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Supplemental information

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The Xa7 Resistance Gene Guards the Susceptibility Gene SWEET14 of Rice Against Exploitation by Bacterial Blight Pathogen

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Files:

Supplemental Table 1. Molecular markers used in this study
Supplemental Table 2. Oligonucleotides used in this study
Supplemental Information 1. The cDNA sequence of *Xa7*Supplemental Information 2. gBlocks of *Xa7* homologous genes
Supplemental File 1. Program for genome assembly
Supplemental Figure 1. Large chromosomal fragment deletion of *Xa7* locus
Supplemental Figure 2. Screenshot of RAMPAGE analysis displayed with the Integrative Genomics Viewer (Thorvaldsdóttir, Robinson et al. 2013)
Supplemental Figure 3. *Xa7* and homologs in diverse grass species

Marker	Primer	Size in IRBB7 (bp)	Size in Nipponbare (bp)
RM7243	RM7243F RM7243R	150	158
RM20571 RM20571F		123	117
RM5509	RM5509F RM5509P	229	255
5610	5610F	261	231
M5	M5-F M5-R	343	1219
M5-3k	M5-3kF M5-3kR	143	122
M5-5k	M5-5kF M5-5kR	107	104
M5-48k	M5-48kF M5-48kR	152	144
M5-72k	M5-72kF M5-72kR	127	136
RM20593	RM20593F RM20593R	314	316
RM3723	RM3723F RM3723R	130	138

Supplemental Table 1. Molecular markers used in this study.

Sequences of each markers in IRBB7 and Nipponbare

RM7243

IRBB7

AAGATGGCGTGCGTACGTACGTACGTGCGGCGCGGGGCGTACAGGCGTACAGGGCGC CGCGCGACGCATGGATGGATGGATCGATGGATCGATGGTCCCGGGCGGCACAGGCA GGGGCTCCCCCGACCGGGCAACCCGTGAAGAACTTCGT

Nipponbare

AAGATGGCGTGCGTACGTACGTACGTGCGGCGCGGGCGTACAGGCGTACAGGGCGC CGCGCGACGCATGGATGGATGGATGGATGGATCGATGGATCGATGGTCCCGGGCGG CACAGGCAGGGGCTCCCCCGACCGGGCAACCCGTGAAGAACTTCGT

RM20571

IRBB7

Nipponbare

RM5509

IRBB7

Nipponbare

5610

IRBB7

Nipponbare

M5

IRBB7

Nipponbare

CGATCTTACTGGCTCTGCAACTCTGTATTGCATGCTAAATCCGTTATGATTTACTACT GGTCGGAAGGTGAGAAAGAGGAGGAGAAAGAAGAGAGAATCTATTATTATTAAAG GAATAGAAAAAGAAGCCTCCACGTTCGCTCTCACGGCCTAGAAATTCTCACATTAAT CGGAGAAAAGAAAAGCAGAGTCCATATAGAAATACAATTTAGAAATAGCTGAAAT TCGGAATTATAAAATAAGGAATATTAGAAGAGGAGACTAGAGTCCATATGGAAATA CAATTTAGAAATAGTTGAAATTCAGAATTAAAAAAATAAGAAATATTAGAAGAGGAG ACTAGAGTCCATATAGAAATATAATTAGGAAATAACTGAAATTCGGAATTAAAAAT AAGGAATATTAGAAGTAGAGTATAGAGTCCATATAAAAATATAATTAGGAAATAAC TGAAATTAGGAATTAATAATAAGGAATATTAGAGATAGAGTATAGAGTCCATATAA AAATACAATTAGTAAATAACTGTAATTCGGAATTAAAAATAAGGAATATTAGAGGT AGAGTATAGAGTCCATATAGAAATACAATTAGAAAATAACTGTAATTCGGAATTAA AAATAAGGAATATTAGAGGTAGAGTATAGAGTCCATATAAAAATACAATTAGGAAA TAACTGAAATTCGGAATTAAAAAATAAGGAATATTAGAGGTAGAGTATAGAGTCCAT ATAGAAATACAATTAGAAAATAATAAAAAATTCGGAATTAAAAAATTTGATATTAAAA ATAATTAATAACTAACACGTATATAATAATACAATATAAATATTACACATTAGTAGTT TTACAAAATTTAAAAATTATATTGTCATTTTAATAAATTTGAATAATACATTGAGAAA ACATATATGCTATTACATGAGAGAGAAAATATAATGATGCTAGCCGCGCAATATGCAC GGGCCACTATGCTAGTTGAAGATGATTAGAAGAAATCTAATCAATTTCAGAGCATTA CTGTATTCATCTTTCAGTGTATATTCGTCTGGGTAGTGCTGATTGTGCCATTATAATT TACTTAGAATTAGTAGCAAAGTCGGTGGGACAAC

