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Article

Novel Sources of Resistance to *Stagonospora nodorum* and Role of Effector-Susceptibility Gene Interactions in Wheat of Russian Breeding

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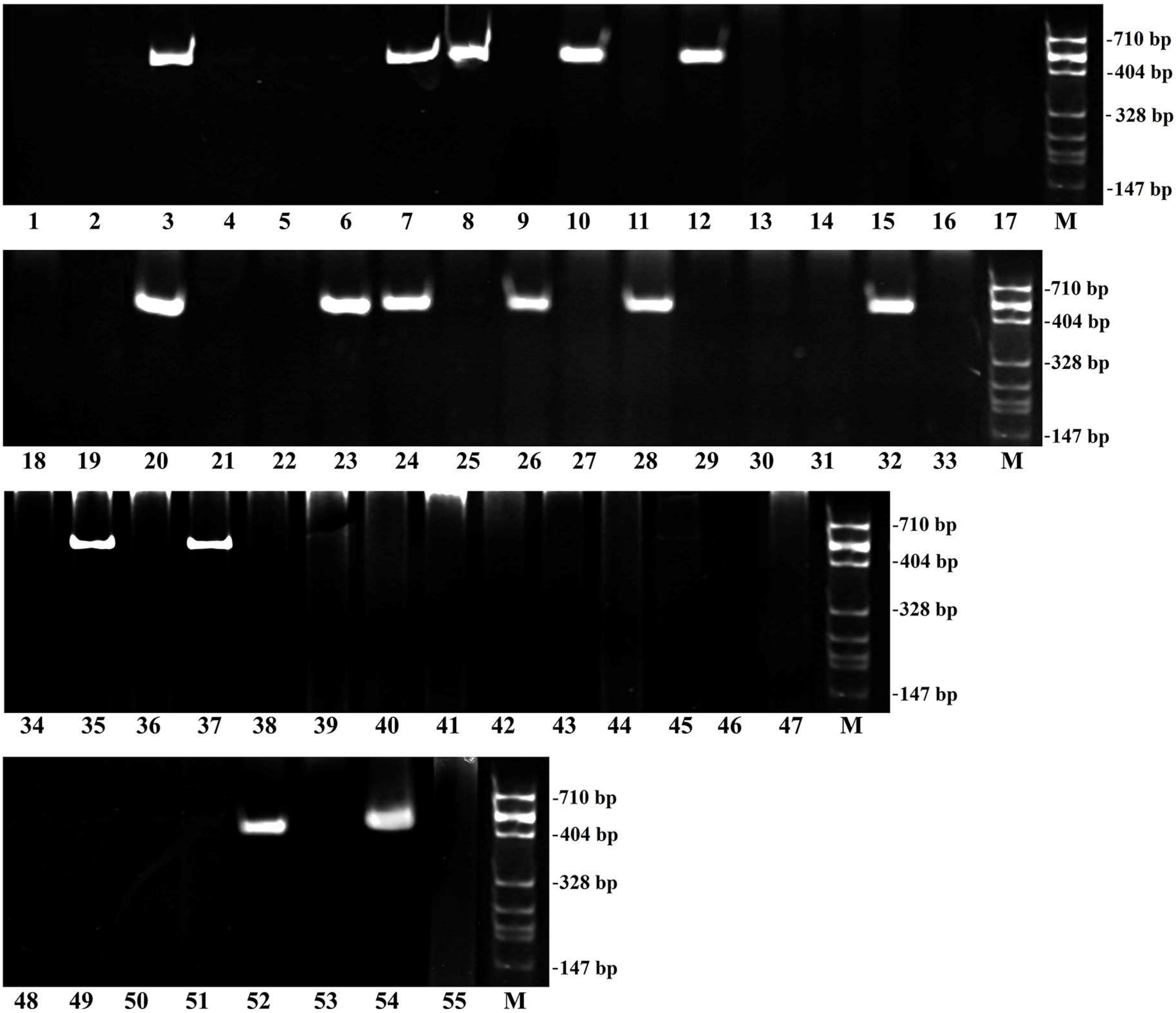
Supplementary Table S1. Primers used to identify pathogen effector genes and plant susceptibility genes from genomic DNA.

