

## New Phytologist Supporting Information Figs S1–S8, Tables S1-S3, and Methods S1

Article title: Accumulation of the RNA polymerase subunit RpoB depends on RNA editing by OsPPR16 and affects chloroplast development during early leaf development in rice Authors: Weifeng Huang, Yang Zhang, Liqiang Shen, Qian Fang, Qun Liu, Chenbo Gong, Chen Zhang, Yong Zhou, Cui Mao, Yongli Zhu, Jinghong Zhang, Hongping Chen, Yu Zhang, Yongjun Lin, Ralph Bock, Fei Zhou Article acceptance date: 11 June 2020

The following Supporting Information is available for this article:

Fig. S1 Phenotype of rice (Oryza sativa) osppr16 mutant and WT plants at the tillering stage.

Fig. S2 Alignment of the amino acid sequences of OsPPR16 and its closest homologues from

Arabidopsis thaliana (NP\_172596.1, CRR22), Nicotiana tabacum (XP\_016505961.1), and Zea

mays (NP\_001356569.1).

Fig. S3 Phylogenetic analysis of OsPPR16 and the nucleotide positions corresponding to the

editing sites rpoB-545, ndhB-746, and ndhD-887 in 79 land plants.

**Fig. S4** Editing efficiency of 24 plastid RNA editing sites in rice (*Oryza sativa*) WT and *osppr16* mutant plants.

**Fig. S5** Splicing analyses of rice chloroplast transcripts in rice (*Oryza sativa*) WT and *osppr16d* mutant plants at the three-leaf stage.

Fig. S6 Expression of *trnN* and *rpl23* in rice (*Oryza sativa*) WT and *osppr16* mutant plants.

Fig. S7 *osppr16* mutants show a gun phenotype on Norflurazon.

Fig. S8 Tissue-specific expression profiles of OsPPR16 in rice (Oryza sativa) plants.

**Table S1** List of oligonucleotides used in this study.

**Table S2** Accession numbers of RpoB, NdhB, NdhD, and homologous of OsPPR16 in 79 landplants.

**Table S3** Genotype and phenotype of  $T_0$  transgenic plants.



## Methods S1 Complementation of the *osppr16* mutant.



**Fig. S1** Phenotype of rice (*Oryza sativa*) *osppr16* mutant and WT plants at the tillering stage. (a) WT plant. (b) *osppr16* mutant plant obtained with Strategy I. (c) *osppr16* mutant generated by Strategy II (cf. Fig. 1).

