

Table S1. Sources, primer sequences, restriction enzymes, annealing temperatures and expected product sizes of the markers in the *Sr60* maps.

Marker	Source gene	Maker type	Forward primer (5'-3')	Reverse primer (5'-3')	Rest. Enz.	Ann. T(°C)	Size(bp) Sus.
Markers in PI 306540 × G3116 population							
<i>puroindoline-b</i> (Grain softness)	AJ242715	CAPS ^a	CCTGATAAACCCAAACGGA	TAGAATCGCAGCGACACTGT	<i>Bam</i> HI	53	761
<i>CJ660676</i> (<i>IWB48091</i>)	<i>Bradi4g00430</i>	CAPS	TGAGGCTTGACATCGACAGT	CAAAGCTGCATCTCCAAGGG	<i>Acc</i> I	55	680
<i>CJ899131</i>	<i>Bradi4g00460</i>	CAPS	ACGACCACGAGGAGGTTACT	CGATGTGCTTGCCATTCTT	<i>Sal</i> I	53	867
<i>CA731405</i>	<i>Bradi4g00480</i>	CAPS	CGAGAACTTCCCCGACCGC	TCAGGAACCCAGCAACAGTAACG	<i>Sty</i> I	56	1042
<i>DK722976</i>	<i>Bradi4g00500</i>	CAPS	ACCGAAGACGATGGAAGAG	TGGATCAATACCCAGAGAG	<i>Hha</i> I	53	513
<i>CA501332</i>	<i>Bradi4g00520</i>	CAPS	GTGGACTTCGGTGGCTTGC	CGTCAGACATAAATGGAACAGAGGA	<i>Ava</i> II	56	450
<i>GH724575</i>	<i>Bradi4g00530</i>	CAPS	AGAAGAAGGACACCAGGAT	AACACCGTTAGTTTTTTGAA	<i>Bsa</i> AI	53	416
<i>CF133295</i> (<i>LRRK123.1</i>)	<i>Bradi4g00550</i>	CAPS	TCGGCGACCTCACTCACCT	AGTTTGCTTGTCATTTTCTCAT	<i>Ple</i> I	55	916
<i>CJ942731</i>	<i>Bradi4g00640</i>	dCAPS	AGAGTAAAGTCATAACTAGGCCTGCA	ACCGACGATGCTAAAAGTCTAACA	<i>Pst</i> I	57	170
<i>CJ884584</i>	<i>Bradi4g00660</i>	CAPS	ATGTGGCATTACTATCGC	CCCTGTTACCCTCACTCA	<i>Nci</i> I	52	753
<i>FD47316</i> (<i>IWB40642</i>)	<i>Bradi2g27230</i>	CAPS	TTCATTGCTGGCACCCTTG	ACTTTGCACGTCCTCCCTTTC	<i>Sap</i> I	55	1383
<i>CJ559150</i>	<i>Bradi4g00800</i>	CAPS	GCGGGAATGGACATAAGGAC	AGCACCAGCAATACGGAA	<i>Aci</i> I	52	1160
<i>CJ671260</i>	<i>Bradi4g00910</i>	CAPS	CAAATGCGATTGTGAATGTGA	GGTAAACGAGCAGGGAGGAT	<i>Spe</i> I	52	492
<i>CJ889659</i>	<i>Bradi4g01250</i>	CAPS	TTATTCTGCGTATTTGGTGG	ACCGTATATCTCGTTTGCCT	<i>Tsp45</i> I	55	525
<i>gwm154</i>		SSR	TCACAGAGAGAGAGGGAGGG	ATGTGTACATGTTGCCTGCA		55	
<i>gwm415</i>		SSR	GATCTCCCATGTCCGCC	CGACAGTCGTCACCTGCCTA		55	
<i>gwm156</i>		SSR	CCAACCGTGTATTAGTCATTC	CAATGCAGGCCCTCCTAAC		60	
<i>gwm186</i>		SSR	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG		60	
Markers in PI 306540 × PI 272557 population							
<i>DK722976</i>	<i>Bradi4g00500</i>	CAPS	ACCGAAGACGATGGAAGAG	TGGATCAATACCCAGAGAG	<i>Hha</i> I	53	513
<i>CA501332</i>	<i>Bradi4g00520</i>	CAPS	GTGGACTTCGGTGGCTTGC	CGTCAGACATAAATGGAACAGAGGA	<i>Bsi</i> EI	56	450
<i>GH724575</i>	<i>Bradi4g00530</i>	CAPS	AGAAGAAGGACACCAGGAT	AACACCGTTAGTTTTTTGAA	<i>Bsa</i> AI	53	416
<i>CF133295</i> (<i>LRRK123.1</i>)	<i>Bradi4g00550</i>	CAPS	TCGGCGACCTCACTCACCT	AGTTTGCTTGTCATTTTCTCAT	<i>Ple</i> I	55	916
<i>CJ942731</i>	<i>Bradi4g00640</i>	dCAPS	AGAGTAAAGTCATAACTAGGCCTGCA	ACCGACGATGCTAAAAGTCTAACA	<i>Pst</i> I	57	170
<i>FD47316</i> (<i>IWB40642</i>)	<i>Bradi2g27230</i>	CAPS	TTCATTGCTGGCACCCTTG	ACTTTGCACGTCCTCCCTTTC	<i>Sap</i> I	55	1383

