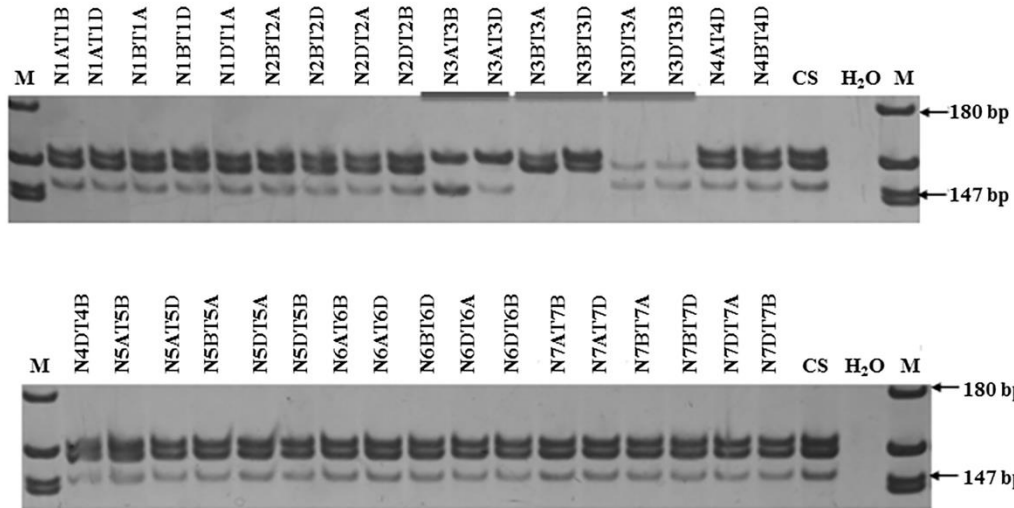


## Appendix A Basic information of 32 accessions

No.	Accession	Origin	Time of release
1	Beijing 10	Beijing, China	1960s
2	An 85 Zhong 124-1	Beijing, China	1970s
3	Dali 1	Shaanxi, China	1970s
4	Dan R 8093	Beijing, China	1970s
5	Jimai 6	Hebei, China	1970s
6	Changle 5	Shandong, China	1970s
7	Jingpin 10	Beijing, China	1970s
8	Beijing 14	Beijing, China	1980s
9	Changwu 131	Shaanxi, China	1980s
10	Fengkang 13	Beijing, China	1980s
11	Jin 2148-7	Shanxi, China	1980s
12	Jinghe 8922	Beijing, China	1980s
13	Pandas	Italy	1990s
14	Yanzhan 1	Henan, China	1990s
15	Jimai 41	Hebei, China	1990s
16	Beijing 8686	Beijing, China	1990s
17	04-044	Beijing, China	1990s
18	04-030	Beijing, China	1990s
19	Neixiang 188	Henan, China	1990s
20	Jing 411	Beijing, China	1990s
21	Chang 6878	Shanxi, China	2000s
22	Linkang 5108	Shanxi, China	2000s
23	Chun 22 9th-25	Beijing, China	2000s
24	Chun 04 9th-5-1	Beijing, China	2000s
25	Chun 45 9th-50-1	Beijing, China	2000s
26	Bawangbian	Hebei, China	Landrace
27	Cangzhouxiaomai	Hebei, China	Landrace
28	Baiqimai	Gansu, China	Landrace
29	Hongheshang	Shannxi, China	Landrace
30	Ziganbaimangxian	Henan, China	Landrace
31	Chinese Spring	Sichuan, China	Landrace
32	Baicaomai	Henan, China	Landrace