	1	10	20	30	40	50	60
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	M S S Q L S F P L L A S	V Q F S T V <b>P</b> Q <b>I P</b> S T T L P P <b>P</b> P <b>L P</b>	N P P S R H R H F L A A I P G Q P P S L	SERNYIPANV AQRIHIPSHI	Y E H P A A L L L E Y K H P T A I L L E	R <u>C S S L K E L</u> H L <u>C T S M K E L</u> H	RQILPLVF HQILPHII
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	70 KNGLYQEH KNGLYNEH	80 MV F FQTKLVSLF LFETKLISLF	90 CSVAATASLL CRYGSVDEAA TKYGSLNDAT MLCCRAT	PSPPPKPTAL RVFEPIDSKL KVFDFAKLKY ASLPSPPPPS	110 A A D D H H A R L R N V L - Y H T M L K D P M - Y H T M L K H A A D P H A R L R	120 A A A A R S D I I G F A K V S D J I A H T R Q S T V I A A A A R Y D J I	PAALAAFV DKALQFFV DSSLSFYS PGALAAFV
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	A M S R M R R D R S M S S P S S A	-         S A G A P P V L R           -         Y D D V E P V V Y           -         Y D D V E P V V Y           -         Y D N V T P V I Y           A H A A A G P V L R	T F T S L L K L C A N F T Y L L K V C G N F G Y L L K A C A T F T A L L K L C A	A R G D L A T G R A D E A E L R V G K E D T S D V L K G K Q A R A D L A T G R A	V H A Q L A A R G I I H G L L V K S G F V H A Q L I L H G F V H A Q L E A R G L	TRU DSEALAATA SLDLFAMT SDNLFAMTS ASESIASTA	190 ALANMYAK GLENMYAK SIVALYAK ALANMYFK
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	C R R P A D A R C R Q V N E A R C G M V G D A C C R R P A D A R	R V F D R M P V R D K V F D R M P E R D K M F D R M P D R D R V F D R M P S R D	ZZ R V A W N A L V A G L V S W N T I V A G L V C W N T V I A G R V A W N A V V A G	Y A R N G L A R M A Y S Q N G M A R M A Y A Q N G M S K R A Y A R N G L P S S A	MEMVVRMQE LEMVKSMCEE LGLVLRMH- MEAVVRMQGE	E G – E R P D S N – – L K P S F E G C H R P D S E G G E R P D S 310	230 [TLVSVLP [TIVSVLP /TVVSILP /TLVSVLP 220
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	A C A N A R A L A V S A L R L I A C A A I G S L A C A D A R A L	A A C R E A H A F A S V G K E I H G Y A R M G K S I H G Y V H A C R E V H A F A	IRSGLEELVN MRSGFDSLVN YRNGFESLVN LRAGLDELVN	V A T A I L D A Y C I S T A L V D M Y A V L T A L V D M Y A V S T A V L D A Y C	K C G D I R A A R V K C G S L E T A R Q K C G S V G T A R L K C G A V E A A R A	VFDWMPTKI LFDGMLERI VFDKIDSKI VFDCMPVRI	340 N S V S W N A M V V S W N S M C V V S L N A M S V S W N A M 280
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	I D G Y A Q N G I D A Y V Q N E I D G Y A R N G I D G Y A D N G	J S R E A L A L F N N P K E A M L I F Q Y Y D E A L I I F R N A T E A M A L F W	R M V E E G V D V T K M L D E G V K P T R M L D E G F K P T R M V Q E G V D V T	DVSVLAALQA DVSVMGALHA NVTIMSTLHA DASVLAALQA	G G E L G C L D E G C A D L G D L E R G C A E S R N L E L G C G E L G Y L D E V	370 M R V H E L L V I R F I H K L S V I Q F V H K L V N ( R R V H E L L V I	380 ELGLDSNV ELGLGSNV RVGLSSNV
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	S V M N A L I T S V V N S L I S A V V N S L I S S V T N A L I T	400 M Y S K C K R V D L M Y C K C K E V D T M F C K C Q R V D I T Y A K C K R A D L	A S H V F D E L - D A A S M F G K L - Q A A G L F K N L - T A A Q V F N E L G N	420 R R T Q V S W N A M S R T L V S W N A M G K T L V S W N A M K K T R I S W N A M	I L G C A Q N G C S I L G F A Q N G C R I L G Y A Q N G C V I L G Y A Q N G C V I L G Y A Q N C C P	E D A V R L F T I D A L N Y F S C M D A L T L F C E D A E R L F A	R M Q L E N V K Q M R S R T V K K M H L L N I K R M Q L E N V R
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	PDSFTLVS PDTFTYVS PDSFTWVS PDSFTLVS	400 VIPALADISD VITALAELSI VVTALAELSV VIPAVADISD	470 PLQARWIHGY THHAKWIHGY LRQAKWIHGF PLQARWIHGY	490 SIRLHLDQDV VMRSCLDKNV AIRTCLNRNV SIRHQLDQDV	Y V L T A L I D M Y F V T T A L V D M Y F A A T A L V D M Y V L T A L I D M Y	A K C G R V N I A A K C G A I M I A A K C G A V H T A S K C G R V S I A	SIU ARILFNSA ARLIFDMM ARKLFDIM ARKLFDSA
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	520 R E R H V I T W S E R H V T T W D D R H V T T W R D R H V I T W	) 530 N A M I H G Y G S H ( N A M I D G Y G T H ( N A M I D G Y G T H ( N A M I H G Y G S H (	54 G F G K A A V E L F G F G K A A L E L F G F G Q A A V E L F G F G Q A A V E L F	U 57 E E M K S I G I V P E E M Q K G T I K P D E M R K G H V K P E E M K G T G S L P	J         50           N E T T F L S V L S         N L S           N G V T F L S V I S         N D I T F L C V I S           N E T T T L S V L A         C20	A C S H A G L V I A C S H S G L V I A C S H S G L V I A C S H S G F V I A C S H A G L V I	570 DEGREYFT 3 AGLKCFY DKGRNYFK DEGQKYFA
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	S M K E D Y G L M M K E N Y S I I M R E E Y S L S M K K D Y G L	E P G M E H Y G T M E L S M D H Y G A M E P S M D H Y G A M E P G M E H Y G T M	V D L L G R A G K L V D L L G R A G R L V D L L G R A G Q L V D L L G R A G K L	DEAWAFIQKM NEAWDFIMQM GEAWDFIDNM DEAWSFIKNM	P M D P G L S V Y G P V K P A V N V Y G P V R P G L N V Y G P I E P G I S V Y G	A M L G A C K L I A M L G A C Q I I A M L G A C K I I A M L G A C K L I	K N V E L A E K N V N F A E R N V E L G E K N V E L A E 700
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	E S A Q K I F E K A A E R L F E K A A D K L F E E S A Q I I F E 710	L G P Q E G V Y H V L N P D D G G Y H V L D P D D G G Y H V L G P E E G V Y H V 720	LLANIYANAS LLANIYRAAS LLANMYATAS LLANIYANAS	M W K D V A R V R T M W E K V G Q V R V M W H K V A K V R T M W K D V A R V R T	A M E K N G L Q K T S M L R Q G L R K T M M E R K G I Q K T A M E K K G L Q K T 750	P G W S I I Q L H P G C S M V E I H P G C S L V D L I P G W S I I Q L H 760	XNEIHTFY XNEVHSFF RNEVHTFY XNEVHTFY
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	S G S T N H Q Q S G S T A H P D S G S T S H P Q S G S T S H P Q S G S T N H Q H	A KE I Y A R L A K S KK I Y A F L E K S E K I Y A Y L E K A K D I Y A R L A K	, 30 L I E E I K A V G Y L I C H I K E A G Y L D R I K A A G Y L E E I K D M G Y	V P D T D S I H D V V P D T N L V L G V I P D T D S I H D V V P D T D S I H D V 800	E D D V K A Q L L N E N D V K E Q L L S E D D V Q E Q L L K E D D V K A Q L L N 810	THSEKLAI THSEKLAIS SHSEKLAIS THSEKLAI	A F G L I R T A S F G L L N T T S F G L L N T T A Y G L I R T A
OsPPR16 CRR22 XP_016505961.1 NP_001356569.1	P G T T I Q I K A G T T I H V R A G T T I H I R P G T T I Q I K	K N L R V C N D C H K N L R V C A D C H K N L R V C G D C H K N L R V C N D C H	N A T K L I S L V T N A T K Y I S L V T S A T K Y I S L V M N A T K L I S L L T	G REIIMRDIQ G REIVVRDMQ K REIIVRDM G REIIMRDIQ	R F H H F K D G K C R F H H F K N G A C R F H H F K D G A C R F H H F K D G K C	S C G D Y W S C G D Y W	

**Fig. S2** Alignment of the amino acid sequences of OsPPR16 and its closest homologues from *Arabidopsis thaliana* (NP\_172596.1, CRR22), *Nicotiana tabacum* (XP\_016505961.1), and *Zea mays* (NP\_001356569.1). The DYW domain is underlined in red.



**Fig. S3** Phylogenetic analysis of OsPPR16 and the nucleotide positions corresponding to the editing sites *rpoB*-545, *ndhB*-746 and *ndhD*-887 in 79 land plants. All protein sequences were downloaded from NCBI, and the phylogenetic tree was constructed by the MEGA 5.0 software using the NJ method with 1000 bootstrap replicates. The nucleotides corresponding to the three editing sites *ropB*-545, *ndhB*-746, and *ndhD*-887 are given in blue. *Oryza sativa Japonica* group was highlighted in the red box.



**Fig. S4** Editing efficiency of 24 plastid RNA editing sites in rice (*Oryza sativa*) WT and *osppr16* mutant plants. Error bars indicate SD (n = 3).