^a Cleaved amplified polymorphic sequence. ^b Derived cleaved amplified polymorphic sequence.

Table S2. Candidate genes of *Sr60* within the colinear regions in *B. distahyon* and the Chinese Spring wheat reference genome.

<i>Brachypodium</i> ^a	Annotation (putative function)	Wheat marker	Wheat gene ^b
<i>Bradi4g00530</i>	Nitrate transporter (NRT1)	<i>GH724575</i>	<i>TraesCS5A01G004400</i>
<i>Bradi4g00540</i>	Cytochrome P450/oxidoreductase		
<i>Bradi4g00550</i>	Leucine-rich repeat protein kinase	<i>CF133295 (LRRK123.1)</i>	<i>TraesCS5A01G005300</i>
<i>Bradi4g00560</i>	Hemiassterlin resistant protein		
<i>Bradi4g00565</i>	NO		
<i>Bradi4g00570</i>	Domain of unknown function		
<i>Bradi4g00580</i>	Calcineurin-like phosphoesterase		
<i>Bradi4g00590</i>	Calcineurin-like phosphoesterase		
<i>Bradi4g00600</i>	NBS-LRR protein		
<i>Bradi4g00610</i>	NBS-LRR protein		
<i>Bradi4g00620</i>	NBS-LRR protein		
<i>Bradi4g00630</i>	NO		
<i>Bradi4g00640</i>	Nitric-oxide synthase (NADPH)	<i>CJ942731</i>	<i>TraesCS5A01G006100</i>

^a *Brachypodium* genome: <https://phytozome.jgi.doe.gov/pz/portal.html>;

^b Wheat reference genome of Chinese Spring: <https://wheat-urgi.versailles.inra.fr/Seq-Repository/BLAST>.

Table S3. Polymorphisms between *SrTm5* (PI 306540) and six previously reported resistant haplotypes of *Sr22*. We showed unique amino acid in *SrTm5* that are polymorphic relative to the other six resistant haplotypes of *Sr22*.

Position ^a (protein level)	<i>Sr22</i> ^b (6 R haplotypes)	<i>SrTm5</i> (PI306540)	Protein change
556	T	S	T556S
580	D	G	D580G
723	L	R	L723R
728	D	H	D728H
751	F	C	F751C
774	L	F	L774F
776	D	N	D776N
777	D/N/Y	Q	D/N/Y777Q
798	T	S	T798S
800	P/Y	S	P/Y800S
822	K/R	S	K/R822S
824	E	R	E824R
825	A/L	V	A/L825V
827	N/R	H	N/R827H
848	L	F	L848F
850	N/H	D	N/H850D
853	E	G	E853G
878	S/G	D	S/G878D
915	N/Y	F	N/Y915F
918	H	D	H918D
921	Q	R	Q921R
926	G	D	G926D
928	T	A	T928A

^a Position is based on protein of *SrTm5* because there are three amino acids insertion or deletion in the six reported resistant haplotypes of *Sr22*;

^b The six reported resistant haplotypes of *Sr22* could have different amino acids at the same position.