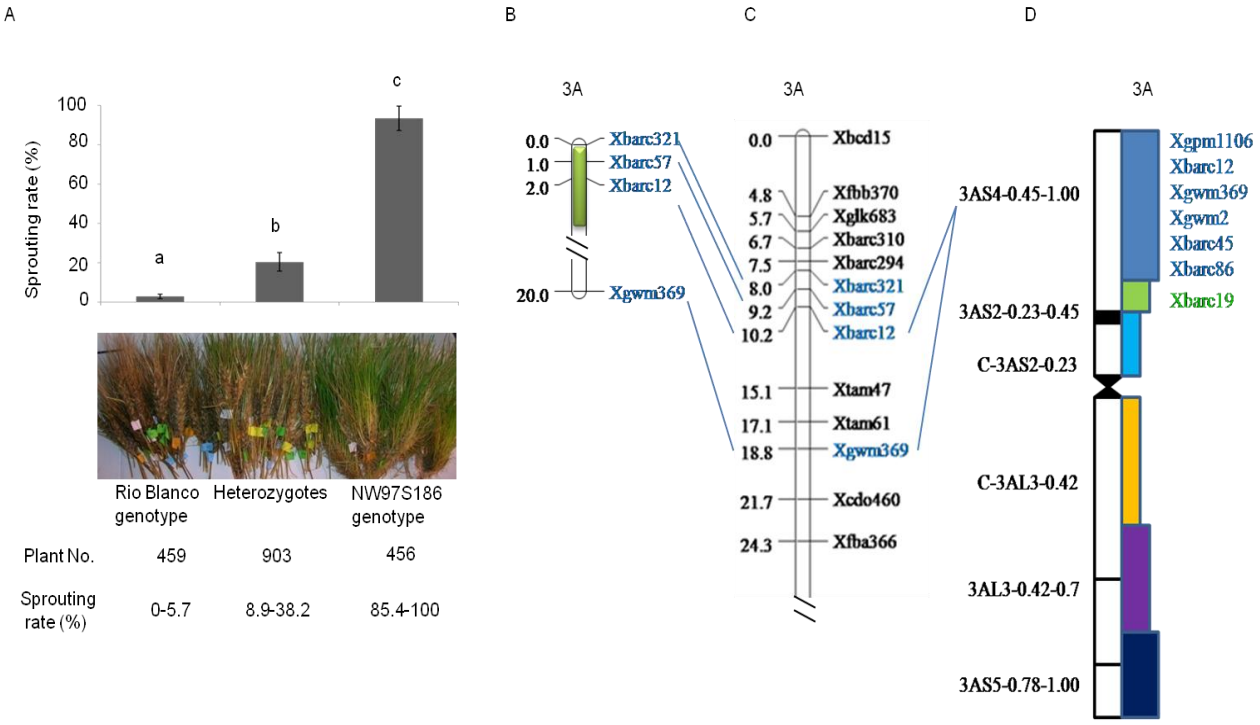

Figure S1 Linkage and physical maps of *Qphs.pseru-3AS*. (A) Segregation of sprouting resistance in the near-isogenic recombinant population. Plants with Rio Blanco genotypes at the three marker loci, *Xbarc321*, *Xbarc57*, *Xbarc12*, were highly sprouting resistant; the heterozygous plants were moderately resistant; plants with all three NW97S186 marker alleles were highly susceptible. Error bar denotes standard deviation, a, b and c indicate significant difference at $P < 0.01$. (B) The QTL map of *Qphs.pseru-3AS* was developed using two recombinant inbred populations derived from Rio Blanco/NW97S186 and Rio Blanco/NW97S078 (17). The green bar represents the QTL interval on the short arm of chromosome 3A (3AS). (C) The four SSR markers (*Xbarc321*, *Xbarc57*, *Xbarc12*, and *Xgwm369)* close to *Qphs.pseru-3AS* show similar order and genetic distance in another map (Song *et al*., 2005). (D) Physical map of 3A (Sourdille *et al*., 2004). Two of the four SSR markers (*Xbarc12*, *Xgwm369*) close to *Qphs.pseru-3AS* are located in the deletion bin 3AS4-0.45-1.00 at the distal end of 3AS.