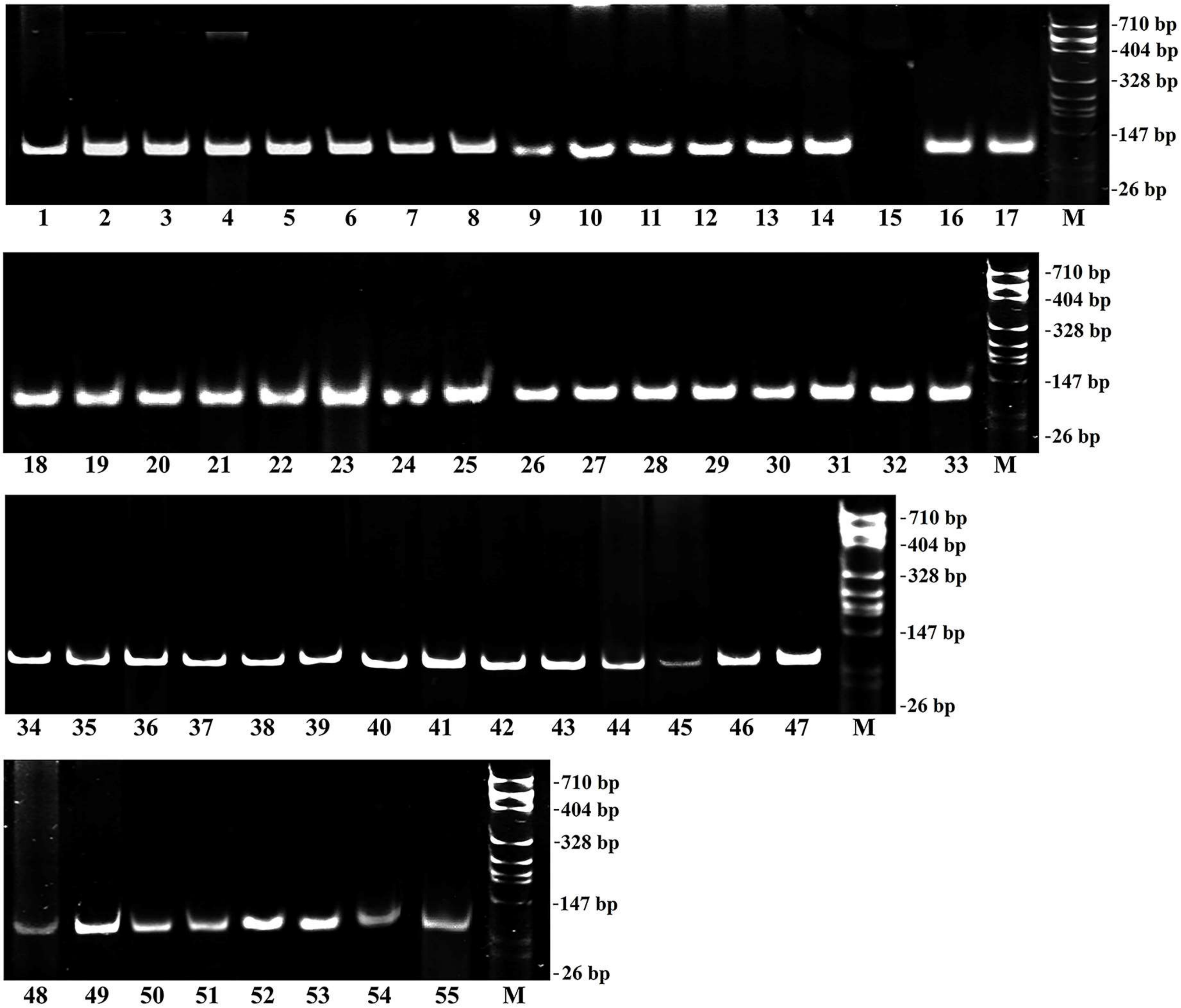
Genes	Strand	5′ to 3′ Primer Sequences	GenBank accession number
TaTsn1-2	Forward	CTCTTTGCCGGAGAGCATAC	GU259657.1
	Reverse	TCGAATCCTCAAAGCCTACC	
TaSnn1	Forward	TGCGCCAACTCAACACATAC	KP085710
	Reverse	GCCAATGGCACCCACAGC	
Xcfd20	Forward	TGATGGGAAGGTAATGGGAG	-
	Reverse	ATCCAGTTCTCGTCCAAAGC	
Xgwm234	Forward	GAGTCCTGATGTGAAGCTGTTG	-
	Reverse	CTCATTGGGGTGTGTACGTG	
SnToxA	Forward	AACGCCAATACAGTGCGAGT	JX997419
	Reverse	GCTGCATTCTCCAATTTTCACG	
SnTox1	Forward	GTACTCCCGTACGTACTCTTCT	JX997402
	Reverse	CGCTTGTTTGCCGTTCTTAC	
SnTox3	Forward	CGAGCTGATATCCCGTTTGA	FJ823644
	Reverse	GGGACAGTGACAATAGGTAAGG	
Snβ-tubulin	Forward	ACACCAGGAACAACTGCTAACAGC	S56922
	Reverse	TATGCGCGCGTGCTGCAAATTCGA	