**Fig. S5** Splicing analyses of rice chloroplast transcripts in rice (*Oryza sativa*) WT and *osppr16d* mutant plants at the three-leaf stage. Splicing efficiency was comparatively assessed by RT-PCR. Transcripts are indicated at the left. Spliced (S) and unspliced (U) transcripts are marked at the right.



**Fig. S6** Expression of *trnN* and *rpl23* in rice (*Oryza sativa*) WT and *osppr16* mutant plants. (a) Motif analysis of the *trnN* promoter. The -35 and -10 boxes (in green box) are typical motifs of PEP promoters, the GAA and YRTA motifs (red text) are typical motifs of NEP promoters (Liere and Börner, 2007). TTS: transcription start site. (b, c) Northern blot analysis to determine accumulation of *trnN* (b) and *rpl23* (c) transcripts in *osppr16* mutants and WT plants at the three-leaf stage and the five-leaf stage. *28S rRNA* accumulation is shown as internal loading control.



**Fig. S7** *osppr16* mutants show a *gun* phenotype in the presence of norflurazon (NF). (a-c) qRT-PCR analysis of the expression of *OsLHCB2* (a), *OsRBCS2* (b), and *OsPP12* (c) in rice seedlings grown on half-strength MS medium under white light with or without 5  $\mu$ M NF for 7 d. *OsPP12* was used as reference gene that is not controlled by retrograde signals. Error bars indicate the SD

(n = 2-4), \* indicates P < 0.05, \*\* indicates P < 0.01. The asterisks denote a significant difference to the WT in the same treatment (-NF or +NF; Student's *t*-test).



**Fig. S8** Tissue-specific expression profiles of *OsPPR16* in rice (*Oryza sativa*) plants. qRT-PCR was used to determine the expression levels of *OsPPR16* in different tissues. *OsTP1* was used as reference gene. The expression level of *OsPPR16* in roots was set as 1.0. YL: young leaves; FL: flag leaves. Error bars indicate SD (n = 3).

Primer	Sequence (5'-3')	Application
Target1-F	GGCACGCGCGGAACGGCCTCGCC	Plasmid construction
Target1-R	AAACGGCGAGGCCGTTCCGCGCG	Plasmid construction
Target2-F	GCCGGCCGCGCGCGCTCCGACCTGC	Plasmid construction
Target2-R	AAACGCAGGTCGGAGCGCGCGGC	Plasmid construction
Target3-F	GCCGGATATTTCAGATCCTCTGC	Plasmid construction
Target3-R	AAACGCAGAGGATCTGAAATATC	Plasmid construction
P1	AGTGGCGGTTGCATGTAGAG	Genotype analysis
P2	ATCAAGACAGCCAAGCTCCC	Genotype analysis
P3	GGGAGCTTGGCTGTCTTGAT	Genotype analysis
P4	CGGCCAAGAAGATCCACCAT	Genotype analysis
RNAi16-F	ACTAGTGGTACCTTGCAAGGGTGAG	Plasmid construction
	AACTGCT	
RNAi16-R	GAGCTCGGATCCGAGCCCAAAAGCA	Plasmid construction
	ATGGCAA	
CO16A-F	GCATTTCTTCCTAGCTCAACCA	Plasmid construction
CO16A-R	GTCGACTCAGCATGGTTCAAAATCG	Plasmid construction
	CA	
CO16B-F	CATGATTACGAATTCGAGCTCAAAG	Plasmid construction
	TAAAGTGCGGGGGATCG	

**Table S1** List of oligonucleotides used in this study.

CO16B-R	CTATGTAAATTATATTAGCTGAGCT CTAGATTTAGAAGAAT	Plasmid construction
Cas9-F	GCATGAAGAGGATCGAGGAG	Genotype analysis
Cas9-R	GATCTCTTGCTCGGACTTGG	Genotype analysis
T-DNA16-F	CTAGCCAATACGCAAACCGC	Genotype analysis
T-DNA16-R	AAGGCCCTATTTGTCCCACG	Genotype analysis
Sbu16YPF-F	GGGGACAAGTTTGTACAAAAAAGC	Plasmid construction
	AGGCTTCACCATGGTGTGCTCCGTG GCTGC	
Sbu16YPF-R	GGGGACCACTTTGTACAAGAAAGCT	Plasmid construction
	GGGTGCCAGTAGTCTCCACATGAGC	
trnE-F	CCCCTATCGTCTAGTGGTTCAG	Northern blot
trnE-R	TACCCCCAGGGGAAGTCG	Northern blot
trnN-F	TCCTCAGTAGCTCAGTGGTAGA	Northern blot
trnN-R	GGATTCGAACCTACGACCAGT	Northern blot
rpl23-F	TGGATGGAATCAAATACGCAGT	Northern blot
rpl23-R	GGGCCCATTCTTCTACCCTT	Northern blot
atpF-F	TTTTAGCTCACTGGCCATCC	RNA splicing
atpF-R	TTCATCGCCCTTTGTTTTTC	RNA splicing
ndhA-F	ATGATAATAGACAGGGTACAGG	RNA splicing
ndhA-R	TTATAGTGAAACAAGTTGGGAAG	RNA splicing
ndhB-F	ATGATCTGGCATGTACAGAATG	RNA splicing
ndhB-R	CTAAAAGAGGGTATCCTGAGCA	RNA splicing
petB-F	TTCTCATATACGGTTCTCGG	RNA splicing
petB-R	TAAAGGGCCCGAAATACCTT	RNA splicing
petD-F	ATGGGAGTAACAAAGAAACC	RNA splicing
petD-R	TGTTGCTCCAATACCTAACC	RNA splicing
rpl2-F	ACGGCGAAACATTTATACAA	RNA splicing
rpl2-R	TTACTTACGGCGACGAAGAATA	RNA splicing
rpl16-F	ATGCTTAGTCCCAAAAGAAC	RNA splicing
rpl16-R	AACCGAAGAAATTGACTTCG	RNA splicing
rps12-F	ACTATCAACCCCAAAAAACC	RNA splicing
rps12-R	TTTGGCTTTTTGACCCCAT	RNA splicing
rps16-F	AAAACGATGTGGTAGAAAGC	RNA splicing
rps16-R	AGAATTCCGCCTTCCTTAAA	RNA splicing
trnA-F	GGGGATATAGCTCAGTTGGT	RNA splicing
trnA-R	TGGAGATAAGCGGACTCGAA	RNA splicing
trnG-F	TCGTTAGCTTGGAAGGCTAG	RNA splicing
trnG-R	GCGGGTATAGTTTAGTGGTA	RNA splicing

