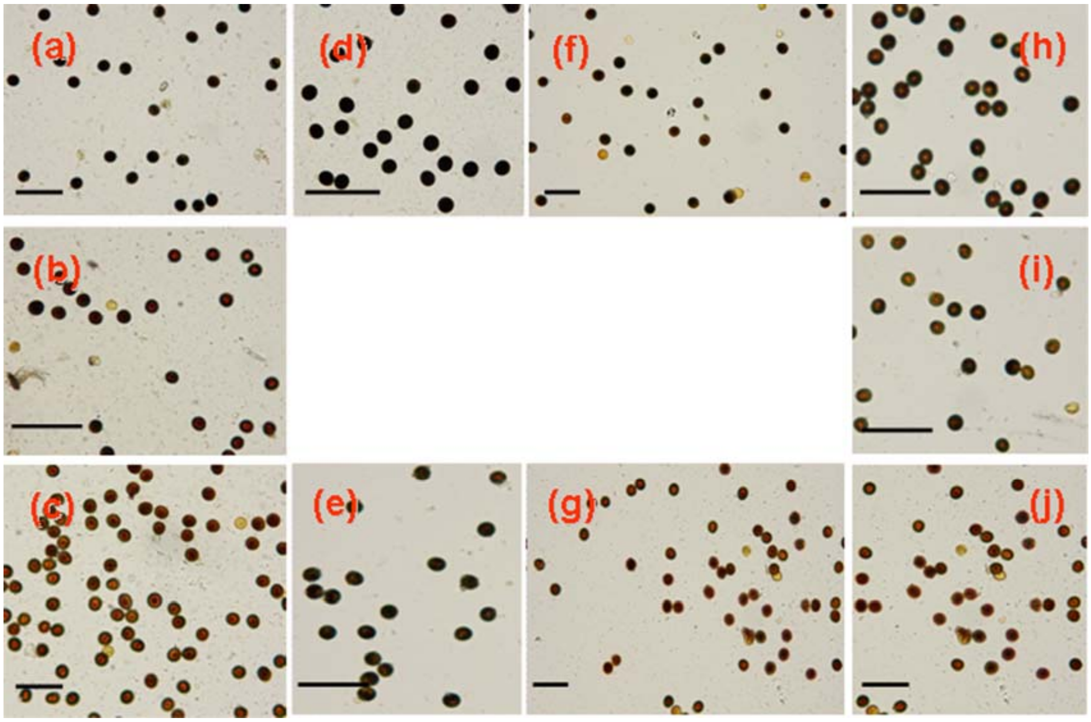


Figure S1 Pollen viability in sped1-D, SPED1-RNAi and control plants. Pollen viability was effected in sped1-D (a, 1 spikelet; b, 2 spikelets; c, 3 spikelets) and in TP309 plants containing *sped1-D-OX* plasmid (h, 1 spikelet; i, 2 spikelets; j, 3 spikelets) compared with that of the wild type (d, TP309 and e, 9311); SPED1-silenced plants had very low pollen viability (f, miR528-sped-1 and g, miR528-sped-2). The pollen had low viability and appeared light brown when assessed by staining the starch in the pollen with a 1% I-KI solution; the mature pollen grains from the control and sped1-D plants were stained dark brown.

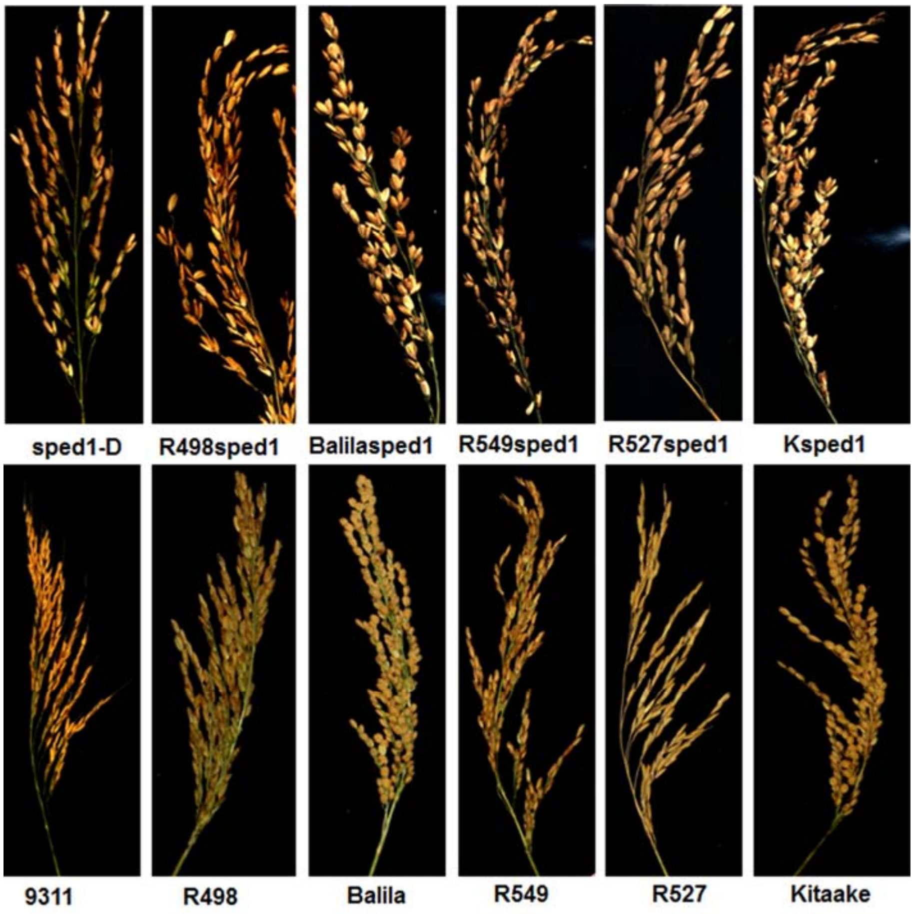


Figure S2 The phenotype of sped1-D isogenic mutants in different genetic backgrounds (Upside is the series isogenic sped1-D mutant, below is the corresponding wild type).