Supplementary Table S2. PCR primers used for qRT-PCR analysis.

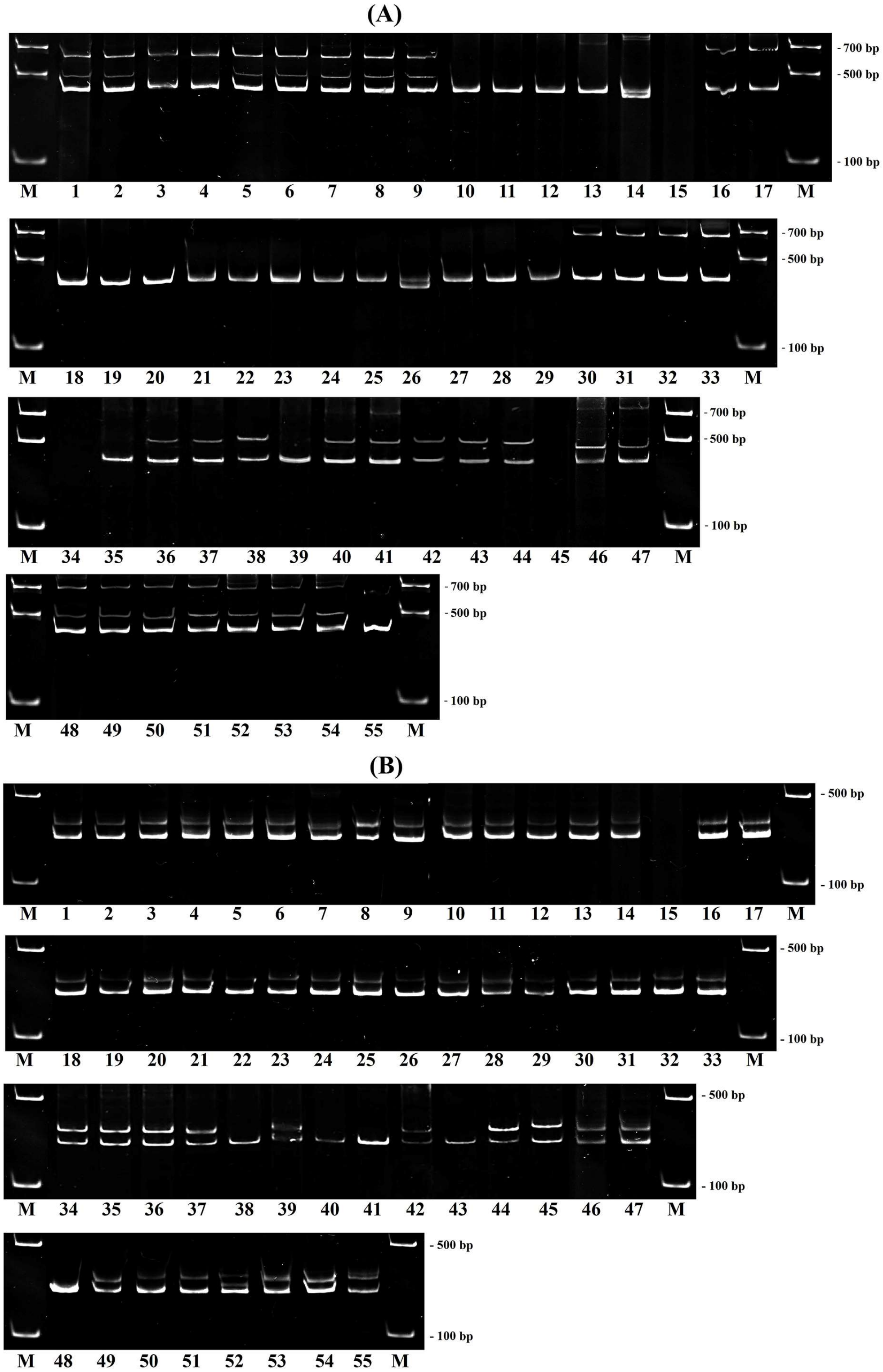
Genes	Strand	5′ to 3′ Primer Sequences	GenBank accession number
TaTsn1-1	Forward	CCTGATTGGCTTCTGGGTATTA	GU259657.1
	Reverse	GTGGTAGGTTCTTGCACTTAGA	
TaSnn1	Forward	CCTATGCTGGTCTACGAGTTTATC	KP085710
	Reverse	TTGAGGGCCACCTTGTTATT	
TaRLI	Forward	TTGAGCAACTCATGGACCAG	AY059462
	Reverse	GCTTTCCAAGGCACAAACAT	



