Table 1 Suppl. Primers used in this study.

Gene name	Primer name	Primer sequences (5' to 3')	Usage
PFPβ	PFPβ conserve F1	CACAATGTGATCTGCGGCATC	pair to mutant identification
	PFPβ conserve R1	CTTGAATCCA TACATGGTGC TG	
	PFPβ conserve F2	TGGAGGAACTGCACTGACTGC	pair to mutant identification
	PFPβ conserve R2	AGCGCCAAGCTCCAACATC	
PFK-1	PFK-1F	TCGTGATGTGGATTGTTGC	qPCR
	PFK-1R	ATGCTTTGGGCGATGAGA	
PFK-2	PFK-2F	TCACCCTGCCTATTCAACTG	qPCR
	PFK-2R	CCACACACCAACTCTCTAATG	
PFK-5	PFK-5F	GAGTCAGACGAAGTGAAAGC	qPCR
	PFK-5R	GAAGCCCTTGTATCCATTCTG	
PFK-6	PFKA-6F	GTTGGTCTTTGGTTGTCTCAG	qPCR
	PFKA-6R	CCTTGTTCTGCTTCTCTGTG	
PFK-7	PFK-7F	ACTTAGCGACATTGGTGTTC	qPCR
	PFK-7R	GCTGTGATGACTTGCGTGA	
PFK-8	PFK-8F	TGTGTGGTTTGTGTGGCT	qPCR
	PFK-8R	ATACCTGATGTGATGCCGC	
PFK-9	PFK-9F	TGTGAATGGCATCGGTCTT	qPCR
	PFK-9R	TCTCAGGAATCAAACAGCAG	
PFK-10	PFK-10F	TTATCAAGTTTGCCTCGCC	qPCR
	PFK-10R	TTCCAGCCTTTACGCACT	
PFPα-1	PFPα-1F	CGACCTGAAAGGGAAGGCATA	qPCR
	PFPα-1R	GCATTGAGAGGGTGGGACAT	
PFPα-2	PFPα-2F	CCAAGCCTATTTCACTGTGCG	qPCR
	PFPα-2R	TGAGAACATCCTGCGAGCA	
PFPα-3	PFPα-3F	GTCGTCTTCAGTGGTAGGCA	qPCR
	PFPα-3R	GAGCAAACAAGCCATCAGTTC	
PFPβ	PFPβ-qF	TGATGTTGTTGACGAGGCAGG	qPCR
	PFPβ-qR	TTCAACCATGGCAATAAGCA	
cpFBP-1	cpFBP-1F	TCGGTCATCTTCGTGCTCAC	
	cpFBP-1R	GCGTAGTTGCCCTCGTTGAA	
cpFBP-2	cpFBP-2F	TCGCAATGAACCC(A/G)AGGGA	qPCR
	cpFBP-2R	CAGGAACAATGGCAGCCTCT	
cyFBP-1	cyFBP-1F	GCTCCGTGTGATGTATGAGGT	qPCR
	cyFBP-1R	ATCTCTCGTGGATGTCGGTG	
cyFBP-2	cyFBP-2F	ATCCTCCTCTCCCACATCGT	qPCR
	cyFBP-2R	CGCCTCGTTCTCTTCAGACA	
Ta26S	26S F	AAGAAGGTCCCAAGGGTTC	internal control
	26S R	TCTCCCTTTAACACCAACGG	

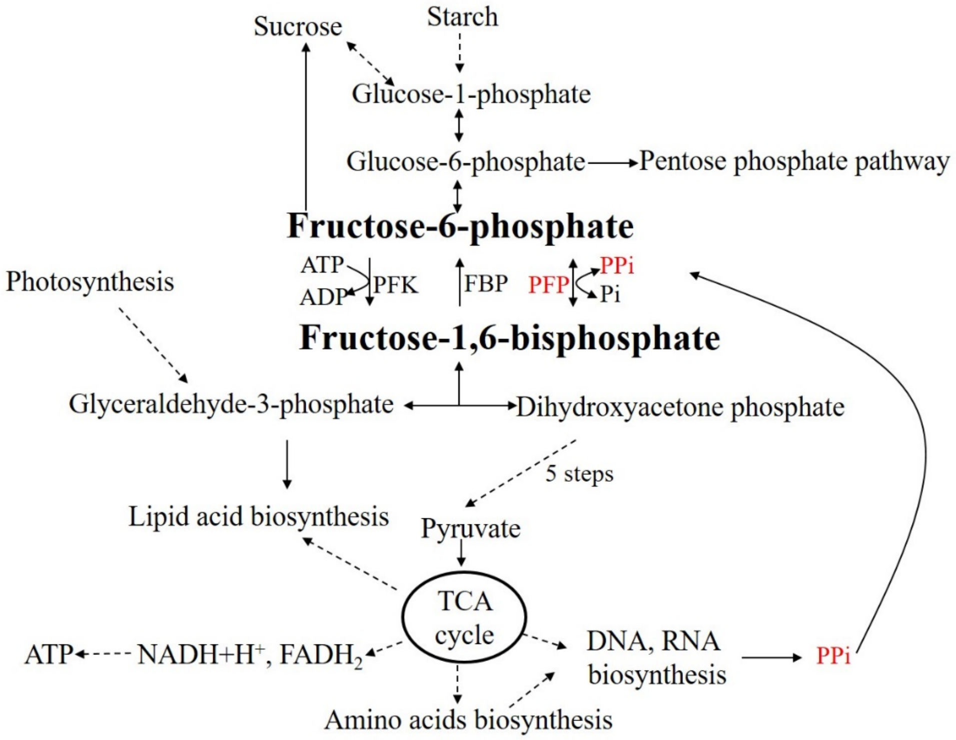

Fig. 1 Suppl. Metabolic pathways involving PFK, FBP, and PFP. FBP - fructose- 1,6-bisphosphatase; PFK - ATP-dependent phosphofructokinase; PFP - pyrophosphate-dependent fructose-6-phosphate 1-phosphotransferase; TCA - tricarboxylic acid; Pi - Phosphoric acid; PPi - pyrophosphoric acid; *Double arrow* lines indicate reactions that are catalyzed reversible by an enzyme, *single arrow* lines indicate the irreversible action. *Dotted* lines indicate more than one step.

