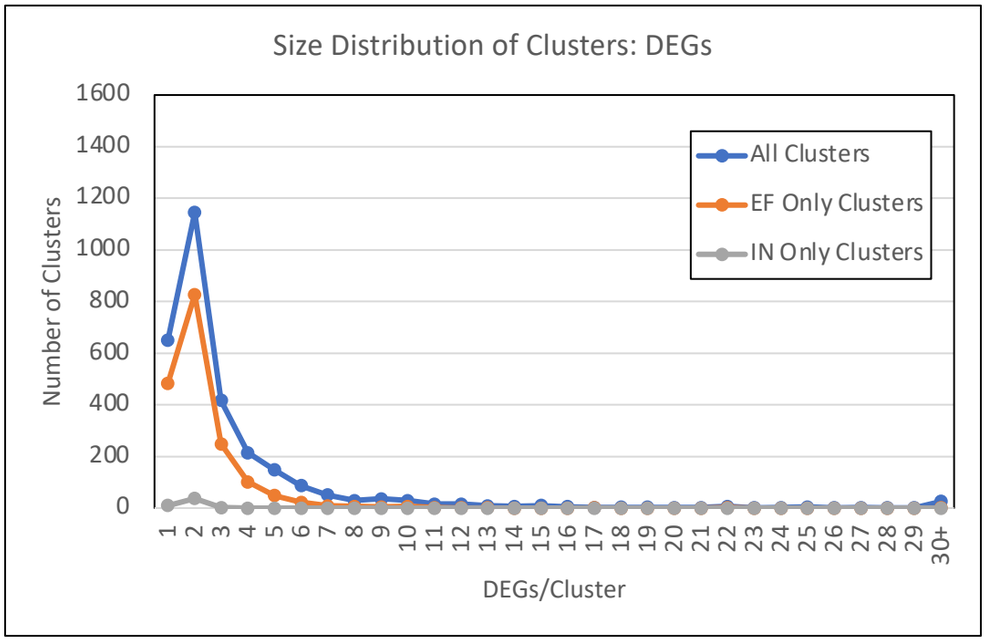


Supplemental figure S3. Differentially expressed transcription factor families in leaf tissue of 18 soybean genotypes. Differentially expressed genes (DEGs) were identified in (A, B) leaf and (C, D) root tissue in response to 60 minutes of iron stress. Transcription factors were identified in each DEG list and then numbers of genes were plotted by transcription factor family (TFF). The percentage relative to the total count of that TFF across the genome was calculated for each genotype with up-regulated genes shown as positive values and down-regulated genes shown as negative values. (B, D) Previous hierarchical cluster analysis based on phenotypic measurements revealed two major clusters of soybean genotypes, iron efficient and iron inefficient, shown in red and blue, respectively.

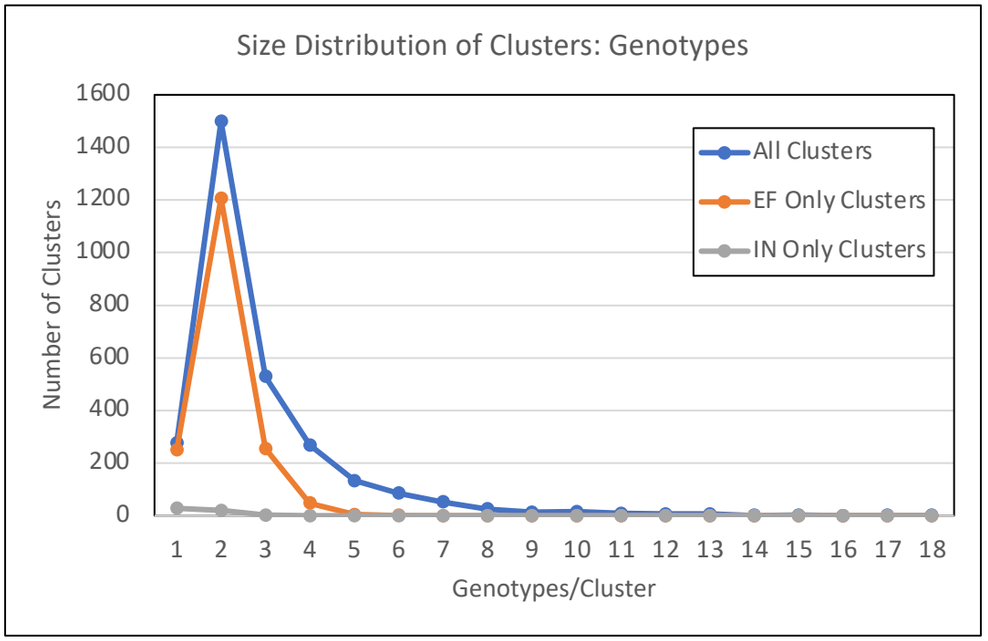


Supplemental figure S4. Differentially expressed transcription factor families in leaf tissue of 18 soybean genotypes. Differentially expressed genes (DEGs) were identified in (A, B) leaf and (C, D) root tissue in response to 60 minutes of iron stress. Transcription factors were identified in each DEG list and then numbers of genes were plotted by transcription factor family (TFF). The number of DEGs associated with each TFF were plotted for each genotype with up-regulated genes shown as positive values and down-regulated genes shown as negative values. (B, D) Previous hierarchical cluster analysis based on phenotypic measurements revealed two major clusters of soybean genotypes, iron efficient and iron inefficient, shown in red and blue, respectively.

A.



B.



C.

	Average DEGs/Cluster	Average Genotypes/Cluster
All clusters	4.14 +/- 39.74	2.85 +/- 1.77
EF clusters	2.28 +/- 1.90	2.07 +/- 0.65
INF clusters	2.02 +/- 1.37	1.48 +/- 0.58

Supplemental figure S5. Distribution and summary of single linkage cluster analysis. Differentially expressed genes (DEGs) were identified across 18 soybean genotypes and two tissue types (leaves and roots) 60 minutes after iron stress. Single linkage clustering was used to identify DEGs with shared sequence homology. Previous hierarchical cluster analysis based on iron stress phenotypic measurements revealed two major clusters of soybean genotypes, iron efficient (EF) and iron inefficient (INF). The distributions of (A) DEGs per cluster and (B) genotypes per clusters were plotted for all clusters (blue), EF-specific clusters (orange), and INF-specific clusters (grey). (C) Average numbers of DEGs and genotypes per cluster were summarized (+/- standard deviation) for all clusters, EF-specific, and INF-specific clusters.