Supplementary Figure S1. Identification of alleles of *Tsn1* gene in 55 wheat accessions by PCR. Amplified fragments with sizes of 467 bp were separated in a polyacrylamide gel. Numbers from 1 to 55 wheat accessions correspond to the numbers in Table S3. M - DNA markers. Marker sizes are indicated on the right. The presence of the amplification product indicates the existence of a dominant allele of the *Tsn1* gene; the absence of the amplification product indicates a null (recessive) *tsn1* allele.



Supplementary Figure S2. Identification of alleles of *Snn1* gene in 55 wheat accessions by PCR. Amplified fragments with sizes of 80 bp were separated in a polyacrylamide gel. Numbers from 1 to 55 wheat accessions correspond to the numbers in Table S3. M - DNA markers. Marker sizes are indicated on the right.The presence of the amplification product indicates the existence of a dominant allele of the *Snn1* gene; the absence of the amplification product indicates a null (recessive) *snn1* allele.



Supplementary Figure S3. Identification of alleles of the *Snn3-B1* locus in 55 wheat samples using primers to SSR markers *Xcfd20* (**A**) and *Xgwm234* (**B**) by PCR. The results show that marker *Xcfd20* amplifies different fragments in wheat (370 bp, 380 bp, 500 bp, about 700 bp), marker *Xgwm234* amplifies two fragments in wheat with sizes of 244 and 255 bp. Numbers from 1 to 55 wheat samples correspond to the numbers in Table S3. M - DNA markers. Marker sizes are indicated on the right. The absence of an amplification product indicates a null (recessive) *snn3-B1* allele.

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Supplementary Table S3. The 55 *Triticum* accessions evaluated for the presence of the *Tsn1*, *Snn1* and *Snn3-B1* DNA sequence.

Number of Accession	Cultivar	Type of development	Source (origin)	*Tsn1*	*Snn1*	*Snn3-B1*	
				GU259657.1	KP085710	*Xcfd20*	*Xgwm234*
1	Afina	winter	NGC* (Krasnodar)	-	+	+	+
2	Bezostay 100	winter	NGC (Krasnodar)	-	+	+	+
3	Esaul	winter	NGC (Krasnodar)	+	+	+	+
4	Laureat	winter	NGC (Krasnodar)	-	+	+	+
5	Sila	winter	NGC (Krasnodar)	-	+	+	+
6	Tanya 1	winter	NGC (Krasnodar)	-	+	+	+
7	Yubileynaya 100	winter	NGC (Krasnodar)	+	+	+	+
8	Ermak	winter	NGC (Krasnodar)	+	+	+	+
9	Etnos	winter	NGC (Krasnodar)	-	+	+	+
10	Trio	winter	NGC (Krasnodar)	+	+	+	+
11	Urup	winter	NGC (Krasnodar)	-	+	+	+
12	Yuka	winter	NGC (Krasnodar)	+	+	+	+
13	Asket	winter	NGC (Krasnodar)	-	+	+	+
14	Gubernator Dona	winter	NGC (Krasnodar)	-	+	+	+
15	Don Mira	winter	NGC (Krasnodar)	-	-	-	-
16	Tabor	winter	NGC (Krasnodar)	-	+	+	+
17	Stan	winter	NGC (Krasnodar)	-	+	+	+
18	Stanichnaya	winter	NGC (Krasnodar)	-	+	+	+
19	Tanya	winter	NGC (Krasnodar)	-	+	+	+
20	Alekseich	winter	NGC (Krasnodar)	+	+	+	+
21	Bagrat	winter	NGC (Krasnodar)	-	+	+	+
22	Antonina	winter	NGC (Krasnodar)	-	+	+	+

