

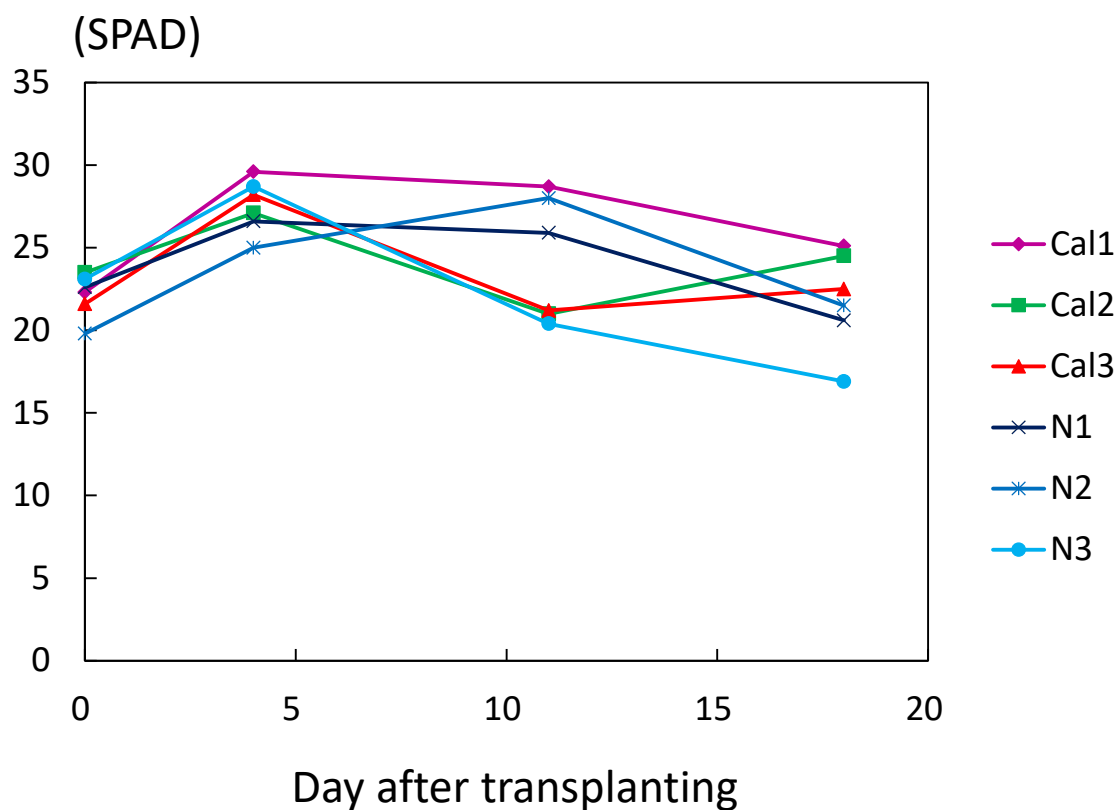
Iron deficiency response and expression of genes related to iron homeostasis in poplars

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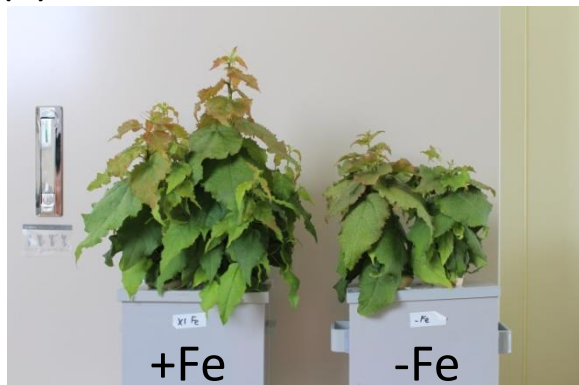
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Supplemental Figures

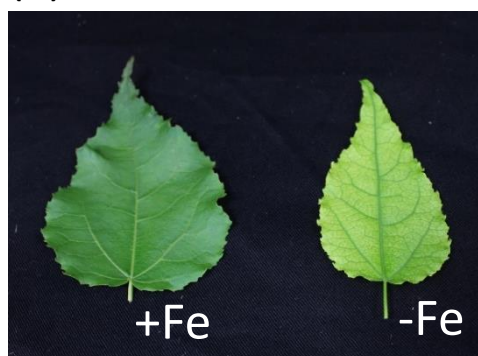


Supplemental Figure 1. SPAD value of poplar plants grown on normal soil or calcareous soil. SPAD value of 4th Newest leaf was measured for individual plant ($n = 1$). Cal1, Cal2, Cal3: each independent plant grown on calcareous soil; N1, N2, N3: each independent plant grown on normal soil.