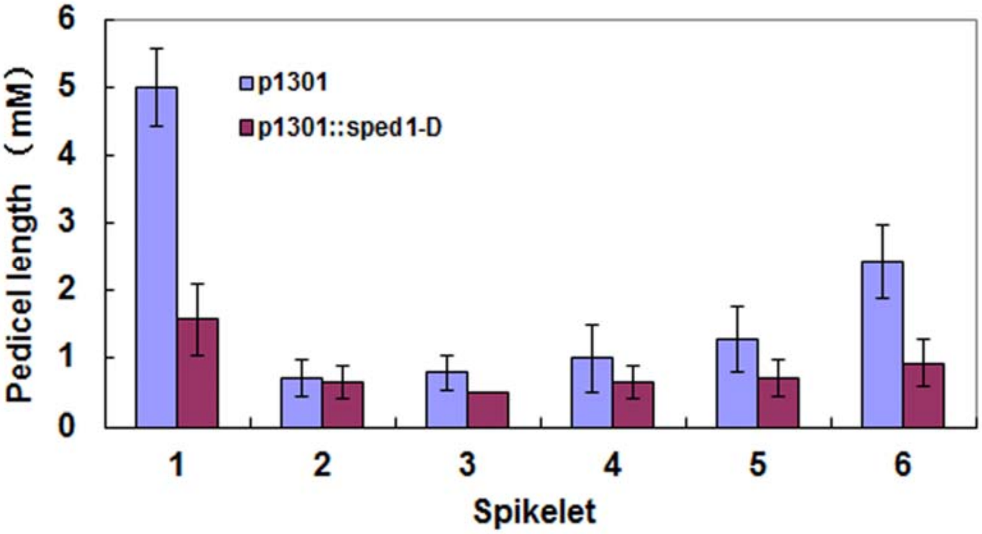
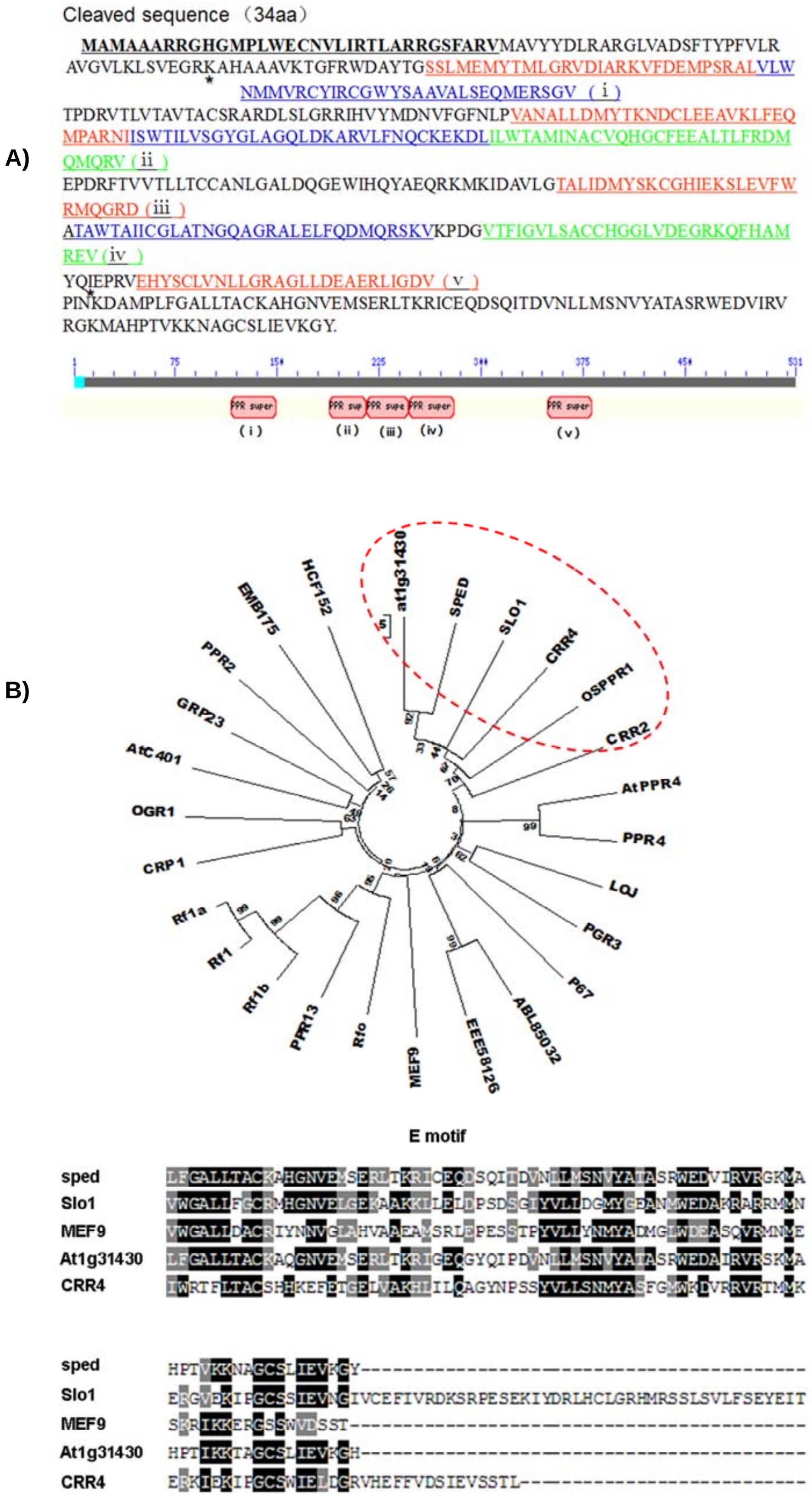


