SUPPLEMENTARY MATERIALS

To the article by O.A. Baranova, S.N. Sibikeev, A.E. Druzhin "Molecular identification of the stem rust resistance genes in the introgression lines of spring bread wheat"

Supplementary Material 1

Molecular markers used to identify *Sr* genes

Sr gene	Marker	Sequence (5′⟶3′)	References
Sr2	csSr2	F – CAA GGG TTG CTA GGA TTG GAA AAC R – AGA TAA CTC TTA TGA TCT TAC ATT TTT CTG	Mago et al., 2011
Sr24/Lr24	Sr24#12	F – CACCCGTGACATGCTCGTA R – AACAGGAAATGAGCAACGATGT	Mago et al., 2005
	Sr24#50	F – CCCAGCATCGGTGAAAGAA R – ATGCGGAGCCTTCACATTTT	
Sr25/Lr19	Gb	F – CAT CCT TGG GGA CCT C R – CCA GCT CGC ATA CAT CCA	Prins et al., 2001
Sr26	Sr26#43	F – AAT CGT CCA CAT TGG CTT CT R – CGC AAC AAA ATC ATG CAC TA	Mago et al., 2005
Sr28	wPt-7004-PCR	F – CTC CCA CCA AAA CAG CCT AC R – AGA TGC GAA TGG GCA GTT AG	Rouse et al., 2012
	Xwmc332	F – CAT TTA CAA AGC GCA TGA AGC C R – GAA AAC TTT GGG AAC AAG AGC A′	
Sr31/Lr26	Scm9	F – TGACAACCCCCTTTCCCTCGT R – TCATCGACGCTAAGGAGGACCC	Weng et al., 2007
Sr32	csSr32#2	F – CAA ATG AAT AGA AAA ACC CGT GCT′ R – CAC ACA CTG TTT TCC GTT GC	Mago et al., 2013
Sr36	Xstm773-2	F – ATGGTTTGTTGTGTTGTGTGTAGG R – AAACGCCCCAACCACCTCTCTC	Tsilo et al., 2008
Sr38/Lr37	VENTRIUP-LN2	VENTRIUP′ – AGG GGC TAC TGA CCA AGG CT LN2 – TGC AGC TAC AGC AGT ATG TAC ACA AAA′	Helguera et al., 2003
Sr39/Lr35	Sr39#22	F – AGAGAAGATAAGCAGTAAACATG R – TGCTGTCATGAGAGGAACTCTG	Mago et al., 2009
Sr57/Lr34	csLV34	F – 5′-GTT GGT TAA GAC TGG TGA TGG-3′ R – 5′-TGC TTG CTA TTG CTG AAT AGT-3′	Lagudah et al., 2006

References

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Mago R., Verlin D., Zhang P., Bansal U., Bariana H., Jin Y., Ellis J., Hoxha S., Dundas I. Development of wheat – *Aegilops speltoides* recombinants and simple PCR-based markers for *Sr32* and a new stem rust resistance gene on the 2S#1 chromosome. Theor. Appl. Genet. 2013;126:2943-2955.