M5-3k

IRBB7

Nipponbare

CATATGTAGCAAGTATGCATCCAGCGAAACCCCTAGATCTCATCATAACAGAGAGT ACGTACGTAGTAGTACTGTAGTACACTTGGATATATACAAATTAAGCCAACACATTA GTCTTCAGA

M5-5k

IRBB7 TGTAGAGATTGTGACGAGGAGCAGTGCAAGTTTCAGGTAGTGATGCATCAGGCGTTT GCCCTCTTTCCTTGCAATGCTACTCGCTGATGATCCATTCTGCAACATCA

Nipponbare

TGTAGAGATTGTGACGAGGAGCAATGCAAATTTCAGGTAGTGATGCGTCAGGCGCT TGCCCTCTTTCCTTGCAGTGCTACTCGCTGATCCATTCTGCAACATCA

M5-48k

IRBB7

CTGTGTGAAAGTTCAGACGGATGGCGATTTCGCAAAGATGTACTCCATTTGAGATCT TTATGTGTATGTATGTATGTATACTTGTCAGGTCTGATGGAGGTCCAAAGAAATGGCAGTC TACTGATGCTGGAATGCAGTCTCATGTGCTCAAAGCAG

Nipponbare

CTGTGTGAAAGTTCAGACGGATGGCGATTTTGCAAAGATGTACTCCATTTGATATCT TTGTGTGTATACTTGCCAGGTCTGATGGAGGTCCAAAGAAATGGCAGTCTACTGATG CTGGAATACAGTCTCATGTGCTCAAAGCAG

M5-72k

IRBB7

Nipponbare

RM20593

IRBB7

Nipponbare

RM3723F

IRBB7

TAGACATGGGTCCCTCACAGATGGAACCCATTCTCATAGGCTCACATGTCAGCGGAC ACATATTCCTCTAACTTAGAGGAGCCTCCCCCTAATGAAAGTGTAATATAGGATCAG AAGCAAGACTTGTGGA

Nipponbare

TAGACATGGGTCCCTCACAGATGGAACCCATTCTCATAGGCTCACATGTCAGCGGAC ACATATTCACATATTCCTCTAACTTAGAGGAGCCTCCCCCTAATGAAAGTGTAATAT AGGATCAGAAGCAAGACTTGTGGA

