A bHLH transcription factor regulates iron intake under Fe deficiency in chrysanthemum

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Supplementary Information

Supplementary Table S1 Primer names and sequences used in this study

Supplementary Fig. S1 A phylogenetic analysis shows that CmbHLH1 is closely related to the *A. thaliana* protein ILR3 (NP_200279.1). *A. thaliana* ILR3 (NP_200279.1) and bHLH (AAM64276.1), *Camellia sinensis* bHLH1 (AEI83428.1), *Fragaria* x *ananassa* bHLH (AFN84533.1), *Glycine max* ILR3-like (XP_003540036.1) and ILR3-like isoform 1 (XP_003527314.1), *Lotus japonicas* bHLH (AFK48825.1), *Malus domestica* bHLH (ADL36593.1), *Medicago truncatula* ILR3 (XP_003596613.1), *Nicotiana tabacum* bHLH (ACG60665.1), *Populus trichocarpa* bHLH (XP_002316706.1), *Ricinus communis* bHLH (XP_002522828.1), *Tamarix hispida* MYC (AEJ88330.1), *Thellungiella halophile* bHLH (BAJ33812.1), *Vitis vinifera* ILR3 (XP_002282727.1) and ILR3-like (XP_002274829.2).

Supplementary Fig. S2 The morphology of sense, antisense transgenic and wild type plants at seedling stage.

Supplementary Fig. S3 The transcription of *CmFRO3* and *CmPP2C* in *CmbHLH1* transgenic plants. (a) *CmFRO3* in plants subjected to Fe deficiency, (b) *CmPP2C* in plants treated with ABA.

Supplementary Fig. S4 *CmPP2C* transcription in response to Fe deficiency. **, difference significant at $P<0.01$.

