

Supplementary

Arabidopsis thaliana 1 -----MEALRYIYWGSLGSLIIVYNSLLEDAALPTTIMOT-----TRSLN
Oryza sativa 1 -----MPPPLPCL-----APALAVLVV-VCAEPADRAALLPLACLR-----GRINW
Brachypodium distachyon 1 -----MPPPLPCL-----AALLL-CAT-----GAPEDPADRAALLPLAVGVGGR-GRINW
Triticum turgidum 1 -----MPPPLPCL-----AALLL-LSAT-----GAPEDPADRAALLPLAVGVGGR-----GRINW
Hordeum vulgare 1 -----MPPPLPCL-----AALLL-SPM-----GAPEDPADRAALLPLAVGVGGR-GRINW
Zea mays 1 MPTPLPSAGLLRLRICPLCLISAPR-----GAPEDPADRAALLPLAVGVGGRATARINW
Sorghum bicolor 1 MPTPLPSAGLLRLRICPLCLISAPR-----GAPEDPADRAALLPLAVGVGGRATARINW
Panicum miliaceum 1 -----MPPPLPCL-----AALLL-LSCLR-----GAPEDPADRAALLPLAVGVGGRARINW
Setaria italica 1 -----MPPPLPCL-----AALLL-LSCLR-----GAPEDPADRAALLPLAVGVGGRARINW
Arabidopsis thaliana 49 NPLSQ-----VCNNTGVTCNQGDSRLVRLPGLVGLSGIERNITSLRLATRVLSLR
Oryza sativa 50 ASS-PR-----VCNNTGVTCNQGDSRLVRLPGLVGLSGIERNITSLRLATRVLSLR
Brachypodium distachyon 49 NPLSQ-----VCNNTGVTCNQGDSRLVRLPGLVGLSGIERNITSLRLATRVLSLR
Triticum turgidum 49 AAA-RP-----VCNNTGVTCNQGDSRLVRLPGLVGLSGIERNITSLRLATRVLSLR
Hordeum vulgare 48 SA-RP-----VCNNTGVTCNQGDSRLVRLPGLVGLSGIERNITSLRLATRVLSLR
Zea mays 59 STHLACSDAGDGG-FWGTGVTCSDAGRVALLPGLVGLSGVPTGLRLATRLVLSLR
Sorghum bicolor 59 PTLACFSSASGAGWTGVTCSDAGRVALLPGLVGLSGVPTGLRLATRLVLSLR
Panicum miliaceum 57 PAAPLACA-----ABGWTGVTCSDAGRVALLPGLVGLSGVPTGLRLATRLVLSLR
Setaria italica 56 PTLACACA-----G-FWGTGVTCSDAGRVALLPGLVGLSGVPTGLRLATRLVLSLR
Arabidopsis thaliana 102 SNLNLGKLVKPVLEKDKLAFLVGLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Oryza sativa 103 ANSLGAGFEBELDLASLALHLLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Brachypodium distachyon 102 ANSLGAGFEBELDLASLALHLLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Triticum turgidum 99 ANSLGAGFEBELDLASLALHLLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Hordeum vulgare 101 ANSLGAGFEBELDLASLALHLLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Zea mays 118 SNLNLGKLVKPVLEKDKLAFLVGLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Sorghum bicolor 119 SNLNLGKLVKPVLEKDKLAFLVGLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Panicum miliaceum 113 ANSLGGLPADLLPLALHLLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Setaria italica 111 ANSLGGLPADLLPLALHLLHLPNAPSGALPLARALLOVLDSFNNGTGLPGL
Arabidopsis thaliana 162 SNLNLKRTGSLNLSLSCPLDLVLSLSCMLNLSNPLASH-PRRSTPFSSVYLD
Oryza sativa 163 SNLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Brachypodium distachyon 162 SNLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Triticum turgidum 159 SNLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Hordeum vulgare 161 SNLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Zea mays 178 ANLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Sorghum bicolor 179 ANLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Panicum miliaceum 173 SNLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Setaria italica 171 SNLNLKVAALNNLSNLSGRVPDLGLPALQFLNLSNNHLDGGEVPSLIRFADAFAGNN
Arabidopsis thaliana 222 IITPFGNNYT-----LVTPFSEBOTHQKPSKAFGLGSEVITLVITAVSVITITALE-VL
Oryza sativa 221 VTPRASASA-----GTFPSGSGAAGAPAKERVLSEAILAIYVGGCVLFAVAVL
Brachypodium distachyon 220 MTPFASASA-----EAPFSGGAPAGAKERVLSEAILAIYVGGCVLFAVAVL
Triticum turgidum 219 MTPFASASA-----EAPFSGGAPAGAKERVLSEAILAIYVGGCVLFAVAVL
Hordeum vulgare 219 MTPFASASA-----EAPFSGGAPAGAKERVLSEAILAIYVGGCVLFAVAVL
Zea mays 236 LTRPAPAQAPVVPVVAPEELAA-----FPARPEELSEAILAIYVGGCVLFAVAVL
Sorghum bicolor 237 LTRPAPAQAPVVPVVAPEELAA-----FPARPEELSEAILAIYVGGCVLFAVAVL
Panicum miliaceum 231 VTRPAPAPVSPAQTPS-EPHAPAGAPARVLSEAILAIYVGGCVLFAVAVL
Setaria italica 229 VTRPAPAPVSPAQTPS-EPHAPAGAPARVLSEAILAIYVGGCVLFAVAVL
Arabidopsis thaliana 278 TVYVRRKL-----RRPGVILDNKLKGGKSPK-FIRMEDVNVNIFFEFGNYSFD