(a)



(b)



(c)



(d)



(e)



0 day

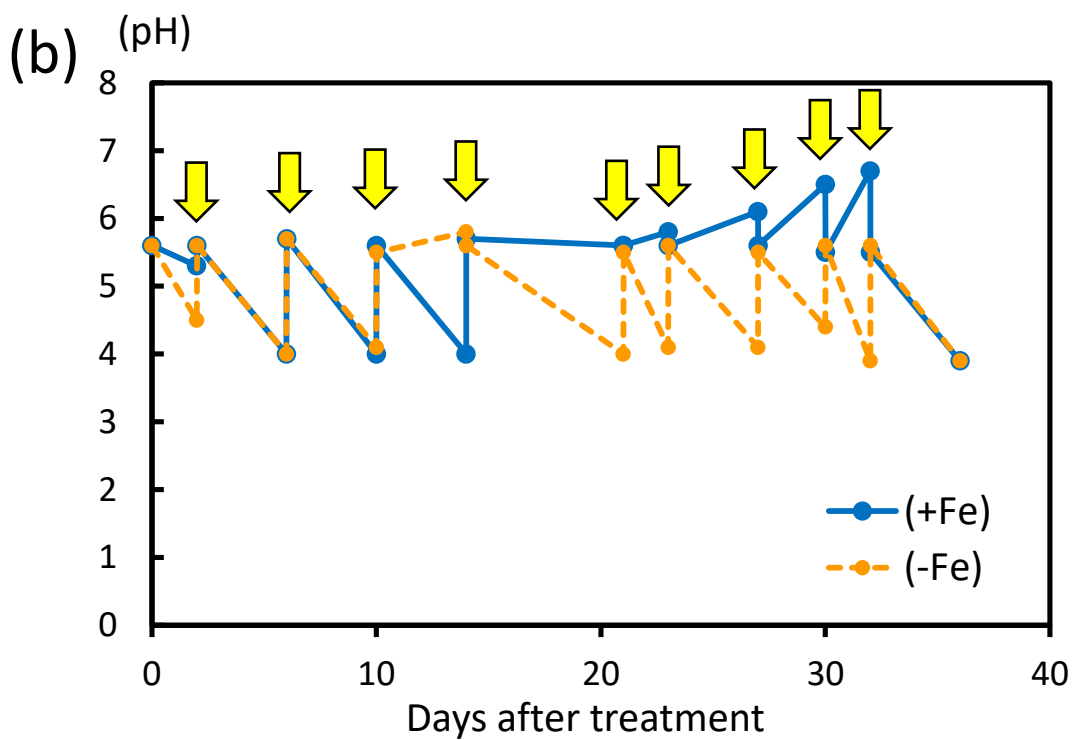
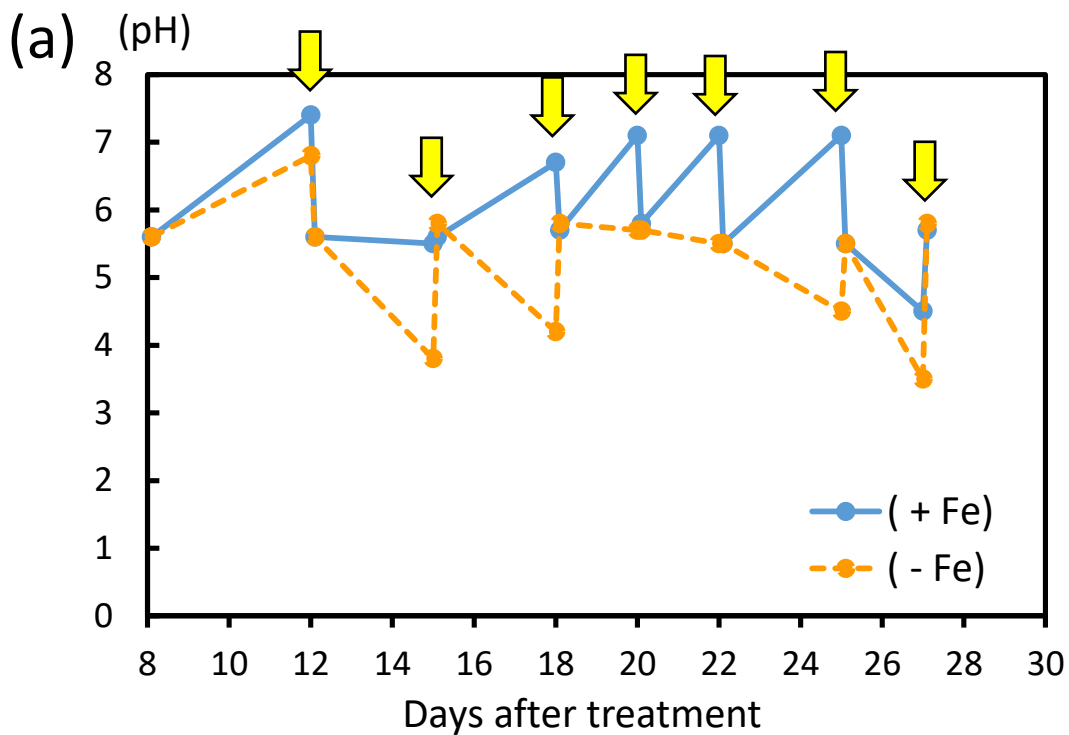
4 days