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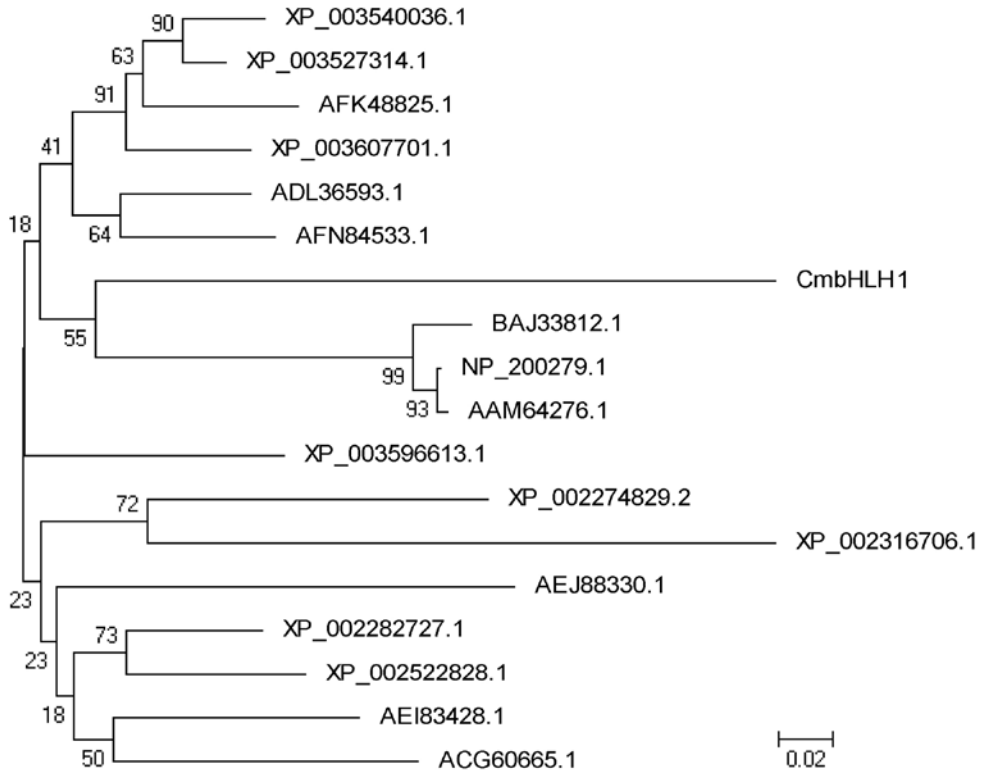
Primer	Sequence (5'-3')	Usage
CmbHLH1-M-F	TACCCGCCACATTTCCAC	Gene Fragment
CmbHLH1-M-R	GCTCCTCGGCTCAGATTA	Gene Fragment
dT-AP	AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTT	3'RACE
AP	AGCAGTGGTATCAACGCAGAG	3'RACE
CmbHLH1-3R-1F	TTGCAGCAGCTCAAGGCCA	3'RACE
CmbHLH1-3R-2F	ATCACGTGCTTCGCCCTCCT	3'RACE
CmbHLH1-5R-1R	AGCTTATCCCTCCTCAAT	5'RACE
CmbHLH1-5R-2R	TCCGCACTTGTGCAATCGTAG	5'RACE
CmbHLH1-5R-3R	GTAGGTAGGCGGCGTTTG	5'RACE
AAP	GGCCACGCGTCGACTAGTACGGGGGGGGGGGGG	5'RACE
AUAP	GGCCACGCGTCGACTAGTAC	5'RACE
CmbHLH1-Full-F	TACCCGCCACATTTCCAC	ORF
CmbHLH1-Full-R	CATTACGCAATGGCAAACC	ORF
CmbHLH1-TP1-1R	GTCCGTAGTCTCCGGTGAAACCATAGT	TAIL-PCR
CmbHLH1-TP1-2R	AAGATTTTCCGGTGGAAATGTGGCGGGT	TAIL-PCR
CmbHLH1-TP1-3R	TGTTGTTCTCGTAAGACGCTGTTTGGTA	TAIL-PCR
CmbHLH1-TP2-1R	ACCCGAAACCGATGGGGAAAACCGAAA	TAIL-PCR
CmbHLH1-TP2-2R	GCGGCTTTCTACTCTACTCCTTAATAGCACATATCTTG	TAIL-PCR
CmbHLH1-TP2-3R	CCCCCGCACTCTTTAATCTTTACACATTACGAATATC	TAIL-PCR
GAPDH-F	GCTGTATCCCCATTCGTT	qPCR
GAPDH-R	AGAAGGCAAGCTCAAGGG	qPCR
CmbHLH1-RT-F	GCCAAACAATGGGCAATA	qPCR
CmbHLH1-RT-R	TACAGGAGGGCGAAGCA	qPCR
CmbHLH1-Sense-F	GCGGATCCATGGTTTCACCGGAGA	Sense vector
CmbHLH1-Sense-R	TCGAGCTCTTAGGCAACAGGAGG	Sense vector
CmbHLH1-Antisense-F	GCGGATCCAATCCGTTGTCCTCC	Antisense vector
CmbHLH1-Antisense-R	TCGAGCTCTACCAAAGTGGCCTCT	Antisense vector
HPTII-F	CGTCTGTCGAGAAGTTTC	Hyg PCR
HPTII-R	TACTTCTACACAGCCATC	Hyg PCR
CmbHLH-Dra-F	AGGCTTTAAAATGGTTTCACCGGAG	Intracellular localization
CmbHLH-Not-R	TTTGCGGCCGCGAGGCAACAGGAGGGCG	Intracellular localization
Pro-SacII-F	TCCCCGCGGTGCCTTAGAGTACCCAC	Promoter analysis
Pro-NheI-R	CTAGCTAGCAGTCGGAAATTGAAGA	Promoter analysis
CmHA-F	AGGATTTCGGTAAGGAACAACG	Transcription analysis
CmHA-R	CAAGACCCTTGAGTCTGACCACT	Transcription analysis
CmFRO3-F	TGTTATCATCCCCAAATCCTGTT	Transcription analysis
CmFRO3-R	ACCATCACTAGCATATCGTGCC	Transcription analysis
CmPP2C-F	TAGTGATGGACTTTGGGATGTGG	Transcription analysis
CmPP2C-R	GCCAATGCCAACTTTGTCAGTAA	Transcription analysis



