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The Use of Genetic Material of Tall Wheatgrass to Protect Common Wheat from *Septoria* Blotch in Western Siberia

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Abstract: The *Septoria* blotch is one of the most economically harmful diseases of common wheat in Russia and the world. The disease is mainly caused by two pathogen species: *Zymoseptoria tritici* that damages the leaves, and *Parastagonospora nodorum* that strikes the leaves and ears. Resistance genes of the alien relatives are traditionally used for genetic defense of cultivars. The aims of the research were to study the resistance of the tall wheatgrass *Thinopyrum ponticum* (Podp.) Z.-W. Liu and R.-C. Wang and perspective introgressive lines of spring common wheat with its genetic material to *Septoria* blotch, and to characterize their agronomical properties to be used in breeding programs in Western Siberia. The studies were carried out in 2015–2019 in the field conditions of the southern forest-steppe (Omsk, Russia) on a natural infection background and according to standard methods. The *Septoria* diseases developed on the wheat in the period of milk-wax ripeness, independently of humid or dry weather conditions. In 2016, a sharp increase in leaf lesion was noted, probably associated with changes in the *Z. tritici* population. In 2017, the ratio of *Z. tritici* and *P. nodorum* was similar, and in 2019 *Z. tritici* prevailed. During the research, the lines that combined leaf and ear resistance to damage with high yield and grain quality were selected.

Keywords: *Triticum aestivum*; *Thinopyrum ponticum*; introgressive lines; *Septoria* blotch; *Zymoseptoria tritici*; *Parastagonospora nodorum*; disease resistance; grain quality

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1. Introduction

Common wheat *Triticum aestivum* L. is one of the most important cereals that provides nutrition to the global population. Its crops occupy more than 200 million hectares in the world. Due to the predicted population growth, it is necessary to increase wheat grain production about by 70% before 2050 [1]. Two directions are proposed to deal with this problem. The first is to increase the potential productivity of cultivars, and the second is to reduce crop losses caused by abiotic and biotic factors. Recently, the *Septoria* blotch has become one of the most economically significant wheat diseases in the world [2–4]. *Septoria* diseases of wheat are caused by hemibiotrophic species of the Ascomycetes, mainly the by the Dothideomycetes subclass: *Zymoseptoria tritici* (Roberge ex Desm.) Quaedvl. and Crous. (syn. *Septoria tritici* Desm., teliomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt.) and *Parastagonospora nodorum* (Berk.) Qua ed vl. (syn. *Septoria nodorum* (Berk.), teliomorph *Leptosphaeria nodorum* E. Mull.); less common is *Parastagonospora* (*Stagonospora*) *avenae* Bissett f. sp. *triticea* T. Johnson (syn. *S. avenae* f. sp. *triticae* [5,6]. Different pathogens can exist on plants simultaneously, while *Z. tritici* damages the leaves, and *P. nodorum* violates the leaves and ears [7,8]. *Z. tritici* causes great harm to wheat in Europe, North America, and Australia. Already in the 1970–80s, epidemics causing crop losses amounting to 50% were noted in humid coastal regions [9]. Due to the disease progression in the 2010s, the annual costs of crop protection in the EU exceeded €400 million (70% of the total cost of fungicides), and $275 million in the USA [5].

The rapid evolution of *Z. tritici* and progressive crop damage is contributed to by biological properties of the species. The pathogen variability is determined by the high

level of mutation process and the accumulation of changed genes during outbreaks of reproduction (under favorable conditions up to 10^{11} spores/ha), which leads to the mass appearance of new phenotypes (up to 20 thousand spores/ha) [3]. Population heterogeneity is enhanced due to the regular sexual process and related chromosome rearrangements and gene recombinations [10–12]. The pathogen has a mixed reproduction system. The sexual process occurs in pseudothecia and air-spread ascospores are formed. After the inoculation of plants with ascospores, the asexual stage begins, and pycnidias with pycnidiospores are developed. The aerial ascospore movement allows rapid migration of new forms, and in the asexual stage, the selection and fixation of the best phenotypes in populations take place [12–15]. A similar genetic basis of cultivars, environmental conditions, and usage of fungicides with equal effect may be the factors that enhance microevolution in the *Z. tritici* populations. The experience of wheat production in the EU has shown that *Z. tritici* is able to quickly overcome the cultivar defense and acquire fungicide resistance [14,16]. The biological features of *P. nodorum* are studied to a lesser extent, but a high adaptive potential to regional conditions has been shown [6,8]. In fungal populations, directional selection for fungicide resistance and existence at high temperature was established [17,18]. Accelerated adaptation of pathogens to the environmental conditions is promoted by use of intensive plant production technologies, such as a no-tillage method, when a large number of propagules with new phenotypes remain on the stubble [5,14,16].

Hemibiotrophs of the Dothideomycetes subclass change the types of interaction with plants during pathogenesis. At the first stage, the pathogens develop as biotrophs without symptoms, but when switching to reproduction, *Z. tritici* and *P. nodorum* secrete dozens of effectors, some of them cause cell necrosis and appear toxin properties (NE-effectors). This leads to the appearance of characteristic disease symptoms on plant organs [18–20]. The complex intergenic interaction occurs in the pathogenesis: in the biotrophic stage, happening in accordance with the "gene-for-gene" theory of H. Flor (for example, for the *AvrStb6*–*Stb6* genes); in the necrotrophic stage, there is an inverse-type interaction [19–21]. As for the *P. nodorum*, it tends to accumulate the genes encoded the toxins, which increase the strain aggressiveness and disease harmfulness [18,19].