23	Grom	winter	NGC (Krasnodar)	+	+	+	+
24	Vassa	winter	NGC (Krasnodar)	+	+	+	+
25	Brigada	winter	NGC (Krasnodar)	-	+	+	+
26	Dmitriy	winter	NGC (Krasnodar)	+	+	+	+
27	Gurt	winter	NGC (Krasnodar)	-	+	+	+
28	Gratsiya	winter	NGC (Krasnodar)	+	+	+	+
29	Zhiva	winter	NGC (Krasnodar)	-	+	+	+
30	Yeremeyavna	winter	NGC (Krasnodar)	-	+	+	+
31	Lebed	winter	NGC (Krasnodar)	-	+	+	+
32	Kalym	winter	NGC (Krasnodar)	+	+	+	+
33	Kuren	winter	NGC (Krasnodar)	-	+	+	+
34	Bashkirskaya 26	spring	IBG UFRC RAS (RB)**	-	+	-	+
35	Zhnitsa	spring	IBG UFRC RAS (RB)	+	+	+	+
36	Kazahstanskaya 10	spring	IBG UFRC RAS (RB)	-	+	+	+
37	Iren	spring	IBG UFRC RAS (RB)	+	+	+	+
38	Omskaya 35	spring	IBG UFRC RAS (RB)	-	+	+	+
39	Vatan	spring	IBG UFRC RAS (RB)	-	+	+	+
40	Salavat Yulaev	spring	IBG UFRC RAS (RB)	-	+	+	+
41	Bashkirskaya 28	spring	IBG UFRC RAS (RB)	-	+	+	+
42	Tulajkovskaya 108	spring	IBG UFRC RAS (RB)	-	+	+	+
43	Ekada 113	spring	IBG UFRC RAS (RB)	-	+	+	+
44	Ekada 70	spring	IBG UFRC RAS (RB)	-	+	+	+
45	Boevchanka	spring	IBG UFRC RAS (RB)	-	+	-	+
46	Bashkirskaya 10	winter	IBG UFRC RAS (RB)	-	+	+	+
47	Bashkirskaya 11	winter	IBG UFRC RAS (RB)	-	+	+	+
48	Saratovskaya 29	spring	IC&G SB RAS***	-	+	+	+
49	Susquehanna	winter	VWC (USA)****	-	+	+	+
50	Mironovskaya 808	winter	VWC (Ukraine)	-	+	+	+

51	Amelio	winter	VWC (France)	-	+	+	+
52	Selkirk	spring	VWC (Canada)	+	+	+	+
53	Atlas 66	spring	VWC (USA)	-	+	+	+
54	Salamoni	spring	VWC (Lebanon)	+	+	+	+
55	Chinese Spring	spring	VWC (China)	-	+	+	+

Note: *NGC - National Grain Center named after P.P. Lukyanenko (Krasnodar); **IBG UFRC RAS (RB) - Institute of Biochemistry and Genetics Ufa Federal Research Centre of the Russian Academy of Sciences (Republic of Bashkortostan); ***IC&G SB RAS - Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences; ****VWC (Vavilov wheat collection) – N.I. Vavilov Institute of Plant Genetic Resources in Russia. Plus (+) and minus (-) indicate presence and absence of the *Tsn1*, *Snn1* and *Snn3-B1* DNA sequence based on PCR assay.

Supplementary Table S4. The 55 *Triticum* accessions evaluated for reaction to SnTox3, SnToxA and SnTox1.