9 days

Supplemental Figure 2. Plant growth and symptoms on leaves of poplar plants grown in hydroponic culture under Fe-sufficient and Fe-deficient conditions (1st cultivation). (a) Plant appearance and growth 18 days after treatment. (b) Normal and chlorotic 5th newest leaves 27 days after treatment, respectively. (c) Severe chlorosis on leaves of Fe-deficient plants 38 days after treatment. (d) Fe-sufficient plants and more severe chlorosis on leaves of Fe-deficient plants 42 days after treatment. (e) New leaves after Fe resupply to 61 days Fe-deficient plants (0, 4, or 9 days after Fe resupply, respectively).

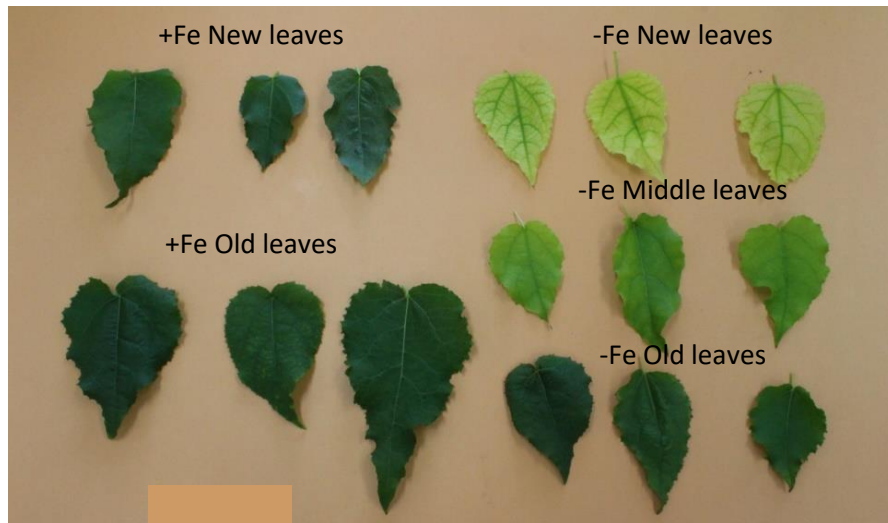


Supplemental Figure 3. Plant growth, appearance, and symptoms on leaves of poplars grown in hydroponic culture 10 days after onset of Fe-sufficient or Fe-deficient conditions (2nd cultivation). Roots and leaves were sampled from these plants for gene expression analysis.