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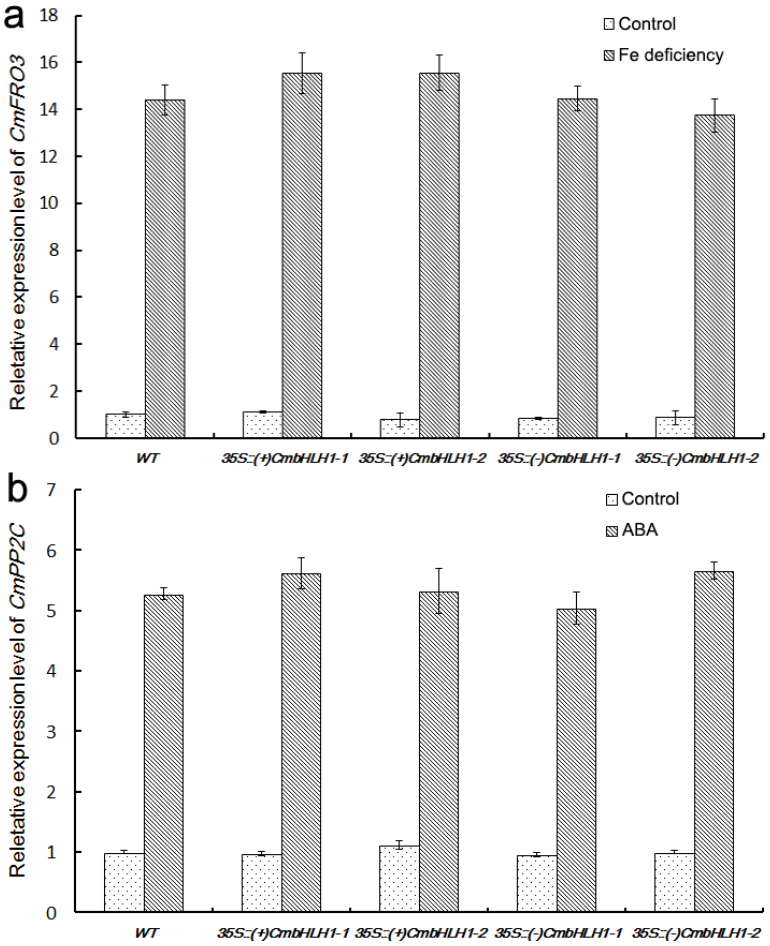

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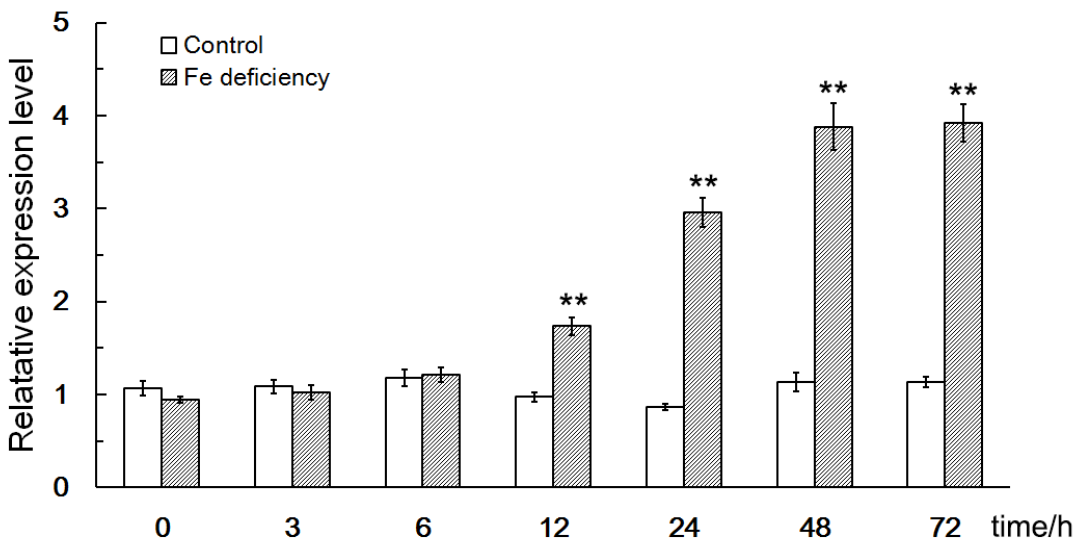

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