Oligos	Sequence (5' TO 3')	Usage	
RM7243F	ACGAAGTTCTTCACGGGTTG		
RM7243R	AAGATGGCGTGCGTACGTAC		
RM20571F	GAGAGGTGGAGAGATGGATGTGG		
RM20571R	GACCAAAGAACCAAACCAAGAACC		
RM5509F	GATGATCCATGCTTTGGCC		
RM5509R	TTCCAGCAGAAAGAAGACGC		
5610F	AAGCTCGTCACCTCACCCGTCAC		
5610R	TGCACAGTAGCAGCATATCTGCAG		
M5-F	GTTGTCCCACCGACTTTGCTA		
M5-R	CGATCTTACTGGCTCTGCAACTCT		
M5-3kF	CATATGTAGCAAGTATGCATCC		
M5-3kR	TCTGAAGACTAATGTGTTGGCT		
M5-5kF	TGTAGAGATTGTGACGAG		
M5-5kR	TGATCCATTCTGCAAC		
M5-48kF	CTGTGTGAAAGTTCAGACGG		
M5-48kR	CTGCTTTGAGCACATGAGAC	Mapping	
M5-72kF	TGGAAAGGAAGCTCCTCGAC		
M5-72kR	TAGGTCTTACCAGGAAGGTC		
RM20593F	AAGGTACACTTGCTCTGACGGTAGC		
RM20593R	AGACCTCAGTGGCAAATCCTACG		
RM3723F	TAGACATGGGTCCCTCACAGATG		
RM3723R	TCCACAAGTCTTGCTTCTGATCC		
3628F	TGTCCGGTGTCTCTAGCTTC		
3628R	ACCAAGCTATACCAAGTACT		
5760-14kF	CTCAACACTCCTCTTCACAG		
5760-14kR	AGTGAGAGAAGCGTAGAGAC		
RM53266F	AGCCTTCCTAACCAATCTGG		
RM53266R	CCATCATTTCGGCTCTTCAC		
3770F	GTCACCAGTCACCACCGATC		
3770R	CGTGATAACCGCGAGACATA		
gRNA1-F	GCAGGTACGTATACCAGTTGTCCG	For the 1^{st} guide RNA to delete the	
gRNA1-R	AAACCGGACAACTGGTATACGTAC	53 kb of <i>Xa</i> 7 locus	
gRNA2-F	GCAGGTTTGTCCAATGAGTTGCTG	For the 2 nd guide RNA to delete the	
gRNA2-R	AAACCAGCAACTCATTGGACAAAC	53 kb of <i>Xa</i> 7 locus	
gRNA3-F	TGTTGGATGATCAGCGGCCGCCA	For guide RNA at the 1 st site of Ya7	
gRNA3-R	AAACTGGCGGCCGCTGATCATCC	FOI guide ANA at the i site of Aa/	
gRNA4-F	GTGTGAGGAAGACGCCGGAGTTGA	For guide PNA at the 2 nd site of Va7	
gRNA4-R	AAACTCAACTCCGGCGTCTTCCTC	FOI guide RNA at the 2 Site of Kar	
Xa7aRT-F	CGTATGCCCGTTGCAGTTGCAG	RT-PCR for Xa7 expression	
Xa7aRT-R	CGGAGTTGACGGTCAGCAGTCG		
Xa27L-F4,	ATAGGCCGGTATCCCAGCTCT	For genotyping Xa7 CRISPR plant	
112.5R	ACTGGAGCAGAGGAATGTGC		
EP53-F	TGTAGAAACCATGTCTCATGC	For genotyping large deletion	
BTB-R1	ACTACCACACCAGCCAGCAAG	For genocyping targe detection	
EP53R	CTTGGATGGAGCTCCAGATG	With EP53-F as internal primers for	
		zygosity of large deletion plants	
Pro2.7k	TATGACCATGATTACGCCAAGCTTC		
HindF	TCATCCCAACCGTTCTG	Amplify 2.7 kb Xa7 promoter	
Xa7aATG-R	AGAAATTTACCCTCAGATCTACCAT		
	GGATGATGGATCCCCCAG		
DelEBEF3	ATACGAACGAAGGCTTTGAAGC	Delete 20 bp for mutant EBE	

Supplemental Table 2. Oligonucleotides used in this study

DelEBER3	TTCAAAGCCTTCGTTCGTATGGGTT	
	ATATATTGGTTTTAGCAG	

Supplemental Information 1. The cDNA sequence of Xa7.

Supplemental Information 2. gBlocks of Xa7 homologous genes.

gBlock cloned into pBY02 at EcoRI-HindIII through Gibson cloning. Sequences shaded in yellow are overlapping regions to facilitate the insertion of gBlocks in pBY02 through Gibson cloning. The start and stop codons are in red. Sequences for EcoRI and HindIII are underlined. The Kozac sequence for optimal expression of gene under 35S promoter is in bold.

*Do_Xa*7 gBlock

Si_Xa7 gBlock

Ph_Xa7 gBlock. (codon optimized for *N. benthamiana* due to high GC of the original sequence)

GAGAACACGGGGGACTCTAGGAATTCGATCTACCATGGCCGGTCTCCAACCAGCAGCCATCCACCTTCAACGTAGAC AACAGGCTATTGCTGGTAGGCGAGTACGTCTCTTAGTAAACGCAGGAGCACTGTTGGTATCCGCAGGAGGCAGTGTC GTTATCATTCACGCAGCTACTCCTTCAGACGACGCCGCAGGAGGGCCCGCCTGCTCTCTGGTGGCCTTTTCTGTCTT TCTGCTGGGAGTCTCTTTAGTCACGCTGGCCTTAGCTGCTGATAGATTCCCACGTGCAGCAAGGGTTGGGGCCGCCG TAGCAACAGCTACTAATAGGTATTTGTTTGGACTCGGCTGGTAATAAGCTTCTAGACTAGTGAGCTCGAAT gBlock cloned into pBY02 at EcoRI-HindIII through Gibson cloning.