In the former USSR countries, single *Septoria* blotch cases were noted in the 1970s [22], and by now the disease has spread in the most regions of wheat cultivation [7,8,23–25]. Three causal agents of *Septoria* diseases were identified on crops in Russia; however, there was an uneven species distribution. *Z. tritici* prevailed in the southern areas (the North Caucasus, the Central Black Earthand the Volga regions). Equal proportions of *Z. tritici* and *P. nodorum* were found in the central zone of European Russia. *P. nodorum* dominated in the northern and eastern regions. The frequency of the *P. avenae* f. sp. *triticae* in the pathogen complex was low in these areas [7,23,24]. In the Central Black Earth region, the *Septoria* blotch appeared annually in 2008–2017, and the average yield losses of spring and winter wheat cultivars reached 18–22% [23]. During epidemics, crop losses can reach 30–50%; at the same time, the seed sowing properties and the grain qualities are decreased [13]. Taking into account the biological properties of *Z. tritici* and *P. nodorum*, the harm from the *Septoria* diseases is predicted to increase, which enhances the risks of food insecurity in the world [2,6,8].

Evaluation of *Stb1*–*Stb8* gene effectiveness in the European part of Russia showed that only two of them (*Stb6* and *Stb8*) provided resistance to all populations of *Z. tritici*, three (*Stb2*, *Stb3* and *Stb4*) to individual regional sub-populations, and the remaining genes had already been overcome by the pathogen [26]. These data indicate that the set of *Stb* genes available for breeding is very limited. In this regard, it is necessary to search for sources of resistance and do advance wheat breeding, taking into account the regional distribution of pathogen species.

Traditionally, alien relatives, and in particular, the genus *Thinopyrum*, are used to enrich the wheat gene pool. The first works on the distant hybridization between wheat and *Thynopyrum* (*Agropyron*) species were carried out in 1920s by a Russian Academician N.V. Tsitsin and coworkers. The most promising species were tall wheatgrass *Thinopyrum*

ponticum (Podp.) Z.-W. Liu & R.-C. Wang (=*Agropyron elongatum* (Host) Beauv.) and intermediate wheatgrass *Th. intermedium* (Host) Barkworth et D.R. Dewey (=*Ag. intermedium* (Host) Beauv.) [27,28]. At the first stages of the work, interspecific wheat–wheatgrass hybrids (WWHs) were created, which were used to transfer the alien genetic material into the wheat genome. Cultivars of perennial, re-growing WWHs and winter-hardy wheat were originated on their basis [27].

The *Th. ponticum* is a decaploid species distinguished from common wheat (BBAADD) by genome composition (2n = 10× = 70, $JJJJJJJ^sJ^sJ^sJ^s$ or $E^eE^eE^bE^bE^xE^xStStStSt$) [29,30]. *Th. ponticum* is considered a valuable source of resistance to abiotic environmental factors: soil salinity, drought, and extreme temperatures [31–33]. However, it is of the greatest interest in terms of its resistance to many wheat diseases. A set of rust resistance genes has already been transferred to the wheat genome (*Lr19*/*Sr25*, *Lr24*/*Sr24*, *Lr29*, *Sr26*, *Sr43*, *Sr61*), and the work continues due to the increased aggressiveness of stem and strip rusts in different regions of the world [34,35]. Beside it, its powdery mildew (*Pm51*), *Fusarium* head blight (*Fhb7*) and viral disease-resistance genes are already introgressed to wheat [34,36]. The interspecific hybrid *Th. ponticum* × *Th. intermedium* also demonstrated resistance to tan spot and *Septoria nodorum* blotch [37]. *Th. ponticum* is of great interest as a source of genetic material to protect wheat from *Septoria* diseases.

The introgressive lines of spring common wheat with genetic material of *Th. ponticum* originated at Omsk State Agrarian University (Omsk, Russia) were preliminarily estimated in terms of their resistance to rust diseases and *Septoria* blotch [38–40]. Due to the rapid evolution of the pathogens and increasing damage of crops with *Septoria* diseases, it is necessary to monitor the line resistance to them, taking into account the pathogen's complex composition.

The aims of this research were to study the resistance of the tall wheatgrass *Th. ponticum* and perspective introgressive lines of spring common wheat with its genetic material to *Septoria* blotch and to characterize their agronomical properties to be used in breeding programs in Western Siberia.

2. Materials and Methods

2.1. Plant Material

The following materials were used in this study:

(1) four accessions of *Th. ponticum* from N.V. Tsitsin's Main Botanical Garden (Moscow, Russia), including two originated from Russia (one of them a parent for the WWHs), the USA and Africa;
(2) introgressive lines of spring common wheat with genetic material of the *Th. ponticum* (in 2015–2016–130, in 2017–2019–93 lines);
(3) four cultivars of spring common wheat viz. Niva 2, Chernyava 13, Golubkovskaya, Sonata susceptible to *Septoria* diseases used for backcrosses;
(4) standard cultivars of spring common wheat viz. Pamyati Azieva (medium-early), Duet (medium-ripe), Serebristaya (medium-late).

The lines originated in the Omsk State Agrarian University (Omsk SAU, Omsk, Russia). At the first step of distant hybridization, the interspecific hybrid (*T. turgidum* × *Th. ponticum*) was obtained, then wheat–wheatgrass hybrids (WWHs) ((*T. turgidum* × *Th. ponticum*) × *T. aestivum* Pyrotrix 28) were made. Later, the spring forms were selected in the self-pollinated WWHs progenies. They were included in the crosses with susceptible to disease- spring common wheat cultivars of local breeding [41]. During breeding, the individual plants resistant to leaf and stem rusts (markers of introgression) with reduced vegetation period and good yield were selected [38,42]. Stable lines obtained as a result of 3–5 backcrosses and five self-pollinations were used for the research (Table 1).

Table 1. The origin of introgressive lines of spring common wheat with genetic material of *Thinopyrum ponticum*.

Line	Origin
2/2015	S_5 [WWH × B_4 Lutescens 444]
9/2015	S_5 [(WWH × Lutescens 444) × B_2 Chernyava 13]
37/2015	S_5 [(WWH × B_3 Lutescens 444) × Chernyava 13]
6/2015, 7/2015, 10/2015, 31/2017	S_5 [WWH × B_4 Chernyava 13]
374/2015, 314/2017, 322/2017, 337/2017	S_5 [WWH × B_5 Chernyava 13]
24/2015	S_5 [WWH × B_5 Sonata]
94/2015	S_5 [WWH × Niva 2) × B_4 Golubkovskaya]

Note: B_2–B_5—number of backcrosses; S_5—five generations of self-pollination; WWH—wheat–wheatgrass hybrid—((*Triticum turgidum* × *Th. ponticum*) × *T. aestivum* Pyrotrix 28).