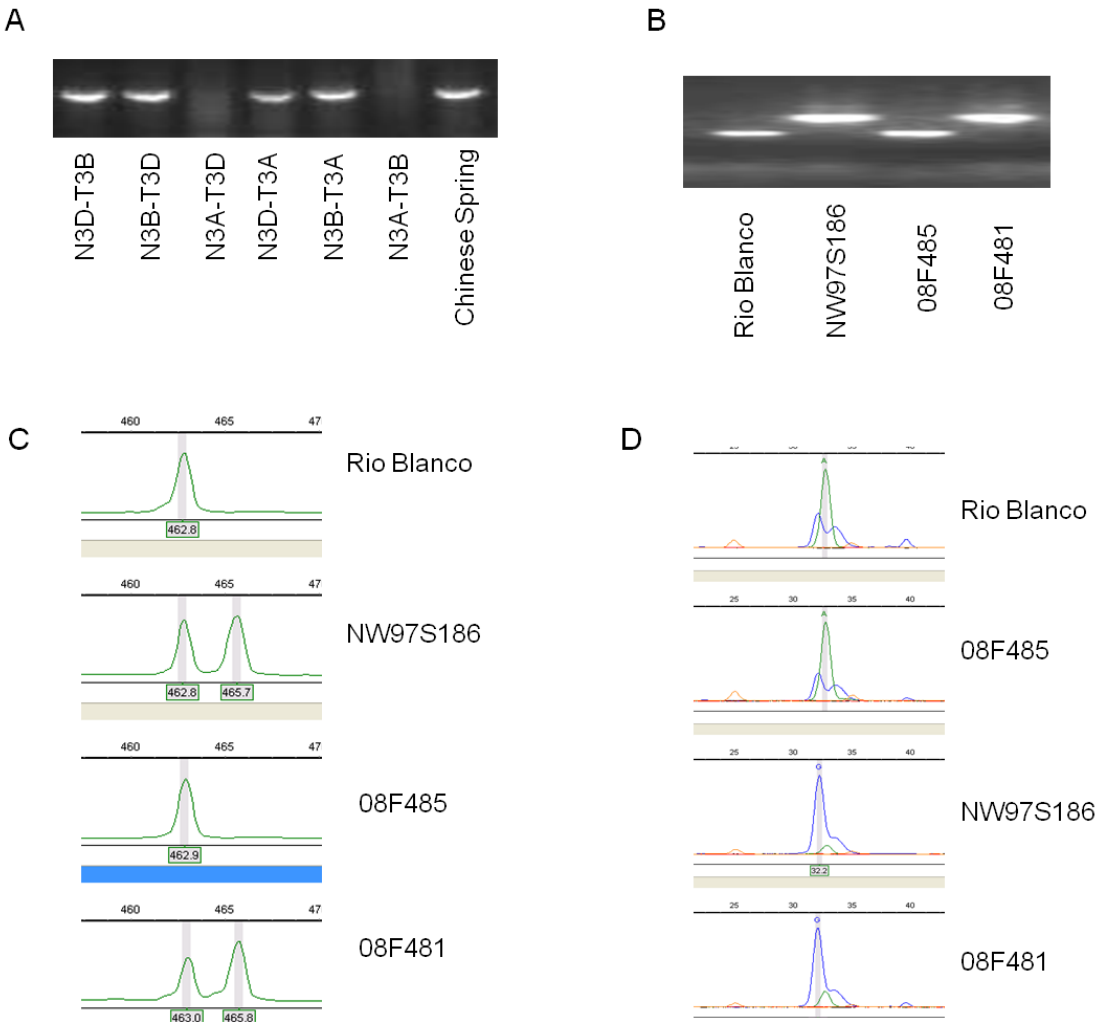

Figure S2 Markers used to determine the chromosome location of *Qphs.pseru-3AS*. (A) An agarose gel image shows *TaPHS1* specific primers amplified only in Chinese Spring nulli-tetrasomic lines carrying 3A chromosome. No PCR product was amplified when the 3A chromosome was replaced by 3B (N3A-T3B) or 3D (N3A-T3D). (B) An agarose gel image shows an STS marker developed from the wheat EST BE423484 and detected polymorphism between the resistant genotypes (Rio Blanco and 08F485) and the susceptible genotypes (NW97S186 and 08F481). (C) An electrophorogram of polymorphic STS marker developed from the EST CA654295 analyzed using capillary electrophoresis in an ABI 3730 DNA analyzer. (D) An electrophorogram of an SNP marker developed by re-sequencing wheat EST CD910417 analyzed using SNPShot in an ABI 3730 DNA analyzer.

