

A Plastid-Localized Pentatricopeptide Repeat Protein Is Required for Both Pollen Development and Plant Growth in Rice

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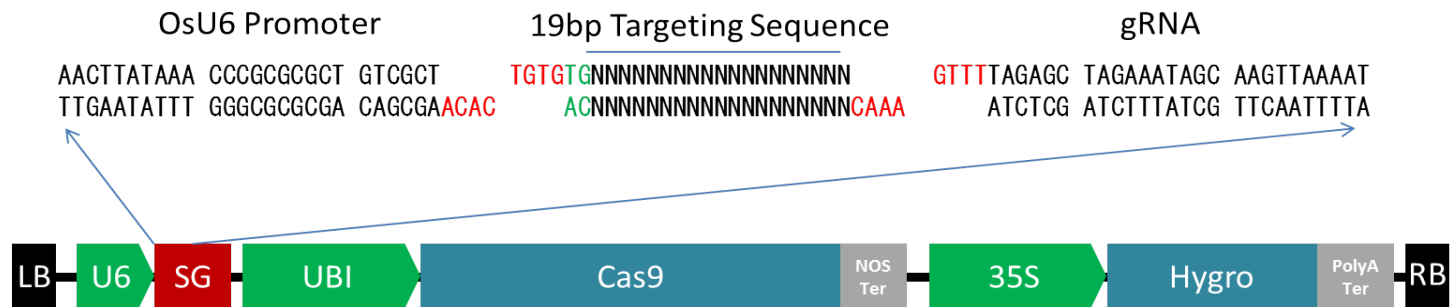
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Feature of plasmid

LB	Left border of T-DNA	Cas9	Optimized Cas9
RB	Right border of T-DNA	NOS Ter	NOS terminator
U6	Rice U6 promoter	35S	CaMV 35S promoter
SG	sgRNA	Hygro	Hygromycin selection marker
UBI	UBI promoter	PolyA Ter	PolyA terminator

Figure S1. sgRNA-CRISPR/Cas9 plant expression vector.

