Supplementary material and methods

Isolation of *Porteresia coarctata iron deficiency responsive cis-acting element binding factor 1*(*PcIDEF1*) gene and cDNA from *Porteresia coarctata* by thermal asymmetric interlaced PCR: The full-length *PcIDEF1* gene and cDNA were isolated using thermal asymmetric interlaced PCR (TAIL-PCR) (Liu *et al*. 2005). An initial degenerate PCR was performed using primers designed in the conserved B3 DNA binding domain of the rice *IDEF1* cDNA to amplify a 1.5 kb product. The 1.5 kb PCR product was cloned in a T/A cloning vector (*Thermo Fisher Scientific*) and sequenced. In order to allow chromosome walking beyond the 1.5 kb known sequence into the unknown 5' and 3' flanking regions, TAIL-PCR was employed. The sequence information of the 1.5 kb clone was used to design three gene specific nested primers, each at 5' (*PcIDEF1-GSP1R*, *GSP2R*, and *GSP3R*) and 3' (*PcIDEF1-GSP1F*, *GSP2F*, and *GSP3F*) ends. Three rounds of PCR were carried out using the product of previous PCR as template for the next with arbitrary degenerate primers (AD1-6) and nested primers in a consecutive manner. The products of the primary, secondary, and tertiary reactions were analyzed on a 1.2% (m/v) agarose gel. Fragments exhibiting a difference in size consistent with the nested gene specific primer positions were selected for cloning in the T/A vector. The PCR clones were sequenced to confirm overlapping of the original PCR product at both the 5' end and 3' end. To obtain the complete *PcIDEF1* gene, additional nested gene specific primers (GSP) were designed at both the 5' (*PcIDEF1-GSP4R*, *GSP5R*, and *GSP6R*) end and the 3' (*PcIDEF1-GSP4F*, *GSP5F*, and *GSP6F*) end. These primers were used in combination with each of the AD primers in TAIL-PCR to complete both the ends. The AD primers and nested GSP primers used are listed in Table 1 Suppl. The *PcIDEF1* cDNA was amplified using *PcIDEF1 RT Fwd* and *PcIDEF1 RT Rev* primers (Table 1 Suppl.) from the cDNA prepared from *P. coarctata* roots grown in an iron deficient medium. The *PcIDEF1RT Fwd* and *PcIDEF1RT Rev* primers were designed in the 5' UTR and 3' UTR, respectively, using the *PcIDEF1* gene sequence. A 1.46 kb product was obtained and cloned in the T/A cloning vector (*Thermo Fisher Scientific*) and sequenced.

PcIDEF1 expression in *E. coli*: For preparation of a hexa histidine (His-6)-tagged PcIDEF1 recombinant protein, *PcIDEF1*open reading frame (ORF) was amplified by PCR using primers *PcIDEF1ORF Fwd* and *–Rev*, incorporating *EcoR1* and *HindIII* sites at its 5' and 3' ends, respectively. The product was cloned in the T/A cloning vector (*Thermo Fischer Scientific*). The *PcIDEF1* ORF region was then excised from the T/A cloning vector using *EcoRI* and *HindIII* and inserted into the same sites in pET32a (*Novagen*).

For immobilized metal affinity chromatography, three different proteins were used. GST (glutathione-S-transferase) as negative control, a PcIDEF1-GSTfusion protein as test protein, and (His-6)-tagged thioredoxine as positive control. For construction of a PcIDEF1-GST fusion product, the ORF region of *PcIDEF1*was amplified by PCR using *PcIDEF1-ORF-EcoRI* Fwd and *PcIDEF1-ORF-XhoI* Rev primers, incorporating *XhoI* and *EcoRI* sites at its 5' and 3' ends, respectively. The amplified product was ligated to the T/A cloning vector. The recombinant plasmid isolated was digested with *XhoI* and *EcoRI* and the excised product was inserted into the same sites in a pGEX4T-3 vector (*GE Healthcare LifeSciences*) in-frame with the GST protein at the C-terminus. The pGEX4T-3 empty vector, which expresses GST, served as negative control. The pET32a empty vector, which expresses (His-6)-tagged thioredoxine served as positive control. All the recombinant plasmids and empty vectors were introduced into *E. coli* BL21 (DE3) cells for protein expression. Isopropyl ß-D-1-thiogalactopyranoside (IPTG) induction and purification of all the recombinant proteins were carried out using divalent cations charged nitrilotriacetic acid (NTA) resin according to the manufacturer's instructions (*Qiagen*). Protein content was determined using Bradford (1976) assay.