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Supplementary Material 2

PCR conditions and compositions of reaction mixtures

Gene (marker)	Composition of reaction mixture	PCR conditions	References
Sr2 (csSr2)	*20 µl of reaction mixture: bidistilled H_2O – 11.9 µl dNTPs mixture (25 mM) – 0.4 µl primer R (10–15 pmol) – 1.6 µl primer F (10–15 pmol) – 1.6 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 0.45 µl Taq-polymerase (5 U) – 0.064 µl genomic DNA – 2 µl	95 °C – 2 min, 30 cycles (95 °C – 30 sec, 60 °C – 40 sec, 72 °C – 50 sec), 72 °C – 10 min Restriction with BspH1: bidistilled H_2O – 12 µl buffer – 2 µl DNA (amplification) – 5 µl Restriction enzyme BspH1 – 0.5 µl Incubate for 1 hour at 37 °C	Mago et al., 2011
Sr24/Lr24 (Sr24#12)	*20 µl of reaction mixture: bidistilled H_2O – 12.64 µl dNTPs mixture (25 mM) – 0.16 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 1.2 µl Taq-polymerase (5 U) – 1 µl genomic DNA – 2 µl	94 °C – 3 min, 7 cycles (94 °C – 30 sec, 65–59 °C (1 °C down each cycle) – 30 sec, 72 °C – 40 sec), 30 cycles (94 °C – 30 sec, 58 °C – 30 sec, 72 °C – 40 sec), 72 °C – 10 min	Mago et al., 2005
Sr24/Lr24 (Sr24#50)	*20 µl of reaction mixture: bidistilled H_2O – 12.64 µl dNTPs mixture (25 mM) – 0.16 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 1.2 µl Taq-polymerase (5 U) – 1 µl genomic DNA – 2 µl	94 °C – 3 min, 30 cycles (94 °C – 30 sec, 63 °C – 30 sec, 72 °C – 40 sec), 72 °C – 10 min	Mago et al., 2005
Sr25/Lr19 (Gb)	*20 µl of reaction mixture: bidistilled H_2O – 11.25 µl dNTPs mixture (25 mM) – 0.4 µl primer R (10–15 pmol) – 2 µl primer F (10–15 pmol) – 2 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 0.6 µl Taq-polymerase (5 U) – 0.08 µl genomic DNA – 2 µl	94 °C – 4 min, 30 cycles (94 °C – 30 sec, 60 °C – 30 sec, 72 °C – 30 sec), 72 °C – 5 min	Prins et al., 2001
Sr26 (Sr26#43)	*20 µl of reaction mixture: bidistilled H_2O – 13.9 µl dNTPs mixture (25 mM) – 0.4 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 1.2 µl Taq-polymerase (5 U) – 0.5 µl genomic DNA – 1 µl	94 °C – 3 min, 35 cycles (94 °C – 1 min, 60 °C – 1 min, 72 °C – 2 min), 72 °C – 10 min	Mago et al., 2005
Sr28 (wPt-7004-pcr wmc 332)	*20 µl of reaction mixture: bidistilled H_2O – 9.52 µl dNTPs mixture (25 mM) – 1 µl primer R (10–15 pmol) – 2 µl primer F (10–15 pmol) – 2 µl 10× PCR buffer – 2.4 µl $MgCl_2$ (50 mM) – 0.72 µl Taq-polymerase (5 U) – 0.36 µl genomic DNA – 2 µl	94 °C – 7 min, 35 cycles (94 °C – 1 min, 60 °C – 1 min, 72 °C – 1 min), 72 °C – 5 min	Rouse et al., 2012

Table (end)