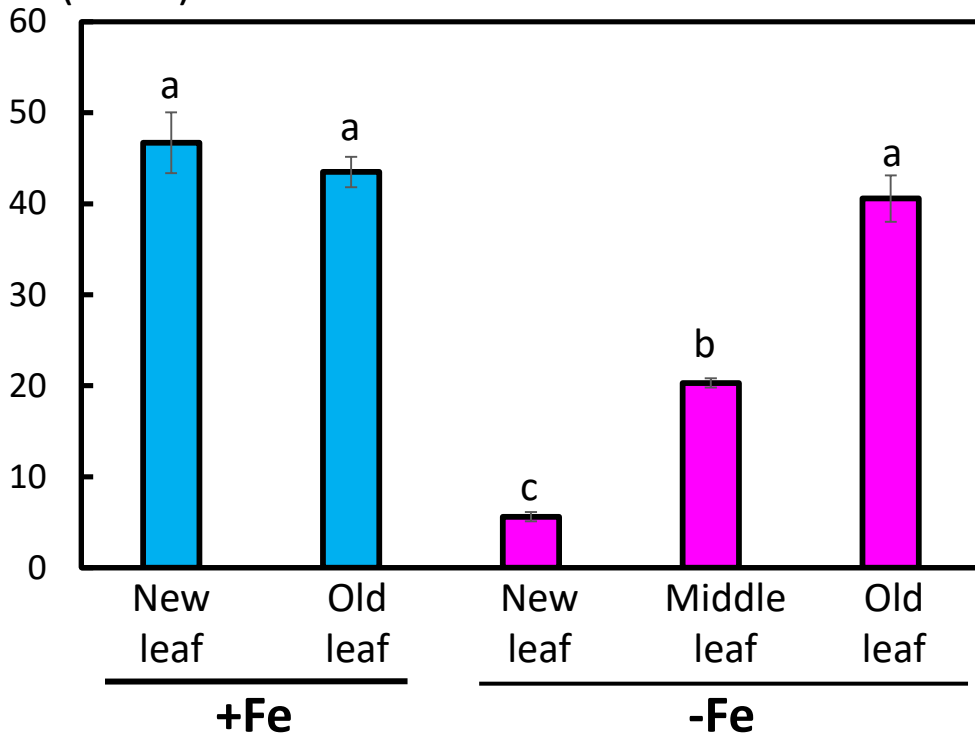


Supplemental Figure 4. Fluctuations in the pH of Fe-sufficient and Fe-deficient hydroponic culture solutions in which poplar plants were grown for the 1st cultivation (a) and 2nd cultivation (b). The pH was recorded every time before and after pH adjustment or changing new solution. Blue line: solution of Fe-sufficient poplar; orange dashed line: solution of Fe-deficient poplar. The pH of the solution was adjusted to 5.6 or renewed on the days indicated by yellow arrows.