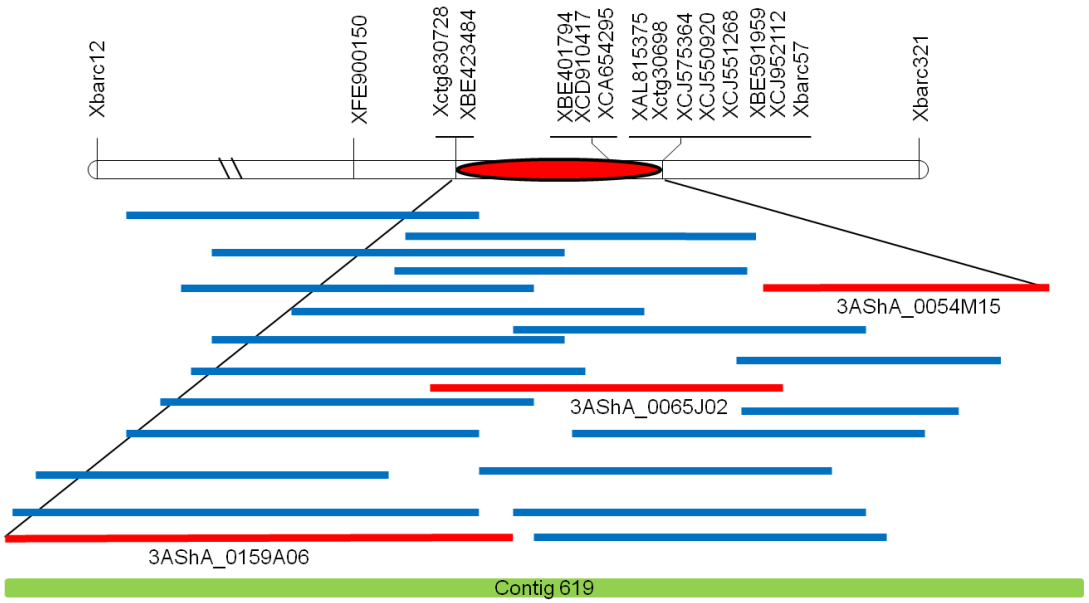

Figure S3 BAC contig Ctg619 (bottom solid bar) spans the entire *Qphs.pseru-3AS* region. Top bar is the linkage map of *Qphs.pseru-3AS* with the *Qphs.pseru-3AS* region labeled by red oval. Solid bars between the linkage map and the contig bar are different BACs in the contig. Three BACs with red color that cover the entire QTL region were selected for sequencing to identify candidate genes for *TaPHS1*.

