

## SUPPLEMENTARY MATERIALS

to the article O.A. Baranova, I.G. Adonina, S.N. Sibikeev  
 “Molecular cytogenetic characteristics of new spring bread wheat  
 introgressive lines resistant to stem rust”

### Supplementary Material 1. Molecular markers used in the work

Sr gene	Marker name	Primer sequence (5'→ 3')	Source of information
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
Sr1A.1R	SCM9	F – TGACAACCCCTTTCCCTCGT 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 – ATGGTTTGTGTGTGTGTGTAGG R – AAACGCCCAACCACCTCTCTC	Tsilo et al., 2008
Sr38/Lr37	VENTRIUP-LN2	F – AGGGGCTACTGACCAAGGCT R – TGCAGCTACAGCAGTATGTACACAAAA	Helguera et al., 2003
Sr39/Lr35	Sr39#22	F – AGAGAAGATAAGCAGTAAACATG R – TGCTGTCATGAGAGGAAGTCTG	Mago et al., 2009
Sr47	Xgwm501	F – GGCTATCTCTGGCGCTAAAA R – TCCACAAACAAGTAGCGCC	Faris et al., 2008
	Xgpw4043	F – ACATATGCACGCACGCAC R – CATTGACACCCCTGACTCTC	Klindworth et al., 2012
	Xgwm47	F – TTGCTACCATGCATGACCAT R – TTCACCTCGATTGAGGTCCT	

- Faris J.D., Xu S.S., Cai X., Friesen T.L., Jin Y. Molecular and cytogenetic characterization of a durum wheat–*Aegilops speltoides* chromosome translocation conferring resistance to stem rust. *Chromosome Res.* 2008;16:1097-1105. DOI 10.1007/s10577-008-1261-3
- Helguera M., Khan I.A., Kolmer J., Lijavetzky D., Zhong-qi L., Dubcovsky J. PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Sci.* 2003;43:1839-1847
- Klindworth D.L., Niu Z., Chao S., Friesen T.L., Faris J.D., Cai X., Xu S.S. Introgression and characterization of a goatgrass gene for a high level of resistance to Ug99 stem rust in tetraploid wheat. *G3: Genes Genomes Genetics.* 2012;2:665-673. DOI 10.1534/g3.112.002386
- Mago R., Zhang P., Bariana H.S., Verlin D.C., Bansal U.K., Ellis J.G., Dundas I.S. Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced *Aegilops speltoides* chromatin and simple PCR markers for marker assisted selection. *Theor. Appl. Genet.* 2009;119:1441-1450
- Mago R., Brown-Guedira G., Dreisigacker S., Breen J., Jin Y., Singh R., Appels R.S., Lagudah E.S., Ellis J., Spielmeier W. An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. *Theor. Appl. Genet.* 2011;122:735-744. DOI 10.1007/s00122-010-1482-7
- 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. DOI 10.1007/s00122-013-2184-8
- Tsilo T.J., Jin Y., Anderson J.A. Diagnostic microsatellite markers for detection of stem rustresistance gene *Sr36* in diverse genetic backgrounds of wheat. *Crop Sci.* 2008;48:253-261
- Weng Y., Azhaguvel P., Devkota R.N., Rudd J.C. PCR-based markers for detection of different sources of 1AL.1RS and 1BL.1RS wheat–rye translocations in wheat background. *Plant Breed.* 2007;126:482-486

**Supplementary Material 2.** Results of cytogenetic analysis of introgressive lines of bread wheat using FISH and GISH methods