Figure S3 Statistical analysis of the lengths of six pedicels on secondary branches of a TP309 plant containing p1301-*sped1-D*. Error bars indicate standard error (SE) for seven replicate experiments.

Figure S4



Cleaved sequence （34aa）

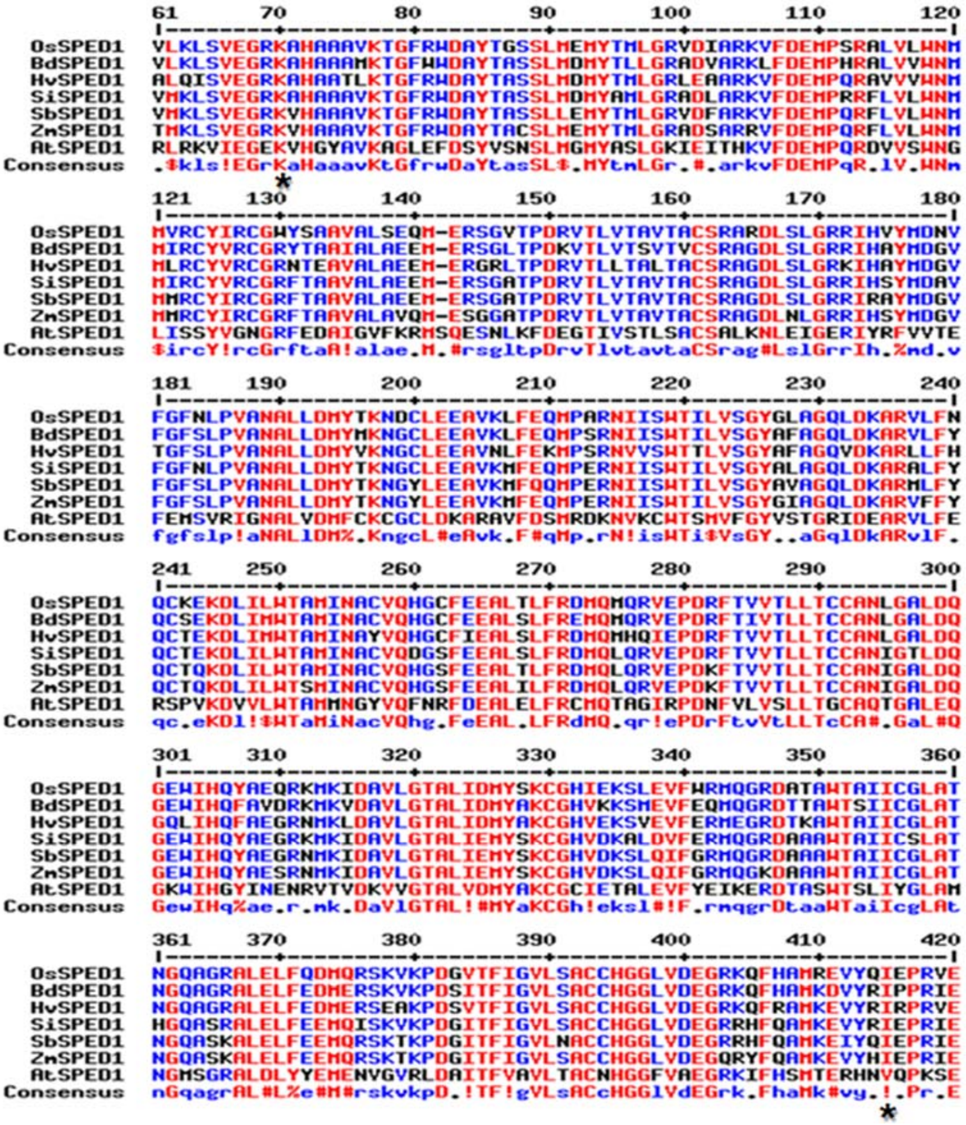
MAMAAARRGHGMPLWECNVLIRTLARRGSFARVMAVYYDLRARGLVADSFTYPFVLR
AVGVLKLSVEGRK*AHAAAVKTGFRWDAYTGSSLMEMYTMLGRVDIARKVFDEMPSRALVLW
NMMVRCYIRCGWYSAAVALSEQMERSGV （i）
TPDRVTLVTAVTACSRARDLSLGRRIHVYMDNVFGFNLPVANALLDMYTKNDCLEEAVKLFEQ
MPARNIISWTILVSGYGLAGQLDKARVLFNQCKEKDLILWTAMINACVQHGCFEEALTLFRDM
QMQRV (ii)
EPDRFTVVTLLTCCANLGALDQGEWIHQYAEQRKMKIDAVLGTALIDMYSKCGHIEKSLEVFW
RMQGRD (iii)
ATAWTAIICGLATNGQAGRALELFQDMQRSKVKPDGVTFIGVLSACCHGGLVDEGRKQFHAM
REV (iv)
YQIEPRVEHYSCLVNLLGRAGLLDEAERLIGDV (v)
PIN*KDAMPLFGALLTACKAHGNVEMSERLTKRICEQDSQITDVNLLMSNVYATASRWEDVIRV
RGKMAHPTVKKNAGCSLIEVKGY.