2.2. Methods

The experiments were carried out in 2015–2019 in field conditions on natural infection background of *Septoria* blotch in the southern forest-steppe of Western Siberia (Omsk, 54.58_ N, 73.24_ E).

The *Th. ponticum* accessions were replanted in 2010 and were maintained thanks to their perennial lifestyle. The other samples were sown in the third ten-days of May at the onset of optimal conditions. In 2015–2016, the samples were sown in rows with 40 seeds/m sowing density fourfold. In 2017–2019, wheat specimens were studied in plot trial (triplicated 1 m^2 plots, sowing density of 500 seeds/m^2). Phenological phases were determined at Zadoks scale. Harvesting was carried out in the third ten-days of August as the plants matured. Yield traits were determined in the sheaf material (except for the perennial *Th. ponticum*), and the grain yield per plant (g) and 1000 grain weight (g) were determined.

The severity of *Septoria* blotch was assessed according to James's scale [43], separately on leaves and ears (in %). The estimations were carried out in dynamics, as disease symptoms appeared in the first-third ten-days of August, the final assessments were made at the beginning of wax ripeness (Zadoks, ph. 82). The samples were divided into groups according to disease severity: high resistant −0–5%; resistant −6–20%; weakly susceptible −21–40%; susceptible −41–65%; and high susceptible −66–100% [43].

Identification of *Z. tritici* and *P. nodorum* infections on wheat was carried out on herbarium material (40 leaves/sample) collected in 2017–2019 at the experimental field of Omsk SAU. Pieces of dry leaves with fruit bodies (0.5 × 0.5 cm) were placed in a drop of water on a slide for 15 min. After the release of pycnidiospores, the fungus species was determined by their shape and size [24,25]. The frequency of occurrence of species on the samples was determined as the proportion of *Z. tritici* or *P. nodorum* sporulations from the total analysed number (in %).

Grain quality parameters such as protein and crude gluten content (in %) were determined in four biological and two analytical repetitions using the INFRALUM FT-10 device (Lumex, St. Petersburg, Russia).

Statistical analysis included calculation of the mean and standard error of the mean ($M \pm SEM$). The annual correlation coefficients (r) between grain yield per plant (average M for 20 plant/sample) and the disease severity (for all cultivars and lines in the experiment) were calculated. The reliability of the r was evaluated by the Student's t-test (at $p \leq 0.05$). The data on grain yield, 1000 grain weight, protein, and crude gluten contents were processed by a one-factor variance analysis (at $p \leq 0.05$) using software package STATISTICA Advanced (StatSoft Russia Moscow).

2.3. Environmental Conditions

Weather conditions differed significantly in July (before the disease) and August (during the disease development) in 2015–2019 (Figure 1). In 2015 and 2018, moderate

precipitation was observed in the third ten-days of July and during August at moderate average ten-day temperatures (13–17 °C). In July 2016 and 2017, sufficient precipitation fell (103 and 71 mm respectively), but in August there was severe drought (17 and 13 mm of precipitation per month) and high air temperatures of 18–20 °C. In 2019, there was little precipitation in July and August at high and moderate temperatures. According to hydrothermal coefficient HTC, the weather conditions in August 2015 and 2018 were characterized as excessive humidification (4.17 and 3.37 respectively); in 2016 and 2017 as insufficient ones (HTC = 0.63 and 0.57 respectively); and in 2019 as satisfactory ones (HTC = 1.67).

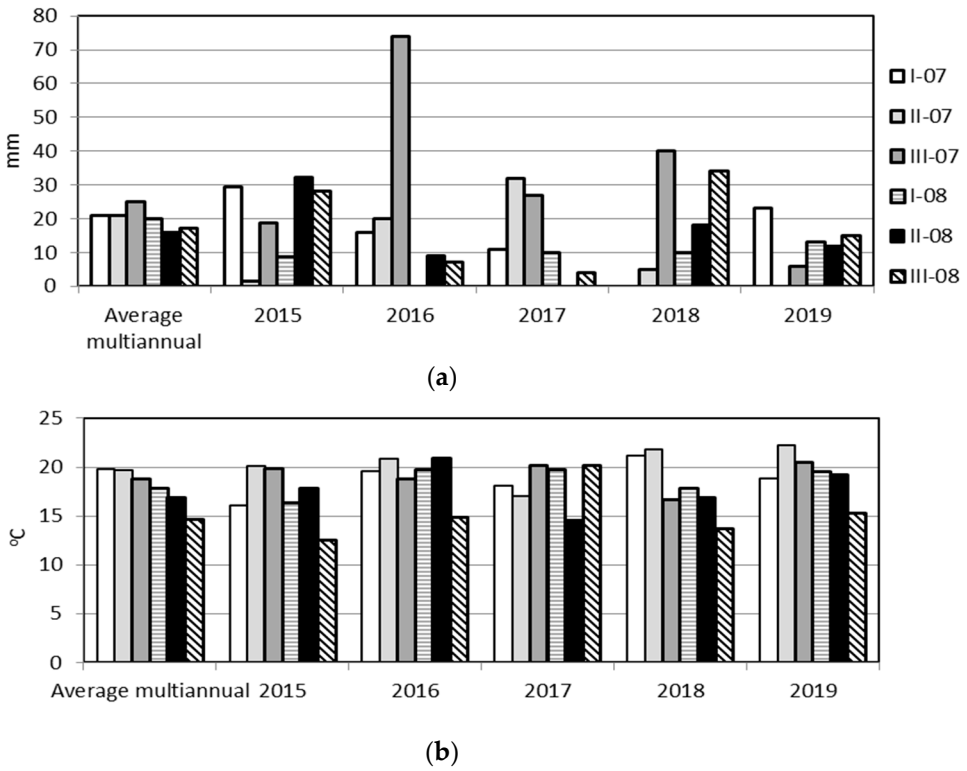

Figure 1. Weather conditions in the southern forest-steppe of Western Siberia: (**a**)—precipitation; (**b**)—average ten-days temperature; I, II, III—ten-days; 7 July, 8 August (Omsk. July–August 2015–2019).