trnI-F	TGGGCCATCCTGGACTTGA	RNA splicing
trnI-R	AGCTCAGTGGTAGAGCGCG	RNA splicing
trnK-F	GGTTGCCCGGGACTCGAA	RNA splicing
trnK-R	GGGTTGCTAACTCAATGGTAGAG	RNA splicing
trnL-F	GGATATGGCGAAATCGGTA	RNA splicing
trnL-R	TGGGGATAGAGGGACTTGA	RNA splicing
trnV-F	TAGGGCTATACGGATTCGAA	RNA splicing
trnV-R	AGGGCTATAGCTCAGTTCGG	RNA splicing
ycf3-F	ATGCCTAGATCCCGTATAAATG	RNA splicing
ycf3-R	TTATTCAAATTCAAAGCGCTTC	RNA splicing
23S-F	TTCAAAAGAGGAAAGGCTTG	RNA splicing
23S-R	AGAGAGCACTCATCTTGGGG	RNA splicing
atpA-F	GTAGCTGAAATGGCGGATTC	RNA editing
atpA-R	ATCTGCTCTTCCACTGGAAG	RNA editing
atpF-F	CGTCCTATCTATAAGAGGAGAGC	RNA editing
atpF-R	CATTCGATACTCATCCGCTTC	RNA editing
ndhA-F	TGTTTGTTGACCCATGAACCC	RNA editing
ndhA-R	TTTATGCAAGAGTGGAAGCCC	RNA editing
ndhB-1F	GGAAATAGCATGGAATAAGGTTTA	RNA editing
ndhB-1R	TTCCTGGGGAGTTATACATTTGTG	RNA editing
ndhB-2F	ATTTACTCATGGGTGGGGGCA	RNA editing
ndhB-a	TCCTTCGTAGACGTCAGGAG	RNA editing
ndhB-b	CCCACTCCAGTCGTTGCTTT	RNA editing
ndhB-2R	ATTACCCTAGCGCCCATTCC	RNA editing
ndhD-F	CCGCAGCATGGCTCTATCTT	RNA editing
ndhD-R	TCTATCACAAGCCGTTCCCG	RNA editing
ndhF-F	GAATTAGTTTGGTGAATCGG	RNA editing
ndhF-R	GGAACTAGTAACCAATCCCAAC	RNA editing
ndhG-F	CGAATACCTACTGCTTATTGC	RNA editing
ndhG-R	TCGGTTGCTAAATGAATCCC	RNA editing
petB-F	TGACGCGATTCCGGTAATAG	RNA editing
petB-R	GAAATACCACAACCTACCCG	RNA editing
psbE-F	GTCAGTACGGGTTTAGCTTA	RNA editing
psbL-R	CCAGTATAGACTGGTACGAT	RNA editing
rpl2-F	GGTGTTAAGGTAGTAGCTGT	RNA editing
rpl2-R	CCTCTATGCCTTGCGGTAATG	RNA editing
rpl20-F	ACCCATGAACCCAGTTACCA	RNA editing
rpl20-R	AATGGGAAAAAGGATGGCTAA	RNA editing
rpoA-F	GAAAGAACAATGGGGAAGAAAA	RNA editing
rpoA-R	AGATGCTTCTCTAGAGTGTC	RNA editing
rpoB-F	GGATGCTGTGTATGAATCAC	RNA editing

rpoB-R	CTTGGCTTGTTGTATCCGTC	RNA editing
rpoC1-F	ACGAGTACCTAATCACGAGG	RNA editing
rpoC1-R	TCCACAAGCACAAATTCTGC	RNA editing
rpoC2-F	GGCACTTTAGAGGAAGACTC	RNA editing
rpoC2-R	TACGACCTCGTAAGGCAGCT	RNA editing
rps2-F	CTCGGCAAAGCGTAAAGGTA	RNA editing
rps2-R	CTTCAAGATTGCCACATCTC	RNA editing
rps8-F	ACCTCTATAAGAAACGCGGACA	RNA editing
rps8-R	AGACTTCTCCCCCAATTCTGTT	RNA editing
rps14-F	CCATCTAGGATTAGAACCGT	RNA editing
rps14-R	ATCTTGTTGCACCCGGTAAC	RNA editing
ycf3-F	GGCTGAGCAGAAATACCTCT	RNA editing
ycf3-R	GTAATGACAGATCACGGCCA	RNA editing
Real16-F	TGTCAAAGCACAGCTGCTCA	qRT-PCR
Real16-R	TGGATTGTTGTGCCAGGGG	qRT-PCR
atpA-F	TCGCGGAGTTACAAGCCTTT	qRT-PCR
atpA-R	ATTCTCGTAATCGTCGGCCC	qRT-PCR
atpB-F	GGGGGAGCTGGAGTAGGTAA	qRT-PCR
atpB-R	CCCCTACTCCGCCAAATACG	qRT-PCR
atpE-F	ACTGACTCCTAAGCGAATT	qRT-PCR
atpE-R	GGCGTGGTTTGGTAATAC	qRT-PCR
atpH-F	CGTAGGTCTTGCTTCTAT	qRT-PCR
atpH-R	GTCTCGCAATACCTTCTA	qRT-PCR
atpI-F	ATTAGTCGTTGTTGTTGTTCTTGT	qRT-PCR
atpI-R	GCTTGAATACCGCTTGTAA	qRT-PCR
petA-F	GCAGTGCTTCCCGATACTGT	qRT-PCR
petA-R	AGCACCCACATTCAACCCTC	qRT-PCR
petB-F	ACGTGGAAGTGCTAGTGTGG	qRT-PCR
petB-R	CGGCAGTAAGCAGAGGAAGT	qRT-PCR
petD-F	CCGACGGGCTTATTGACAGT	qRT-PCR
petD-R	GGTTGTAGCTACTGGGCGAC	qRT-PCR
petG-F	TTTCTATTTGGAATCGTCTTAGG	qRT-PCR
petG-R	ATCCCCACGTCTGTATTG	qRT-PCR
petL-F	CTAGTTATTTCGGTTTTCTATTGG	qRT-PCR
petL-R	GACGTATCTTGTTCAAGCC	qRT-PCR
petN-F	GGATATAGTAAGTCTCGCTTGG	qRT-PCR
petN-R	CTAGAGTCCACTCCTCCC	qRT-PCR
psaA-F	GCAGTCGGATGTTTGGGGGTA	qRT-PCR
psaA-R	GAAATCTCGGAGCCACCCAT	qRT-PCR
psaB-F	ATGAACGGACACCTTTGGCT	qRT-PCR
psaB-R	CCCACCAATCTTGCTTGCAC	qRT-PCR