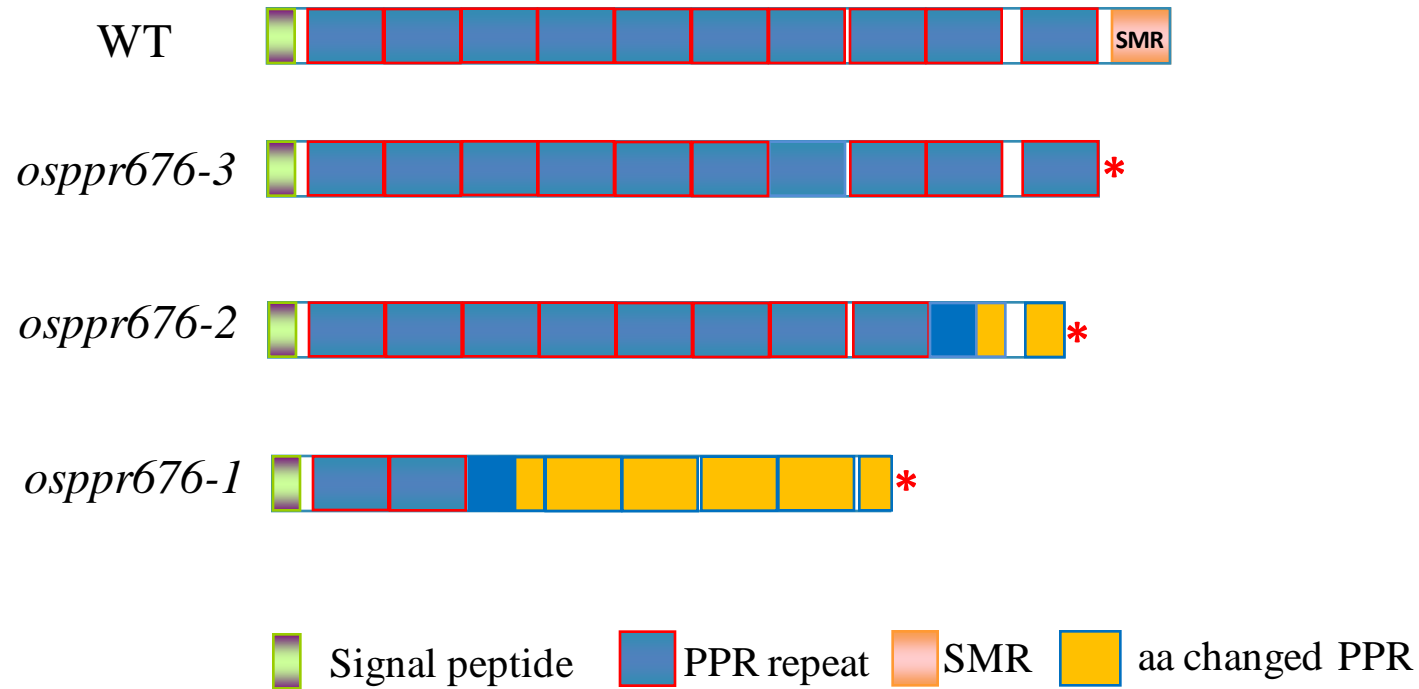


Figure S2. Truncated proteins structure of *osppr676-1*, *osppr676-2*, *osppr676-3*.