## Appendix B Primers used in this research

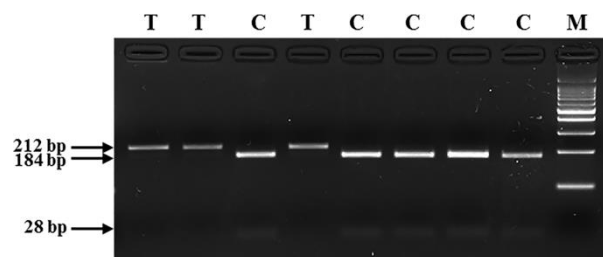
Primer set	Nucleotide sequence (5' to 3')	Experimental purpose
GF	GCAGAAGTTCACGATAGGCC	A, B and D genomic fragment amplification
GR	CCAACCTACCGACCCAACGAAC	
M13F	TGTA AACGACGGCCAGT	Sequencing primers for <i>TaSnRK2.4</i>
M13R	CAGGAAACAGCTATGACC	
G-SeqF1	GGGGTCATCCTGGTCTTC	
G-SeqF2	ATAACTGGCTTCATTTGAT	
G-SeqF3	ACATCTTACCTCGACAAACC	
G-SeqF4	ATACTCACGGCGGGATA	
G-SeqF5	ACAGATCAACTGCTGAAAGAAA	
AGF	CATGAGTAAAACCTGCATACTATATTCTC	A genomic fragment amplification
AGR	TCCGCGGCTCCGGC	
AG- <i>HpaII</i> -F	CAAACCTACCTTTCCATCATACTACGCC	Marker 2.4AM1 development for SNP-1,780 (T/C)
AG- <i>HpaII</i> -R	ATATGCGATTTCCGGCTACTCCAAGTTG	
GF	GCAGAAGTTCACGATAGGCC	B genomic fragment amplification
BGR	CATCTTACATATGGGACGGAG	
BG- <i>BmlI</i> -F	TTCTTGCGTTATTGAAGTTTCAGTGTGCT	Marker 2.4BM1 development for SNP-3,318 (T/C)
BG- <i>BmlI</i> -R	TTTACGGGAATGGAGAAGCTGTAGCA	
BG-InDel-F	AACCTCCTTGACCCTCTGTGCTG	Marker 2.4BM2 development for InDel-4,325 (15 bp/-)
BG-InDel-R	CTTCTTTCTCGGAGCAGACTTCA	
Y2H-AF	<u>CGGAATTC</u> CTGAAGAACCTGCCAAGGGA ( <i>EcoRI</i> cut site underlined)	BD vector construction for <i>TaSnRK2.4-C</i>
Y2H-AR	<u>CGGGATC</u> CTCATGATATGCGTAGCGAGCT ( <i>BamHI</i> cut site underlined)	
nLUC-AF	<u>CACGGGGACGAGCTCGGTACC</u> ATGCTGAAGAACCTGCCAAGGGA (overlapping sequence underlined)	nLUC vector construction for <i>TaSnRK2.4-C</i>
nLUC-AR	<u>ACGCGTACGAGATCTGGTCGACT</u> GATATGCGTAGCGAGCTCATGC (overlapping sequence underlined)	
cLUC-LTP3F	<u>ACGCGTCCCGGGCGGTACC</u> ATGGCTCGTCTCAACAGCAAGGCT (overlapping sequence underlined)	cLUC vector construction for <i>TaLTP3</i>
cLUC-LTP3R	<u>GTTGCTGCAGGTCGACCT</u> AGTTGACATTGTTGCAGTTGGT (overlapping sequence underlined)	
NT-F	GCACGATCCCCGTGGGA	Chromosomal location for <i>TaSnRK2.4</i>
GR	CCAACCTACCGACCCAACGAAC	



**Appendix C** Chromosome location of *TaSnRK2.4s*. NT, Nulli-tetrasomic lines of Chinese Spring; CS, Chinese Spring; H<sub>2</sub>O, negative control; M, pBR322 DNA/*Msp*I ladder.

**Appendix D** Variant combinations for *TaSnRK2.4-3B*

No.	1977	2064	2389	2539	2647	2834	2892	2897	2980	3318	3477	4084	4325
1	Del	C	A	C	G	A	A	G	T	C	A	T	In
2	Del	C	A	C	G	A	A	G	T	C	A	T	Del
3	In	T	G	T	A	G	C	A	A	T	C	C	In
4	In	T	G	T	A	G	C	A	A	T	C	C	Del



**Appendix E** Functional marker development for *TaSnRK2.4-3A*. A dCAPS marker 2.4AM1 was developed based on SNP-1780 nt (T/C) using restriction endonuclease *Hpa*II, which cleaved only the sequence with SNP-1,780 C. M, 100 bp DNA ladder.