psaC-F	ACCGAAGATTGTGTGGGGTTGT	qRT-PCR
psaC-R	AAGATAGAGCCATGCTGCGG	qRT-PCR
psaI-F	AACTTACCCTCTATTTTCGTGCC	qRT-PCR
psaI-R	TGCACATAAAGAAATAAAGAAGCC	qRT-PCR
	Α	
psaJ-F	CATATCTCTCTGTAGCACCCGT	qRT-PCR
psaJ-R	GGGAATGACAAAGCATCTGGG	qRT-PCR
psbA-F	TGTGGCCGCTCATGGTTATT	qRT-PCR
psbA-R	CCCTACTACAGGCCAAGCAG	qRT-PCR
psbB-F	GTTGAACAAGTAGGCGTAA	qRT-PCR
psbB-R	ACAGTAGCAGGATCAGAATA	qRT-PCR
psbC-F	GCTCCTTTAGGCTCTTTA	qRT-PCR
psbC-R	GTCGCTAACCAACTTCTA	qRT-PCR
psbD-F	GGCCCAATCGCTGTTTTTGT	qRT-PCR
psbD-R	CCAAAACTCGGCGCAAAGAA	qRT-PCR
psbE-F	CCCTATTCATTGCGGGTTGG	qRT-PCR
psbE-R	ACGGAATTCCTTGTCGGCTT	qRT-PCR
psbF-F	TTTTACAGTGCGATGGCTGG	qRT-PCR
psbF-R	CGTTGGATGAACTGCATTGCT	qRT-PCR
psbI-F	ACAGTAGTGATATTCTTTGTTTCCC	qRT-PCR
psbI-R	TCGTCACGCCCAGGATTA	qRT-PCR
psbJ-F	TGGCCGATACTACTGGAAGA	qRT-PCR
psbJ-R	CCCAATCCAGAATATGAACCGT	qRT-PCR
psbK-F	TTTCTTCGCCAAATTGCCCG	qRT-PCR
psbK-R	CAGCTTGCCAAACAAAGGCT	qRT-PCR
psbL-F	ATGACACAATCAAACCCGAATG	qRT-PCR
psbL-R	ACAAAAATGAGTAATAAACCCCAGT	qRT-PCR
psbM-F	ATATTCTCGCATTTATTGCTACTG	qRT-PCR
psbM-R	AATCATTTTGGCTGACTGTTTT	qRT-PCR
psbN-F	GGAAACAGCAACTTTAGTCG	qRT-PCR
psbN-R	CGTGTTCTTCGAATGGATCT	qRT-PCR
psbZ-F	GCTTCTCCTGATGGTTGGTCA	qRT-PCR
psbZ-R	AGCTACCAGAAAGACCAGTCC	qRT-PCR
rbcL-F	AGGGGAACGCGAAATGACTT	qRT-PCR
rbcL-R	AAAGATACCGCGAGCACGAT	qRT-PCR
rpoA-F	CCGCCTTCCGTAGAAATCGT	qRT-PCR
rpoA-R	CGCGATCTCTCTTGATCCGT	qRT-PCR
rpoB-F	GGCAGAAGAACTTGAGAA	qRT-PCR
rpoB-R	GGAAGAATAGGTGAGTGATT	qRT-PCR
rpoC1-F	CGTGCTACTTCTAATGTT	qRT-PCR
rpoC1-R	CCTGTATGCCTAATCTATG	qRT-PCR

GGCTGATGATGAGGTAAG	qRT-PCR
TGAAGAATAGAAGGATAGACAA	qRT-PCR
ATTATATCTGTACTTCCATCTCA	qRT-PCR
GTCATAACCACTGCCTAC	qRT-PCR
CGCTATTTCTGATGCTATGGGT	qRT-PCR
TCTTGGGTGATGATTGTTTGG	qRT-PCR
GCTTGCAGAAAGCTACACAAGC	qRT-PCR
AGGCCGTGAGCTAAAGGAGC	qRT-PCR
ATGAACCTTACCGTCCTATTCCTT	qRT-PCR
GGACCCACCAACAGCAAGAT	qRT-PCR
TGTACTGGAGCTGGAACAACAA	qRT-PCR
GAGCACAGCAAAATCCTAGACG	qRT-PCR
GATCCATACCCGATCGACAT	qRT-PCR
CGAGAGACATCCGGTAGAGC	qRT-PCR
CGAGCCCAGGTTCATCAAGGTGC	qRT-PCR
CCTCCCGATCTTGCCGAACTCC	qRT-PCR
TGTGTACACTTGTAGTAGCCAC	qRT-PCR
CACACACACACACAATTAAGGT	qRT-PCR
CATCTCAAGAAGTACTCGAGCA	qRT-PCR
GAACTTCTTGATGCCCTCAATC	qRT-PCR
AAGGTTCTGCTAGTGTTAGTCC	qRT-PCR
GAATCATCAAGGACAATCTGCC	qRT-PCR
	GGCTGATGATGAGGGTAAGTGAAGAATAGAAGGATAGACAAATTATATCTGTACTTCCATCTCAGTCATAACCACTGCCTACCGCTATTTCTGATGCTATGGGTTCTTGGGTGATGATGATTGTTTGGGCTTGCAGAAAAGCTACACAAGCAGGCCGTGAGCTAAAGGAGCATGAACCTTACCGTCCTATTCCTTGGACCCACCAACAGCAAGATTGTACTGGAGCTGGAACAACAAGAGCACAGCAAAAATCCTAGACGGATCCATACCCGATCGACATCGAGAGACATCCGGTAGAGCCGAGCCCAGGTTCATCAAGGTGCCCTCCCGATCTTGCCGAACTCCTGTGTACACTTGTAGTAGCACACCACACACACACACACAATTAAGGTCATCTCAAGAAGTACTCGAGCAGAACTTCTTGATGCCCTCAATCAAGGTTCTGCTAGTGTTAGTCCGAATCATCAAGGACAATCTGCC