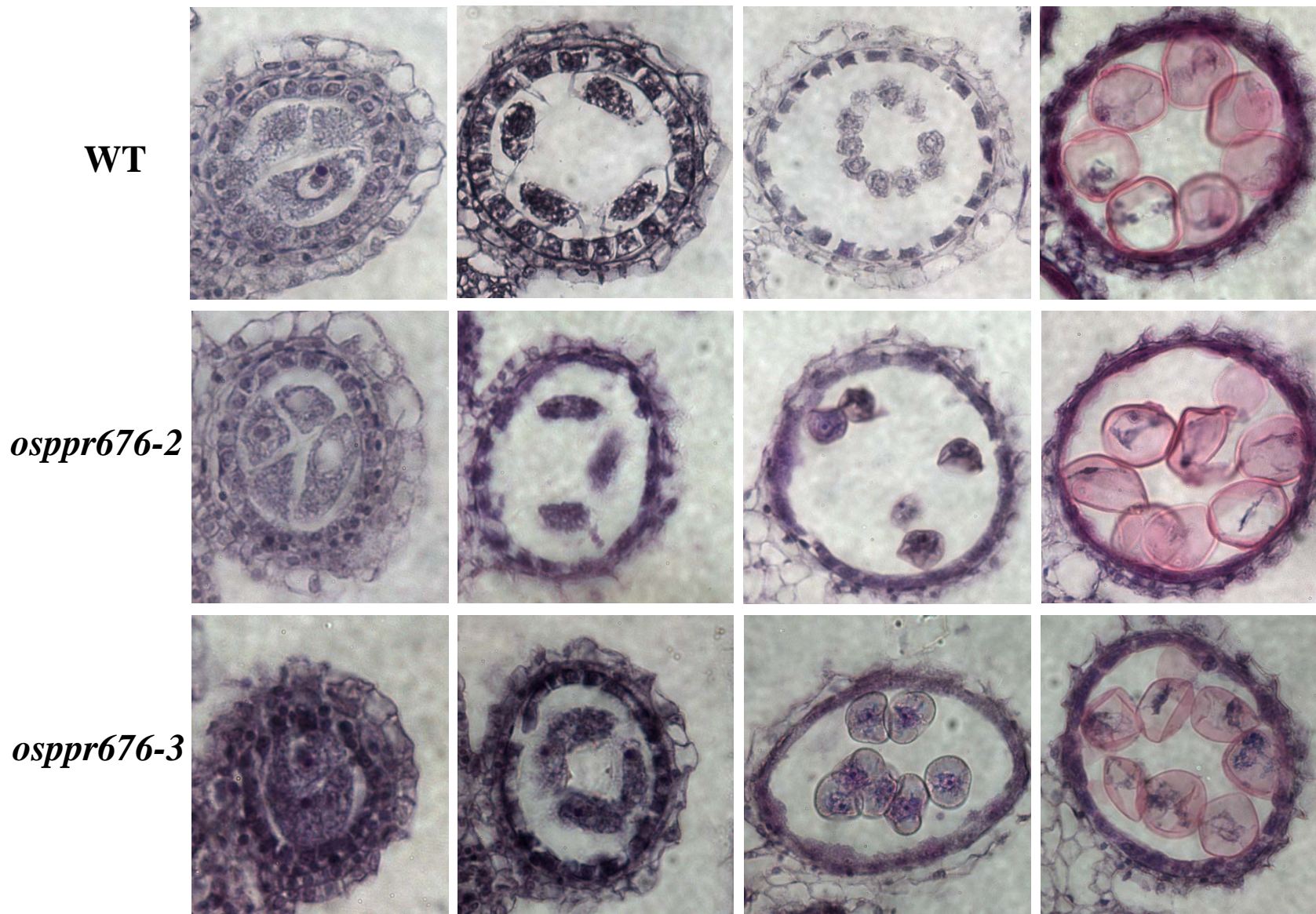


Figure S3. Comparison of cross sections of anthers from wild type and *osprr676-2* and *osprr676-3* mutants.

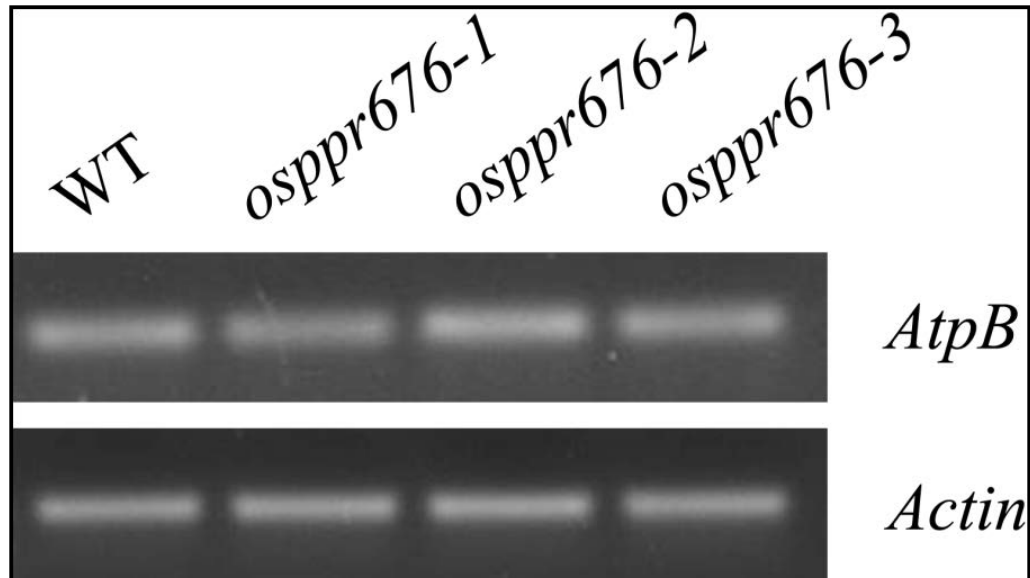


Figure S4. No significant difference was detected in mRNA accumulation of *atpB* in WT and *osppr676* mutants.