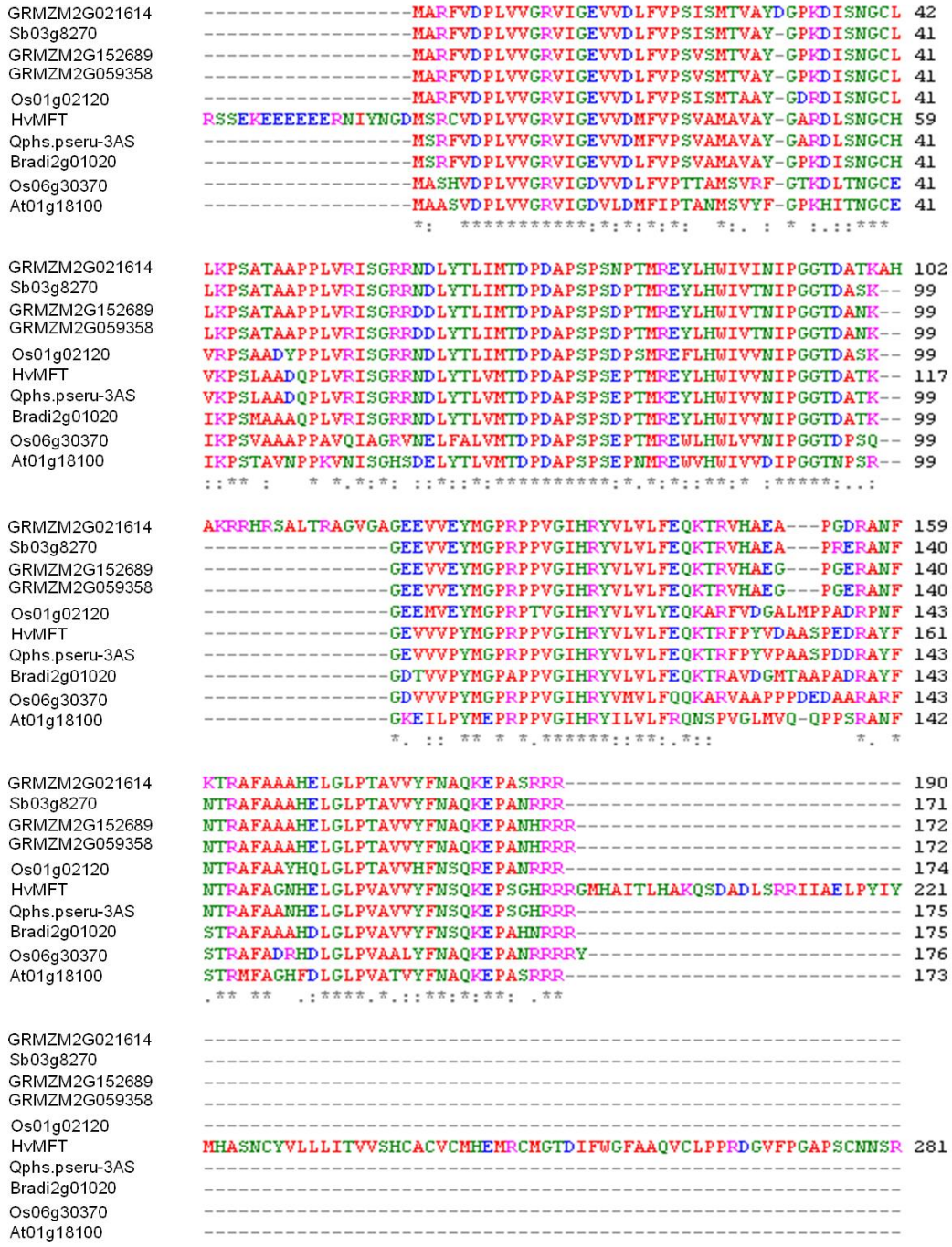

Figure S4 Sequence alignment of the deduced amino acids of *TaPHS1* homologs from maize (GRMZM2G021614, GRMZM2G152689, GRMZM2G059358), sorghum (Sb03g8270), *Brachypodium* (Bradi2g01020), barley (*HvMFT*), rice (Os01g02120, Os06g30370), *Arabidopsis* (At01g18100), and wheat (*Qphs.pseru-3AS*).

Figure S5 Comparison of the promoter sequences between Rio Blanco and NW97S186. Identical sequences are labeled by *. ABRE CEs are indicated by blue or yellow boxes if two ABRE CEs overlapped. RY repeats are indicated by green letters. Motif IIB is underlined. Two SNPs in the ABRE CEs at -222 and -314 positions are shown in red letters.