Gene (marker)	Composition of reaction mixture	PCR conditions	References
Sr31/Lr26 (scm9)	*20 µl of reaction mixture: bidistilled H_2O – 12.4 µl dNTPs mixture (25 mM) – 1.6 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 0.8 µl Taq-polymerase (5 U) – 0.2 µl genomic DNA – 2 µl	95 °C – 3 min, 30 cycles (94 °C – 45 sec, 60 °C – 1 min, 72 °C – 90 sec), 75 °C – 1 min	Weng et al., 2007
Sr32 (csSr32#2)	*20 µl of reaction mixture: bidistilled H_2O – 12.4 µl dNTPs mixture (25 mM) – 1.6 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 0.8 µl Taq-polymerase (5 U) – 0.2 µl genomic DNA – 2 µl	95 °C – 2 min, 30 cycles (95 °C – 30 sec, 60 °C – 40 sec, 72 °C – 50 sec), 72 °C – 5 min	Mago et al., 2013
Sr36 (STM773-2)	*20 µl of reaction mixture: bidistilled H_2O – 12.4 µl dNTPs mixture (25 mM) – 1.6 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 0.8 µl Taq-polymerase (5 U) – 0.2 µl genomic DNA – 2 µl	94 °C – 10 min, 7 cycles (92 °C – 1 min, 64 °C – 1 min, 72 °C – 1 min), 5 cycles (92 °C – 1 min, 57 °C – 1 min, 72 °C – 1 min), 25 cycles (92 °C – 1 min, 55 °C – 1 min, 72 °C – 1 min), 72 °C – 10 min	Tsilo et al., 2008
Sr38/Lr37 (VENTRIUP-LN2)	*20 µl of reaction mixture: bidistilled H_2O – 12.4 µl dNTPs mixture (25 mM) – 1.6 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 0.8 µl Taq-polymerase (5 U) – 0.2 µl genomic DNA – 2 µl	94 °C – 45 sec, 30 cycles (94 °C – 45 sec, 65 °C – 30 sec, 72 °C – 60 sec), 72 °C – 7 min	Helguera et al., 2003
Sr39/Lr35 (Sr39#22)	*20 µl of reaction mixture: bidistilled H_2O – 8.8 µl dNTPs mixture (25 mM) – 0.4 µl primer R (10–15 pmol) – 0.5 µl primer F (10–15 pmol) – 0.5 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 1.2 µl Taq-polymerase (5 U) – 1 µl genomic DNA – 2 µl	94 °C – 5 min, 30 cycles (92 °C – 30 sec, 58 °C – 30 sec, 72 °C – 40 sec), 72 °C – 10 min	Mago et al., 2009
Sr57/Lr34 (csLV34)	*20 µl of reaction mixture: bidistilled H_2O – 12.54 µl dNTPs mixture (25 mM) – 0.16 µl primer R (10–15 pmol) – 0.8 µl primer F (10–15 pmol) – 0.8 µl 10× PCR buffer – 2 µl $MgCl_2$ (50 mM) – 1.2 µl Taq-polymerase (5 U) – 0.5 µl genomic DNA – 2 µl	94 °C – 5 min, 40 cycles (94 °C – 45 sec, 55 °C – 30 sec, 72 °C – 60 sec), 72 °C – 7 min	Lagudah et al., 2006

* With modifications.

Supplementary Material 3

The wheat lines and cultivars carrying known genes resistant to stem rust

Gene	Sample	Gene	Sample
Sr2-complex	Pavon76 Arthur Oasis	*Sr31*	Avrora
		Sr32	C77.19.SR32 CnsSr32AS
Sr2+Sr23	Buck Buck	*Sr36*	W2691SR36TT1 Sr36(CI12632)/8*LMPG Cook
Sr24	BTSR24AG	*Sr38*	RL6081
Sr25	LC-SR25-ARS	*Sr39*	RL6082
Sr26	Sr26/9*LMPG Eagle	*Sr57(Lr34)*	line Th+Lr34 Glenlea

Supplementary Material 4

North American differential set: *Sr5*, *Sr21*, *Sr9e*, *Sr7b*, *Sr11*, *Sr6*, *Sr8a*, *Sr9g*, *Sr36*, *Sr9b*, *Sr30*, *Sr17*, *Sr9a*, *Sr9b*, *Sr10*, *SrTmp*, *Sr24*, *Sr31*, *Sr38*, *SrMcN*. Additional isogenic lines (lines with *Sr* genes) – *Sr2-complex*, *Sr8b*, *Sr13*, *Sr15*, *Sr20*, *Sr22*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr29*, *Sr32*, *Sr35*, *Sr37*, *Sr39*, *Sr40*, *Sr44*, *Sr26+9g*, *Sr31+ Sr36*, *Sr31+ Sr24*, *Sr24+ Sr36*.

Wheat lines and cultivars with known *Sr* genes used for virulence analysis of the *P. graminis* f. sp. *tritici* populations

Gene	Line/cultivar	Gene	Line/cultivar	Gene	Line/cultivar
Sr2-complex	Pavon76	*Sr26*	SR26/9*LMPG	*Sr39*	RL6082
Sr8b	Barleta Benventuro	*Sr27*	Coroong	*Sr40*	RL6088
Sr13	W2691Sr13	*Sr28*	W2691SR28KT	*Sr44*	Taf-2
Sr15	W2691'2/Norka-Sr15	*Sr29*	Pusa/EDCH-Sr29	*Sr26+Sr9g*	EAGL-SR29.SR9G
Sr20	LC-Sr20-MQ	*Sr32*	CnsSr32AS	*Sr31+Sr36*	PI675465TR13AZSD
Sr22	SWSR22TB	*Sr35*	Mq(2)5*G2919	*Sr31+Sr24*	PI675464TR13AZSD
Sr25	LC-SR25-ARS	*Sr37*	W2691SrTt-2	*Sr24+Sr36*	PI675466TR13AZSD