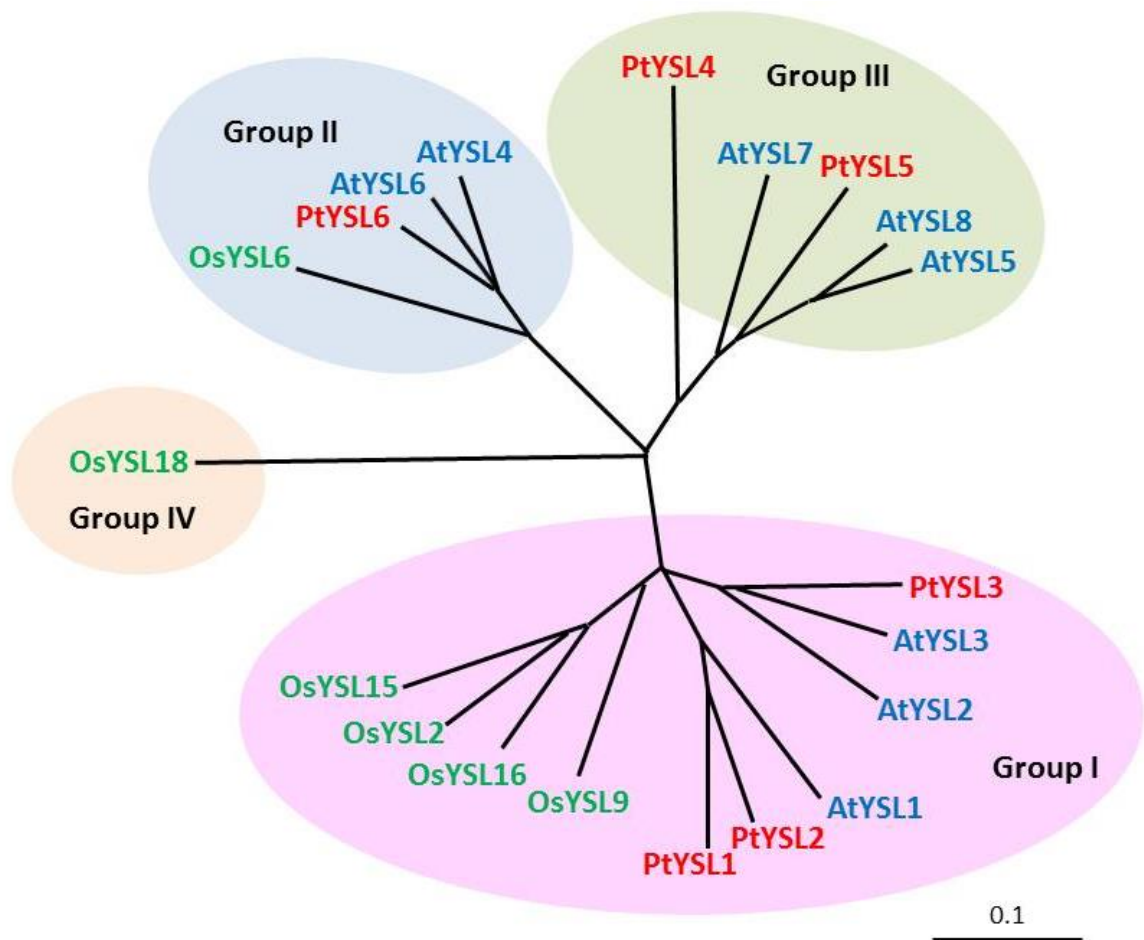
(a)



(b) (SPAD)



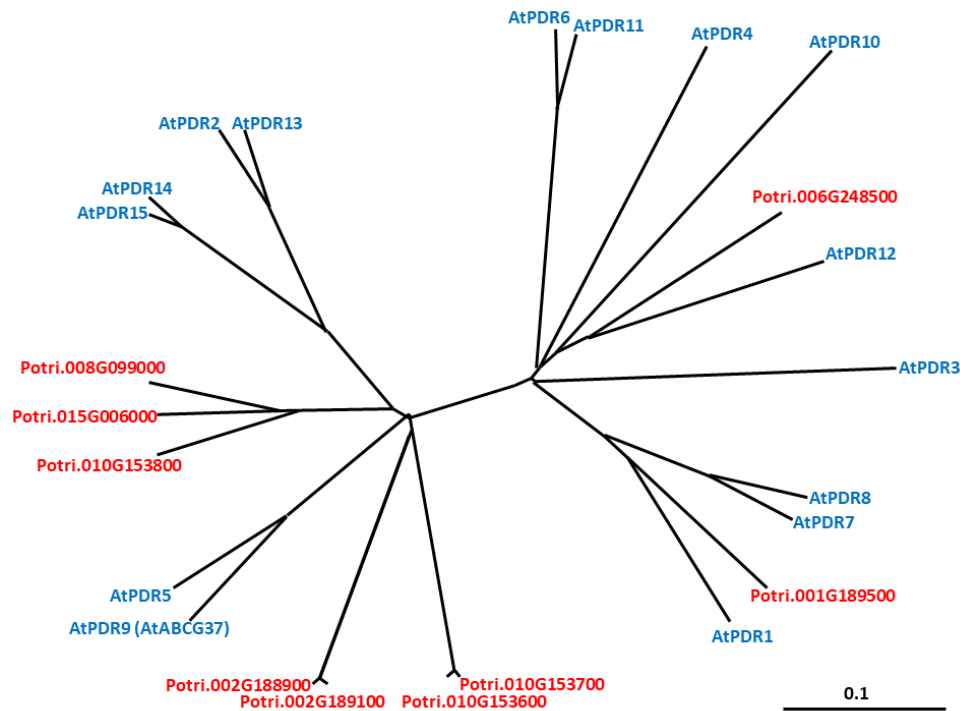
Supplemental Figure 5. Leaves sampled after 74 days of Fe-sufficient or Fe-deficient poplar growth in hydroponic culture (1st cultivation) for metal concentration analysis and their SPAD values. (a) New and old leaf samples from Fe-sufficient plants (left). Samples of new leaves (SPAD value less than 10), middle leaves (leaves from middle positions; SPAD value around 20), and old leaves (leaves from lower positions; SPAD value above 40) from Fe-deficient plants (right). (b) SPAD values of the sampled leaves (n=3). Values followed by different letters differed significantly according to Student's *t*-test ($p < 0.05$).