SVR7	MSFHLLCS SPSSLLHDPFLPCNLLSVYEKSTPRSFSSYNENSSHFHSRNLLQATHVSVQEAIEQSEKSKIVDVDFPIPEPTASKSYVWVNEKSPRASQL	100
ATP4	MASLPLCR SPSSLLPSWEHRPISASENFKNES.SFVAAHVSVQETPPQPQDPSPEFSDS...NENGRTPSSSSNTRFIVWVNEKSPRAADV	85
OsPPR676MAS SPSSLLSWEHRAISLSFQPKNESEFATARVSVQDPPP.....EESDA...NPSFGR..SSNTRSRYVWVNEKSPRAZAGL	71
SVR7	RRKSYDS.RYSS LTKLAESLDACKFNADVCDVITG.FGGKLEEQDAVVLIN.NMINPETAFIVINNLETMKFSREVILYNVIMVVERKSKDLEKSEK	196
ATP4	ARARAGSGRRAR IASPAAAGACETTESAVEAALQAAEPEPPSEQDAVIVLNTAAATRAETAVALRWFELGNKAVRKKVILYNVVLKLRKKRIWSETEA	185
OsPPR676	ARARAGSGRRAR IAAPAAATAACEAGAEVAALAAEAPEPPSEQDAVIVLN.TTSAREAAVVIAVWVFLRNAEVRKEVILYNVALKLRKRRRFSWDAEA	170
SVR7	LFDMLERGIKIPDNATETLISCARQNCVPKFAVEWEKMSFQCEPDNVMAAMIDAYGRAGNVDMAISLYDRARTEKRRIDAVTFSLIRIYGVSGNY	296
ATP4	LWAEMLRDGVCQPDNATESIVLSCARACCLHSAVEWEDKMPFEGCSPDMIYSAVIDAYGHAGNSEAALRLYDRARAERWQDDEVICSTVIVKHVHSTSGNF	285
OsPPR676	LWBEMLRECVCQPDNATESIVLSCARACCMPGRAVEWEKMPFEGCSPDMIYSAVIDAYGRAGDAETALRLYDRARAERWQDDEVICATVIRVHSSSGNF	270
SVR7	DGCINLYEEMKAGVKFNIVVYNRLIDSMGRAKREFCAKIIYKDLITNGFTENWSTYAAIVFAYCRARYGDDATAIYREMKKGLSLTVILYNLLSMCA	396
ATP4	DGCINLYEEMKAGVRFNIVVYNIMLIDMGRALREFVVKTIHREMVDQCVQESFATYCCILHAYTRARYGEDAMAVYRUMKDEAMGIDVLYNMLLSMCA	385
OsPPR676	DGCINLYEEMKAGVKFNIVVYNIVLIDMGRAMREFVVKTIHRELVSQEAVENTKATYCCILHAYTRARYGEDAMAVYRUMKDEVMIDVLYNMLLSMCA	370
SVR7	DNRVYDEAEELIQDMKN.CETCDPDSWTFSSLLTVYACSGFVSEAEAPLQMRAGEEETLEVLTSVIOCYGKAKQVDDVVRTEFCVLELGIIPDDRFC	494
ATP4	DICVYDEAEELIRDMKASMGASHKPDSSYSSMVLLYSSTANVLSAEGIINEMVEAGEKENIEVLTSILRCYGVGRITDDVVRSEGMQLDLGIIPDDRFC	485
OsPPR676	DICVYEEAEELIRDMKASMDRSRSPDSSYSSMVLLYSCTGNVACAEGIINEMVEAGEKENIEVLTSILRCYGVGRITDDVVRSEAMLEDLGIIPDDRFC	470
SVR7	GCLINVMQTQTESEIIGKILICVEKAKPKLGVVVMVVEEQNCEEGVFKKEASELIDSIGSDVKKAMINCLIDICVNINKLEFACETLQGLGYDIYTGILQ	594
ATP4	GCLISVAANTEAEELGKVISCIERSNVQLGAVVKLIVD.RSS.SESFREARELLRSSRGVVKMEVQNCIMDIOVNINQMEKACALLDAAQQLGIYANIQ	583
OsPPR676	GCLITVAACTEAEELGKVICCIDRSSAQLGAVVRIIVD.AAAPSEPLREAPAGELLGGARGVVRMEVQNCIMDIAVNLSQMEKACALLDVALRLGIYSNVQ	569
SVR7	SKSATQWSLHLKSLSLGAALTALHVVMNDISEAALESGEFPPLLGINTCGKHKYSKGLIAPVFESHLKEINAPFHEAPDKVGFLLTTSVAAKAWLESR	694
ATP4	TRTQTQWSLHLRCLSVGAALTTLHVVMNDLYTSLQTGNEGIPPLLGITHTCGKNTYSRGLIAPMFAHLKELDAPFHEAPDKAGWFLTTVAAKQWLESK	683
OsPPR676	TRTQTQWSLHLRCLSVGAALTTLHVVMNDLYAALQAG.DELPPLLGITHTCGKNTYSYKGLIAPVFESHLKELDAPFHEAPDKAGWFLTTSVAAREWLETK	668
SVR7	RSAGCVSA	702
ATP4	AASEIVTV	691
OsPPR676	KSAEIVAV	676

Figure S5. Protein sequences alignment of SVR7, ATP4 and OsPPR676.