Supplementary Material 5

Types of reaction to infection (IT) with *P. graminis* f. sp. *tritici* according to the Stackman scale (Stackman et al., 1962): IT 0 = no symptoms, IT; = necrotic flecks, IT 1 = minute pustules barely sporulating; IT 2 = necrotic halo surrounding small pustules; IT 3 = chlorotic halo; IT 4 = well-formed pustules with no associated chlorosis or necrosis. Designations of "+" and "–" were added to indicate larger and smaller size of uredinia pustules; "X" – heterogeneous type of reaction – on one plant different types of reaction.

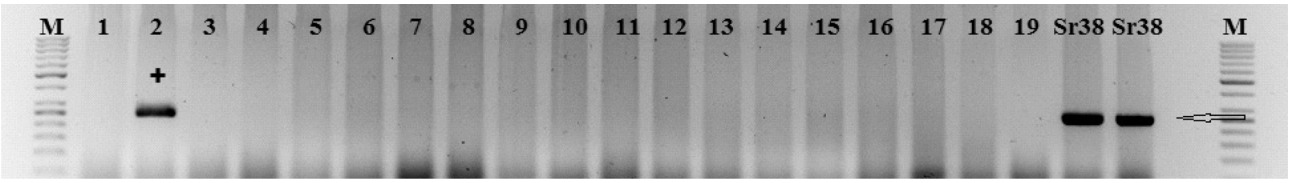
Supplementary Material 6

Results of phytopathological analysis and identification of *Sr* genes (resistant lines)

Pedigree	Resistance to *P. graminis* (type of reaction)				Identified genes
	Saratov (field)	Laboratory evaluation to *P. graminis* populations at the seedling stage			
		Derbent	Lysogorsk (from cultivar Favorit)	Omsk	
L2032*6/Curinda87	R	0;	1	1	*Sr25* + *Sr31*
Dobrynya*4/TsLr25	R	0	2–	1	*Sr25*
L503Lr19Lr26	R	2	X	2	*Sr25* + *Sr31*
L505//L503//L583/Kukushka//L505L200	10MR	0	2–	2–	*Sr25*
S55*3/T.dic-s//L2032	R	1	0;1	1	*Sr31*
L2032*5/Seri82	R	0	1–	1	*Sr25* + *Sr31*
L505*2//Croc/Ae.squar(224)//Yaco	R	0	1–	1	*Sr25* + *Sr57(Lr34)*
L505/3/Croc/Ae.squar(205)//Weaver/4/L505/5/S68	R	0	2–	1;	*Sr25* + *Sr31*
L505/3/Croc/Ae.squar(205)//Weaver/4/L505/5/L505	R	0	10;	1;	*Sr25* + *Sr31*
Bel/3/Croc/Ae.squar(205)//Weaver/4/Bel	R	1+	0;	0	*Sr31*
L12(DobrLr24)/S68//S68	R, 20MR	2+	2+	2	*Sr25*
L505*2/Prokh//Bel(L496/16)	R	1	0;	1	*Sr31*
S55*3/T.dic-s//L2032(L501/16)	R	1	0;	1	*Sr31*
Dobr/Zol.volna//DobrLr24/3/Dobrynya	R	2+	2–	2	*Sr25*
Prokh/MultiLr6R//S68/3/Dobr	20MR	0	0	1	*Sr25*
L505/S42/4/L505*3//Prokh//L505/3/S70/4/DobrLr24	R	0	0;1=	1	*Sr25* + *Sr31*
L505/L164/4/L503//Trap#1/Bow/3/L503/5/L505/6/S68	R	1	0;	1	*Sr25* + *Sr31*
Yu-V-2/L505//L503Lr26/3/L505/4/S68	R, MR	0	10;	1+	*Sr25* + *Sr31*
Croc/Ae.squar(205)//Weaver/3/L505/4/DobrLr25	R, 20MR	2	2+	2–	*Sr25*
Croc/Ae.squar(205)//Weaver/3/L505/4/Bel	R	0	1+	1;	*Sr25* + *Sr31*
Dobr*5/TcLr9//L505//L503*3/TRAP≠BOW//Prokh/S55	R	0	1=	1	*Sr25* + *Sr31*
Sar.zol/T.dic-s//S58/3/*2Bel/4/Voevoda	R	0	1–	1	*Sr25* + *Sr31*
L503Lr26/Ottan(Rl1,Rl2)//Revansh	R, 20MR	2–2+	2+	1	*Sr25*
L18(L503Lr26)/S68//Revansh	R	0	0	1;	*Sr25* + *Sr31*
Tulaykovskaya10//Agis181/S29+Agis181/S58	R	0	0	1	*Sr25* + *Sr31* + *Sr28*
ThatcherLr37*4/L503	R, 25MR	2+	1	1	*Sr25*
L503Lr26/Ottan(Rl1,Rl2)//Revansh	R	1	0	0	*Sr25* + *Sr31*
Yu-V-2/L505//L503Lr26/3/L505/4/S68	R	1	1	1	*Sr25* + *Sr31*
Milan/Prinia//*4Dobr/3/Favorit	R, 25MR	0	2+	2–	*Sr25*
Tselinnaya20/Dobr//Dobr/3/DobrLr9/4/Milan/Prinia//*4Dobr	R	0	2	X	*Sr25* + *Sr38*
Dobr*5//Milan/Prinia/3/Belyanka/4/S68	R	2–	2–	2.	*Sr25*
L503/Sr35//L503/3/L503	R	1+	2	1	*Sr25*
Satu/S70//S74/3/S74	–	0	0	0;	–
LC-SR25-ARS •	–	1,2	3–	1,2	*Sr25*
Avrora •	–	1,1–	0;1	0;1	*Sr31*
W2691SR28KT •	–	3	3	3	*Sr28*
lineTh+Lr34 •	–	3	3	3	*Sr57(Lr34)*
RL6081 •	–	3	3–	3	*Sr38*