E motif

sped LFGALLTACKAHGNVEMSERLTKRICEQDSQITDVNLLMSNVYATASRWEDVIRVRGKMA
Slo1 VWGALLFGCRMHGNVELGEKAAKKILELDPSDSGIYVLLDGMYGEANMWEDAKRARRMMN
MEF9 VWGALLDACRIYNNVGLAHVAAEAMSRLEPESSTPYVLLYNMYADMGLWDEASQVRMNME
At1g31430 LFGALLTACKAQGNVEMSERLTKRIGEQGYQIPDVNLLMSNVYATASRWEDAIRVRSKMA
CRR4 IWRTFLTACSHHKEFETGELVAKHLIILQAGYNPSSYVLLSNMYASFGMWKDVRRVRTMMK

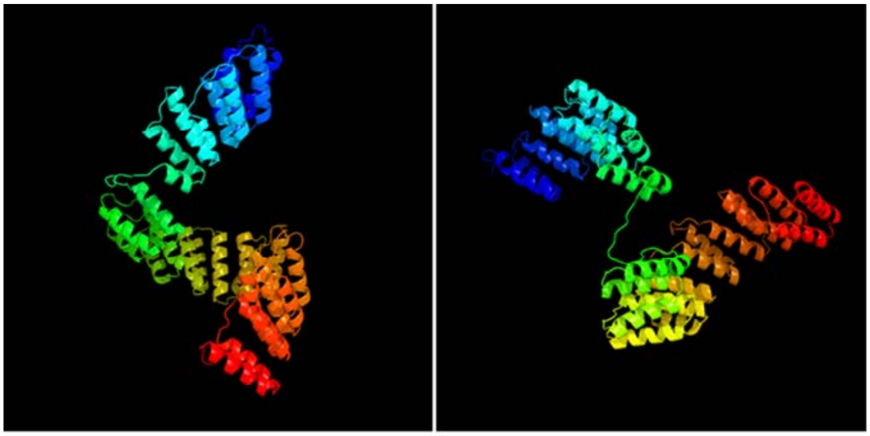
sped HPTVKKNAGCSLIEVKGY---
Slo1 ERGVEKIPGCSSIEVNGIVCEFIVRDKSRPESEKIYDRLHCLGRHMRSSLSVLFSEYEIT
MEF9 SKRIKKERGSSWVDSST--
At1g31430 HPTIKKTAGCSLIEVKGH---
CRR4 ERKIEKIPGCSWIELDGRVHEFFVDSIEVSSTL---------------------------