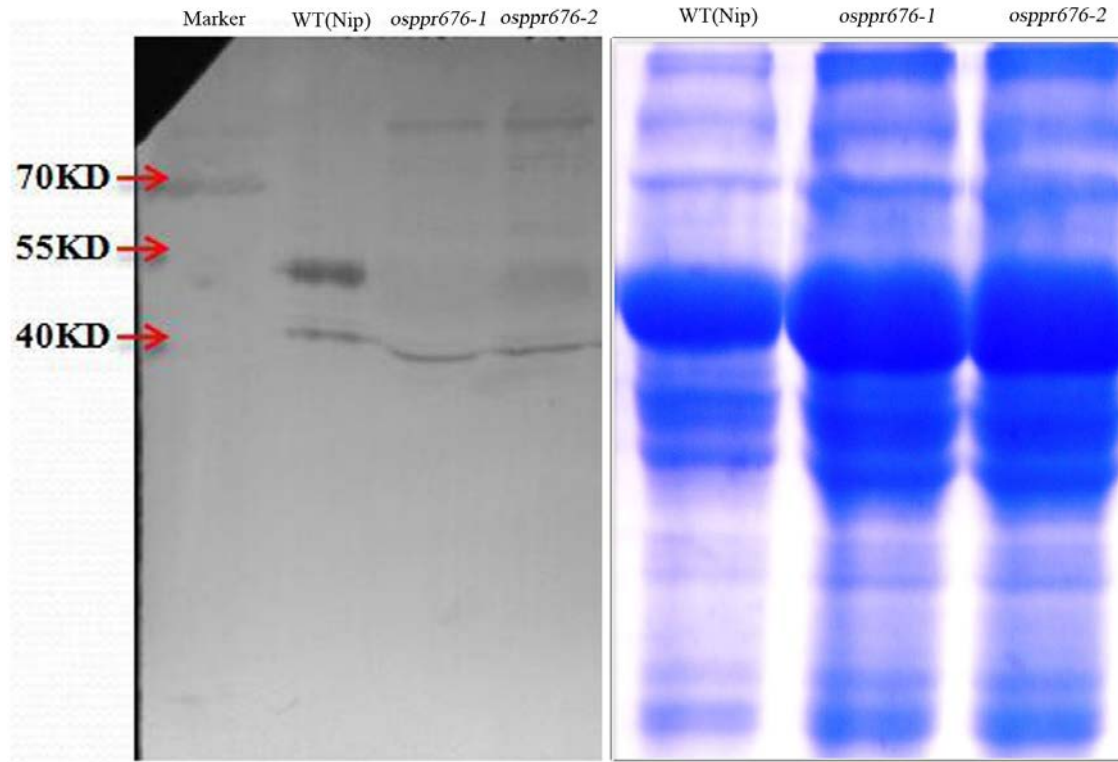


Figure S6. Absence of AtpB subunit production in rice *osppr676* mutants. Western blot analysis with AtpB antibody on total proteins from the *osppr676-1* and *osppr676-2* mutants and WT seedlings and Coomassie Blue staining of the loading amount of samples. The size of target atpB band is 54KD, and these bands about 40KD are unspecific bands.

Table S1. Primers used in this study.