Notes: R – resistance reaction; MR – medium resistance; • – control.

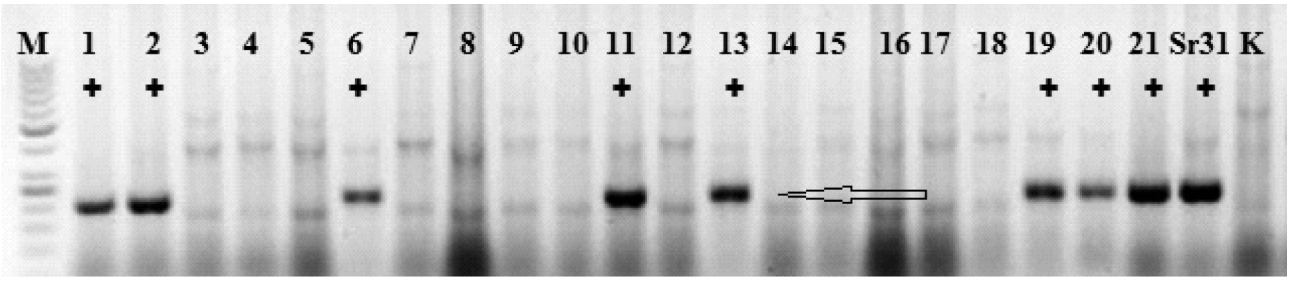
Supplementary Material 7



Identification of the gene *Sr38* using the molecular marker VENTRIUP-LN2.

M – molecular weight marker 50 bp "Fermentas", *Sr38* – positive control (line RL6081). The arrow indicates diagnostic fragment with the molecular weight of 259 bp. Amplification products were separated in 2 % agarose gel. "+" – the presence of diagnostic fragment 259 bp.

Supplementary Material 8



Identification of the gene *Sr31* using the molecular marker Scm9.

M – molecular weight marker 50 bp "Fermentas", *Sr31* – positive control (cultivar Avrora). The arrow indicates diagnostic fragment with the molecular weight of 207 bp. Amplification products were separated in 2 % agarose gel. "+" – the presence of diagnostic fragment 207 bp.