**Table S2** Accession numbers of RpoB, NdhB, NdhD, and homologous of OsPPR16 in 79 landplants.

Organism	OsPPR16	RpoB	NdhB	NdhD
Aegilops tauschii subsp.	XP_02018508	YP_008474	YP_0084743	YP_0084743
tauschii	3.1	290.1	44.1	53.1
Amborella trichopoda	XP_01162433 7.1	NP_904091 .2	NP_904144.1	NP_904149.1
Ananas comosus	XP_02011448 6.1	YP_009116 334.1	YP_0091163 87.1	YP_0091163 92.1
Arabidopsis lyrata subsp. lyrata	XP_00289263 5.1	YP_009357 259.1	YP_0093573 27.1	YP_0093573 17.1
Arabidopsis thaliana	NP_172596.1	NP_051051 .1	NP_051103.2	NP_051109.2
Asparagus officinalis	XP_02024133 3.1	YP_009370 013.1	YP_0093700 65.1	YP_0093700 70.1
Brachypodium distachyon	XP_01022811 2.1	YP_002000 478.1	YP_0020005 29.1	YP_0020005 35.1

Buggging ugnug	XP_02254704	YP_005089	YP_0050900	YP_0050900
Brassica napus	6.1	944.1	13.1	03.1
Brassica olaracaa yar olaracaa	XP_01360425	YP_009564	YP_0095646	YP_0095646
Drussicu oieruceu vur. oieruceu	1.1	567.1	20.1	26.1
Brassica rana	XP_01850999	YP_009553	YP_0095538	YP_0095538
Brassica rapa	0.1	759.1	12.1	18.1
Caianus caian	XP_02022521	YP_009309	YP_0093091	YP_0093092
	8.1	154.1	98.1	08.1
Camalina sativa	XP_01909894	YP_009231	YP_0092311	YP_0092311
	4.1	067.1	19.1	25.1
Camallia sinansis	XP_02808961	YP_007317	YP_0073173	YP_0073172
	6.1	240.1	09.1	99.1
Cannahis sativa	XP_03048059	YP_009123	YP_0091231	YP_0091231
	6.1	065.1	16.1	22.1
Cansella rubella	XP_00630449	YP_009161	YP_0091619	YP_0091619
Cupsena rasena	4.1	913.1	65.1	70.1
Capsicum annuum	XP_01656001	YP_006666	YP_0066660	YP_0066660
	7.1	022.1	75.1	80.1
Carica nanava	XP_02190133	YP_001671	YP_0016717	YP_0016717
	8.1	675.1	27.1	32.1
Chenopodium auinoa	XP_02174522	YP_009380	YP_0093801	YP_0093801
	1.1	122.1	74.1	80.1
Cicer arietinum	XP_00449978	YP_002149	YP_0021497	YP_0021497
	2.1	733.1	75.1	85.1
Citrus sinensis	XP_00649462	YP_740467	YP 740521.1	
	9.1	.1		YP_740527.1
Coffea arabica	XP_02711431	YP_817474	YP 817527.1	ND 015500 1
	2.1	.l	-	<u>YP_817532.1</u>
Coffea canephora	GDD101(0.1	YP_009251	YP_0092511	YP_0092511
	CDP18169.1	11/.1 ND 047501	70.1	76.1
Cucumis sativus	XP_00414712	I YP 247591		
	( )	1	YP 247644.1	VD 047(50 1
	6.2	.1	YP_247644.1	YP_247650.1
Cucurbita maxima	6.2 XP_02296703	.1 YP_009447	YP_247644.1 YP_0094474	YP_247650.1 YP_0094475
Cucurbita maxima	6.2 XP_02296703 2.1	.1 YP_009447 442.1	YP_247644.1 YP_0094474 95.1	YP_247650.1 YP_0094475 01.1
Cucurbita maxima Cucurbita moschata	6.2 XP_02296703 2.1 XP_02294517	.1 YP_009447 442.1 YP_009447	YP_247644.1 YP_0094474 95.1 YP_0094475	YP_247650.1 YP_0094475 01.1 YP_0094475
Cucurbita maxima Cucurbita moschata	6.2 XP_02296703 2.1 XP_02294517 8.1	.1 YP_009447 442.1 YP_009447 527.1	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1
Cucurbita maxima Cucurbita moschata Durio zibethinus	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569	.1 YP_009447 442.1 YP_009447 527.1 YP_009457	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579
Cucurbita maxima Cucurbita moschata Durio zibethinus	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01004070	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 VP_000072	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_006073 006_1	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0060731 40_1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54_1
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079 0.1 XP_01006767	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_006073 096.1 VP_002022	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0060731 49.1 VP_0020240	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54.1 VP_0020240
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis Eucalyptus grandis	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079 0.1 XP_01006767 1.1	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_006073 096.1 YP_003933 054_1	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0060731 49.1 YP_0039340 03_1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54.1 YP_0039340 08_1
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis Eucalyptus grandis	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079 0.1 XP_01006767 1.1 XP_00620271	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_006073 096.1 YP_003933 954.1 VP_000175	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0060731 49.1 YP_0039340 03.1 YP_0001757	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54.1 YP_0039340 08.1 YP_0001757
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis Eucalyptus grandis Eutrema salsugineum	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079 0.1 XP_01006767 1.1 XP_00639371 7.1	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_006073 096.1 YP_003933 954.1 YP_009175 667_1	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0060731 49.1 YP_0039340 03.1 YP_0091757 36.1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54.1 YP_0039340 08.1 YP_0091757 26.1
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis Eucalyptus grandis Eutrema salsugineum	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079 0.1 XP_01006767 1.1 XP_00639371 7.1 XP_00252634	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_006073 096.1 YP_003933 954.1 YP_009175 667.1 YP_528762	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0060731 49.1 YP_0039340 03.1 YP_0091757 36.1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54.1 YP_0039340 08.1 YP_0091757 26.1
Cucurbita maxima Cucurbita moschata Durio zibethinus Elaeis guineensis Eucalyptus grandis Eutrema salsugineum Glycine max	6.2 XP_02296703 2.1 XP_02294517 8.1 XP_02276569 7.1 XP_01094079 0.1 XP_01006767 1.1 XP_00639371 7.1 XP_00352634 0.1	.1 YP_009447 442.1 YP_009447 527.1 YP_009457 853.1 YP_009457 853.1 YP_006073 096.1 YP_003933 954.1 YP_009175 667.1 YP_538763 1	YP_247644.1 YP_0094474 95.1 YP_0094475 97.1 YP_0094579 06.1 YP_0094579 06.1 YP_0060731 49.1 YP_0039340 03.1 YP_0091757 36.1 YP_538808.1	YP_247650.1 YP_0094475 01.1 YP_0094475 86.1 YP_0094579 11.1 YP_0060731 54.1 YP_0039340 08.1 YP_0091757 26.1