Number of Accession	Cultivar	Isolate of *S. nodorum*					
		SnB(SnTox3/SnToxA)		Sn9MN-3A(SnTox3/SnToxA)		Sn1SP (SnTox1)	
		Damage zone, %	Resistance group*	Damage zone, %	Resistance group	Damage zone, %	Resistance group
1	Afina	17 ± 1.5	M	20 ± 2	M	45 ± 4	S
2	Bezostay 100	18 ± 1.4	M	34 ± 2.1	S	52 ± 3.7	S
3	Esaul	14 ± 0.9	R	32 ± 2.6	S	48 ± 4.3	S
4	Laureat	13 ± 1.5	R	47 ± 3.8	S	42 ± 2.1	S
5	Sila	31 ± 2.8	S	63 ± 5.7	S	25 ± 2	M
6	Tanya 1	36 ± 2.9	S	55 ± 4.4	S	17 ± 1.4	M
7	Yubileynaya 100	16 ± 1.1	M	21 ± 1.9	M	31 ± 2.5	S
8	Ermak	35 ± 2.8	S	42 ± 2.1	S	38 ± 3.4	S
9	Etnos	17 ± 1	M	32 ± 2.6	S	55 ± 3.9	S
10	Trio	3 ± 0.3	RR	35 ± 3.2	S	28 ± 1.1	S
11	Urup	10 ± 0.9	R	25 ± 2	M	36 ± 2.9	S
12	Yuka	n/d**	n/d	n/d	n/d	n/d	n/d
13	Asket	n/d	n/d	n/d	n/d	n/d	n/d
14	Gubernator Dona	n/d	n/d	n/d	n/d	n/d	n/d

15	Don Mira	1.5 ± 0.05	RR	2 ± 0.08	RR	2 ± 0.2	RR
16	Tabor	1 ± 0.03	RR	30 ± 2.7	S	46 ± 3.7	S
17	Stan	5 ± 0.3	RR	27 ± 1.6	S	51 ± 4.6	S
18	Stanichnaya	n/d	n/d	n/d	n/d	n/d	n/d
19	Tanya	4 ± 0.5	RR	36 ± 2.9	S	53 ± 2.7	S
20	Alekseich	13 ± 1.2	R	31 ± 1.9	S	18 ± 1.6	M
21	Bagrat	15 ± 1.2	R	38 ± 3.4	S	36 ± 2.1	S
22	Antonina	3 ± 0.2	RR	23 ± 1.4	M	48 ± 2.4	S
23	Grom	5 ± 0.3	RR	14 ± 0.9	R	4 ± 0.3	RR
24	Vassa	4 ± 0.3	RR	15 ± 1.2	R	3 ± 0.3	RR
25	Brigada	13 ± 0.9	RR	22 ± 1.3	M	32 ± 2.6	S
26	Dmitriy	26 ± 2.1	S	36 ± 3.3	S	58 ± 4.1	S
27	Gurt	12 ± 0.6	R	25 ± 2.1	M	60 ± 4.8	S
28	Gratsiya	13 ± 1.1	R	29 ± 2.7	S	53 ± 3.2	S
29	Zhiva	2 ± 0.08	RR	9 ± 0.5	R	7 ± 0.2	R
30	Yeremeyavna	1 ± 0.1	RR	15 ± 1.2	R	5 ± 0.3	RR
31	Lebed	2 ± 0.15	RR	15 ± 1.3	R	3 ± 0.2	RR
32	Kalym	21 ± 1.7	M	28 ± 2.5	S	3 ± 0.1	RR
33	Kuren	2 ± 0.2	RR	24 ± 1.7	M	42 ± 3	S
34	Bashkirskaya 26	15 ± 1.4	R	64 ± 5.1	S	86 ± 5.2	SS
35	Zhnitsa	80 ± 5.6	SS	85 ± 7.7	SS	8 ± 0.6	R
36	Kazahstanskaya 10	52 ± 3.2	S	54 ± 3.3	S	14 ± 1.1	R
37	Iren	22 ± 0.9	M	33 ± 2.7	S	15 ± 1.2	R
38	Omskaya 35	4 ± 0.2	RR	22 ± 1.3	M	60 ± 5.4	S
39	Vatan	14 ± 1	R	34 ± 2	S	53 ± 3.2	S
40	Salavat Yulaev	15 ± 1.4	R	35 ± 2.8	S	46 ± 3.7	S
41	Bashkirskaya 28	15 ± 1.5	R	41 ± 3.3	S	52 ± 2.5	S
42	Tulajkovskaya 108	52 ± 4.2	S	59 ± 4.2	S	76 ± 6.1	SS