Supplemental Figure 6. Phylogenetic tree of *PtYSLs*.

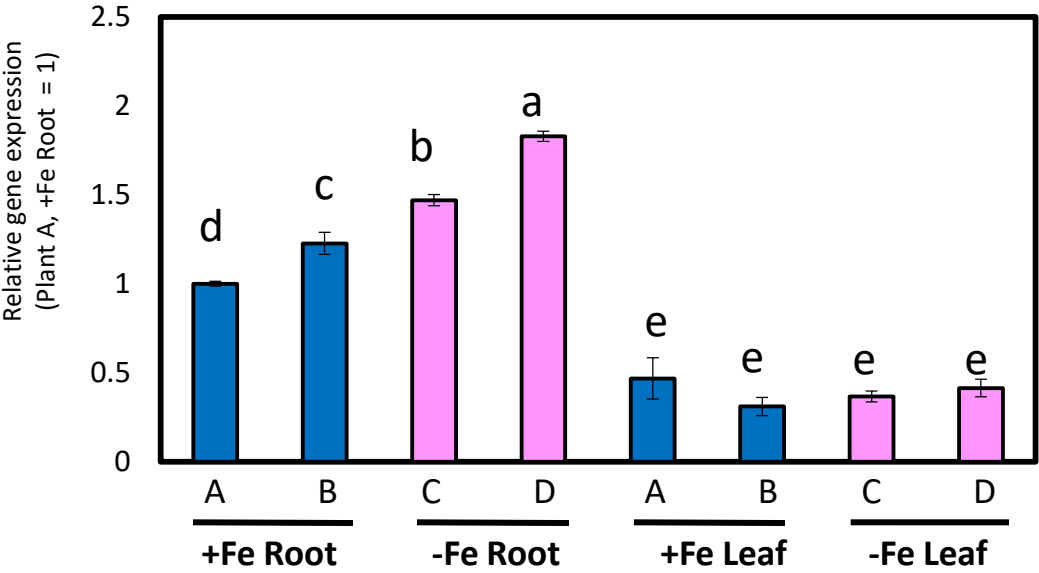
An unrooted phylogenetic tree of the YSL family from rice (green), *Arabidopsis* (blue), and poplar (red). Accession numbers of the amino acid sequences used for this phylogenetic tree are listed in Supplemental Table 1. The phylogenetic tree was obtained using DNASIS Pro software (Hitachi Solutions, Ltd. Tokyo, Japan). The four groups are indicated by different colors.