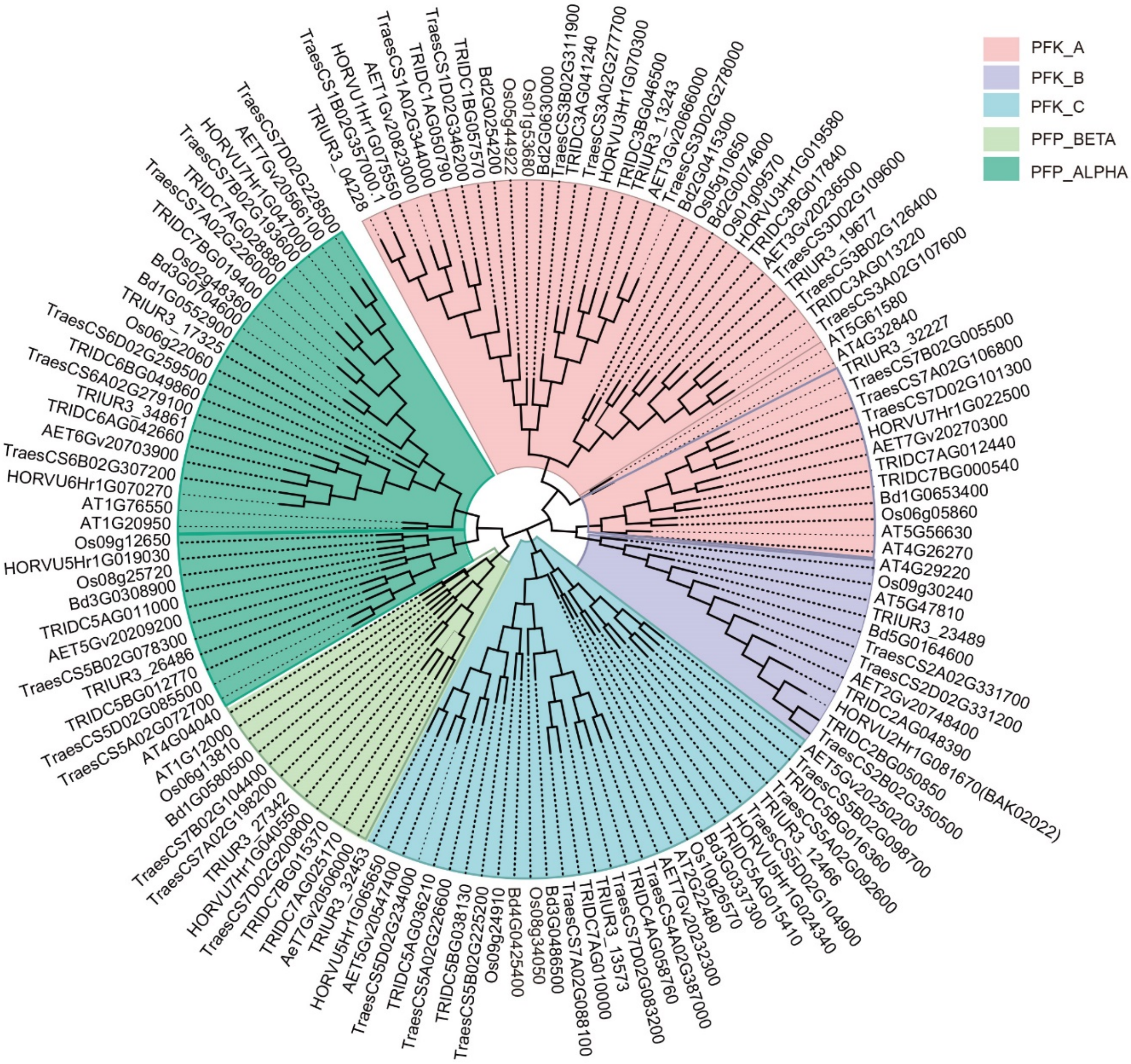

Fig. 2 Suppl. Phylogenic tree of PFKs and PFPs in eight plant species. PFK - ATP-dependent phosphofructokinase; PFP - pyrophosphate-dependent fructose-6-phosphate 1-phosphotransferase. The evolutionary history was inferred using the neighbor-joining method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in *MEGA7*. *AET* - *Aegilops tauschii*; *AT* - *Arabidopsis thaliana*; *Bd* - *Brachypodium distachyon*; *HORVU* - *Hordeum vulgare*; *Os* - *Oryza sativa*; *TRIUR* - *Triticum urartu*; *TRIDC* - *Triticum dicoccoides*; *Traes* - *Triticum aestivum.* As *HORVU2Hr1G081670* (*HvPFK_B*) annotated in the barley genome encodes truncated proteins at the N-terminus, we *BLAST*-searched its homologs in *NCBI*, and we found a full-length peptide (BAK02022.1) that was 100 % similar in the query regions and was therefore used to reconstruct the tree. As expected, it belongs to the PFK_B subfamily.