Oryza sativa 277 ACFNRSGGGD-----EEVSVRVSGSKKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Brachypodium distachyon 276 ACFNRSGD-----EEVSVRVSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Triticum turgidum 273 ACFNRSGD-----EESSRAGSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Hordeum vulgare 274 ACFNRSGD-----EESSRAGSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Zea mays 293 ACFNRSGD-----EESSRAGSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Sorghum bicolor 297 ACFNRSGD-----EESSRAGSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Panicum miliaceum 294 ACFNRSGD-----EESSRAGSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Setaria italica 284 ACFNRSGD-----EESSRAGSGKGGKRGRESPEKAVIGKAGDGNRIVFEFGSLAFDL
Arabidopsis thaliana 333 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Oryza sativa 331 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Brachypodium distachyon 331 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Triticum turgidum 328 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Hordeum vulgare 331 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Zea mays 353 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Sorghum bicolor 357 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Panicum miliaceum 345 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Setaria italica 340 EDLLRASAELVGKAGFTAYRAVEDATTVVVRKLEVNAGRDFEQOMELGRIHRIDNV
Arabidopsis thaliana 393 VELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Oryza sativa 395 AELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Brachypodium distachyon 391 AELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Triticum turgidum 391 AELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Hordeum vulgare 391 AELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Zea mays 413 VELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Sorghum bicolor 417 VELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Panicum miliaceum 405 VELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Setaria italica 400 VELRAYYSKDEKLLVYDYSRGVSNNMLHGKRGEDTPTLFDWETRKIALGAARGIAHTH
Arabidopsis thaliana 453 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Oryza sativa 455 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Brachypodium distachyon 451 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Triticum turgidum 448 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Hordeum vulgare 451 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Zea mays 473 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Sorghum bicolor 477 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Panicum miliaceum 465 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Setaria italica 460 TENNGKFVHGNIKASNVFLNNOCGGICSDGLASLMPNITA-RSRLSGYCAPEVTRKKA
Arabidopsis thaliana 513 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Oryza sativa 514 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Brachypodium distachyon 510 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Triticum turgidum 507 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Hordeum vulgare 510 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Zea mays 532 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Sorghum bicolor 536 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Panicum miliaceum 524 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Setaria italica 519 SCSSDVYSGFVLELLTGKSPVQITGG-NEVVHLVRWQSVVREWTAAYFVDELLRY
Arabidopsis thaliana 571 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSDT-----STIPSTEA
Oryza sativa 573 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSDT-----STIPSTEA
Brachypodium distachyon 569 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSDT-----STIPSTEA
Triticum turgidum 566 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSDT-----STIPSTEA
Hordeum vulgare 569 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSDT-----STIPSTEA
Zea mays 592 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSGGTATGT-----STIPSTEA
Sorghum bicolor 595 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSDT-----STIPSTEA
Panicum miliaceum 583 PNIEEEMVELQIAMCVSRTPERRRKAADVITIEVRRSD