 Sb_Xa7 gBlock (codon optimized for N. benthamiana due to high GC of the original sequence)

GAGAACACGGGGGGACTCTAGGAATTCGATCTACCATGTCTCGACCCGCCGAGATGCGTAACCCCCGTCACGCAGACG ATGGCGATTTGCAGTTAGCTTGGATTGGTAGAAGGCGAAGGGGCCTTCAACAACAACTCCTGTTGGATTCCGGAGGC AGGGCCTGCATGTTGTTAGGGGGCTCTGGTCCTCACTTGGGATCAGCTCGCCCTCGCAAGTTCTTCCAGCCCCGAGCA TGTCCTGCTGGCTGCATTTGTTCTTTGGTTACTGGGGGGCCGCCCTCGTGATGCTTAGTCTGGTAAGCCGAAGGTTTC CAAGACTGGCATCCGCCGGTGCAGCCCTGGTAATGGCACTTAGGAATTATTTGTTAGGGGGGTGGTGGGCTCT<u>AAGCT</u> TCTAGACTAGTGAGCTCGAAT

Op_Xa7 gBlock

Ol_Xa7 gBlock

GAGAACACGGGGGACTCTAGGAATTCGATCTACCATGGCGAATCGGACAGCTGCTCATCCGCGGATGCCCGTACAGG GGCTGCGCCGCAATCAAATCGCGCGGGGGCCCGCCGCTCGCCGTTCTCGACTGCTGATCGTCAACACCGGCGTTTTC CTCATCTCCACCTCCGGGGCCATCGTCGTCGTCGTCCACACCGCTGGAAACCCCTCCTCCGCCATCGACGACGGTCCGTC CTCCGCCCTCGTCGCATTCGTGCTCTTCCTACTTGGCATCTGGCTCGTGCTTCTTGCCCTCGTCGCCGACAAGTTCC CGCGAGCCGCCAGGGTCGCCGTCGCCATTGCTAGTGCACTGCAGGATCACCTCATAGGCGGCAACTAGT<u>AAGCTTCT</u> AGACTAGTGAGCTCGAAT

>Ol_XA7 KN541332.1_FGP003 (Oryza longistaminata)

MANRTAAHPRMPVQGLRRNQIARGPAARRSRLLIVNTGVFLISTSGAIVVVHTAGNPSSAIDDG PSSALVAFVLFLLGIWLVLLALVADKFPRAARVAVAIASALQDHLIGGN

>Op_XA7 OPUNC06G22220 (Oryza punctata)

MVNRTAAHPQMPVQALRRNRIAQVNRVPAARRARARLLIVNSGVFLISTSGAIVVVHTAGNPFA LVAFVLFLLGIWLVLLALVADKFPRAARVAVAIASALQDHLIGGN

>Do_XA7 BAE44_0010678 (*Dichanthelium oligosanthes*) (NCBI accession: OEL28303.1) MADRPAAIHQGLAPVGFVVRQQRAIAPRRARLLMINSGTLLISAAWSVIIIHSTSSGTTAGGGR AFALVTFLLFLLGVSLVMVALVAYRFRRAAAVGVAIARALRRYLLGLGW

>SI_XA7 LOC105914264 (*Setaria italica*) (NCBI accession: XP_012700797.1) MAGRPAELHLQRVIAGRRARLLVNAGALLISAAGSVIIHAAATPSDASSGPARPLIAFCIFVLG VSLVMSALVADRFPRAARAGVAIARALQRYVFGLVGW