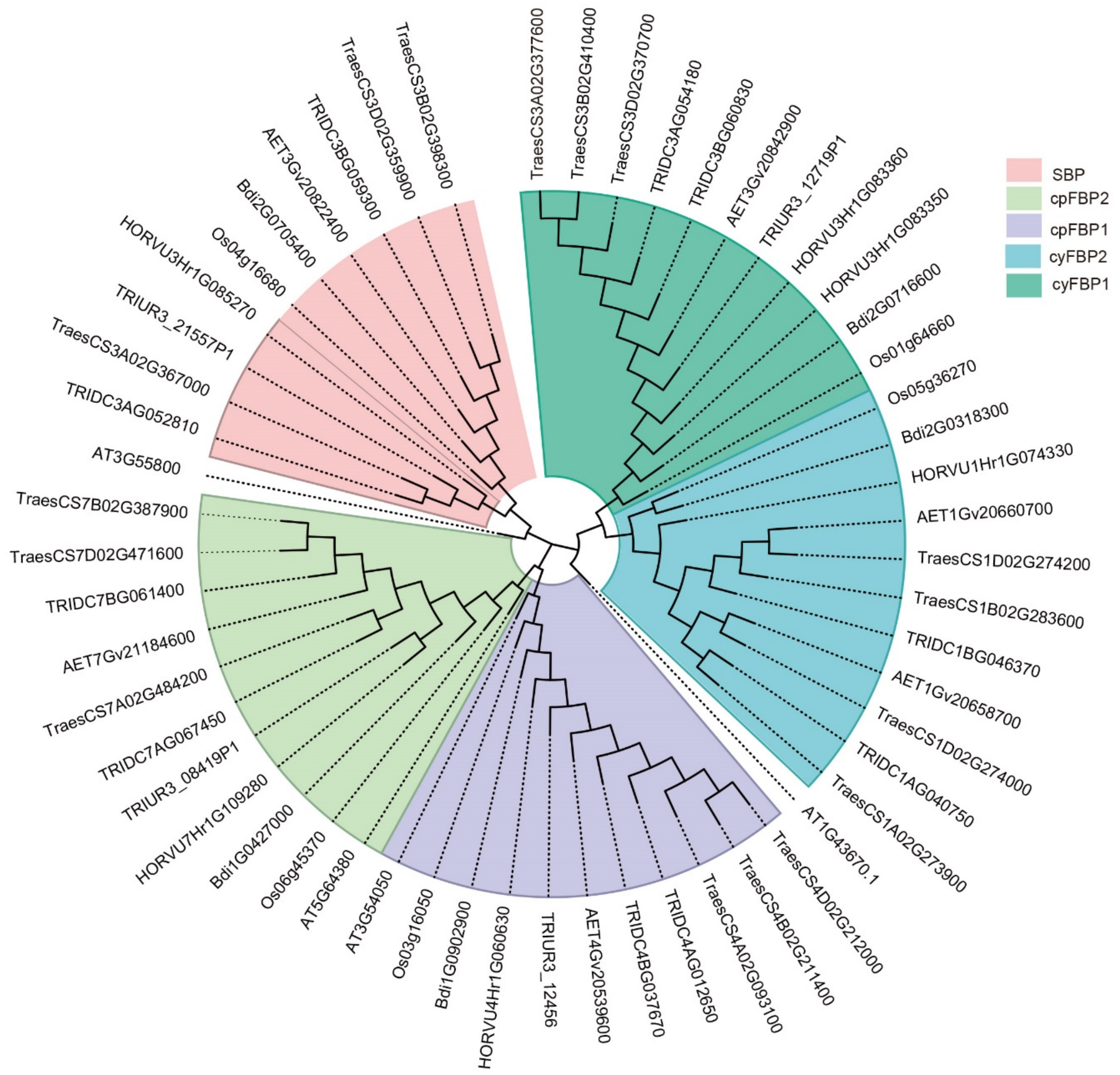

Fig. 3 Suppl. Evolutionary relationships of FBP in eight plant species. The evolutionary history was inferred using the neighbor-joining method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in *MEGA7*. *AET* - *Aegilops tauschii*; *AT* - *Arabidopsis thaliana*; *Bd* - *Brachypodium distachyon*; *HORVU* - *Hordeum vulgare*; *Os* - *Oryza sativa*; *TRIUR* - *Triticum urartu*; *TRIDC* - *Triticum dicoccoides*; *Traes* - *Triticum aestivum;* FBP - fructose- 1,6-bisphosphatase; SBP - sedoheptulose-1,7-bisphosphatase; cy - cytoplasmic; cp - chloroplast.