Line	Probe combinations used	Research result
657	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	Substitution of chromosome 6D with chromosome 6A <i>T. dicoccum</i> – 6A(6D)
664	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Substitution of <i>T. aestivum</i> chromosome 2A with <i>T. dicoccum</i> chromosome 2A or translocation T2AS.2A <sup>dic</sup> L 2. Substitution of chromosome 2D with chromosome 2S <i>Ae. speltoides</i> – 2S(2D) 3. Translocation from <i>Th. ponticum</i> in the long arm of chromosome 7D
758	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Translocation from <i>T. timopheevii</i> – T2A <sup>t</sup> .2A 2. Translocation from <i>Th. ponticum</i> in the long arm of chromosome 7D
935	pSc119.2 + pAs1; GISH with rye DNA + pAs1	Chromosomal substitution 3R(3D)
960	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Substitution of <i>T. aestivum</i> chromosome 2A with <i>T. kiharae</i> chromosome 2A <sup>t</sup> – 2A <sup>t</sup> (2A) 2. Translocation T3BS.3GL 3. Chromosomal substitution 4G(4B) 4. Translocation T2D <sup>(T. aestivum)</sup> S.2D <sup>(T. kiharae)</sup> L 5. Translocation from <i>Th. ponticum</i> in the long arm of chromosome 7D
968	pSc119.2 + pAs1; GISH with rye DNA + pAs1	Chromosomal substitution 3R(3D)
971	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Substitution of <i>T. aestivum</i> chromosome 2A with <i>T. timopheevii</i> chromosome 2A <sup>t</sup> – 2A <sup>t</sup> (2A) 2. Chromosomal substitution 2G(2B) or translocation T2BS.2GL 3. Chromosomal substitution 6G(6B) 4. Translocation from <i>Th. ponticum</i> in the long arm of chromosome 7D
995/1	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Translocation from <i>T. timopheevii</i> – 2A <sup>t</sup> .2A 2. Chromosomal substitution 2G(2B) or translocation T2BS.2GL
997	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Substitution of <i>T. aestivum</i> chromosome 2A with <i>T. timopheevii</i> chromosome 2A <sup>t</sup> – 2A <sup>t</sup> (2A) 2. Chromosomal substitution 2G(2B) or translocation T2BS.2GL 3. Translocation from <i>Th. ponticum</i> in the long arm of chromosome 7D
1110	pSc119.2 + pAs1; Spelt1 + pSc119.2; Spelt52 + pSc119.2	1. Substitution of <i>T. aestivum</i> chromosome 2A with <i>T. timopheevii</i> chromosome 2A <sup>t</sup> – 2A <sup>t</sup> (2A) 2. Chromosomal substitution 2G(2B) or translocation T2BS.2GL 3. Translocation from <i>Th. ponticum</i> in the long arm of chromosome 7D

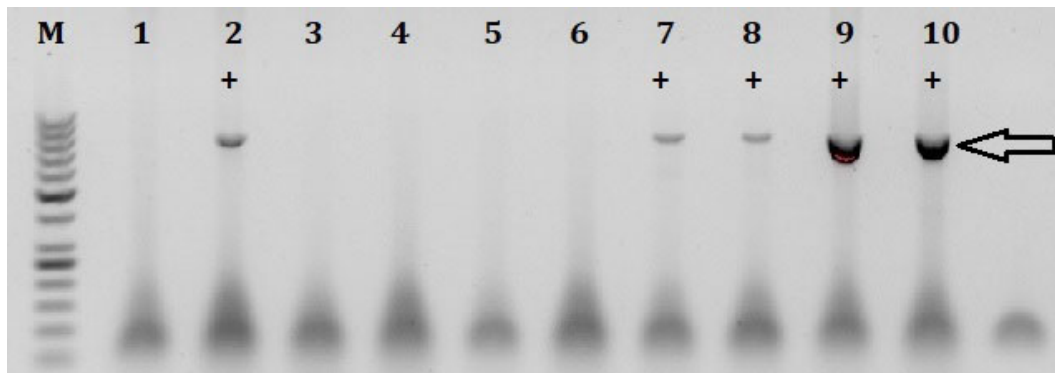
**Supplementary Material 3.** Analysis of stem rust resistance and identification of *Sr*-genes in introgressive wheat lines

Line	Pedigree	Resistance to <i>P. graminis</i> *		Identified <i>Sr</i> -genes (marker)									
		I	II	<i>Sr25</i> ** (Gb)	<i>Sr38</i> (VENTRIUP-LN2)	<i>Sr36</i> (Xstrm773-2)	<i>Sr32</i> (csSr32#2)	<i>Sr47</i> (Xgwm501)	<i>Sr47</i> (Xgpm4043) 115 bp	<i>Sr47</i> (Xgpm4043) 95 bp	<i>Sr47</i> (Xgwm47)	<i>Sr39</i> (Sr39#22)	
657	L505*2//L503/3/L528// AD <i>T.dic/Ae.spelt</i> *5S29/4/Th <i>Lr28</i>	3	0;	-	-	-	-	-	-	-	+	-	-
664	S55//Dobrynya/L164//Agr139/ L528*2//AD <i>T.dic/Ae.spelt</i> *5S29// Dobrynya	1	0;	+	-	-	-	-	-	-	+	+	+
758	L XI S29 imm/L2870	2	3	+	-	-	-	-	-	-	+	-	-
935	Satu/S70//S70	0;	1	-	-	-	-	-	-	-	-	-	-
960	S68/ <i>T.kiharae</i> //S70/3/S68	0;	3	+	-	-	+	-	-	-	-	-	-
968	Satu/S70//S74/3/S70/4/S70	0;	0;	-	-	-	+	-	-	-	-	-	-
971	S68/ <i>T.timopheevii</i> *4//Dobrynya	23-	12	+	+	-	-	+	-	-	+	-	+
995/1	S70/Pamyati Maistrenko//S68	2+	0	-	-	-	+	+	-	-	-	-	+
997	S70/Pamyati Maistrenko// Dobrynya	0	0;	+	-	+	-	+	-	-	-	-	+
1110	L VI S29 imm/L2032//L2032/3/ L2032	0;	2++	+	-	+	-	+	-	-	-	-	+

\* I – laboratory assessment of resistance to the Tatarstan population of *Pgt* from the Nadira variety 2022. II laboratory assessment of resistance to the Saratov population *Pgt* from the Voevoda variety 2022 (Baranova et al., 2023a).