Table 1 Suppl. List of primers used in this study.

SL No.	Primername	Sequence (5'-3')
1	*IDEF1ORFFwd1*	GCTATGCCTTCTGGACACATTACA
2	*IDEF1ORFRev1*	CCATGTCGTCCATGTGCAGTATC
3	*PcIDEF1-GSP1 F*	GCGCAAGGAGAAGACAAAGAG
4	*PcIDEF1-GSP2 F*	CATGCGGTCTAAACTATGCTTC
5	*PcIDEF1-GSP3 F*	GCTGCATTGATCCTGTGATACTG
6	*PcIDEF1-GSP4 F*	CTGCGGATGTGGACATCGGTAG
7	*PcIDEF1-GSP5 F*	GCATCTCTGACTTAGCATTTATGGC
8	*PcIDEF1-GSP6 F*	GATAAGCCCTCCTAAACAAACC
9	*PcIDEF1-GSP1 R*	CAGCTCTGAGCAATGTAACGGAAG
10	*PcIDEF1-GSP2 R*	GAAGGAGCAGAGTGAGGGAATG
11	*PcIDEF1-GSP3 R*	AAGTGGTCGTCGTGTGGCGC
12	*PcIDEF1-GSP4 R*	GTGGTGGTGGTGGTGAGGTGAC
13	*PcIDEF1-GSP5 R*	CCATCCATTCTCTCCTCTCTCC
14	*PcIDEF1-GSP6 R*	GGACCATTTCTTCTCCCCCATTC
15	AD1	NGTCGASWGANAWGAA
16	AD2	TGWGNAGSANCASAGA
17	AD3	WGTGNAGWANCANAGA
18	AD4	STTGNTASTNCTNTGC
19	AD5	WTCGASTWTSGWGTT
20	AD6	AGWGNAGWANCAWAGG
21	*PcIDEF1RTFwd*	CTCCTCTCTGCGAGTGAGCG
22	*PcIDEF1RTRev*	CCCAAGATAAGCTGTAGAAGCC
23	*PcIDEF1 5' UTRFwd*	CAAGACAAGACAGGAGAAGAGAAGAG
24	*PcIDEF1 5' UTRRev*	TGTTTTGATTGTGATTGTGATTGTG
25	*Pcβ-Actin1Fwd*	GAAAGGAAGTACAGTGTCTGGATTG
26	*Pcβ-Actin1Rev*	AAGCATTTCCTGTGCACAATGGAT
27	*PcIRT1Fwd*	ATCCTTGTCTCCAGCGTCATC
28	*PcIRT1Rev*	GACGACGACGAAGAGGTTACG
29	*PcIDEF1-EcoR1-ORFFwd*	GGAATTCATGGGCGAAATGGACGGCGG
30	*PcIDEF1-HindIII-ORFRev*	CCCAAGCTTCTAGGGATTTGTTGTCTGCTG
31	*PcIDEF1-ORF-EcoRIFwd*	GGAATTCCATGGGGCAAATGGACGGC
32	*PcIDEF1-ORF-XhoIIRev*	CCGCTCGAGCGGGATTTGTTGTCTGCTG
33	*PcIDEF1-KpnI-Fwd*	GGGGTACCCCCTCCTCTCTGCGAGTGAGCG
34	*PcIDEF1-SacI-Rev*	CGAGCTCGCCCAAGATAAGCTGTAGAAGCC
35	*PcIDEF-Kpn I Fwd*	GGGGTACCATGGGGCAAATGGACGGCG
36	*PcIDEF-Hind III Rev*	GGGAAGCTTGGGATTTGTTGTCTGCTGATG
37	*NtYSLFwd*	CCTCTAATTGGTGAACGCAAAG
38	*NtYSLRev*	CAGGATGAGAGCAATGGAGATAA
39	*NtPD3Fwd*	GTCATTGAGACCATTGAGCTAGA
40	*NtPD3Rev*	CAAGCTCCACAGCAATTGTAAG
41	*NtNAS1Fwd*	CTTGCTTGAGTTCAACATCCTTAC
42	*NtNAS1Rev*	ACAAGAGAAGTGAGA
43	*NtIRT1Fwd*	CATTCTCCAGGCTGAGTACAAG
44	*NtIRT1Rev*	AAGTGCTATCCCAAGTGCTATAC
45	*NtUbqFwd*	TCCAGGACAAGGAGGGTATC
46	*NtUbqRev*	CATCAACAACAGGCAACCTAG

Table 2 Suppl. The 5' upstream region of *Porteresia coarctata* iron regulated transporter 1 showing iron deficiency responsive elements *IDE1* and *IDE2*. *IDE1* elements are *boxed* and *IDE2* elements are *underlined*.