>Ph_XA7 LOC112891075 (*Panicum hallii*) (NCBI accession: XP_025813762.1) MAGLQPAAIHLQRRQQAIAGRRVRLLVNAGALLVSAGGSVVIIHAATPSDDAAGGPACSLVAFS VFLLGVSLVTLALAADRFPRAARVGAAVATATNRYLFGLGW

>Sb_XA7 SORBI_3K004400 (Sorghum bicolor) (NCBI accession: OQU75609.1) MSRPAEMRNPRHADDGDLQLAWIGRRRRGLQQQLLLDSGGRACMLLGALVLTWDQLALASSSSP EHVLLAAFVLWLLGAALVMLSLVSRRFPRLASAGAALVMALRNYLLGGGGL

Supplemental File 1. Program for genome assembly.

###The script uses illumina reads dowloaded form the 3K rice genomes project #Raw sequence data is also available from the http://gigadb.org/dataset/200001 - SRA at PRJEB6180 #deposited the reads in a new folder rice3k ## use the Xa7 region as reference and create an index using bowtie2 bowtie2-build complete Xa7.fasta cXa7 ##create an accession list from the reads dictionary that contains the sample accession and the run id ## sample accession (1st collumn) run accession (2dn collum) in reads dictionary for x in `cut -f 1 reads dictionary` do grep \$x reads dictionary | sed 's/ $\frac{y}{g}$ | cut -f 2 > copy list #### for i in `cat copy list` do cp "/rice3k/"\$i" 1.fastq.gz" ./ cp "/rice3k/"\$i" 2.fastq.gz" ./ gunzip *.fastq.gz done ### ##generate temporary files to map cat * 1.fastq > x'' 1.fq" cat * 2.fastq > \$x" 2.fq" rm * 1.fastq rm * 2.fastq rm *.fastq.gz ### bowtie2 -x cXa7 -1 \$x" 1.fq" -2 \$x" 2.fq" -S \$x" results.sam" -p 8 rm *.fq samtools view -bS -o \$x" results.bam" \$x" results.sam" rm \$x" results.sam" samtools view -b -F 4 \$x" results.bam" > \$x" mapped.bam" rm * results.bam samtools sort \$x" mapped.bam" -o \$x" results mapped.sorted.bam" rm \$x"_mapped.bam" samtools index \$x" results mapped.sorted.bam" #### done

###Generate a consensus Xa7 for each mapped rice3k
sam_list is the list of bam files generate above

for x in `cat sam_list` do samtools mpileup -uf complete_Xa7.fasta \$x"_results_mapped.sorted.bam" | bcftools call -c | vcfutils.pl vcf2fq > \$x"_cns.fq" done



Supplemental Figure 1. Large chromosomal fragment deletion of Xa7 locus.

(A) Schematic map of the CRISPR/Cas9 construct used for genome editing of *Xa7* locus. Guide RNA guides are under rice U6 promoters and Cas9 under maize ubiquitin 1 gene promoter. Hygromycin resistance gene is under the CaMV 35S promoter.

(B) *Xa7* locus delimited by two markers with guide RNA target sites. P1 and P2 are two deletion-specific primers to screen and identify the CRISPR-induced large deletion lines. Guide RNA target sites are in shaded with adjacent Cas9 PAM (protospacer adjacent motif) shaded in red.

(C) Sequencing chromatogram of PCR-amplicon from the T0 nb7-1 line. PCR-amplicon with two primers (P1 and P2) and genomic DNA of nb7-1 was subjected to Sanger sequencing.



Supplemental Figure 2. Screenshot of RAMPAGE analysis displayed with the Integrative Genomics Viewer (Thorvaldsdóttir, Robinson et al. 2013).

RAMPAGE reads from two RNA samples derived from IRBB7 leaves inoculated with MX53 (Control 1 and 2) and three RNA samples (Inoculation 1, 2, and 3) derived from IRBB7 leaves inoculated with PXO86 were used for analysis. The tracks show read coverage per position. Note that Control 1 and 2 and Inoculation 2 coverage is shown on a scale 0-4000, compared to 0-16000 for Inoculation 1 and 3, in order to accommodate different library sizes (read pairs aligned are 7,021,714; 8,098,348; and 6,507,082 for Control 1 and 2 and Inoculation 2, respectively; compared to 32,296,182 and 37,344,426 for Inoculation1 and 3, respectively); Alignment and analysis were done with GoRAMPAGE (https://github.com/BrendelGroup/GoRAMPAGE). Counts are displayed on a log scale. For example, site 66,859 has coverage 9 and 31 for Control, compared to 9144, 217, and 6100 for Inoculated.