Fig. 4 Suppl.

```
cpFBP2-B                --------MTLSPRTLRPPLPL--PLPP--TSNRRPPPRPSRPRLPALARR---RSSSVL 45
cpFBP2-D                --------MTLSPHTLRPPLPLSLPLPP--TSNRRPPPRPLHPSLA----R---RRRSVL 43
cpFBP2-A                --------MTLSPHTLRPPLPLPLPLPP--TSNRRHHRPPPPPSRS----R---PRA--- 40
cpFBP1-D                --------MAAATTTASRPLLLSRQQAAASSLQCRLPRRPGSSLFAGQGQASTPNVRCMA 52
cpFBP1-A                --------MAAATTTTSRPLLLSRQQAAASSLQCRLPRRPGSSLFAGQGQASTPNVRCMA 52
cpFBP1-B                --------MAAATTTTSRPLLLSRQQAAASSLQCRLPRRPGSSLFAGQGQASTPNVRCMA 52
AT1G43670(cyFBP)        ------------------------------------------------------------ 0
cyFBP1-D                ------------------------------------------------------------ 0
cyFBP1-A                ------------------------------------------------------------ 0
cyFBP1-B                ------------------------------------------------------------ 0
cyFBP2-D2               ------------------------------------------------------------ 0
cyFBP2-D1               ------------------------------------------------------------ 0
cyFBP2-B                ------------------------------------------------------------ 0
cyFBP2-A                ------------------------------------------------------------ 0


cpFBP2-B                LARPPRA---G-AGAGDGG--MA--SHPTLLE-HMGRAGAPADLAVLVAHIQAACKRIAA 96
cpFBP2-D                LPKPPRA---G-AGAGDGMATHG--AHPTLLE-HMGRAGAPADLAVLVAHIQAACKRIAA 96
cpFBP2-A                ---VRRS---E-LLAGDGMASHG--SHPTLLE-HMGRTGAPADLAVLVAHIQAACKRIAA 90
cpFBP1-D                VVDTPSAPAVAPARKRSSYDMIT--LTTWLLK-QEQEGVIDNEMTIVLSSISTACKQIAS 109
cpFBP1-A                VVDTASAPAPAAARKRSSYDMIT--LTTWLLK-QEQEGVIDNEMTIVLSSISTACKQIAS 109
cpFBP1-B                VVDTASAPAPAAARKRSSYDMIT--LTTWLLK-QEQEGVIDNEMTIVLSSISTACKQIAS 109
AT1G43670(cyFBP)        ---------MDHAADAHRTDLMT—ITRFVLNEQSKYPESRGDFTILLSHIVLGCKFVCS 49
cyFBP1-D                ---------MDHAADTFRTDLMT—ITRHVLNEQSRHPESRGDLTILLSHIVLGCKFVAS 49
cyFBP1-A1               ---------MDHAADTFRTDLMT—ITRHVLNEQSRHPESRGDLTILLSHIVLGCKFVAS 49
cyFBP1-B                ---------MDHAADTFRTDLMT—ITRHVLNEQSRHPESRGDLTILLSHIVLGCKFVAS 49
cyFBP2-D2               ---------MDHAAEAQRTDLMT—ITRHALNEQGRHPESRGDFTILLSHIVLGCKFVAS 49
cyFBP2-D1               ---------MDHAAEAQRTDLMT—ITRHALNEQGRHPESRGDFTILLSHIVLGCKFVAS 49
cyFBP2-B                ---------MDHAAEAQRTDLMT—ITRHALNEQGRHPESRGDFTILLSHIVLGCKFVAS 49
cyFBP2-A                ---------MDHAADAQRTDLMT—ITRHALNEQGRHPESRGDFTILLSHIVLGCKFVAS 49
                                                      *:          ::  ::  :  . : :.