3. Results

The first symptoms of *Septoria* blotch on wheat leaves appeared in beginning of August, during milk ripeness (Zadoks, ph. 71–75), and the damage of the ears occurred 7–10 days later. The maximum disease severity on both organs was noted in the third ten-days of August at wax ripeness (Zadoks, ph. 81–85). In 2015–2019, all *Th. ponticum* accessions (including the parent one) did not have visible symptoms of the disease (severity 0%, immunity) (Table 2). In 2013–2014, it had been shown that standard medium-ripe cv. Duet and medium-late cv. Serebristaya were resistant or weakly susceptible to leaf and ear *Septoria* blotch (severity 10–25%), and about half of the introgressive lines was resistant to the disease [40]. In the humid conditions of August 2015, the medium-early cv. Pamyati Azieva showed susceptibility to leaf blotch (severity 50%), but other standard and parent cultivars were resistant or weakly susceptible (severity 5–25%) (Table 2). Among a set of introgressive lines, 30% were resistant, 15% were weakly susceptible, and others were susceptible and highly susceptible (Figure 2a). However, in the very dry conditions of August 2016, the final assessment revealed a sharp increase in leaf damage. It is possible that the disease was to a certain degree provoked by heavy precipitation in the third ten-days of July and regular dew-fall after them. All cultivars and 95% of the lines showed high

susceptibility (severity 75%). At the same time, an insignificant negative effect of leaf lesion on grain yield was established ($r = -0.11$), which can be explained by a late outbreak of the disease. In the following years the cultivars showed high susceptibility to *Septoria* leaf blotch in the droughts of 2017 and 2019 (severity 50–75%), and a slight damage reduction was noted in humid August 2018 (severity 40–60%).

Table 2. Results of assessment of *Septoria* blotch severity on *Thinopyrum ponticum*, cultivars of spring common wheat and introgressive lines, and the frequency of occurrence of *Z. tritici* and *P. nodorum* (field conditions, natural infection background, Omsk, Russia, 2017–2019).

Sample	Average Vegetation Period, Days	Severity, %										Frequency of Occurrence, *Z. tritici*/*P. nodorum*, %/ %		
		2015		2016		2017		2018		2019				
		Leaf	Ear	Leaf	Ear	Leaf	Ear	Leaf	Ear	Leaf	Ear	2017	2018	2019
Th. ponticum	**Perennial**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**-**	**-**	**-**
Medium-early														
Pamyati Azieva—standard	77 ± 3.5	50	25	75	50	75	50	60	50	60	50	52.5/47.5	37.5/62.5	57.5/43.5
Chernyava 13	77 ± 3.3	50	25	75	25	75	50	50	60	60	75	57.5/42.5	52.5/47.5	62.5/37.5
2/2015	77 ± 3.9	50	25	75	50	5	10	10	10	20	10	5.0/95.0	5.0/95.0	5.0/95.0
6/2015	73 ± 3.7	10	5	50	0	10	0	5	10	10	10	100/0.0	95.0/5.0	100/0.0
7/2015	75 ± 3.6	25	10	75	25	10	0	10	5	10	10	100/0.0	92.5/7.5	100/0.0
9/2015	76 ± 3.3	50	25	75	50	75	30	25	20	50	30	67.5/32.5	52.5/47.5	67.5/32.5
10/2015	77 ± 3.5	10	5	50	10	60	30	20	20	50	30	62.5/37.5	57.5/42.5	65.0/35.0
94/2015	76 ± 3.6	10	5	25	10	10	10	10	20	5	10	5.0/95.0	5.0/95.0	10.0/90.0
322/2017	76 ± 2.8	-	-	-	-	10	0	10	20	5	10	100/0.0	92.5/7.5	100/0.0
337/2017	76 ± 3.1	-	-	-	-	20	10	20	20	20	5	77.5/22.5	67.5/32.5	82.5/17.5
374/2015	77 ± 3.4	10	25	75	25	10	0	10	10	20	10	100/0.0	92.5/7.5	100/0.0
Medium-ripe														
Duet—standard	82 ± 4.4	5	25	75	25	60	50	50	60	75	50	70.0/30.0	62.5/37.5	77.5/22.5
Sonata	79 ± 3.9	10	25	75	25	60	50	40	40	75	50	52.5/47.5	47.5/52.5	62.5/37.5
31/2017	79 ± 38	-	-	-	-	5	0	5	5	10	5	100/0.0	90.0/10.0	100/0.0
314/2017	79 ± 3.6	-	-	-	-	10	5	10	5	20	10	75.0/25.0	65.0/35.0	82.5/17.5
24/2017	80 ± 3.8	-	-	-	-	10	10	10	20	5	5	5.0/95.0	5.0/95.0	10.0/90.0
37/2015	80 ± 3.9	25	25	75	5	20	10	20	20	20	10	70.0/30.0	62.5/37.5	72.5/27.5
Medium-late														
Serebristaya—standard	87 ± 4.4	10	10	50	10	50	50	50	60	75	40	87.5/12.5	77.5/22.5	90.0/10.0
Niva 2	84 ± 4.1	10	25	75	50	50	50	50	60	75	50	18.5/81.5	12.5/87.5	15.0/85.0
Golubkovskaya	85 ± 2.1	25	25	60	25	75	50	50	50	50	40	51.0/49.0	45.5/54.5	57.5/42.5
Average ratio	-	-	-	-	-	-	-	-	-	-	-	532/46.8	45.1/54.9	59.8/40.2

According to the assessment held in 2016, 55 lines susceptible to leaf damage were rejected, and 18 new ones were added to the set. In 2017, in the updated set (93 lines) the lines No. 2/2015 and 31/2017 with high resistance (severity 5%) to leaf blotch were revealed (Table 2). Of the nine lines with severe leaf damage in 2016, three restored resistance in 2017–2019 (No. 6/2015, 7/2015, 374/2015), and six (including No. 9/2015, 10/2015) remained susceptible (Table 2). In 2017, the total proportion of highly resistant and resistant lines was 18%, and weakly susceptible ones accounted for 31%. In the following years, the total proportion of resistant lines increased to 49% (Figure 2a).