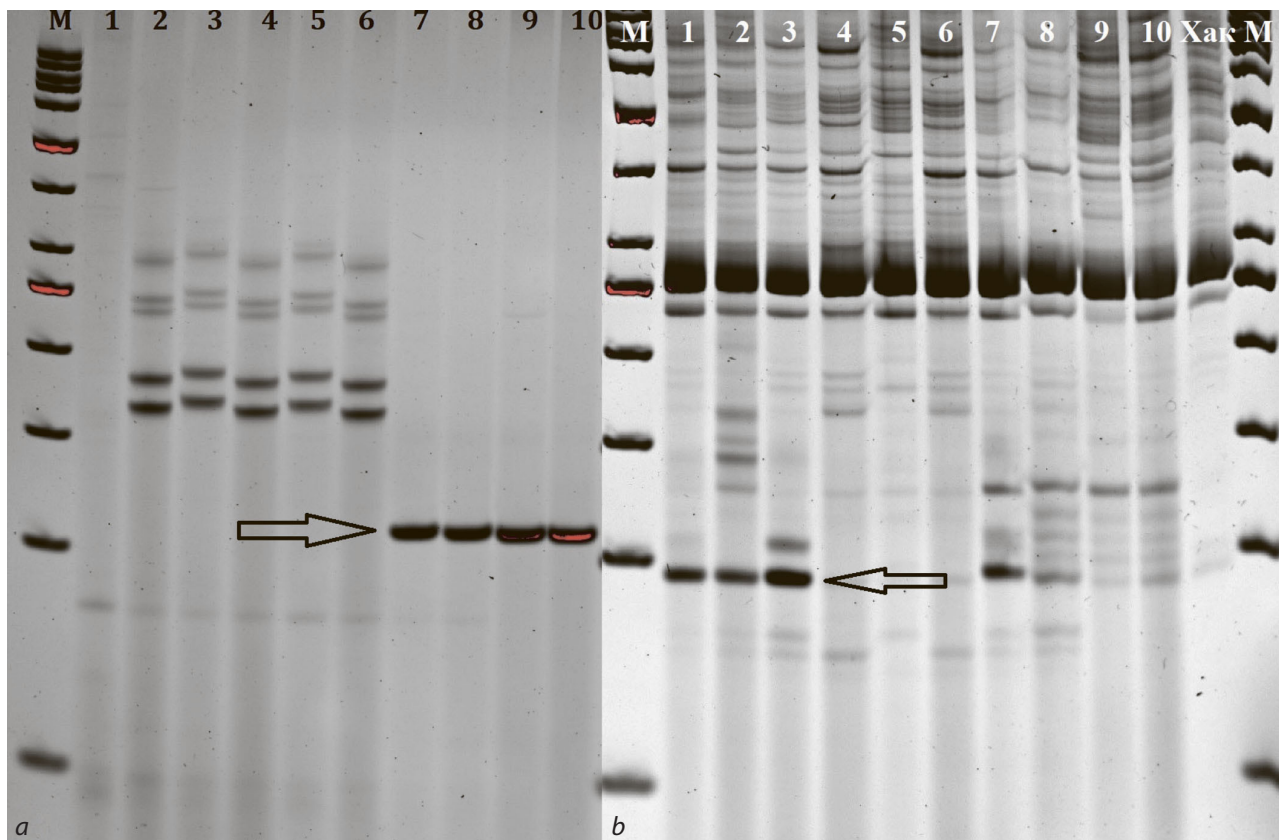
\*\* Data (Baranova et al., 2023a).

**Supplementary Material 4. Identification of the *Sr39* using the *Sr39#22* marker.**



M – molecular weight marker 50bp, 1–10 – lines No. 657, 664, 758, 935, 960, 968, 971, 995/1, 997 and 1110; the arrow indicates a diagnostic fragment of 800 bp.

**Supplementary Material 5. Identification of the *Sr47* gene using markers *Xgwm501* (a) and *Xgpw4043* (b).**



M – molecular weight marker 50 bp, 1–10 – lines No. 657, 664, 758, 935, 960, 968, 971, 995/1, 997 and 1110; arrows indicate diagnostic fragments of markers *Xgwm501* – 109 bp (a) and *Xgpw4043* – 95 bp (b).