Glycine soja	XP_02823833	YP_008816	YP_0088162	YP_0088162
	5.1	245.1	89.1	99.1
Gossypium arboreum	XP_01764351 3.1	YP_005088 991.1	YP_0050890 42.1	YP_0050890 52.1
Gossypium hirsutum	XP_01674657 8.1	YP_538927	YP_538980.1	YP 538990.1
Gossypium raimondii	XP_01244936	YP_005087 685.1	YP_0050877 36.1	YP_0050877 46.1
Helianthus annuus	XP_02197002 9.1	YP_588104	YP_588162.1	YP 588173.1
Hevea brasiliensis	XP_02163937 7.1	YP_004327 649.1	YP_0043277 21.1	YP_0043277
Ipomoea nil	XP_01917514 8.1	YP_009295 096.1	YP_0092951 48.1	YP_0092951 57.1
Jatropha curcas	XP_01206874 0.1	YP_002720 104.1	YP_0027201 57.1	YP_0027201 62.1
Juglans regia	XP_01884937 0.1	YP_009186 160.1	YP_0091862 28.1	YP_0091862 18.1
Lactuca sativa	XP_02376860 2.1	YP_398316 .1	YP_398373.1	YP 398378.2
Manihot esculenta	XP_02162810 8.1	YP_001718 429.1	YP_0017184 81.1	YP_0017184 86.1
Medicago truncatula	XP_01345890 7.1	YP_001381 728.1	YP_0013816 86.1	YP_0013816 74.1
Momordica charantia	XP_02213909 3.1	YP_009456 053.1	YP_0094561 05.1	YP_0094561 12.1
Morus notabilis	XP_01009132 4.1	YP_009139 671.1	YP_0091397 23.1	YP_0091397 28.1
Musa balbisiana	THU44351.1	YP_009180 363.1	YP_0091804	YP_0091804 24.1
Nelumbo nucifera	XP_01024361	YP_009093 942 1	YP_0090939 94 1	YP_0090939
Nicotiana attenuata	XP_01922418	YP_009429 442 1	YP_0094294 94 1	YP_0094294 99.1
Nicotiana sylvestris	XP_00979958	YP_358664	YP 3587271	YP 358737 1
Nicotiana tabacum	XP_01650596	NP_054488	NP_054547.1	NP 054557.2
Nicotiana tomentosiformis	XP_00963133 5.1	YP_398851	YP_398913.1	YP 398923.1
Oryza brachyantha	XP_00665315 5.1	YP_009266 412.1	YP_0092664 65.1	YP_0092664 71.1
Oryza sativa Japonica Group	XP_01564385 1.1	NP_039373 .1	NP_039432.1	NP 039444.1
Papaver somniferum	XP_02642434 0.1	YP_009234 974.1	YP_0092350 27.1	YP_0092350 32.1

Phoenix dactylifera	XP_02665606	YP_003540	YP_0035409	YP_0035409
	5.1	923.1	76.1	83.1
Physcomitrella patens	XP_02438979	NP_904223	NP 904166.1	
	4.1	.1		NP_904238.1
Populus euphratica	XP_01102049	YP_009054	YP_0090544	YP_0090544
	0.1	421.1	73.1	78.1
Populus trichocarpa	XP_00230396	YP_001109	YP_0011095	YP_0011095
	0.2	491.1	48.1	57.2
Prunus avium	XP_02183293	YP_009691	YP_0096919	YP_0096919
	9.1	926.1	77.1	82.1
Prunus mume	XP_00823160	YP_009020	YP_0090200	YP_0090200
	9.1	030.1	83.1	88.1
Prunus persica	XP_00722025	YP_004021	YP_0040217	YP_0040217
	5.1	657.1	09.1	14.1
Raphanus sativus	XP_01843996	YP_009046	YP_0090469	YP_0090469
· · · · · · · · · · · · · · · · · · ·	3.1	906.1	59.1	65.1
Ricinus communis	XP_01557872	YP_005090	YP_0050902	YP_0050902
	0.1	170.1	22.1	28.1
Selaginella moellendorffii	XP_02453738	YP_003097	YP_0030974	YP_0030975
	9.1	4′/6.1	73.1	25.1
Sesamum indicum	XP_01110186	YP_004935	YP_0049357	YP_0049357
	1.1	658.1	12.1	17.1
Setaria italica	XP_01270061	YP_008815	YP_0088157	YP_0088158
	3.1	741.1	94.1	00.1
Setaria viridis	<b>TUUUUUUUUUUUUU</b>	YP_009172	YP_0091721	YP_0091721
	TKW19248.1	130.1	83.1	90.1
Solanum lycopersicum	XP_00423381	YP_008563	YP_0085631	YP_0085631
	2.1	080.1	34.1	39.1
Solanum pennellii	XP_01506361	YP_009421	YP_0094214	YP_0094214
	6.1	349.1	03.1	08.1
Solanum tuberosum	XP_00634715	YP_635631	YP 635684.1	
	9.1	.l	_	YP_635689.2
Sorghum bicolor	XP_02131854	YP_899397	YP 899451.1	
	0.1	.1	_	YP_899457.1
Spinacia oleracea	XP_02183836	NP_054924	NP 054977.1	
	1.1	.1		NP_054988.2
Tarenava hassleriana	XP_01054019	YP_009356	YP_0093565	YP_0093565
	4.1	519.1	71.1	77.1
Theobroma cacao	XP_01798484	YP_004021	YP_0040213	YP_0040213
	4.1	309.1	59.1	69.1
Triticum aestivum		NP_114249	NP 114301.1	
	SP116892.1			NP_114308.1
Vigna radiata var. radiata	XP_01449960	YP_003434	YP_0034343	YP_0034343
	0.1	344.1	84.1	89.1
Vigna unguiculata	XP_02793995	YP_006460	YP_0064603	YP_0064603
· ·Sin miSmennin	1 1	343.1	82.1	88.1