TaSnRK2.4-3A	MEKYEAVRDIGSGNFGVARLMRNRETRELVAVKCIERGHRIDENVYREIINHRSLRHPNIIRFKEVVLTPTNL	73
TaSnRK2.4-3B	MEKYEAVRDIGSGNFGVARLMRNRETRELVAVKCIERGHRIDENVYREIINHRSLRHPNIIRFKEVVLTPTNL	73
TaSnRK2.4-3D	MEKYEAVRDIGSGNFGVARLMRNRETRELVAVKCIERGHRIDENVYREIINHRSLRHPNIIRFKEVVLTPTNL	73
TaSnRK2.4-3A	MIVMEFAAGGELFERICDRGRFSEDEARYFFQQLICGVSYCHHMQICHRDLKLENVLLDGSAAPRLKICDFGY	146
TaSnRK2.4-3B	MIVMEFAAGGELFERICDRGRFSEDEARYFFQQLICGVSYCHHMQICHRDLKLENVLLDGSAAPRLKICDFGY	146
TaSnRK2.4-3D	MIVMEFAAGGELFERICDRGRFSEDEARYFFQQLICGVSYCHHMQICHRDLKLENVLLDGSAAPRLKICDFGY	146
TaSnRK2.4-3A	SKSSVLHSRPKSAVGTPAYIAPEVLSRREYDGLADVWSCGVTLVYVMLVGGYPFEDQDDPKNIRKTIQRIMSV	219
TaSnRK2.4-3B	SKSSVLHSRPKSAVGTPAYIAPEVLSRREYDGLADVWSCGVTLVYVMLVGGYPFEDQDDPKNIRKTIQRIMSV	219
TaSnRK2.4-3D	SKSSVLHSRPKSAVGTPAYIAPEVLSRREYDGLADVWSCGVTLVYVMLVGGYPFEDQDDPKNIRKTIQRIMSV	219
TaSnRK2.4-3A	QYTI PDHVHISMPCRQLMARIFVNVPSKRITMREIKSHPWFLKNLPREL TETAQAMYFRRDNAVPSFSEQTSE	292
TaSnRK2.4-3B	QYTI PDHVHISMPCRQLMARIFVNVPSKRITMREIKSHPWFLKNLPREL TETAQAMYFRRDNAVPSFSEQTSE	292
TaSnRK2.4-3D	QYTI PDHVHISMPCRQLMARIFVNVPSKRITMREIKSHPWFLKNLPREL TETAQAMYFRRDNAVPSFSEQTSE	292
TaSnRK2.4-3A	EIMKIVQEARTMPKSSRPSYGWGDEGSDDEEKEEEEEERPEVVEEEEEDEYDKRVKEVHASGELRMSSLRIS	363
TaSnRK2.4-3B	EIMKIVQEARTMPKSSRPSYGWGDEGSDDEEKEEEEEERPEVVEEEEEDEYDKRVKEVHASGELRMSSLRIS	363
TaSnRK2.4-3D	EIMKIVQEARTMPKSSRPSYGWGDEGSDDEEKEEEEEERPEVVEEEEEDEYDKRVKEVHASGELRMSSLRIS	363

**Appendix F** Amino acid alignment of TaSnRK2.4s. The numbers on the right indicate the amino acid position. Identical amino acids are shown with black background.

### Appendix G Candidate genes screened by yeast two-hybrid assays

Contig	Number of clones	Blast hit	Annotation
1	5	AY226580.1	Lipid transfer protein 3 (LTP3)
2	3	XM_020317866.1	Phosphate starvation response 1 protein (PHR1)
3	3	XM_020317884.1	CBS domain-containing protein CBSX3
4	1	AY914051.1	Leucine zipper protein (zip1)
5	1	XM_020295641.1	Late embryogenesis abundant protein Lea14-A-like
6	3	AY589586.1	Endotransglucosylase/hydrolase XTH2
7	1	XM_020321719.1	Sucrose 1-fructosyltransferase-like
8	2	XM_020343752.1	DnaJ protein A6
9	1	XM_020293067.1	Chaperone protein dnaJ 16-like
10	1	XM_020342661.1	DEAD-box ATP-dependent RNA helicase 15
11	1	XM_020306191.1	Cyclin-B1-2-like
12	1	KC521451.1	Copper transporter (CT1-5A) gene
13	1	XM_020342975.1	40S ribosomal protein S30
14	1	XM_020334995.1	40S ribosomal protein S25-2
15	2	XM_020315926.1	40S ribosomal protein Sa-2-like
16	2	XM_020312228.1	Protein TIFY 10b-like
17	1	XM_020328819.1	Mitochondrial outer membrane porin
18	1	XM_020294557.1	BTB/POZ domain-containing protein POB1-like
19	1	XM_020306959.1	Ricin B-like lectin R40C1
20	1	XM_020297963.1	T-complex protein 1 subunit epsilon
21	3	XM_020310019.1	Coatomer subunit delta-1
22	3	XM_020329301.1	Glucose-6-phosphate 1-epimerase

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23	1	XM_020309683.1	Glutathione S-transferase TCHQD
24	1	XM_020338660.1	Proteinase inhibitor 3-like
25	1	XM_020310427.1	Protease aleurain
26	1	XM_020333520.1	Epoxide hydrolase A
27	1	XM_020331189.1	Oxygen-evolving enhancer protein 1
28	1	XM_020298855.1	Transcription factor ABORTED MICROSPORES-like
29	1	XM_020318228.1	Elongation factor 1-alpha
30	1	XM_020324646.1	Elongation factor G-2
31	1	XM_020314756.1	Serine/arginine-rich SC35-like splicing factor SCL28
32	1	XM_020336921.1	Obg-like ATPase 1
33	1	XM_020336063.1	GDP-mannose 3,5-epimerase 2
34	1	XM_020299402.1	Chloroplastic protein SPA
35	1	XM_020307408.1	Allene oxide cyclase, chloroplastic-like
36	1	XM_020338108.1	Allene oxide synthase 1, chloroplastic
37	1	XM_020330960.1	Chlorophyllase-2, chloroplastic-like

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