D)



```
          61       70        80        90        100       110       120
          |--------+---------+---------+---------+---------+---------|
OsSPED1   VLKLSVEGRKAHAAAVKTGFRWDAYTGSSLMEMYTMLGRVDIARKVFDEMPSRALVLWNM
BdSPED1   VLKLSVEGRKAHAAAMKTGFWWDAYTASSLMDMYTLLGRADVARKLFDEMPHRALVVWNM
HvSPED1   ALQISVEGRKAHAATLKTGFRWDAYTASSLMDMYTMLGRLEAARKVFDEMPQRAVVVWNM
SiSPED1   VMKLSVEGRKAHAAAVKTGFRWDAYTASSLMDMYAMLGRADLARKVFDEMPRRFLVLWNM
SbSPED1   VMKLSVEGRKVHAAAVKTGFRWDAYTASSLLEMYTMLGRVDFARKVFDEMPQRFLVLWNM
ZmSPED1   TMKLSVEGRKVHAAAVKTGFRWDAYTACSLMEMYTMLGRADSARRVFDEMPQRFLVLWNM
AtSPED1   RLRKVIEGEKVHGYAVKAGLEFDSYVSNSLMGMYASLGKIEITHKVFDEMPQRDVVSWNG
Consensus .$kls!EGrKaHaaavKtGfrwDaYtasSL$.MYtmLGr.#.arkvFDEMPqR.lV.WNm
                   *
          121      130       140       150       160       170       180
          |--------+---------+---------+---------+---------+---------|
OsSPED1   MVRCYIRCGWYSAAVALSEQM-ERSGVTPDRVTLVTAVTACSRARDLSLGRRIHVYMDNV
BdSPED1   MIRCYVRCGRYTAAIALAEEM-ERSGLTPDKVTLVTSVTVCSRAGDLSLGRRIHAYMDGV
HvSPED1   MLRCYVRCGRNTEAVALAEEM-ERGRLTPDRVTLLTALTACSRAGDLSLGRKIHAYMDGV
SiSPED1   MIRCYVRCGRFTAAVALAEEM-ERSGATPDRVTLVTAVTACSRAGDLSLGRRIHSYMDAV
SbSPED1   MMRCYIRCGRFTAAVALAEEM-ERSGATPDRVTLVTAVTACSRAGDLSLGRRIRAYMDGV
ZmSPED1   MMRCYIRCGRFTAAVALAVQM-ESGGATPDRVTLVTAVTACSRAGDLNLGRRIHSYMDGV
AtSPED1   LISSYVGNGRFEDAIGVFKRMSQESNLKFDEGTIVSTLSACSALKNLEIGERIYRFVVTE
Consensus $ircY!rcGrftaA!alae.M.#rsgltpDrvTlvtavtaCSrag#LslGrrIh.%md.v

          181      190       200       210       220       230       240
          |--------+---------+---------+---------+---------+---------|
OsSPED1   FGFNLPVANALLDMYTKNDCLEEAVKLFEQMPARNIISWTILVSGYGLAGQLDKARVLFN
BdSPED1   FGFSLPVANALLDMYMKNGCLEEAVKLFEQMPSRNIISWTILVSGYAFAGQLDKARVLFY
HvSPED1   TGFSLPVANALLDMYVKNGCLEEAVNLFEKMPSRNVVSWTTLVSGYAFAGQVDKARLLFH
SiSPED1   FGFNLPVANALLDMYTKNGCLEEAVKMFEQMPERNIISWTILVSGYALAGQLDKARALFY
SbSPED1   FGFSLPVANALLDMYTKNGYLEEAVKMFQQMPERNIISWTILVSGYAVAGQLDKARMLFY
ZmSPED1   FGFSLPVANALLDMYTKNGYLEEAVKMFEQMPERNIISWTILVSGYGIAGQLDKARVFFY
AtSPED1   FEMSVRIGNALVDMFCKCGCLDKARAVFDSMRDKNVKCWTSMVFGYVSTGRIDEARVLFE
Consensus fgfslp!aNALlDM%.KngcL#eAvk.F#qMp.rN!isWTi$VsGY..aGqlDkARvlF.

          241      250       260       270       280       290       300
          |--------+---------+---------+---------+---------+---------|
OsSPED1   QCKEKDLILWTAMINACVQHGCFEEALTLFRDMQMQRVEPDRFTVVTLLTCCANLGALDQ
BdSPED1   QCSEKDLIMWTAMINACVQHGCFEEALSLFREMQMQRVEPDRFTIVTLLTCCANLGALDQ
HvSPED1   QCTEKDLIMWTAMINAYVQHGCFIEALSLFRDMQMHQIEPDRFTVVTLLTCCANLGALDQ
SiSPED1   QCTEKDLILWTAMINACVQDGSFEEALSLFRDMQLQRVEPDRFTVVTLLTCCANIGTLDQ
SbSPED1   QCTQKDLILWTAMINACVQHGSFEEALTLFRDMQLQRVEPDKFTVVTLLTCCANIGALDQ
ZmSPED1   QCTQKDLILWTSMINACVQHGSFEEALILFRDMQLQRVEPDKFTVVTLLTCCANIGALDQ
AtSPED1   RSPVKDVVLWTAMMNGYVQFNRFDEALELFRCMQTAGIRPDNFVLVSLLTGCAQTGALEQ
Consensus qc.eKDl!$WTaMiNacVQhg.FeEAL.LFRdMQ.qr!ePDrFtvVtLLTcCA#.GaL#Q

          301      310       320       330       340       350       360
          |--------+---------+---------+---------+---------+---------|
OsSPED1   GEWIHQYAEQRKMKIDAVLGTALIDMYSKCGHIEKSLEVFWRMQGRDATAWTAIICGLAT
BdSPED1   GEWIHQFAVDRKMKVDAVLGTALIDMYAKCGHVKKSMEVFEQMQGRDTTAWTSIICGLAT
HvSPED1   GQLIHQFAEGRNMKLDAVLGTALIDMYAKCGHVEKSVEVFERMEGRDTKAWTAIICGLAT
SiSPED1   GEWIHQYAEGRKMKIDAVLGTALIEMYSKCGHVDKALDVFERMQGRDAAAWTAIICSLAT
SbSPED1   GEWIHQYAEGRNMKIDAVLGTALIEMYSKCGHVDKSLQIFGRMQGRDAAAWTAIICGLAT
ZmSPED1   GEWIHQYAESRNMKIDAVLGTALIEMYSKCGHVDKSLQIFGRMQGKDAAAWTAIICGLAT
AtSPED1   GKWIHGYINENRVTVDKVVGTALVDMYAKCGCIETALEVFYEIKERDTASWTSLIYGLAM
Consensus GewIHq%ae.r.mk.DaVlGTAL!#MYaKCGh!eksl#!F.rmqgrDtaaWTaiIcgLAt

          361      370       380       390       400       410       420
          |--------+---------+---------+---------+---------+---------|
OsSPED1   NGQAGRALELFQDMQRSKVKPDGVTFIGVLSACCHGGLVDEGRKQFHAMREVYQIEPRVE
BdSPED1   NGQAGRALELFEDMERSKVKPDSITFIGVLSACCHGGLVDEGRKQFHAMKDVYRIPPRIE
HvSPED1   NGQAGRALELFEDMERSEAKPDSVTFIGVLSACCHGGLVDEGRKQFRAMKEVYRIRPRVE
SiSPED1   HGQASRALELFEEMQISKVKPDGITFIGVLSACCHGGLVDEGRRHFQAMKEVYRIEPRIE
SbSPED1   NGQASKALELFEEMQRSKTKPDGITFIGVLSACCHGGLVDEGRRHFQAMKEIYQIEPRIE
ZmSPED1   NGQASKALELFEEMQRSKTKPDGITFIGVLSACCHGGLVDEGQRYFQAMKEVYHIEPRIE
AtSPED1   NGMSGRALDLYYEMENVGVRLDAITFVAVLTACNHGGFVAEGRKIFHSMTERHNVQPKSE
Consensus nGqagrAL#L%e#M#rskvkpD.!TF!gVLsACcHGGlVdEGrk.FhaMk#vy.!.Pr.E
                                                                *
```

E)



SPED1

sped1-D

Figure S4 Sequence and structure of SPED 1

A) SPED 1 is a PPR protein containing 12 PPR motifs. The positions of the two changed amino acid, K70T and 414L in sped1-D are indicated by asterisks.