Figure S1. Amino acid sequence alignment of RLK protein. The amino acid sequences for OsSRLK proteins of other plant species homologous to rice SRLK were obtained from NCBI through BLAST analysis. Sequence alignment was performed using ClustalW with default parameters. The sequences are as follows: *Arabidopsis thaliana*, NM_118505.5; *Oryza sativa*, LOC_Os01g12390.1; *Brachypodium distachyon*, XP_003565740; *Triticum turgidum*, VAH59131; *Hordeum vulgare*, BAJ86409; *Zea mays*, NP_001168844; *Sorghum bicolor*, XP_021312403; *Panicum miliaceum*, RLN23550; *Setaria italic*, XP_004967473. Red and blue bars represent the leucine rich repeats and catalytic domain of serine/threonine kinases, respectively.

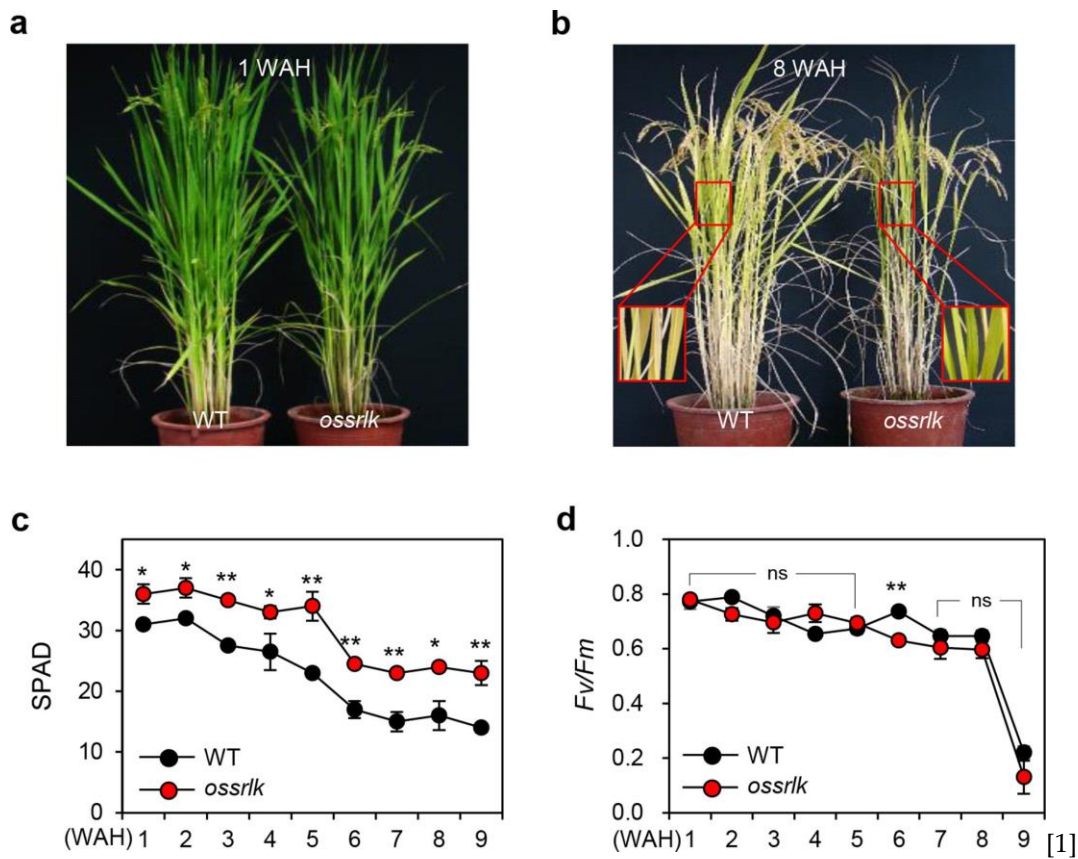


Figure S2. The senescence phenotype of *ossrlk* under natural senescence conditions in the field. The plants were photographed 1 week after heading (WAH) (a) and 