                                                   M                            MM
cpFBP2-B                LVASPGNADLSRAKPAAAGGASAAAGRDAPKPLDELSNEIILSSLQSSGKVAVVASEEND 156
cpFBP2-D                LVASPGNADLSRAKPAAAGAA-SAAGRDAPKPLDELSNEIILSSLQSSGKVAVVASEEND 155
cpFBP2-A                LVASPGNADLSRAKPAAAGAA-SAAGRDAPKPLDELSNEIILSSLQSSGKVAVVASEEND 149
cpFBP1-D                LVQRAPISNLTGVQ-----GA-TNVQGEDQKKLDVISNEVFSNCLRWSGRTGVIASEEED 163
cpFBP1-A                LVQRAPISNLTGVQ-----GA-TNVQGEDQKKLDVISNEVFSNCLRWSGRTGVIASEEED 163
cpFBP1-B                LVQRAPISNLTGVQ-----GA-TNVQGEDQKKLDVISNEVFSNCLRWSGRTGVIASEEED 163
AT1G43670(cyFBP)        AVNKAGLAKLIGLA-----GE-TNIQGEEQKKLDVLSNDVFVNALVSSGRTSVLVSEEDE 103
cyFBP1-D                AVNKAGLAKLTGLA-----GE-TNVQGEEQKKLDVLSNEVFVNALVSSGRTCVLVSEEDE 103
cyFBP1-A                AVNKAGLAKLTGLA-----GE-TNVQGEEQKKLDVLSNEVFVNALVSSGRTCVLVSEEDE 103
cyFBP1-B                AVNKAGLAKLTGLA-----GE-TNVQGEEQKKLDVLSNEVFVNALVSSGRTCVLVSEEDE 103
cyFBP2-D2               AVNKAGLAKLIGLA-----GE-TNVQGEEQKKLDVLSNEVFVKALVSSGRTCVLVSEENE 103
cyFBP2-D1               AVNKAGLAKLIGLA-----GE-TNVQGEEQKKLDVLSNEVFVKALVSSGRTCVLVSEENE 103
cyFBP2-B                AVNKAGLAKLIGLA-----GE-TNVQGEEQKKLDVLSNEVFVKALVSSGRTCVLVSEENE 103
cyFBP2-A                AVNKAGLAKLIGLA-----GE-TNVQGEEQKKLDVLSNEVFVKALVSSGRTCVLVSEENE 103
                         *                         :    :* :::.:: ..*  *       ***


                                         M MF F
cpFBP2-B                LPVWMC--DDGPYVVVTDPLDGSRNIEVSIPTGTIFGIYDRLVELD------QLPVEEKA 208
cpFBP2-D                LPVWMC--DDGPYVVVTDPLDGSRNIEVSIPTGTIFGIYDRLVELD------QLPVEEKA 207
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cpFBP2-A                LPVWLC--DDGPYVVVTDPLDGSRNIEVSIPTGTIFGIYDRLVELD------QLPVEEKA 201
cpFBP1-D                VPVAVEESYSGNYIVVFDPLDGSSNIDAAVSTGSIFGIYSPSDECHIGDDATLDEVTQMC 223
cpFBP1-A                VPVAVEESYSGNYIVVFDPLDGSSNIDAAVSTGSIFGIYSPSDECHIGDDATLDEVTQMC 223
cpFBP1-B                VPVAVEESYSGNYIVVFDPLDGSSNIDAAVSTGSIFGIYSPSDECHIDDDATLDEVTQMC 223
AT1G43670(cyFBP)        EATFVEPSKRGKYCVVFDPLDGSSNIDCGVSIGTIFGIYTLDHTD------------EPT 151
cyFBP1-D                KATFVDPKLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKNQD------------TVT 151