Primer name	Primer Sequence (5'–3')	Use for
OsPPR676 MF	CTCAACGTGTTCGAGGAAAT	Confirm <i>osprr676-1</i> mutant
OsPPR676 MR	CAGTTGCAGTACGGCATCCT	Confirm <i>osprr676-1</i> mutant
GT638-F	CACCTTCTCGACCGTCATCA	Confirm <i>osprr676-2</i> mutant
GT638-R	CGCCTTCATTCCTCGAACA	Confirm <i>osprr676-2</i> mutant
GT640-F	CCTCACCTCGCTCATCCGTT	Confirm <i>osprr676-3</i> and <i>676-4</i> mutant
GT640-R	AGCAGCGGGCGGAGCTCGT	Confirm <i>osprr676-3</i> and <i>676-4</i> mutant
OsPPR676-ENF1	CACCATGGCTTCCCCTTCCTCCCT	Construct pENTR vector
OsPPR676-ENR1	CACCGCTACTAACTCTGCCG	Construct pENTR vector
OsBTF3-ENF1	CACCATGAATGTTGACAAGCTCAA	Construct pENTR vector
OsBTF3-ENR1	TGATTCCTTTTCTCTTCTG	Construct pENTR vector
OsPPR676 probe F	GAGGACGCCATGGCCGTG	RNA in situ hybridization
OsPPR676 probe R	TCACCATGGATGAGTAGC	RNA in situ hybridization
OsPPR676 RF1	GACCATCCACAGGGAGCTAGT	Real-time PCR
OsPPR676 RR1	ACAAGGCGACGTACTGCTG	Real-time PCR
OsPPR676- OE-F	CTAGTCTAGACAGAGAGCATCGCGC	Construct over-expression vector
OsPPR676- OE-R	CGGGTACCCATTGTTGCGTTTTACAC	Construct over-expression vector
OsPPR676- KpnI	GATCGGTACCGGATGACCTCCTTGCGAACCTC	Construct RNAi vector
OsPPR676-BamHI	GATCGGATCCCGCCCGTGTCTCCGTCCAA	Construct RNAi vector
OsPPR676- SpeI	GATCACTAGTGGATGACCTCCTTGCGAACCTC	Construct RNAi vector
OsPPR676- SacI	GATCGAGCTCCGCCCGTGTCTCCGTCCAA	Construct RNAi vector

Table S2. Multiple *osprr676* lines created by CRISPR/Cas9 mutation system.

Mutation site	Mutation type
<i>OsPPR676</i> SG638 site	
GGTGCTCGCCGGACATGCTCACGTACT-CGGCGGTGATCGACGC	WT
GGTGCTCGCCGGACATGCTCACGTACC-CGGCGGTGATCGACGC	D1, +1
GGTGCTCGCCGGACATGCTCACGTACTTCGGCGGTGATCGACGC	+1
GGTGCTCGCCGGACATGCTCACGTCT-CGGCGGTGATCGACGC	D1, +1
GGTGCTCGCCGGACATGCTCACGT----CGGCGGTGATCGACGC	D3
GGTGCTCGCCGGACATGCTCAGGTA---CGGCGGTGATCGACGC	D6, +4
GGTGCTCGCCGGACATGCTCACGTACT-CGGGGGTGATCGACGC	D1, +1
GGTGCTCGCCGGACATGCTCACGTA---CGGCGGTGATCGACGC	D2
GGTGCTCGCCGGACATGCTCACGTCT----GGCGGTGATCGACGC	D4, +1

***OsPPR676* SG640 site**

CGTCCGGGGT GATGCCGAGGTCCT -CGA- GCATGGCGAATGACCT	WT
CGTCCGGGGT GATGCCGAGGTGCTCAAT -GCATGGCGAATGACCT	D6, +7
CGTCCGGGGT GCCGCAGAGGTCCT -CAA- GAATGGCGAATGACCT	D18, +18
CGTCCGGGGT GATGCCGAGGTGCT - CAATGCC TGGCGAATGACCT	D10, +10
CGTCCGGGGTGATGCCGAGGTCCT-C---GCATGGCGAATGACCT	D2
CGTCCGGGGT GATGCCGAGGTCCT -CGA A GCATGGCGAATGACCT	+1
CGTCCGGGGT GATGCCGAGGTCC ----A-GCATGGCGAATGACCT	D3
CGTCCGGGGT GATGCCGAGGTC -----GCATGGCGAATGACCT	D5
CGTCCGGGGT GATGCCGAGGTCCT -CGA-- T ATGGCGAATGACCT	D2, +1