Fragment name	Sequence (5'-3')
Fragment 1 (*IDE1* and *IDE2*) (-119 to -269)	CTCTACGAGCAGATGAAAGATCAAAGCAAGATT AATTAAAGTCAACTTCTCAG**CACGCAC**CTCTCTC TGTGTCAGTTAGCAGCAGCA**GCATG**AATTCGCTT GTCTGGCTCATGGCGTCACCGAAAGATCACCAG TTGCTCAGCTCCCAAG
Fragment 2 (*IDE2*) (-528 to -599)	ATTATAACGGCCCCGAAAAATCAACCGTGC **CACGCAC**TGTCAGACACGATAGCACCACCG CCACCGCCGCC
Fragment 3 (*IDE2*) (-725 to -797)	GTGACGTCTAATGATGATGATCGTCCCAAA **CCACGTG**AAATACTTACTTTATCAATCTAAA ATATAAACACG
Fragment4 (*IDE1*) (-1017 to -1104)	CTACAATCACCATAACTCGATTTCACATTA TGAC**CATGCATG**TCTCTTATATTACCTATTAG ATTACTCATATCTATTAGGCAATAGC
Fragment 5 (*IDE1*) (-1524 to -1588)	CAACGGAATACGTAGAGCAGGTTGGGACAC **GCATG**GTCTCGCCGAGCCATGGCCCCTCTGA GCGA