B) Phylogenetic analysis showed that SPED or sped has the highest sequence similarity with at1g31430 and SLO1 in Arabidopsis. Sequences used in this analysis were as follows: At1g31430, MEF9 (At1g62260), SLO1 (At2G22410), CRR4 (At2g45350), CRR2-At3g46790, AtPPR4 (At5g04810), LOJ (At2g39230), PGR3 (At4g31850), P67 (At4g16390), AtC401 (At5g21222), GRP23 (At1g10270), EMB175 (At5g03800) and HCF152 (At3g09650) from Arabidopsis; sped (NP_001057989), OSPPR1 (AAS93059), Rf1a (ABC42330), Rf1b (ABC42331), EEE58126 and OGR1 (ACL79585) from rice; PPR4 (NP_001105869), CRP1(NP_001105879) and PPR2 (ACG30078) from maize; ABL85032 from *Brachypodium sylvaticum*; Rfo (ACJ70132) from *Brassica napus*; PPR13 (XP_002436844) from sorghum; and Rf1 (AAM52341) from petunia.

C) Sequence alignment of sped 1, Slo1, MEF9, At1g31430 and CRR4 also showed that the sped 1 contains a truncated E motif.

D)Protein sequence alignment of SPED1 homologs from Arabidopsis (AtSPED1, At1g31430), Setaria italic (SiSPED1, XP_004967201.1), Sorghum bicolor (SbSPED1, XP_002438633), Zea mays (ZmSPED1, AFW76438) and Brachypodium distachyon (BdSPED1, XP_003563682).The two changed amino acid,K70T and I414L in sped1-D are both located in a conservative domain. Their positions are indicated by asterisks.

E) Prediction of 3-D structures of SPED1 and sped1-D proteins by using phyre2.

A)

B)

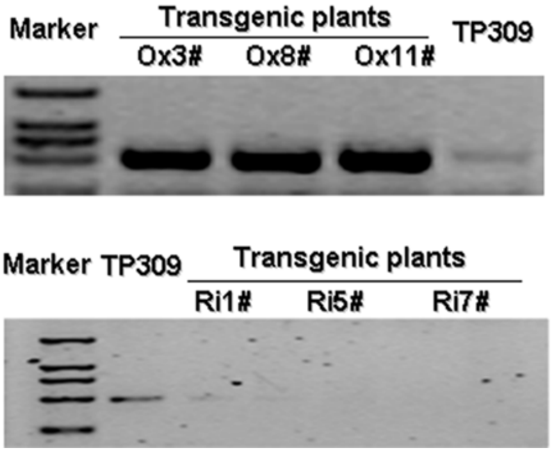


Figure S5 The phenotypes of TP309 plants containing SPED-OX or miR528-sped-2.

A) Two to three spikelets clustered in TP309 transgenic plant #8 containing *SPED-OX*; Statistical analysis of the spikelet sterility of TP309 transgenic plant containing *miR528-sped-2* showed that the number of fertile spikelets or grain yield per panicle was about reduced to 5% (The phenotype of Ri5# is shown).

B) RT-PCR analysis showed that the expression levels of *SPED1* are much higher in plants with *SPED-OX*, while they are lower in plants with *miR528-sped-2* than that in TP309