***OsPPR676* SG641 site**

GGACGCAGAC GCAGTGGTCGCTGCACCTGA GGG GCCTCTCCGT	WT
GGACGCAGACGCAGTGGTCGCTGCACC-GAGGGGCCTCTCCGT	D1
GGACGCAGACGCAGTGGTCG TTG --- G TGAGGGGCCTCTCCGT	D7, +4

GGGGCCTCACCGTCGGGTCCGTGC---TGAGGGGCCTCTCCGT	D25, +22
GGACGCAGACGCAGTGGTCGCTGCAC-TGAGGGGCCTCTCCGT	D1
GGACGCAGACGCAGTGGTCGCTGTAC-TGAGGGGCCTCTCCGT	D3, +2
GGACGCAGACGCAGT-----GGCCTCTCCGT	D17
GGACGCAGACGCAGTGGTCGCTGCC-TGAGGGGCCTCTCCGT	D3, +2
GG-----GGCCTCTCCGT	D30
GGACGCAGACGCAGTGGTCGCTGCAT-GGAGGGGCCTCTCCGT	D3, +2

The wild type sequence is shown at the top with the PAM sequence highlighted in brown and the target sequence in blue. Red dashes, deleted bases; red bases, insertions or mutations. Green sequences, *ospPr676-1* mutation; yellow sequences, *ospPr676-2* mutation; purple sequences, *ospPr676-3* mutation. The net change in length is to the right of each sequence (+, insertion; D, deletion). Note that some alterations have both sequence insertions and deletions.

Table S3. Predicted possible off-target sites of SG638, SG640 and SG641 by CRISPR-P (<http://cbi.hzau.edu.cn/crispr/>).

SG638 position: Chr3:-6090845		Guide sequence: GGACATGCTCACGTACTCGG CGG				
Predicted off-target sites for SG638 site:						
Sequence	Score	MMs	Locus	Gene	Region	
GGACT TTGC CACGTACTCGG GGG	0.6	4MMs	Chr12: -2141485	LOC_Os12g04960	exon	
GGTCAT CCGC AGGTACTCGG CGG	0.3	4MMs	Chr1: +33066673	LOC_Os01g57230	exon	
C GACATGC CC CACGC AC AGG CGG	0.1	4MMs	Chr11: -3818498	LOC_Os11g07530	exon	
GGTC AGG CTC AAGT GCTCGG TGG	0.1	4MMs	Chr6: -19265675	LOC_Os06g33090	intron	
GGTC AGG CTC AAGT GCTCGG TGG	0.1	4MMs	Chr6: -19271440	LOC_Os06g33100	exon	
GGTC AGG CTC AAGT GCTCGG TGG	0.1	4MMs	Chr6: -19258290	LOC_Os06g33080	exon	
GGAC AGA CTCACGTACTC CCAGG	0.1	4MMs	Chr10: +4041974	LOC_Os10g07542	exon	
SG640 position: Chr3:+6090129		Guide sequence: GATGCCGAGGTCTCGAGCA TGG				
Predicted off-target sites for SG640 site:						
Sequence	Score	MMs	Locus	Gene	Region	
GGAGT CGAGGG CCT CGAGCAC CGG	0.8	4MMs	Chr3: +862609	LOC_Os03g02430	exon	
GATGCCGAGGT CCTTGAGC TGG	0.7	2MMs	Chr4: +1026229	LOC_Os04g02690	exon	
GAGGCCGAGG GA CTCGAGCAC CGG	0.7	3MMs	Chr6: -3650122	LOC_Os06g07560	exon	
ATTG CCGAG CTC CTCGAG CTCGG	0.7	4MMs	Chr4: +7155034	LOC_Os04g12960	exon	
ATTG CCGAG CTC CTCGAG CTCGG	0.7	4MMs	Chr4: +7026047	LOC_Os04g12690	exon	
GATG AGG AGGT CTC CGAGCAC CGG	0.6	3MMs	Chr3: +5590948	LOC_Os03g10880	exon	
GATG AGG AGGT CTC CGAGCAC CGG	0.6	3MMs	Chr5: -26749777	LOC_Os05g46120	exon	
GGCGC AGAGG AC CTCGAGCAC CGG	0.5	4MMs	Chr1: +40677374	LOC_Os01g70250	exon	
GATG AC GAGGT CTC CGAG CACGG	0.4	3MMs	Chr1: +21944968	LOC_Os01g39040	exon	
SG641 position: Chr3:-6089774		Guide sequence: GCAGTGGTCGCTGCACCTGA GGG				
Predicted off-target sites for SG641 site:						
Sequence	Score	MMs	Locus	Gene	Region	
GC GG AGGTCGCTGCAC CTCACGG	1	3MMs	Chr8: -20013729	LOC_Os08g32310	exon	
GCAG AGG AGCTGCAG CTGAAGG	0.2	4MMs	Chr2: -31996582	LOC_Os02g52250	exon	
GC TGG GT CG TGCT CTG TAT G	0.2	4MMs	Chr4: +11721509	LOC_Os04g20880	exon	
GA ACTGGTCGCTGCAG CTCAAGG	0.1	4MMs	Chr2: +32995893	LOC_Os02g53890	exon	
GC CGT GGT CCT GCAT CTG CGG	0.1	4MMs	Chr6: +597759	LOC_Os06g02054	exon	
GG AGTGGT CA CTGCAG CTGG CGG	0.1	4MMs	Chr1: +1869038	LOC_Os01g04230	exon	
GC G GTGGT CGC AG AGC CTG ACGG	0	4MMs	Chr5: -1541161	LOC_Os05g03610	exon	