8 WAH (b). SPAD value (c) and F_v/F_m (efficiency of photosystem II) (d) were measured in WT and *ossrlk* every 1 week from 1 WAH to 9 WAH. Mean and standard deviation values were obtained from 10 measurements. Asterisks indicate a significant difference between WT and *ossrlk* mutant (Student's *t*-test, * $P < 0.05$, ** $P < 0.01$). n.s., not significant.

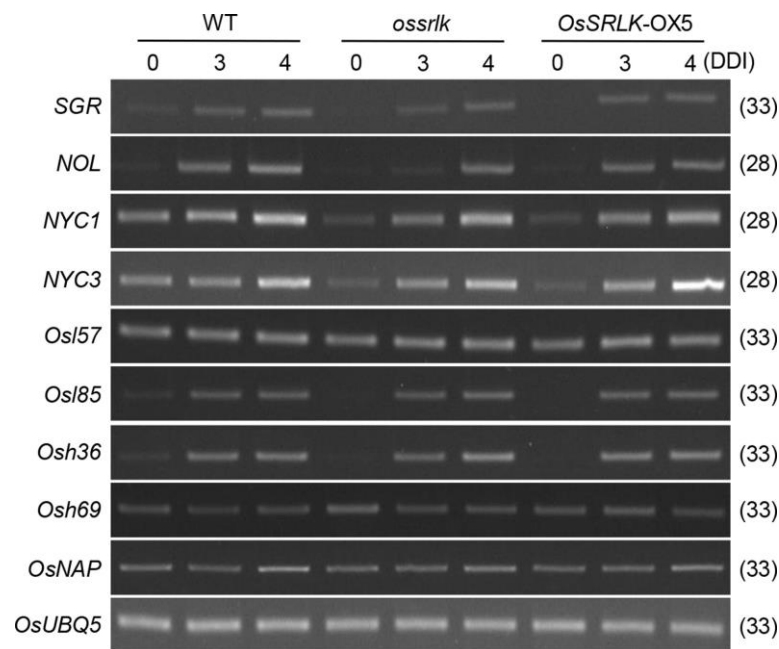


Figure S3. Expression of CDGs and SAGs in *ossrlk* and *OsSRLK-OX5* during DIS. Total RNA was isolated from the detached leaves of WT, *ossrlk*, and *OsSRLK-OX5* plants under DIS, as presented in Figure 2 (2 c,h). Transcripts of CDGs (*SGR*, *NOL*, *NYC1*, and *NYC3*) and SAGs (*Osl57*, *Osl85*, *Osh36*, *Osh69*, and *OsNAP*) were determined by reverse-transcriptase PCR analysis. *OsUBQ5* was used as a loading control. Numbers in parentheses indicate the numbers of PCR cycles. DDI, day(s) of dark incubation.