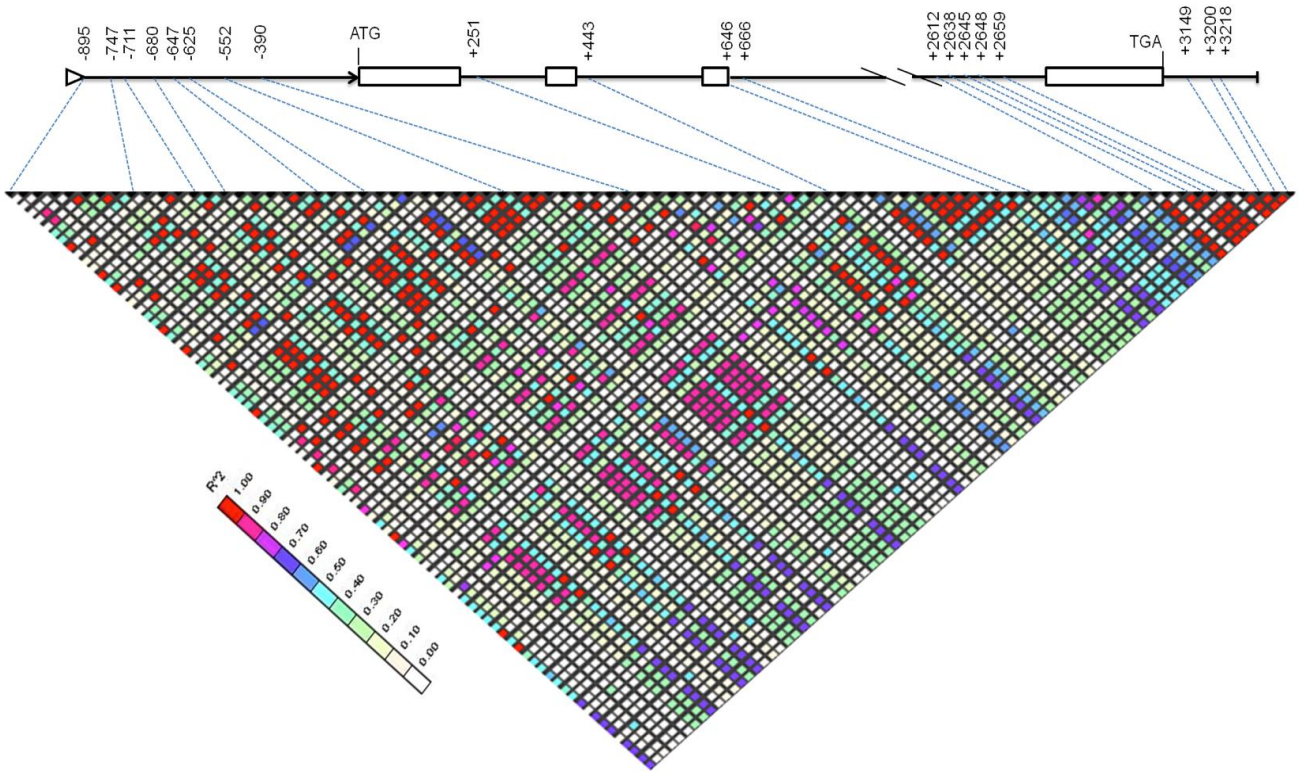

Figure S6 Linkage disequilibrium (LD) between the two causal SNPs (+646 and +666) in intron 3 and 18 polymorphic sites of *TaPHS1* analyzed in 82 wheat cultivars. The upper part is the gene structure of *TaPHS1*, and the lower part is the LD graph. From left to right, each grid indicates one SNP or InDel of the 84 SNPs or InDels in the order from the 5'-end of *TaPHS1* promoter. Among the 84 sequence variations, only 20 variations that were significantly associated with PHS resistance were labeled on the graph, including two causal SNPs (+646 and +666) in intron 3 and the 18 non-causal polymorphic sites (Figure 5A) associated with sprouting resistance due to their LD to the two causal SNPs.

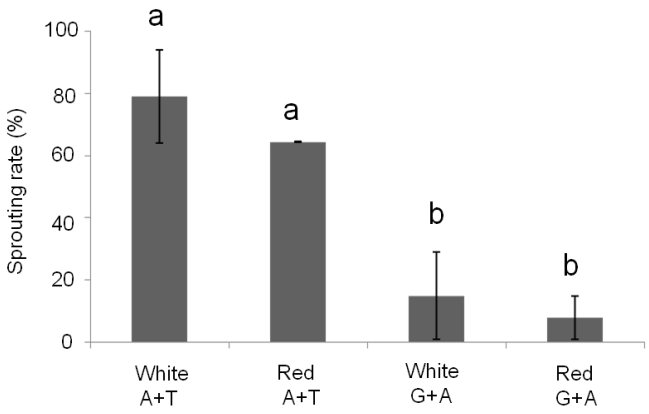

Figure S7 Two causal SNPs determine PHS resistance. White wheat had slightly higher spike sprouting rates than red wheat in both haplotypes of *TaPHS1* based on the two causal SNPs loci (+646 and +666), but the difference was not significant (a and b indicate significant difference at $P < 0.05$) as the two causal SNPs ($P=2.98 \times 10^{-5}$, Figure 5B).