Table S4. The seed-setting rate was reduced in *osprr676* RNAi lines 15 and 19.

Lines	No.of filled grains per plant	No.of total grains per plant	seed-setting rate ^a (%)
WT	801.9 ± 18.2	960.7 ± 12.4	83.5 ± 1.4
RNAi line15	358.1 ± 8.1	764.8 ± 13.2	46.9 ± 1.4 ^b
RNAi line19	674.1 ± 6.8	995.6 ± 7.5	67.7 ± 0.3 ^b

^aThe seed-setting rate was measured based on three replications, each replication has three plants.

^bSignificantly different from control at $p < 0.01$ by two-tailed *t*-test.

Table S5.**Total Soluble Fatty Acids of Wild-Type and Mutant Anthers.**

Acids	Wild-Type	<i>Osppr676-2</i>		<i>Osppr676-1</i>	
	Mean±SD (ug/mg Dry Weight)		up		up
C12 acid	0.394 ± 0.055	0.104 ± 0.057	-73.60%	0.090 ± 0.009	-77.16%
C14 acid	0.484 ± 0.171	0.103 ± 0.013	-78.72%	0.372 ± 0.127	-23.14%
C16 acid	7.183 ± 1.223	5.230 ± 0.403	-27.19%	3.564 ± 0.809	-50.38%
C18 (2/3)acid	7.534 ± 0.513	4.273 ± 0.562	-43.28%	3.530 ± 0.120	-53.15%
C18 (0/1)acid	3.190 ± 0.797	2.239 ± 0.065	-29.81%	1.515 ± 0.025	-52.51%
C20(1/2)acid	2.918 ± 0.546	0.715 ± 0.014	-75.50%	0.121 ± 0.049	-95.85%
C20 acid	0.250 ± 0.020	0.183 ± 0.003	-26.80%	0.135 ± 0.004	-46.00%
C22 acid	0.141 ± 0.065	0.069 ± 0.024	-51.06%	0.039 ± 0.006	-72.34%
C24 acid	0.129 ± 0.032	0.054 ± 0.011	-58.14%	0.041 ± 0.008	-68.22%
C26 acid	0.155 ± 0.033	0.086 ± 0.011	-44.52%	0.134 ± 0.053	-13.55%
Total acids	22.377 ± 2.769	13.057 ± 0.171	-41.65%	9.541 ± 0.670	-57.36%