cyFBP1-A                KATFVDPKLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKNQD------------TVT 151
cyFBP1-B                KATFVDPKLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKNQD------------TVT 151
cyFBP2-D2               AAIFVDAPLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKDKD------------NAT 151
cyFBP2-D1               AAIFVDAPLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKDKD------------NAT 151
cyFBP2-B                AAIFVDAPLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKDKD------------NAT 151
cyFBP2-A                AAIFVDAPLRGKYCVCFDPLDGSSNIDCGVSIGTIFGIYMIKDKD------------NAT 151
                            :     * : *  ******  ::  .  *:***::

cpFBP2-B                QLNSLQSGSRLVAAGYVLYSSATIFCISFG--AGTHGFTLDRSTGEFVLTHPSMQIPPRG 266
cpFBP2-D                QLNSLQSGSRLVAAGYVLYSSATIFCISFG--AGTHGFTLDRSTGEFVLTHPSMQIPPRG 265
cpFBP2-A                QLNSLQSGSRLVAAGYVLYSSATIFCISFG--AGTHGFTLDRSTGEFVLTHPSMQIPPRG 259
cpFBP1-D                IVNVCQPGSNLLAAGYCMYSSSVIFVLTIG--TGVYVFTLDPMYGEFVLTQEKVQIPKSG 281
cpFBP1-A                IVNVCQPGSNLLAAGYCMYSSSVIFVLTIG--TGVYVFTLDPMYGEFVLTQEKVQIPKSG 281
cpFBP1-B                IVNVCQPGSNLLAAGYCMYSSSVIFVLTIG--TGVYVFTLDPMYGEFVLTQEKVQIPKSG 281
AT1G43670(cyFBP)        TADVLKPGNEMVAAGYCMYGSSCMLVLSTG--TGVHGFTLDPSLGEFILTHPDIKIPNKG 209
cyFBP1-D                LEEVLQPGKDMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFIMTHPDIKIPPKG 209
cyFBP1-A                LEEVLQPGKDMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFIMTHPDIKIPPKG 209
cyFBP1-B                LEEVLQPGKDMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFIMTHPDIKIPPKG 209
cyFBP2-D2               LEDVLQPGTDMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFILTHPNIKIPNKG 209
cyFBP2-D1               LEDVLQPGTHMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFILTHPNIKIPNKG 209
cyFBP2-B                LEDVLQPGTDMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFILTHPNIKIPNKG 209
cyFBP2-A                LEDVLQPGTNMIAAGYCMYGSSCTLVLSTG--NGVNGFTLDPSLGEFILTHPNIKIPNKG 209
                               *   :**.  :*.    : ::     *.  * *    *::  .:        *
                                                             FF F
cpFBP2-B                QIYSVNDARYFDWPEGLKKYIDTIRQGKGQHPKKYSARYVCSLVADFHRTIIYGGVAMNP 326