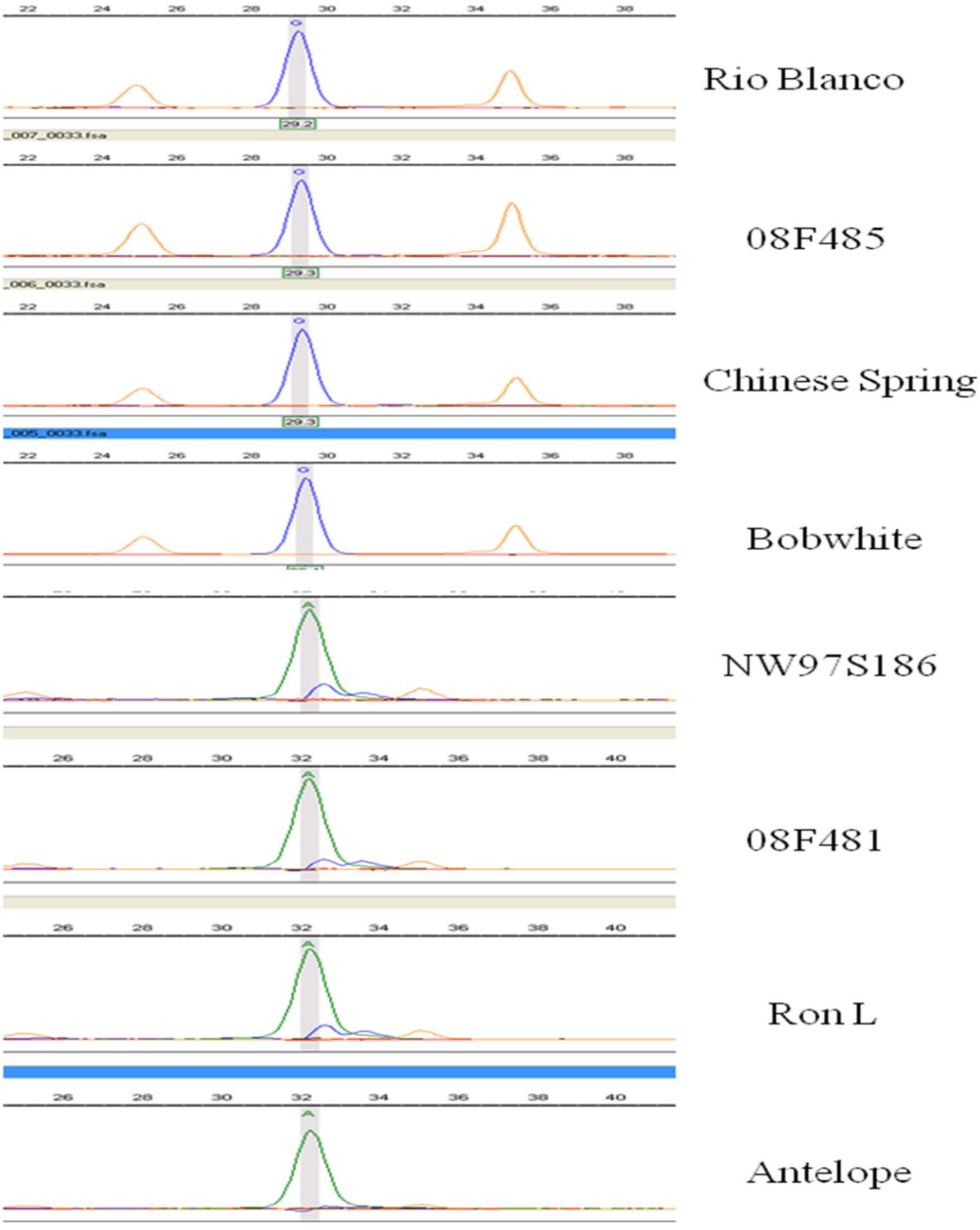

Figure S8 One diagnostic SNP markers for *TaPHS1* (*TaPHS1-SNP1*) was developed based on the GT-to-AT mutation at the mis-splicing site (+646) in susceptible cultivars. The SNP was analyzed using SNPShot via an ABI3730 DNA analyzer. The top four cultivars with blue (G) peaks are sprouting resistant, and the bottom four cultivars with green (A) peaks are sprouting susceptible.