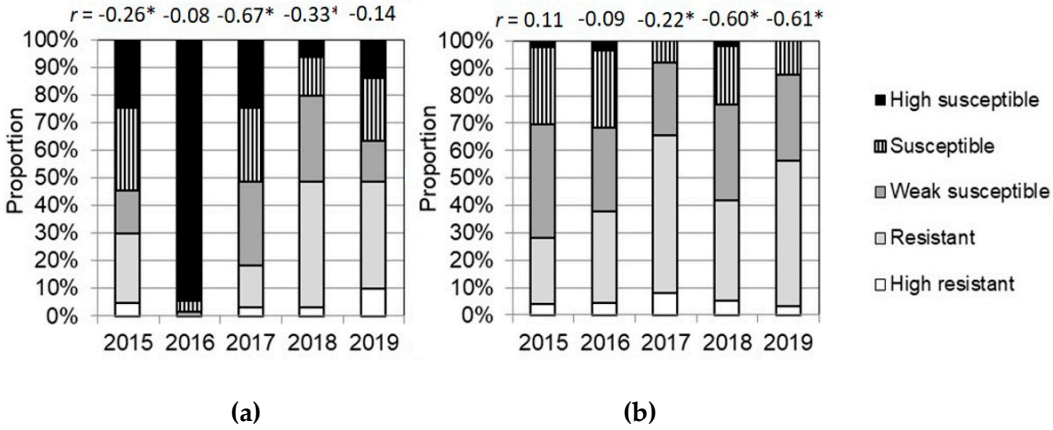

Figure 2. Distribution of introgressive lines of spring common wheat with genetic material of *Thinopyrum ponticum* on resistance to *Septoria* leaf (**a**) and ear (**b**) blotch and correlations between grain yield per plant and disease severity (*r*). (Omsk, Russia, field conditions, 2015–2019). * reliable (at $p \leq 0.05$).

In 2015–2016, the ear damage of standard and parent cultivars by *P. nodorum* blotch did not exceed 10–25%, except for cv. Pamyati Azieva (50%). In the following years, the severity increased up to 40–75%, with slight fluctuations among ripeness groups (Table 2). In 2016, there was no increase in ear damage of introgressive lines, by comparison with 2015. Among the lines, 29% were highly resistant or resistant to ear lesion, and 40% were weakly susceptible. At the same time the effect of ear damage on grain yield was insignificant ($r = -0.09$) (Figure 2b). The best proportion of the lines resistant to ear blotch was noted in the arid year 2017, and in the following years the resistant/susceptible ratios were better than in 2016.

In 2017, the greatest negative impact of leaf damage on grain yield was noted ($r = -0.67$), but in 2018 and 2019, the effect was decreased ($r = -0.33$ and $r = -0.14$, respectively). However, in 2018 and 2019, the negative correlations between grain yield and ear blotch increased ($r = -0.60$–0.61), which may be explained by stronger injury of susceptible lines.

In 2017–2019, for the first time in the Omsk region, the composition of the *Septoria* complex on wheat was studied. The *Z. tritici* and *P. nodorum* were determined in the *Septoria* pathogen complex, while *P. avenae* f. sp. *triticea* was not detected in the probs. In 2017, the average frequency of species occurrence was similar (53% and 47%, respectively). In the humid 2018, the ratio of species slightly shifted towards *P. nodorum* (45% and 55%, respectively), but in 2019 *Z. tritici* became dominant (60% and 40%, respectively) (Table 2).

In 2017, the ratio of *Z. tritici* and *P. nodorum* on the cvs. Pamyaty Azieva, Chernyava 13, Sonata, and Golubkovskaya was approximately equal. *Z. tritici* dominated on the cv. Duet, while *P. nodorum* dominated on the cv. Niva 2. The frequency of species occurrence varied significantly on the introgressive lines. *Z. tritici* prevailed on ten lines (62.5–100%), and *P. nodorum* on three ones (67.5–100%). Low leaf damage of the lines No. 6/2015, 7/2015, 322/2017 and 31/2017 was determined by only *Z. tritici*, while *P. nodorum* mostly damaged leaves and ears of the lines No. 2/2015, 94/2015, 24/2017. In the following years, the ratio of pathogens on the lines varied to some extent, but general patterns in the dominance of one fungus and the suppression of the other one were maintained. Such results are probably related to different distribution of resistance genes in the lines.

Among the best lines, seven (No. 2/2015, 322/2017, 337/2017, 31/2015, 314/2017, 374/2015, 37/2015) combined leaf and ear resistance to *Septoria* blotch with high grain yield; the remaining ones were close in the yield to the standards (Tables 2 and 3). In 2017, the ears of some lines were not infected (No. 6/2015, 7/2015, 322/2017, 374/2015, 31/2017), and later their damage was low (5–10%) (Table 2). Three lines with a low lesion of two organs ($\leq$ 10%) are of particular interest (No. 6/2015, 7/2015, 31/2017). It was previously

shown that lines No. 2/2015, 6/2015, 9/2015, 31/2017 were also resistant to stem rust, but the No. 7/2015 and 374/2015 were susceptible to it [39].

Table 3. Grain yield of spring common wheat cultivars and introgressive lines with genetic material of *Thinopyrum ponticum* on *Septoria* blotch natural infection background (Omsk, Russia, 2017–2019).