43	Ekada 113	15 ± 1.2	R	25 ± 2	M	49 ± 3.9	S
44	Ekada 70	43 ± 2.6	S	48 ± 3.4	S	22 ± 1.1	M
45	Boevchanka	9 ± 0.5	R	15 ± 1.4	R	79 ±3.9	SS
46	Bashkirskaya 10	23 ± 1.9	M	33 ± 2.7	S	25 ± 1.8	M
47	Bashkirskaya 11	75 ± 3	SS	81 ± 6.5	SS	16 ± 1.3	M
48	Saratovskaya 29	15 ± 1.4	R	22 ± 2	M	4 ± 0.3	RR
49	Susquehanna	30 ± 1.8	S	51 ± 4.1	S	3 ± 0.2	RR
50	Mironovskaya 808	29 ± 1.5	S	28 ± 1.9	S	2 ± 0.1	RR
51	Amelio	27 ± 1.62	S	26 ± 1.8	S	9 ± 0.5	R
52	Selkirk	30 ± 2.4	S	20 ± 1.6	M	8 ± 0.4	R
53	Atlas 66	32 ± 2.6	S	34 ± 3.1	S	3 ± 0.2	RR
54	Salamoni	20 ± 1.4	M	20 ± 1.7	M	6 ± 0.6	R
55	Chinese Spring	15 ± 1.1	R	23 ± 1.9	M	52 ± 3.6	S

Note: *RR (0 - 5%) - varieties with very high and high resistance; R (6 - 15%) - resistant varieties; M (16 - 25%) - slightly susceptible varieties; S (26 - 65%) - susceptible varieties; SS (66 - 100%) - varieties with very high and high susceptibility; **n/d – no data - these accessions were omitted from analysis due to poor growth or lack of seeds during different stages of the experiment.

Supplementary Table S5. Regression statistics for 32 wheat accessions that were resistant to one or two pathogen isolates.

Regression Statistics	
Multiple R	0,583423
R Square	0,340382
Adjusted R Square	0,318395

Standard Error	12,95647
Observations	32

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2598,773	2598,773	15,48086	0,000456923
Residual	30	5036,102	167,8701		
Total	31	7634,875			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	31,71997	4,238575	7,483639	2,43E-08	23,06364045	40,37629	23,06364	40,37629
X Variable 1	-0,39355	0,100023	-3,93457	0,000457	-0,597823195	-0,18927	-0,59782	-0,18927

Supplementary Table S6. Regression statistics for 22 wheat accessions, in which the expression of the *Snn1* gene and damage zones was analyzed during infection with the Sn1SP isolate.

Regression Statistics	
Multiple R	0,923312002
R Square	0,852505053
Adjusted R Square	0,845130305
Standard	0,169938247

Error	
Observations	22

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3,338351661	3,338352	115,5979	9,23E-10
Residual	20	0,577580157	0,028879		
Total	21	3,915931818			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	0,025206344	0,053203076	0,473776	0,640791	-0,08577	0,136186	-0,08577	0,136186
X Variable 1	0,014512536	0,001349797	10,75164	9,23E-10	0,011697	0,017328	0,011697	0,017328

Supplementary Table S7. Regression statistics for 13 wheat accessions, in which the expression of the *Tsn1* gene and damage zones was analyzed during infection with the SnB isolate.

Regression Statistics	
Multiple R	0,642054
R Square	0,412234
Adjusted R Square	0,358801

Standard Error	0,660263
Observations	13

ANOVA

	df	SS	MS	F	Significance F
Regression	1	3,363295	3,363295	7,714925	0,017982
Residual	11	4,795412	0,435947		
Total	12	8,158708			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95,0%	Upper 95,0%
Intercept	0,233656	0,291567	0,801383	0,439878	-0,40808	0,87539	-0,40808	0,87539
X Variable 1	0,0274	0,009865	2,777575	0,017982	0,005688	0,049111	0,005688	0,049111