Table S1 Primers used for sequencing, gene expression, and gene transformation

	Forward	Reverse	SNP primer
Ta PHS1-GS	GCGACGAAGACCCGAGGAGG	GCACGACTGAATGAAAATCT	-
TaPHS1- P	CCTGCTTTTCTCTGGACGAG	CATCGAGTTGTGGAGCAAGA	-
TaPHS1-17F18	TTCAGCACCGTGATAAGAGG	GGCGCTCAATTTCATGTTGT	-
TaPHS1-18F18	CAAGATATCGCAAATCGAAAGA	ACGACAGTGCTCCAA	-
TaPHS1-19F18	TGCATGCTCGAAAGTTTGTG	CACCTAAGACCCAAC	-
TaPHS1-20F18	ACATGGGGTGACATGAGCTA	TCACTGCGTGCGTTTTA	-
*TaPHS1-21*F18	TACCGTGAGAGACACGCAAG	CCGTGACAAAACCCT	-
TaPHS1-SNP1 (+646)	GTGAGAGACACGCAAGAACG	TTTGTACAGGTCGTCGTTGG	TGGAACAGATGCAACTAAAG
TaPHS1-SNP2 (+666)	GTGAGAGACACGCAAGAACG	TTTGTACAGGTCGTCGTTGG	GTTAGTACTTTATTTATGAC
TaPHS1-CDS	(P1) ATGTCCCGGTTCGTTGATCCGCTG	(P6)TCAACGCCGGCGGTGTCCGGAC	-
ACTIN	ACCTTCAGTTGCCCAGCAAT	CAGAGTCGAGCACAATACCAGTTG	-
AK330655 full length cDNA	TGTCCCGGTTCGTTGATCCGCTG	TTTGAGCGCCAACTGCATGCTA	-
TaPHS1 P1 and P2	The same as P1	(P2) TCTGTTCCACCCGGTATGTT	-
Taphs1- CDS (P3, P4)	(P3)GAGGTGGTGGACATGTTCGT	(P4)TGATCTTTAGTTGCATCTGTTCC	-
PHS1 P5 and P6	(P5)TGTCCCGGTTCGTTGAT	(P6)CTAGGGCTAGGCGCGTCA	-
for RNAi and over-expression			
RNAi	RNAi-F TGCTCACCGACCAACAATAG	RNAi-R: TTTTTGAGCGCCAACTGC	-
	Gus-F1: CACGTAGTCCGCATCTTCA	Gus-R2: GTGGAGTGAAGAGTATCAGTGTGC	-
Bar	CCTGCCTTCATACGCTATTTATTTGC	CTTCAGCAGGTGGGTGTAGAGCGT G	-

Table S2 Genotypic data and sprouting resistance levels of the 56 recombinants analyzed with the markers in the QTL *Qphs.pseru-3AS* region

No. of RSLs	Xbarc12	XFE900150	Xctg830728	XBE424484	XBE401794	XCD910417	XCA654295	XAL815375	Xctg30698	XCJ575364	XCJ550920	XCJ551268	XCJ952112	XBE591959	Xbarc57	Xbarc321	PHS rate
08F485	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	L
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	L
16	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	L
1	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	L
1	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	L
1	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	H
2	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H
3	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	H
31	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H
08F481	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H

A=Rio Blanco genotype; B=NW97S186 genotype. L=low PHS rates as in NW97S186; H=high PHS rates as in Rio Blanco.

Those red letters are the markers co-segregated with *TaPHS1.*

Table S3 Thirteen genes that were mapped in the QTL *Qphs.pseru-3AS* region by comparative mapping

Wheat ESTs	Rice annotated genes	*Branchypodium* annotated gene	Forward primer	Reverse primer	SNP primer*
XFE900150	*LOC_Os01g15209*	*Bradi2g00780*	TTTTTGTGCCCTCGTATGTTT	AGCTGAACAATGGCGAAGAT	-
Ctg830728	*LOC_Os01g02050*	*Bradi 2g00910*	CAACCTAGGCGGCAGTTTTA	ACCGAGACgGGAATCCTAAT	-
BE423484	*LOC_Os01g02070*	no	CCACGGTTCTCGTCATTCTT	TTTCGAAACGGAAACTAGCC	-
BE401794	*LOC_Os01g02090*	*Bradi 2g00980*	GGGCCATCTTGGTAGGAAAT	TGCTCACCAGGCATGAGATA	-
CD910417	*LOC_Os01g02100*	*Bradi 2g00990*	CATGCCTGTGAAGAGCTCAG	AAGGTACGGGTGCAATGAAT	AGCACTGGACTGAAGTT TACCA
CA654295	*LOC_Os01g02110*	*Bradi 2g01000*	GTCCGAAGAGAGCGCATCA	GGAGAATTGATCTAAAAGGAGTGA	-
AL815375	*LOC_Os01g02130*	*Bradi 2g01030*	CCCTCTTCGCTGCTAACCAC	GTACGTACACGCGCACCA	-
Ctg30698	no	*Bradi 2g01070*	GTATTGCCAGCCCCTGACT	GAACCCTGAGGGATGTCAAA	-
CJ575364	*LOC_Os01g02170*	*Bradi 2g01090*	TCACTGCAACCAAAATTCTCA	GGTGTGCCTGCACTACCTCT	-
CJ550920	*LOC_Os01g02190*	*Bradi 2g01090*	CGGCTAGCTGGCTAGTTCGT	AAGGGGCAGGACCTAGAGC	-
CJ551268	*LOC_Os01g02200*	*Bradi 2g01100*	CGCTTATGATGGGCTTATTTG	ATCCTTGGGATTGCATCATC	-
CJ952112	*LOC_Os01g02300*	*Bradi 2g01120*	ATCGGAAGCATTCACCATGT	GCCAGCTTCAAGCATGTCTA	-
BE591959	*LOC_Os01g02310*	no	TTTCAGGGTCAATTTGGTGA	TTGCATAGTCCAAAATCAGAAA	-

* indicates the gene that was mapped using the SNP marker.