Cultivar, Line	Grain Yield g/m^2			
	2017	2018	2019	Average
Medium-early				
Pamyati Azieva-standard	481	393	390	421 ± 30
Chernyava 13	453	362	372	396 ± 29
2/2015	652 *	582 *	575 *	603 ± 25
6/2015	470	410 *	406 *	429 ± 21
7/2015	441	393	404 *	413 ± 15
9/2015	549 *	416 *	435 *	467 ± 42
10/2015	577 *	472 *	480 *	510 ± 34
94/2015	497 *	427 *	403	442 ± 28
322/2017	665 *	619 *	594 *	626 ± 21
337/2017	598 *	490 *	471 *	520 ± 40
374/2015	627 *	595 *	564 *	595 ± 18
$SSD_{0.05}$	15.6	11.3	13.3	-
Medium-ripe				
Duet-standard	541	377	472	463 ± 48
Sonata	372	305	330	336 ± 29
31/2017	572 *	423 *	507 *	501 ± 43
314/2017	594 *	586 *	528 *	569 ± 21
24/2017	395	348	371	371 ± 14
37/2015	541	468 *	492 *	500 ± 21
$SSD_{0.05}$	16.2	14.4	12.1	-
Medium-late				
Serebristaya-standard	414	310	387	370 ± 31
Niva 2	372	305	330	336 ± 20
Golubkovskaya	355	315	325	332 ± 12
$SSD_{0.05}$	10.1	14.6	12.6	-

Note: * reliably exceeded the standard ($p \leq 0.05$).

Additionally, the effect of *Septoria* blotch on the main grain parameters of introgressive lines was studied. A middle negative correlation was established between the 1000 grain weight and leaf damage in 2017 ($r = -0.51$), as well as with ear damage in 2018 and 2019 ($r = -0.45$–0.49) (Table 4). At the same time, correlation between protein content and damage of leaves and ears was weak. In 2018 and 2019, the middle negative correlation between crude gluten content and ear damage was noted ($r = -0.44$ and -0.35, respectively). The correlation between the parameter hectolitre weight of grain with leaf lesion was weak and fluctuated in 2017, 2018, and 2019 ($r = +0.35$, ·0.28, and 0.30, respectively). The other correlations between yield traits and *Septoria* diseases were insignificant. The positive relationships of the hectolitre weight of grain with the lesion may be due to the decreased grain volume of infected plants.

Table 4. Parameters of grain quality of spring common wheat cultivars and introgressive lines with genetic material of *Thinopyrum ponticum* and correlation with *Septoria* blotch severity (natural infection background, Omsk, Russia, 2017–2019).

Cultivar, Line	Weight of 1000 Grains, g				Protein Content, %				Crude Gluten, %				Hectoliter Weight of Grain, g/L			
	2017	2018	2019	Average	2017	2018	2019	Average	2017	2018	2019	Average	2017	2018	2019	Average
Medium-early																
Pamyati Azieva-standard	35.8	37.3	28.2	33.8 ± 2.8	16.4	14.2	14.8	15.1 ± 0.7	27.7	31.5	30.7	30.0 ± 1.2	758	751	720	743 ± 12
Chernyava 13	37.2	38.1	35.9	37.1 ± 0.6	14.5	12.5	13.0	13.3 ± 0.6	25.1	23.4	23.0	23.8 ± 0.6	731	746	761 *	746 ± 9
2/2015	38.6 *	40.3 *	42.4 *	40.4 ± 1.1	16.2	15.8 *	16.4 *	16.1 ± 0.2	32.5 *	29.4	30.8	30.9 ± 0.9	750	721	728	733 ± 9
6/2015	38.8 *	42.5 *	40.9 *	40.7 ± 1.1	19.6 *	15.0	14.8	16.5 ± 1.6	32.9 *	28.4	29.1	30.1 ± 1.4	785 *	761	730	759 ± 16
7/2015	32.9	34.2	36.9 *	34.7 ± 1.2	17.5 *	14.7 *	16.2 *	16.1 ± 0.8	33.9 *	28.8	29.3	30.7 ± 1.6	842 *	821 *	778 *	814 ± 19
9/2015	35.0	38.1	36.1 *	36.4 ± 0.9	16.4	15.1 *	13.1	14.9 ± 1.0	32.8 *	29.6	26.3	29.6 ± 1.9	794 *	761	792 *	782 ± 11
10/2015	39.3 *	38.3	40.2 *	39.3 ± 0.5	13.7	13.3	15.1	14.0 ± 0.5	27.7	26.2	28.8	27.6 ± 0.8	737	757	718	737 ± 11
94/2015	30.7	33.7	33.1 *	32.5 ± 0.9	16.0	15.8 *	16.8 *	16.2 ± 0.3	30.8 *	29.7	29.9	30.1 ± 0.3	852 *	820 *	805 *	826 ± 14
322/2017	44.2 *	48.8 *	41.3 *	44.8 ± 2.2	16.1	15.6 *	16.3 *	16.0 ± 0.2	32.3 *	28.8	32.0 *	31.0 ± 1.1	652	682	759 *	698 ± 32
337/2017	41.6 *	39.7 *	43.5 *	41.6 ± 1.1	15.6	14.9 *	16.2 *	15.6 ± 0.4	32.1 *	28.6	32.7 *	31.1 ± 1.3	705	715	744 *	721 ± 12
374/2015	41.3 *	39.2 *	40.4 *	39.8 ± 0.5	15.2	15.5 *	14.7	15.1 ± 0.2	31.3 *	29.8	28.7	29.9 ± 0.8	753	788	768 *	770 ± 10
$SSD_{0.05}$	1.9	1.8	2.1	-	0.4	0.4	0.3	-	0.6	0.5	0.3	-	15	19	21	-
Medium-ripe																
Duet-standard	34.5	43.8	37.2	38.5 ± 2.8	16.6	15.2	15.4	15.7 ± 0.4	31.8	30.0	30.6	30.8 ± 0.5	758	698	693	716 ± 21
Sonata	36.9 *	35.3	36.4	36.2 ± 0.5	14.5	13.0	13.9	13.8± 0.4	28.2	26.8	27.5	27.5 ± 0.4	735	749 *	753 *	746 ± 5
31/2017	52.0 *	44.6 *	39.0 *	45.2 ± 3.8	15.0	14.6	15.6	15.1 ± 0.3	29.7	28.1	31.5 *	29.8 ± 1.0	698	730 *	747 *	725 ± 14
314/2017	46.4 *	46.2 *	42.0 *	44.9 ± 1.4	15.5	14.7	15.6	15.3 ± 0.3	30.4	29.2	31.5 *	30.4 ± 0.7	658	718 *	736 *	704 ± 24
24/2017	39.1 *	40.3	38.7	39.4 ± 0.5	16.4	15.9 *	16.2 *	16.2 ± 0.1	30.6	29.8	32.1 *	30.8 ± 0.7	741	720 *	772 *	744 ± 15
37/2015	48.1 *	44.2	40.9 *	44.4 ± 2.1	16.2	15.2	16.9 *	16.1 ± 0.5	32.3	30.5 *	33.5 *	32.1 ± 0.9	709	739 *	757 *	735 ± 14
$SSD_{0.05}$	1.8	1.5	1.6	-	0.3	0.5	0.4	-	0.6	0.4	0.7	-	21	17	19	-