cpFBP2-D                QIYSVNDARYFDWPEGLKKYIDTIRQGKGQHPKKYSARYVCSLVADFHRTIIYGGVAMNP 325
cpFBP2-A                QIYSVNDARYFDWPEGLKKYIDTIRQGKGQHPKKYSARYVCSLVADFHRTIIYGGVAMNP 319
cpFBP1-D                KIYSFNEGNYALWDDKLKKYMDSLKEPG-TSGKPYSARYIGSLVGDFHRTMLYGGIYGYP 340
cpFBP1-A                KIYSFNEGNYALWDDKLKKYMDSLKEPG-TSGKPYSARYIGSLVGDFHRTMLYGGIYGYP 340
cpFBP1-B                KIYSFNEGNYALWDDKLKKYMDSLKEPG-TSGKPYSARYIGSLVGDFHRTMLYGGIYGYP 340
AT1G43670(cyFBP)        NIYSVNEGNAQNWDGPTTKYVEKCKFPK-DGSPAKSLRYVGSMVADVHRTLLYGGIFLYP 268
cyFBP1-D                KIYSVNEGNAKNWDTPTAKYVEKCKYPT-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
cyFBP1-A                KIYSVNEGNAKNWDTPTAKYVEKCKYPT-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
cyFBP1-B                KIYSVNEGNAKNWDTPTAKYVEKCKYPT-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
cyFBP2-D2               KIYSVNEGNARNWDAPTAKYAERCKFPQ-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
cyFBP2-D1               KIYSVNEGNARNWDAPTAKYVERCKFPQ-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
cyFBP2-B                KIYSVNEGNARNWDAPTAKYVERCKFPQ-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
cyFBP2-A                KIYSVNEGNARNWDAPTAKYVERCKFPQ-DGSSPKSLRYIGSMVADVHRTLLYGGIFLYP 268
                        :::* .: .   :      * .             : **  .:* *.:: ::


                                F F   F
cpFBP2-B                R------DHLRLVYEANPLSFLAEQAGGRGSDGKTRILSIQPVKLHQRLPLFLGSMEDML 380
cpFBP2-D                R------DHLRLVYEANPLSFLAEQAGGRGSDGKTRILSIQPVKLHQRLPLFLGSMEDML 379
cpFBP2-A                R------DHLRLVYEANPLSFLAEQAGGRGSDGKTRILSIQPVKLHQRLPLFLGRMEDML 373
cpFBP1-D                SDQKSKNGKLRLLYECAPMSFIAEQAGGKGSDGHQRVLDIMPTEVHQRVPLYVGSVEEVE 400
cpFBP1-A                SDQKSKNGKLRLLYECAPMSFIAEQAGGKGSDGHQRVLDIMPTAVHQRVPLYVGSVEEVE 400
cpFBP1-B                SDQKSKNGKLRLLYECAPMSFIAEQAGGKGSDGHQRVLDIMPTAVHQRVPLYVGSVEEVE 400
AT1G43670(cyFBP)        ADKKSPNGKLRVLYEVFPMSFLMEQAGGQAFTGKKRALDLVPEKIHERSPIFLGSYDDVE 328
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cyFBP1-D                ADKKSPSGKLRVMYEVFPMSFLMEEAGGQSFTGKGRSLDLIPTDIHERSPIFLGSSDDVE 328
cyFBP1-A                ADKKSPSGKLRVMYEVFPMSFLMEEAGGQSFTGKGRSLDLIPTDIHERSPIFLGSSDDVE 328
cyFBP1-B                ADKKSPSGKLRVMYEVFPMSFLMEEAGGQSFTGKGRSLDLIPTDIHERSPIFLGSSDDVE 328