(a)



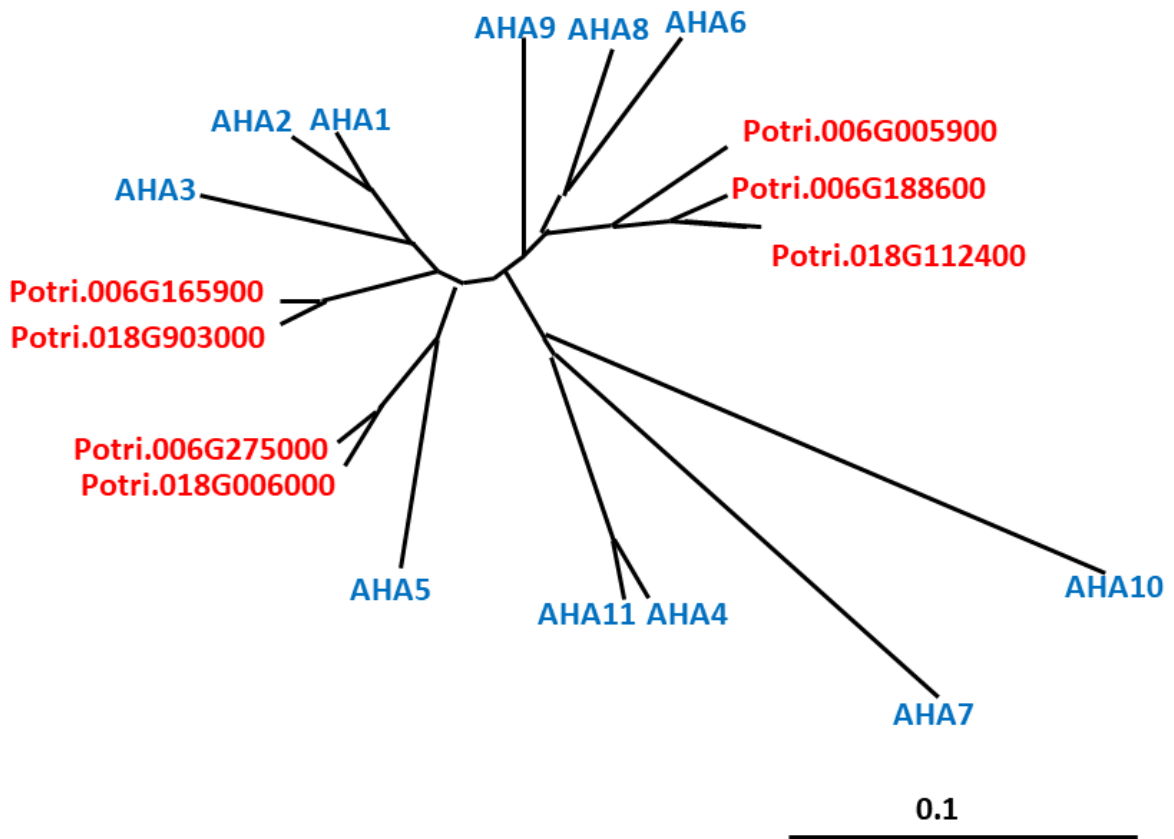
(b)

Potri.006G248500



Supplemental Figure 7. Phylogenetic tree and relative expression level of *AtPDR9* (*AtABCG37*) homologous genes in *Poplar*.

(a) An unrooted phylogenetic tree of the PDA family from *Arabidopsis* (AtPDR1–15), and nine poplar homologs. Homologs of AtPDR9 were searched based on amino acid sequences and a phylogenetic tree was obtained using DNASIS Pro software (Hitachi Solutions, Ltd. Tokyo, Japan). Accession numbers of the amino acid sequences used to construct this phylogenetic tree are shown in Supplemental Table 1. (b) *Potri.006G248500* expression in roots or 5th newest leaves of Fe-sufficient and Fe-deficient poplar plants grown in hydroponic culture 10 days after treatment (2nd cultivation). Error bar shows the technical error, SE; n=3. Data were normalized to the observed expression levels of *PtTIF5α* and displayed as relative gene expression (plant A, +Fe root = 1). Values followed by different letters differed significantly according to Student's *t*-test ($p < 0.05$).



Supplemental Figure 8. Phylogenetic tree of *AHA2* homologous genes in *Poplar*.

An unrooted phylogenetic tree of the HA family from *Arabidopsis* (AHA1–11), and seven poplar homologous genes. Homologs of *AHA2* were searched based on amino acid sequences and a phylogenetic tree was obtained using DNASIS Pro software (Hitachi Solutions, Ltd. Tokyo, Japan). Accession numbers of the amino acid sequences used to construct this phylogenetic tree are shown in Supplemental Table 1.