Table 4. *Cont.*

Cultivar, Line	Weight of 1000 Grains, g				Protein Content, %				Crude Gluten, %				Hectoliter Weight of Grain, g/L			
	2017	2018	2019	Average	2017	2018	2019	Average	2017	2018	2019	Average	2017	2018	2019	Average
Medium-late																
Serebristaya-standard	36.3	36.8	30.6	34.6 ± 2.0	13.8	13.0	14.2	13.7 ± 0.4	27.7	25.8	26.2	26.6 ± 0.6	736	730	768	745 ± 12
Niva 2	37.7	34.1	33.2 *	35.0 ± 1.4	13.5	12.5	12.9	13.0 ± 0.3	25.0	23.5	24.2	24.2 ± 0.4	778 *	751 *	743	757 ± 11
Golubkovskaya	35.2	32.0	315	32.9 ± 1.2	13.1	12.6	12.9	12.9 ± 0.1	24.9	24.0	23.5	24.1 ± 0.4	761 *	775 *	784	773 ± 7
$SSD_{0.05}$	1.5	1.6	1.3	-	0.4	0.3	0.5	-	0.5	0.6	0.6	-	19	15	25	-
r - leaf damage - ear damage	 −0.51 * −0.15	 −0.29 * −0.45 *	 −0.19 −0.49 *	-	 −0.29 * −0.18	 +0.17 −0.28 *	 −0.23 −0.33 *	-	 +0.09 −0.24 *	 −0.15 −0.44 *	 −0.28 * −0.35 *	-	 +0.35 * +0.11	 −0.28 * +0.23	 +0.09 +0.30 *	-

Note: * reliably exceeded the standard; *r* reliable ($p \leq 0.05$).

The climate of the steppe and forest-steppe zones of Western Siberia makes it possible to obtain grain with high quality. In Russia, the quality of wheat grain is determined according to the "National Standard of the Russian Federation. Wheat. Specifications" [44]. The most important parameters of grain quality are as follows: protein content in dry weight, crude gluten content, hectolitre weight of grain, and vitreousness. The content and quality of gluten affect the baking parameters, and the hectolitre weight of grain and vitreousness influence the milling properties of the grain. According to quality specifications, wheat grain is divided into groups: strong (first and second class), valuable (third class), and weak fodder (fourth and fifth class). The grain of strong wheat can be used to improve the baking properties of weak wheat. Bread of standard quality can be obtained from valuable wheat, but its additives do not improve the properties of weak grain. According to "Wheat. Specifications" the protein content in dry matter and crude gluten in grain of the first class should be 14.5% and 32.0%, the second class 13.5% and 28.0%, the and third class 12.0% and 23.0%, respectively. The hectolitre weight of grain for the first and second classes should be at least 750 g/L, and for the third class at least 730 g/L.

The standard cultivars showed the best parameters for protein and crude gluten content in 2017, while the worst were noted in 2018 (Table 4). During these years the cvs. Pamyati Azieva (attributed to strong wheat) and Duet (attributed to valuable class) for the content of protein and crude gluten corresponded to strong wheat of the second class, and Serebristaya to the valuable wheat of the third class. The parent varieties susceptible to *Septoria* blotch were inferior to the standards in terms of protein and crude gluten content. The best introgressive lines demonstrated good grain quality. The line No. 37/2015 resistant to leaf and ear damage had the highest parameters corresponding to strong wheat of the first class (protein content 16.1%, crude gluten content 32.1%). Other resistant lines (No. 2/2015, 6/2015, 7/2015, 94/2015, 322/2017, 337/2017, 24/2017) showed a high protein content (15.6–16.5%), and in terms of crude gluten content they were equal to or exceeded the cv. Pamyati Azieva, i.e., they corresponded to strong wheat of the second class. The lines No. 9/2015 and 10/2015, being susceptible to leaf damage and weakly susceptible to ear damage, were inferior to resistant ones in terms of protein and gluten content. On average they corresponded to valuable wheat of the third class. The highest parameter of hectolitre weight of grain, which corresponded to the strong wheat of the first and second classes, had lines with small grains (No. 6/2015, 7/2015, 9/2015, 10/2015, 11/2015, 94/2015).

4. Discussion

Previously, it had been shown that development of *Septoria* blotch on wheat in Europe, North America and Australia was strongly dependent on the amount and duration of precipitation, and therefore crops in coastal zones were most severely damaged [3,5]. In summer, the disease progress was mainly connected with infection by asexual pycnidiospores (6–12 generations per season) [8,45]. It was shown that drip liquid water is needed to release asexual spores from pycnids; therefore, precipitation of at least 10 mm, continuous three-day 1 mm rainfall at a moderate temperature, high humidity (98%), or regular dewfall was necessary to trigger wheat infection. Spread of propagules in leaf canopy is accelerated if they are splashed with raindrops [46]. Examples of wheat crops in the Moscow and Kirov regions showed that in rainy weather leaf lesion occurred during the tillering stem elongation period and could lead to plant death [8]. Release of spores from pycnidias did not occur without liquid water and at low humidity, and led to the suppression of the disease [13,46].