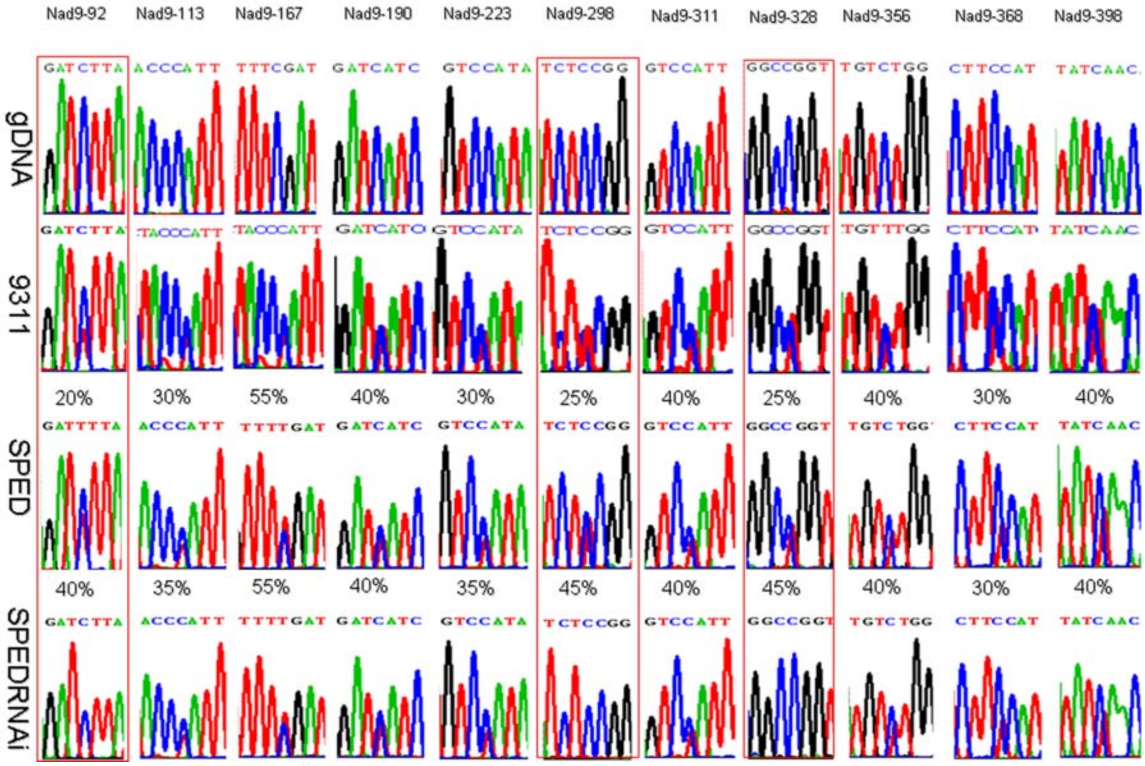


Figure S6 SPED is involved in the editing of Nad9 gene. RT-PCR products containing the 11 partial editing sites of Nad9 gene were directly sequenced. Besides the editing efficiency of C92, C298 and C328 are 50% increase in average in SPED plant, that of all the 11 sites are increased to 70-80% in sped-RNAi plant. Asterisks indicate the editing sites.

Table S1 Nine plant hormones that were examined to determine the effects of hormones on the elongation of sped1-D's pedicels and peduncles.

Phytohormone	Concentration	Panicle type
IAA	20mg/L and150mg/L	Clustered panicle
ZT	20mg/L and150mg/L	Clustered panicle
ABA	20mg/L and150mg/L	Clustered panicle
6-BA	20mg/L and150mg/L	Clustered panicle
NAA	20mg/L and150mg/L	Clustered panicle
Epibrassinolide	0．01mg／L	Clustered panicle
Cytokinin	0.001g/L	Clustered panicle
2，4-D	40ppm,80ppm, 120ppm, 160ppm	Clustered panicle
GA3	50mg/L,100mg/L,150mg/L	Clustered panicle

Table S2 The incompletely and completely dominant inheritance of sped1-D in different background.

Cross combination	Number of F1 plant (Mutant type: wild type)	Number of F2 plant (Mutant type: wild type)	Dominant or recessive
9311×sped1-D	6:0	252:88	incomplete dominant
R498×sped1-D	10:0	137:43	incomplete dominant
Balila×sped1-D	7:0	110:39	incomplete dominant
R549×sped1-D	9:0	66:21	complete dominant
R527×sped1-D	9:0	178:53	incomplete dominant
TP309×sped1-D	11:0	112:42	complete dominant
Kitaake×sped1-D	6:0	100:30	complete dominant

Table S3 Molecular markers used for map-based cloning of *sped1-D* gene.

Molecular Marker	Forward primer（F）	Reverse primer （R）
RM5957	ACTGCTGCACTGCACAAGAC	AGCTAGCTAGGCGTGAGCTG
RM3287	GGACGGATTGTAGGTAGGAC	CCTTTCTTCAATCTGCATTC
RM275	GCATTGATGTGCCAATCG	CATTGCAACATCTTCAACATCC
RM20384	TCAGATCACTGTGCTCCAACTCC	TCTATCTATCATGCCGACCTTGC
RM20297	TTGGCACGGCCATATAACAAGC	AAGTTGATGGCCTTTGGTTTGC
RM20311	ATAATTCATCCGGCCACCAACACC	CTCCGGGACAAGGTTGCTGAGG
RM20315	CGTCCTCCAGGAAACCCTGTAAGC	CAGAAACTCGCCGAAGCAGAGC
RM1340	TCCAAACTAGTGGGAACGC	CTCAACGCCATGAACCTC
RM20303	ACCTCCGCGTCGTAGAAGTAGC	CAAACCCAAACCCAAGGAGAGG
CSP1	AGGCTTCTTGGAATGGAACTGC	GGGAATATACGTGGATGTGAGAGG
CSP4	AGCGTCCTTCTCCATCAT	TTTGTCACTTGTCCCGTA
CSP7	TGTGACAGCATAGGAGTG	AGGAGTAGCTTTTGGTTC
CSP8	ACGCCTCTCCTTCACGCT	GTATTCCCCATCTCCAGTACG
CSP25	TGCCAGTGCCAGCCATCAAAC	GCCAAGCCGGTGCGACGAG
CSP30	CAATTCGCGGCCCTATGAT	GGCGGCCGAGCTGGTGGAC
CSP320B	CAATGAGGGAGGTTTATCAGC	CCAAACAGTGGCATAGCATCCTTT

Table S4 Flowering-related genes used for RT-PCR analysis.