Thorvaldsdóttir, H., J. T. Robinson and J. P. Mesirov (2013). "Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration." <u>Brief Bioinform</u> **14**(2): 178-192.

Α					
Sb_XA7 Ph_XA7 Si_XA7 Do_XA7 Op_XA7 OL_XA7 XA7	MAA MS-RPAEMRN MAG MAG MAD MVNRTAAHP- MANRTAAHP- MANRTAAHP- MA	PA PRHADDGDLQLAWIGR LQPAAIHL -RPAELHL -RPAAIHQGLAPVGFV QMPVQALRRNRIAQ RMPVQGLRRNQIA- RMPVAVAGLRHHYAF-	RR GLQQ QRRQQAIAGRRVR QR VIAGRRAR VRQQRAIAPRAR VNRVPAARRARAR - RGPAARRSR PANLRPAAR	QLLLDSGGRACMLLG QLLLDSGGRACMLLG LL-VNAGALLVSAGG LL-VNAGALLISAAG LLIVNSGTLLISAAW LLIVNSGVFLISTSG LLIVNTGVFLISTSG	A V H A ALVLTWDQLALA-SSS 62 SVVIIHAATPSD-DAA 53 SVIIHAAATPSD-AS 48 SVIIIHSTSSGT-TAG 61 AIVVVHTAGNPF 67 AIVVVHTAGNPSSAID 62 AIVLVHTAGNPP-AID 62
Sb_XA7 Ph_XA7 Si_XA7 Do_XA7 Op_XA7 Ol_XA7 XA7	XGPAXALVAF SPEHVLLAAF GGPACSLVAF SGPARPLIAF GGRAFALVTF ALVAF DGPSSALVAF NDPAYALVAF	VLFLLGXXLVXLALVA VLWLLGAALVMLSLVS SVFLLGVSLVTLALAA CIFVLGVSLVMSALVA LLFLLGVSLVMVALVA VLFLLGIWLVLLALVA VLFLLGIWLVLLALVA	DRFPRAARVGVAI RRFPRLASAGAAL DRFPRAARVGAAV DRFPRAARAGVAI YRFRRAAAVGVAI DKFPRAARVAVAI DKFPRAARVAVAI DQFPRAAGVAVAI	A×ALQ×YL×G×-G×- VMALRNYLLGG-GGL ATATNRYLFGL-GW- ARALQRYVFGLVGW- ARALRRYLLGL-GW- ASALQDHLIGGN- ASALQDHLIGGN- ARALQDYLIGGN-	115 105 101 113 109 113 113
E	3		Ор ХА7	c	
	0.3	98 0.3 63 0.3 0.0 67 0.2 0.1 0.2 Si X	0.1 OI XA7 0.3 OI XA7 0.3 Do XA7 	SI_Xa - xa7 Sb_Xa	a7 Xa7 Do_Xa7 7 Ol_Xa7
·	0.20	1.0		- Sb XA7	Op_Xa7

Supplemental Figure 3. Xa7 and homologs in diverse grass species.

(A) Amino acid alignment of seven XA7 homologs generated by ClustalX. Sequences are from *Do_Xa7*, *Dichanthelium oligosanthes*; *Si_Xa7*, *Setaria italica*; *Ph_Xa7*, *Panicum hallii*; *Sb_Xa7*, *Sorghum bicolor*; *Op_Xa7*, *Oryza punctata*; *Ol_Xa7*, *Oryza longistaminata*.

(B) Phylogenetic tree of seven XA7 homologs. The unrooted phylogenetic tree of 7 proteins was generated by using the Maximum Likelihood method and JTT matrix-based model. The tree with the highest log likelihood (-1290.17) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analyses were conducted in MEGA X (http://www.megasoftware.net).

(C) Cell death phenotypes caused by overexpression of Xa7 and its homologs in *N*. *benthamiana* through agroinfiltration. Leaf image was taken three days post infiltration.