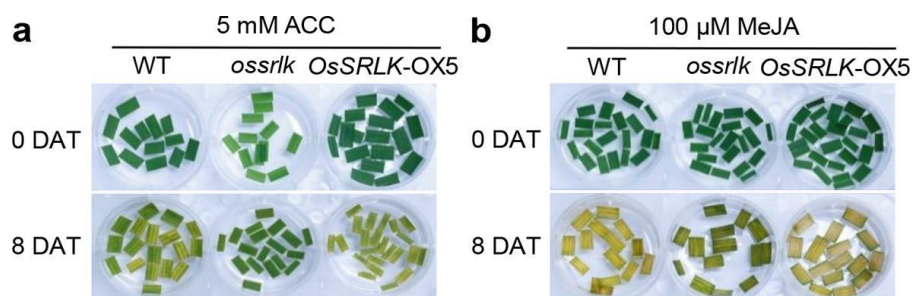


Figure S4. Phytohormone hypersensitivity of *ossrlk*. Detached leaves of 3-week-old WT, *ossrlk*, and *OsSRLK-OX5* plants were treated with 3 mM MES buffer (pH 5.8) containing 5 mM ACC (1-aminocyclopropane-1-carboxylic acid) (a) and 100 μ M MeJA (b) under continuous light conditions at 28 $^{\circ}$ C. DAT, day(s) after treatment.

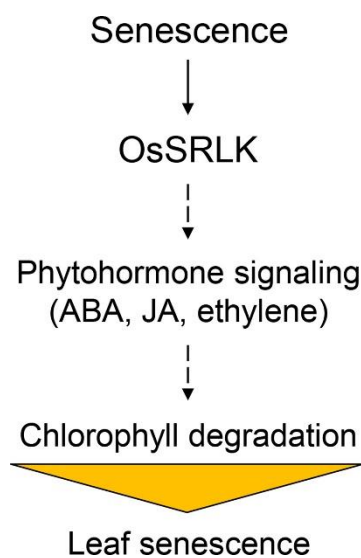


Figure S5. Proposed model for the role of OsSRLK in leaf senescence. Solid and dashed arrows represent direct and indirect activation, respectively.

Supplemental Table S1. Primers used in this study

A. Primers for verification of T-DNA insertion		
Primer name	Left primers (5' → 3')	Right primers (5' → 3')
PFG_1A-15835	TCTCCCTCATCAAAACGTCC	AGTGAGAGGAGCTCCTCCG
pGA2717	ACGCTGAACTTGTGGCCGTT	AACGCTGATCAATTCCACAG
B. Primers for RT-qPCR		
Genes	Forward primers (5' → 3')	Reverse primers (5' → 3')
<i>OsSRLK</i>	GACGTCGAGCTGATGAGGTA	CACCACGTCCGACATCTTAG
<i>OsUBQ5</i>	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
C. Primers for RT-PCR		
Genes	Forward primers (5' → 3')	Reverse primers (5' → 3')
<i>OsSRLK</i>	TTGTTGGACAGATTCAAGAA	ATAGAGACTACTGTGAAACA
<i>SGR</i>	AGGGGTGGTACAACAAGCTG	GCTCCTTGCGGAAGATGTAG
<i>NOL</i>	GAAAGGGTAGAATCTGCGGTG	CTGCAGAGATTTTGTAAGGTG
<i>NYC1</i>	GAATCCGTAATTGGGCTGAA	CTGGAAGAGGTCCACCTGAG
<i>NYC3</i>	TGCTGCATCCTGTCCACACCTTG	GATGCAAATGATGCAGCAGCTGC
<i>OsI57</i>	ACCCTAAAGTAAATGAAGTC	CCTGCTCTTGTCTTGTTA
<i>OsI85</i>	CGTCACGGACACGTTCGC	GCAAGAACATGGCTGCTGC
<i>Osh36</i>	GTTGAGGCGATGGTCAACC	CAGTGTAAGCCGGGCAATC
<i>Osh69</i>	CCTGCTCTTGTCTTGTTA	GGTGAACACTATGGAACA
<i>OsNAP</i>	AACCATTTCATCGCGAACAAC	CAGTGACGATCCCTGCAAGG
<i>OsUBQ5</i>	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
D. Primers for plant transformation		
Primer name	Forward primer (5' → 3') ^a	Reverse primer (5' → 3') ^a
<i>OsSRLK_HindIII</i> and <i>HpaI</i>	<u>AAGCTT</u> ATGCCGCCGCGAGGCTG	<u>GTAACT</u> CAGAGGAGCTCCTCCG

^aThe underlined nucleotides represent the restriction site for restriction enzymes.