Gene	Forward primer	Reverse primer	Annealing temperature (℃)	PCR fragment (bp)
RFL	CAGAGGGAGCATCCTTTCGTG	CGCATCTTGGGGCTTGTTGA	62	227
FCP1	GTCTCATGGGATTCTGGGT	GTACACGATTGAGGCGCGG	62	626
OMADS3	CCTGTTGCTGCTTCTGCC	CGGTTCGTCGTGTTCTCG	62	264
OsWUS	CCGCATCGAGGGCAAGAA	GCCGACTGGGAAGAGTGGAA	62	397
WOX3	TCGACCACGCTAATTCCCTTCT	CATGCTGCTCTTCCTTGAGGC	62	438
OSH1	AAGGTAAACAACAAGGCACA	GCTCAAGACACGCAGGATA	62	201
Fon4	TGTTTGGTGGTTGCATGGTGTT	TGCTTCGTCTTCGGCTCTGTC	52	146
Fon1	GGATCAGGCACCGGAACA	AGCCGTAGGAGCCAGCAAT	60	348
Fon2	GTTAGCCGAAGACGAAGC	TCCACTATGCAGGAGCAG	58	382
OsMADS6	CACCAGCAACTACAGAGCC	CCACGCAAGACCATTAGG	55	341
OsPck1	GTTACTGAAGAATAAAAAAT	GATCAAATCAAGATGCTTTC	43	253
OsMe1	CGCCGCGGAGGTCAAATTTT	ATGCTGCCCATTACAATGGG	55	265
OsRAA1	ATGTCAGGGGTTTGGGTGTTC	GGCGTCGACGACGCGGAAGGA	52	340
LAX1	TTCCTCAAGGCGCAGGTCA	ATCTCCAGCGTCGTCATCCC	55	176
SP1	CGGTAACCAAGAGGAAACAAGTG	CACCACGCACAGTAGCACCTT	56	246
APO1	CCGCCGGCCCGACCTCCATCATC	CTCCACCAGCGCCGGCGACCTCAG	62	317
AK070205	GCCACTGCGCCAACCTGCTCTC	TGTCCGGATTGCTTGCCTTGATGC	56	402
AK106784	TCGTCGTCGGCTGTCTTCCCACTG	GCGCTCGCGGCCTGCTCCATC	62	329
AK120659	ACCAACGGCCGGCCCCTCTCG	CGCCCCTTCCCCCGCATCTCG	62	404
SPEDJ	GAAGCAATTCCATGCAATGAGG	GGTCCAGTCAAACTAATGG	60	450
HYG	GACGGTGTCGTCCATCACAGTTT	ACTCACCGCGACGTCTGTCGAGAA	56	495
Actin	AGCAACTGGGATGATATGGA	CAGGGCGATGTAGGAAAGC	56	450

Table S5 Rice materials used in this study

Rice variety and material	Rice type	Inflorescence phenotype	Genotypes	Source or reference
9311	*Indica*	Normal	Sped1	Restorer line (Yu *et al.* 2002)
TP309	*Japonica*	Normal	Sped1	Taipei 309 (Song *et al.* 1995)
R948	*Indica*	Normal	Sped1	Restorer line, from Sichuan Agricultural University, China
R549	*Indica*	Normal	Sped1	Restorer line, from Sichuan Agricultural University, China
Ballila	*Japonica*	Normal	Sped1	Introduced from Itali (Ji *et al.* 2012)
R527	*Indica*	Normal	Sped1	Restorer line, from Sichuan Agricultural University, China
Kitaake	*Japonica*	Normal	Sped1	Introduced from USA (Chen *et al.* 2014)
sped1-D	*Indica*	clustered spikelets	sped1-D	The rice spontaneous mutant of 9311(This study)
Tsped1	*Japonica*	clustered spikelets	sped1-D	BC3 plant from the backcross of TP309 with sped1-D.This study (This study)
R948sped1	*Indica*	clustered spikelets	sped1-D	BC3 plant from the backcross of R948 with sped1-D.This study (This study)
R549sped1	*Indica*	clustered spikelets	sped1-D	BC3 plant from the backcross of R549 with sped1-D.This study (This study)
Ballilasped1	*Japonica*	clustered spikelets	sped1-D	BC3 plant from the backcross of Ballila with sped1-D.This study (This study)
R527sped1	*Indica*	clustered spikelets	sped1-D	BC3 plant from the backcross of R527 with sped1-D.This study (This study)
Ksped1	*Japonica*	clustered spikelets	sped1-D	BC3 plant from the backcross of Kitaake with sped1-D (This study)

Files S1-S4

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.163931/-/DC1

File S1 672 genes that were up-regulated in sped1-D plant.

File S2 574 genes that were down regulated in sped1-D plant.

File S3 Secondary structure and disorder prediction of SPED1.

File S4 Secondary structure and disorder prediction of sped1-D.

References

CHEN, X., S. ZUO, B. SCHWESSINGER, M. CHERN, P. E. CANLAS *et al.*, 2014 An XA21-Associated Kinase (OsSERK2) Regulates Immunity Mediated by the XA21 and XA3 Immune Receptors. Mol Plant **7:** 874-892.

JI, Q., M. ZHANG, J. LU, H. WANG, B. LIN *et al.*, 2012 Molecular basis underlying the S5-dependent reproductive isolation and compatibility of indica/japonica rice hybrids. Plant Physiol **158:** 1319-1328.

SONG, W. Y., G. L. WANG, L. L. CHEN, H. S. KIM, L. Y. PI *et al.*, 1995 A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science **270:** 1804-1806.

YU, J., S. HU, J. WANG, G. K. WONG, S. LI *et al.*, 2002 A draft sequence of the rice genome (Oryza sativa L. ssp. indica). Science **296:** 79-92.