Our research was carried out in the forest-steppe zone of South-Western Siberia, an area characterized by a sharply continental climate. During the vegetation period the plants are affected by such stresses as large temperature fluctuations and drought. In recent years, long periods without precipitation have been observed in every third season, while spring and early summer droughts in April–June are the most typical for the region [47]. Due to climate peculiarities of Western Siberia and neighboring regions of Kazakhstan, spring

common wheat is mainly grown there, forming a "wheat belt" covering several million hectares [48]. Asian populations of parasitic fungi that adapted to the wheat cultivars and climate exist on these crops [49].

Unlike the European regions of Russia [8,46], in Western Siberia, *Septoria* blotch developed on adult plants during milk-wax ripeness stages, which may be due to the general patterns of precipitation and temperature in the region. The disease damaged wheat under different hydrothermal conditions, both with excessive and insufficient humidity and high temperatures. This indicates that parasitic fungi *Z. tritici* and *P. nodorum* have sufficiently adapted to regional climate. In the northern regions of Kazakhstan, annual damage of crops by *Septoria* blotch was observed in various weather conditions, except severe drought [25]. The increased harmfulness of the *Septoria* blotch was also observed in dry and hot climate of Tunisia, and the airborne ascospores of *Z. tritici* played an important role in spreading the disease [15]. The pathogen's ability to adapt to stress conditions was confirmed by the fact that genes increasing the resistance to extreme air temperature were identified in the genome of *Z. tritici* [50]. Earlier, it had been shown that resistance to high temperature strengthened the adaptive properties of the fungus *P. striiformis* f. sp. *tritici*, which contributed to the rapid spread of stripe rust on wheat in the Mediterranean Region and Western Europe [51].

The studies of *Septoria* blotch development in Northern Kazakhstan showed that all commercial wheat cultivars were susceptible to the disease [52]. The accessions of *Th. ponticum* were immune to the disease during the research, while cultivars and lines were affected to different extent. The quantitative differences can be explained by the complex control of resistance to *Septoria* blotch. Previously it had been shown that polygenic control and quantitative appearance of cultivar resistance can be connected with the action of major and minor plant genes, as well as the release of a set of pathogen effectors [16,20,53,54]. Wheat resistance to *P. nodorum* is controlled by at least nine QTLs with minor action, with five of them expressed in the flag leaf, and four in the spikelet [54].

Among the studied introgressive lines with genetic material of *Th. ponticum*, more than 40% were resistant or poorly susceptible to the disease in 2015. The sharp increase in the leaf lesion of wheat in 2016, probably, was connected with changes in *Z. tritici* population, since no significant sustained damage of the ears was noted. The ratio of pathogens on introgressive lines fluctuated: *Z. tritici* dominated on ten ones, and *P. nodorum* prevailed on three ones. Such results may be related to the different distribution of resistance genes in the lines. The best lines maintained high resistance to *Septoria* leaf and ear blotch during the observations.

The *Septoria* complex on wheat was first studied in the Omsk region. In 2017, the ratio of *Z. tritici* and *P. nodorum* was similar. In 2018, it shifted slightly towards *P. nodorum*, and in 2019, *Z. tritici* prevailed. Judging by restoration of the resistance of some lines and reduction in the proportion of susceptible ones in 2018–2019, some pathogen strains were eliminated from the population. It had been previously shown that *P. nodorum* prevailed in Northern-eastern Kazakhstan in 1994–2011 [25], but in 2018–2019, it was *Z. tritici* that dominated in Northern Kazakhstan [52]. These confirms our conclusion that the increased harmfulness of *Septoria* leaf blotch in the Omsk region was caused by changes in *Z. tritici* population. At the same time, *P. nodorum* prevailed in the neighboring Novosibirsk region, Altai Krai, and some districts of the Tyumen region in 2016–2018 [24]. Due to the increased harmfulness of *Septoria* diseases, the evolutionary processes in the populations of *Z. tritici* and *P. nodorum* need additional study.

The Omsk region specializes in wheat cultivation, and its soil and climatic conditions allow obtaining grain with high quality. It is known that the main reserve substances in grain are carbohydrates (more than 80%) and proteins [55]. Efficiency of grain filling, determined by the 1000 grain weight, is provided mainly by accumulation of carbohydrates. At the same time, spare proteins that determine the baking properties are formed. The obtained results showed that damage of leaves and ears negatively affected the weight of 1000 grain, but at the same time the parameter hectoliter weight of grain increased. The

ear damage had a stronger effect on the formation of spare proteins, which was reflected in the content of protein and crude gluten. In general, previously established negative impact of *Septoria* diseases on yield, weight of 1000 grain and baking qualities [25,56,57], was confirmed. However, the negative effect was lower than that previously described, i.e., during epidemics, which can be explained by late plant injury. During the breeding process, eight introgressive lines with stable resistance to *Septoria* blotch, high yield, and grain quality were selected.

5. Conclusions

Accessions of *Th. ponticum* and introgressive lines of spring common wheat with its genetic material were studied for resistance to *Septoria* blotch in the southern forest -steppe of Western Siberia (Omsk, Russia). *Th. ponticum* showed immunity to the disease in 2015–2019. In 2016, a sharp increase in leaf lesion of wheat without significant increase in ear damage was noted. Probably, the increased *Septoria* blotch damage to leaf was connected with changes in the *Z. tritici* population. We noted quantitative differences in the ratio of pathogens on introgressive lines, which may be due to different distributions of resistance genes. Introgressive lines resistant to leaf and ear damage were selected. The best lines with the genetic material of *Th. ponticum*, combining resistance to *Septoria* blotch with high yield and grain quality, represent a valuable material for breeding common wheat.

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