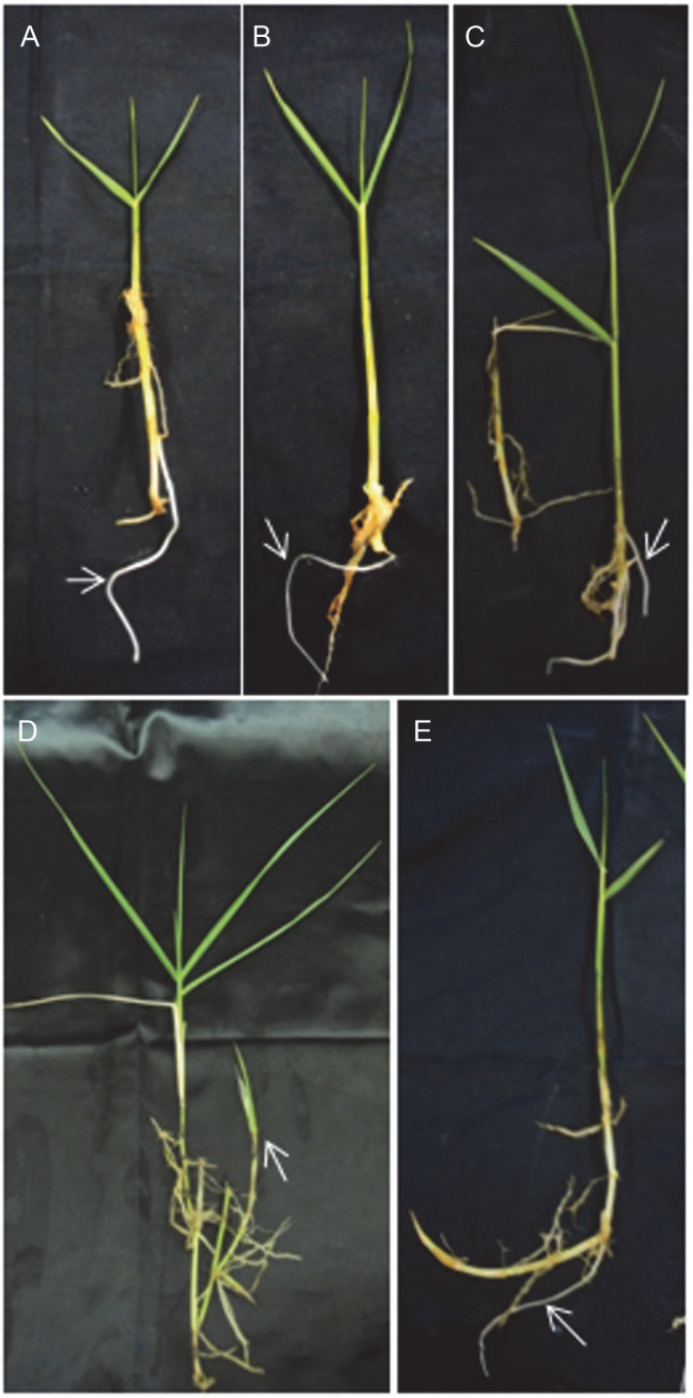

Fig. 1 Suppl. The phenotype of *Porteresia coarctata* three weeks after growth in different treatment conditions: a half strength Murashige and Skoog medium (½ MS) (*A*); ½ MS without iron (*B*); ½ MS without iron and with 100 mM NaCl (*C*); ½ MS without iron and with 150 mM NaCl (*D*); ½ MS without iron and with 200 mM NaCl (*E*). The *arrows* indicate new roots and leaves emerging from a rhizome.

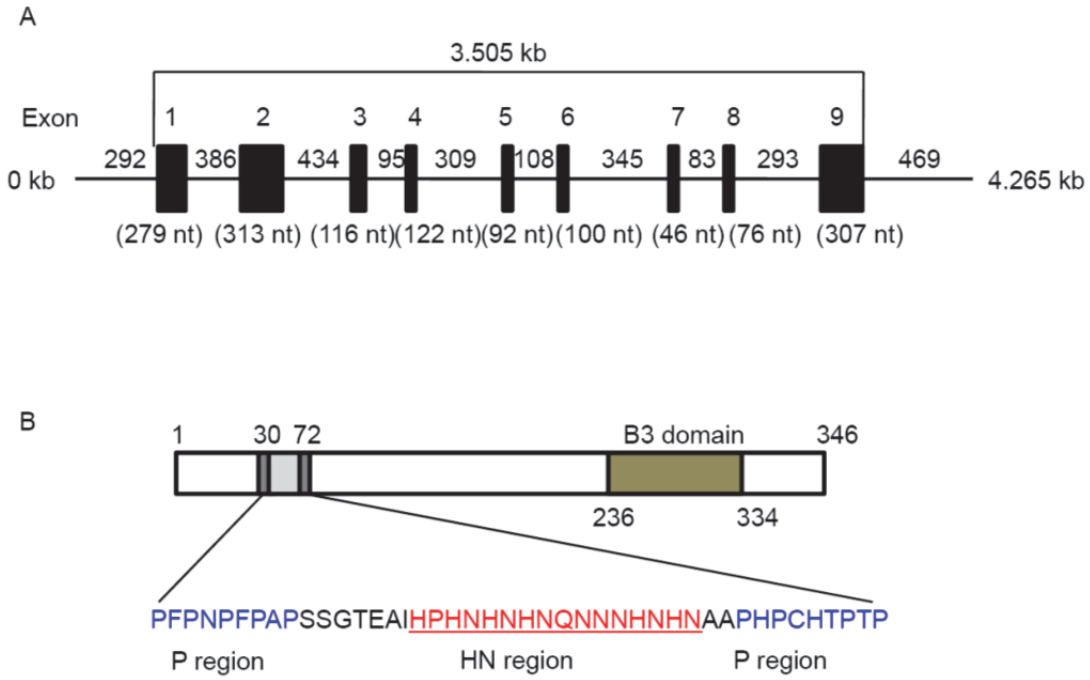

Fig. 2 Suppl. Sequence characteristics of *Porteresia coarctata* iron deficiency responsive *cis*-acting element binding factor 1 (PcIDEF1). The genomic organization of the *PcIDEF1*gene (*A*), the *black boxes* represent exons, the *numbers* on the bottom of the *boxes* indicate exon length [nt], the *lines* connecting the *boxes* represent introns, and length of each intron [nt] isindicated *above* the *lines*. A schematic representation of PcIDEF1 showing a metal binding HN domain *underlined* and marked in *red* flanked by proline rich P regions (*blue*) at the N terminal end, and the B3 domain (the *brown box*) towards the C terminal end (*B*). Comparison of the HN domain of PcIDEF1 with its homologs from other graminaceous species namely *Oryza brachyantha (ObIDEF1)*, *Oryza sativa* (*IDEF1*), and *Hordeum vulgare* (*HvIDEF1*) (*C*).