Table S4 Genes identified by sequencing the three BACs spanning the *Qphs.pseru-3AS* region

Brachypodium homologs	Rice homologs	Predicted gene function
Bradi2g00950	LOC_Os01g02080	Peptidyl-prolyl cis-trans isomerase
Bradi2g00980	LOC_Os01g02090	Uncharacterized mRNA-associated protein RAP55
Bradi2g00990	LOC_Os01g02100	Tm3 transposase
Bradi2g01000	LOC_Os01g02110	HLH DNA-binding domain containing protein
Bradi2g01010	no	Cell division cycle
Bradi2g01020	LOC_Os01g02120	Homologous to Mother of FT and TFL1 gene

Table S5 Relationship among two structure groups, seed color, and causal SNPs for PHS resistance in *TaPHS1*.

Cultivars	Seed color	Structure group	SNP at +646	SNP at +666
Trego	white	1	G	A
SD07W041	white	1	G	A
NW05M6015-25-4	white	1	G	A
KS07HW25	white	1	G	A
KS07HW81	white	1	G	A
Snowmass	white	1	G	A
OK Rising	white	1	G	A
Rio Blanco	white	1	G	A
Danby	white	1	G	A
Avalanche	white	1	G	A
CO05W101	white	1	G	A
Mesa	white	1	G	A
KS07HW52-5	white	1	G	A
NE08452	red	1	G	A
Jagger	red	1	G	A
Overland	red	1	G	A
Intrada	white	1	G	A
KS05HW136-3	white	1	G	A
CO02W237	white	1	G	A
CO03W139	white	1	G	A
KS05HW121-2	white	1	G	A
CO03W043	white	1	G	A
SD05W030	white	1	G	A
SD05W148-1	white	1	G	A
Tiger	white	1	G	A
Lakin	white	1	G	A
Hartog	white	1	G	A
KS010990M-8	white	1	G	A
Infinity CL	red	1	G	A
Thunder CL	white	1	G	A
Recital	white	1	G	A
NW03Y2016	white	1	G	A
OK Bullet	red	1	G	A
Postrock	red	1	G	A
Santa Fe	red	1	G	A
NX03Y2489	white	1	A	T
NX04Y2107	white	1	A	T
Antelope	white	1	A	T
KS05HW15-2	white	1	A	T
CO04W210	white	1	A	T
KS07HW117	white	1	A	T
RonL	white	1	A	T
NW97S078	white	1	A	T

Grandin	red	1	A	T
CO03W054	white	2	G	A
NW04Y2188	white	2	G	A
Janz	white	2	G	A
Spica	white	2	G	A
Overlay	red	2	G	A
Jackpot	red	2	G	A
Jagalene	red	2	G	A
AC Taber	red	2	G	A
ZenkojiKomugi	red	2	G	A
Bobwhite	red	2	G	A
Karl92	red	2	G	A
Chinese Spring	red	2	G	A
Aus1408	white	2	G	A
Bungulla	white	2	G	A
NW96S016	white	2	G	A
TutoumaiA	white	2	G	A
Betty	white	2	G	A
Champlain	white	2	G	A
Halberd	white	2	G	A
Jabim	white	2	G	A
Kite	white	2	G	A
Lerma Rojo	white	2	G	A
Siyang936	white	2	G	A
2137	red	2	G	A
Bill Brown	red	2	G	A
Hatcher	red	2	G	A
Losprout	red	2	G	A
Heyne	white	2	G	A
99ID536	white	2	G	A
Protection CL	red	2	G	A
Renan	red	2	G	A
Jing411	white	2	A	T
NW05M6011-6-1	white	2	A	T
NW97S186	white	2	A	T
John	white	2	A	T
Klasic	white	2	A	T
Geneva	white	2	A	T
Xinchun9	white	2	A	T