Vitis vinifera	XP_01907316 3.1	YP_567068 .1	YP_567123.1	YP_567128.1
Zea mays	NP_00135656 9.1	NP_043015 .1	NP_043076.1	NP_043087.1
Ziziphus jujuba	XP_01587614 2.1	YP_009256 319.1	YP_0092563 72.1	YP_0092563 77.1

**Table S3** Genotype and phenotype of  $T_0$  transgenic plants.

Line	Genotype	Phenotpye
osppr16d-1	Biallelic mutant	Pale
osppr16d-2	Biallelic mutant	Pale
osppr16d-3	Biallelic mutant	Pale
osppr16d-4	Biallelic mutant	Pale
OsPPR16d-5	Wild-type	Green
osppr16d-6	Biallelic mutant	Pale
OsPPR16d-7	Heterozygous	Green
OsPPR16d-8	Heterozygous	Green
OsPPR16d-9	Wild-type	Green
osppr16d-10	Biallelic mutant	Pale
OsPPR16d-11	Heterozygous	Green
OsPPR16d-12	Wild-type	Green
OsPPR16d-13	Heterozygous	Green
OsPPR16d-14	Heterozygous	Green
osppr16d-15	Biallelic mutant	Pale
osppr16d-16	Biallelic mutant	Pale
OsPPR16d-17	Wild-type	Green
OsPPR16d-18	Heterozygous	Green
OsPPR16d-19	Heterozygous	Green
OsPPR16d-20	Wild-type	Green
osppr16d-21	Biallelic mutant	Pale
OsPPR16d-22	Heterozygous	Green
OsPPR16d-23	Heterozygous	Green
osppr16d-24	Biallelic mutant	Pale
OsPPR16d-25	Heterozygous	Green
OsPPR16d-26	Wild-type	Green
OsPPR16d-27	Heterozygous	Green
OsPPR16d-28	Wild-type	Green
OsPPR16d-29	Heterozygous	Green
OsPPR16d-30	Heterozygous	Green

OsPPR16d-31	Heterozygous	Green
OsPPR16d-32	Heterozygous	Green
OsPPR16d-33	Wild-type	Green
OsPPR16d-34	Heterozygous	Green
OsPPR16d-35	Heterozygous	Green
osppr16d-36	Homozygous	Pale
OsPPR16s-1	Wild-type	Green
OsPPR16s-2	Heterozygous	Green
OsPPR16s-3	Heterozygous	Green
osppr16s-4	Biallelic mutant	Pale
OsPPR16s-5	Wild-type	Green
OsPPR16s-6	Heterozygous	Green
osppr16s-7	Biallelic mutant	Pale
OsPPR16s-8	Heterozygous	Green
OsPPR16s-9	Heterozygous	Green
OsPPR16s-10	Wild-type	Green
osppr16s-11	Biallelic mutant	Pale
OsPPR16s-12	Wild-type	Green
OsPPR16s-13	Heterozygous	Green
osppr16s-14	Biallelic mutant	Pale
OsPPR16s-15	Heterozygous	Green
OsPPR16s-16	Heterozygous	Green
osppr16s-17	Homozygous	Pale
OsPPR16s-18	Wild-type	Green
OsPPR16s-19	Heterozygous	Green
OsPPR16s-20	Heterozygous	Green
OsPPR16s-21	Wild-type	Green
OsPPR16s-22	Heterozygous	Green
osppr16s-23	Biallelic mutant	Pale
OsPPR16s-24	Heterozygous	Green
OsPPR16s-25	Heterozygous	Green
OsPPR16s-26	Wild-type	Green
osppr16s-27	Biallelic mutant	Pale
OsPPR16s-28	Heterozygous	Green
OsPPR16s-29	Heterozygous	Green
OsPPR16s-30	Heterozygous	Green
osppr16s-31	Biallelic mutant	Pale
OsPPR16s-32	Heterozygous	Green
osppr16s-33	Homozygous	Pale

## Methods S1 Complementation of the *osppr16* mutant.

For mutant complementation, an 8300-bp WT genomic DNA fragment comprising the entire *OsPPR16* coding region, 4731 bp of the 5' upstream region and 1364 bp of 3' downstream sequence was cloned into the pCAMBIA2300 vector (CAMBIA, Canberra, Australia) in two steps. In the first step, a 5533 bp DNA fragment (3' portion of the 8300-bp fragment) was amplified with primers CO16A-F and CO16A-R, digested with *Sac* I and *Sal* I, and ligated into pCAMBIA2300 to generate pCAMBIA2300-OsPPR16A. In the second step, a 2767 bp DNA fragment (5' portion of the 8300-bp fragment) was amplified with primers CO16B-F and CO16B-R and subcloned into the pCAMBIA2300-OsPPR16A vector (digested with *Sac* I) by using the ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China). The pCAMBIA2300-OsPPR16 vector was transformed into callus tissue induced from mature seeds of line *osppr16d-36-6*, which was free of the CRISPR/Cas9 construct. The sequences of all primers are listed in Table S1.

## Reference

Liere K, Börner T 2007. Transcription and transcriptional regulation in plastids. *Cell and molecular biology of plastids*: Springer, 121-174.