cyFBP2-D2               ADKKSPSGKVRVLYEDFPMSFLMEQAGGQSFTGKQRALEIVPSEIHQRSPIFLGSYDDVE 328
cyFBP2-D1               ADKKSPNGKLRVLYEVFPMSFLMEQAGGQSFTGKHRALEIVPSEIHQRSPIFLGSYDDVE 328
cyFBP2-B                ADKKSPNGKLRVLYEVFPMSFLMEQAGGQSFTGKQRALEIVPAEIHQRSPIFLGSYDDVE 328
cyFBP2-A                ADKKSPNGKLRVLYEVFPMSFLMEQAGGQSFTGKQRALEIVPYEIHQRSPIFLGSYDDVE 328
                                ::*:::*  *:.*: *:***:.  *:   *.     :.:*  :  *  :::

cpFBP2-B                ELESYGDVQQKVNPGYDV----     398
cpFBP2-D                ELESYGDVQQKVNPGYDV----     397
cpFBP2-A                ELESYGDVQQKVNPGYDV----     391
cpFBP1-D                KVEKFLSSE-------------     409
cpFBP1-A                KVEKFLSSE-------------     409
cpFBP1-B                KVEKFLSSE-------------     409
AT1G43670(cyFBP)        EIKALYAEEEKKN---------     341
cyFBP1-D                EIKALYAEEAKKA---------     341
cyFBP1-A                EIKALYAEEAKKA---------     341
cyFBP1-B                EIKALYAEEAKKE---------     341
cyFBP2-D2               EIKSLYASESSTA---------     341
cyFBP2-D1               EIKSLYAAESSTA---------     341
cyFBP2-B                EIKSLYASESSTA---------     341
cyFBP2-A                EIKSLYASESSTA---------     341
                                .. :      .
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Fig. 4 Suppl. Multiple alignments of TaFBPs with AtcyFBP. * and : indicate conserved and semi-conserved amino acid residues among FBP, respectively. FBP-binding sites are highlighted in *yellow*, whereas Mg^{2+}-bind sites are highlighted in *gray*. F above residues indicates FBP, whereas M indicates magnesium ions. *Clustal OMEGA* was used (https://www.ebi.ac.uk/Tools/msa/clustalo/).

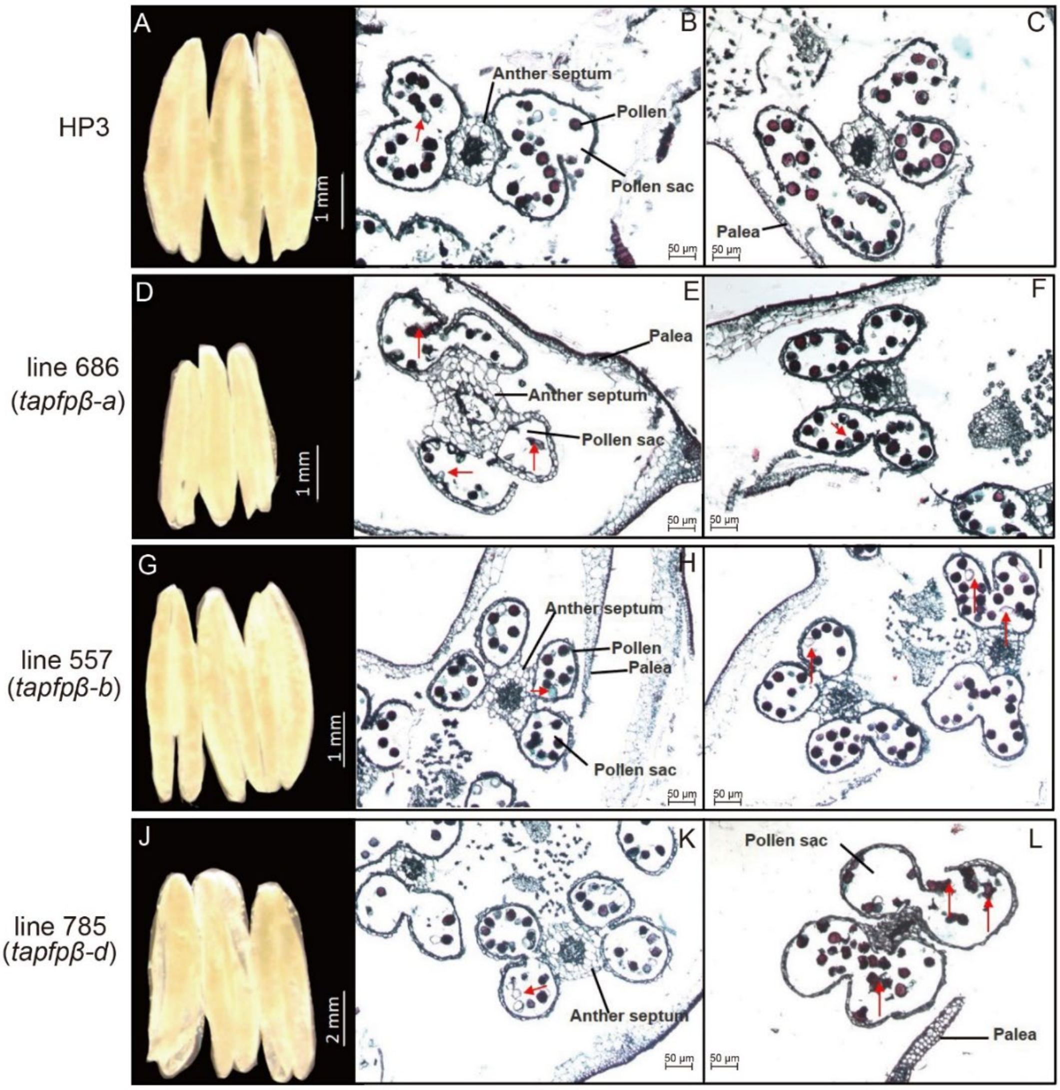

Fig. 5. Suppl. Morphology and transverse sections of anther. *A* to *C* - HP3; *D* to *F* - *pfpβ-a* mutant (line 686); *G* to *I* - *pfpβ-b* (line 557); *J* to *L* - *pfpβ-d* (line 785). Abnormal pollen grains are indicated by *red arrows*. Palea, anther septum, and anther sac are indicated by black lines.

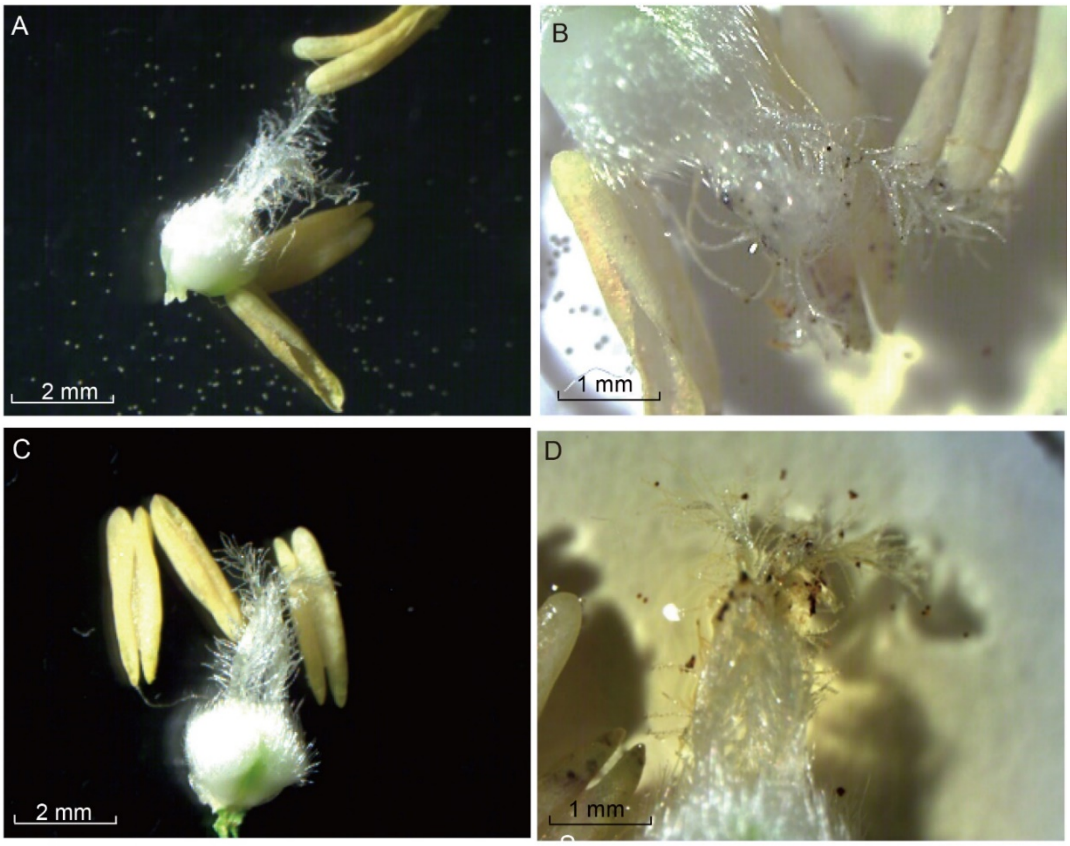

Fig. 6 Suppl. Ovary and stigma of HP3 and mutant. *A* to *B* - HP3; *C* to *D* - *pfpβ-b* (line 557). Pollen was cohesive together on some of the stigma. Data of line 686 and line 785 are not shown.