Characteristics of *Zymoseptoria tritici* isolates from the Russian Federation and the Republic of Kazakhstan by azoxystrobin sensitivity

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Abstract. Septoria tritici blotch is one of the harmful diseases of wheat cultivars cultivated in the Russian Federation and the Republic of Kazakhstan. The purpose of the research was to study a sample of *Zymoseptoria tritici* isolates obtained from the Saratov and Tambov (Russian Federation) and Almaty (Republic of Kazakhstan) regions according to the degree of the azoxystrobin sensitivity, as well as to evaluate these isolates for the presence of the G143A mutation using PCR-RFLP analysis. The azoxystrobin sensitivity of *Z. tritici* isolates was evaluated by studying the growth restriction of cultures on a nutrient medium (concentrations of active drug in the medium were 100, 10, 1, 0.1, 0.01 mg/l.). In the control, on the 21st day of cultivation, the diameter of the colonies of *Z. tritici* strains varied from 14.24±1.43 mm to 19.53±0.66 mm. Azoxystrobin had a significant inhibitory effect on the growth of fungal isolates from the Tambov region: EC50 is 1.72 μg/l, from the Almaty region EC50 is 2.36 μg/l, from the Saratov region EC50 is 2.63 μg/l. The results of biotesting made it possible to identify isolates of *Z. tritici* resistant to azoxystrobin: 277-22-5, 277-22-13 (from Kazakhstan); 104-23-9, 104-23-10 (from the Saratov region); 19-23-5, 19-23-8 (from the Tambov region). PCR-RFLP method made it possible to identify mitochondrial mutations G143A in selected fungal isolates, which are associated with the development of resistance to fungicides in the causative agent of septoria. This work is the beginning of a more in-depth study of the sensitivity of the economically significant phytopathogen *Z. tritici* to fungicides in the territories of Russia and the Republic of Kazakhstan.

1 Introduction

Septoria tritici blotch is a dangerous wheat disease of mycotic etiology. Disease pathogens cause serious damage to agricultural production in grain-producing countries [1, 2]. Leaf spot diseases are a problem in wheat production in the Pacific Northwest and northern

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Great Plains of the United States, Europe, Central and Western Asia, Russia, and Kazakhstan [3 – 6].

On the territory of the Russian Federation and the Republic of Kazakhstan, Septoria tritici blotch (STB) causes *Zymoseptoria tritici* (Desm.) Quaedvl. & Crous. [7, 8].

Direct losses of agricultural products by courtesy of Septoria spot during epiphytotics can reach 50%. In moderate years, damage can range from 5 to 20% depending on weather conditions and varietal characteristics of the host. It is evaluated that up to 70% of fungicides in Europe are used to control Septoria spot [9, 10].

To protect grain crops from diseases in Russia and Kazakhstan, the chemical method is widely used.

Strobilurins are a broad class of fungicides that are effective at the early stages of the pathological process - during spore germination, as well as at the very beginning of the penetration of infectious hyphae into the cells of the host plant [11]. Strobilurins are preventive fungicides; they do not stop mycelial growth [12].

This class of fungicides has good antisporulant properties: without preventing the appearance of infectious spots, fungicides suppress the formation of secondary spores on them [13].

The active substances of strobilurins inhibit the respiration of fungi by connecting to a specific site in mitochondria - quinol oxidation - Qo, therefore in the English literature they are called QoIs (QoI fungicides - Quinone outside Inhibitors). By binding to the quinol oxidation site in cytochrome b, strobilurins disrupt the transfer of electrons from cytochrome b to cytochrome c, which leads to disruption of NADP oxidation and ATP synthesis, which causes the cessation of energy synthesis and the fungus death [14].

Treatment of plants with strobilurins causes the so-called “greening effect”, which manifests itself externally and at the biochemical level (increasing the rate of photosynthesis, preventing leaf aging) and increases yield [14, 15].

Strobilurins have high rain resistance, which is ensured by lipophilic particles that hold the drug on the leaf surface in the form of a hardened sediment [13]. Azoxystrobin, picoxystrobin and pyraclostrobin are capable of translaminar movement [14].

Depending on the chemical structure, the class of strobilurins is divided into 10 groups, of which active substances from the groups of methoxy-acrylates (azoxystrobin, picoxystrobin), methoxy-carbamates (pyraclostrobin), oxymino-acetates (cresoxim-methyl), dihydro-dioxazine (fluoxastrobin) are used to protect grain crops [16].

Since the late 1990s, QoIs have become a key component of cereal disease management strategies in northwestern Europe due to their persistent activity and potential for additional yield by prolonging ground greenness [17].

Isolates of *Z. tritici* with reduced strobilurins sensitivity were first discovered in 2002. The G143A mutation has become widespread in the gene pool of fungal populations [18, 19]. However, despite this, some QoI fungicides are still effective and are widely used both in Russia and in other grain-producing countries [20 – 22].

Following the discovery of resistant isolates in field populations of *Z. tritici* [23], QoIs were allowed to be used only in mixtures with fungicides, demethylation inhibitors (DMIs), particularly triazoles, with a maximum of two sprays per season to slow the development of resistance and ensure effective disease control.

The main reason for the fungi resistance to QoI are single nucleotide polymorphisms (SNP) in the cytochrome b gene, leading to the substitution of glycine with alanine at the 143 amino acid position (G143A) [24], as well as the substitution of phenylalanine with leucine at the 129 amino acid position (F129L), and adaptation mechanisms, in particular overexpression of an alternative oxidase that bypasses respiratory complex III [25, 26].

The purpose of the work is to study a sample of *Z. tritici* isolates obtained from the Saratov and Tambov (Russian Federation) and Almaty (Republic of Kazakhstan) regions

according to the degree of azoxystrobin sensitivity, as well as to evaluate these isolates for the presence of the G143A mutation using PCR-RFLP analysis.

2 Materials and methods

2.1 Experimental site and plant materials

In 2022 and 2023, wheat plants infected with the fungus *Z. tritici* were collected in Russia, in the Tambov and Saratov regions, and in the Almaty region of Kazakhstan. Infectious samples were leaves with characteristic disease signs. Leaves were collected at the stage of milky-wax plant ripeness according to the Zadoks growth scale (75-85). In total, material was collected from 7 wheat varieties and 1 hybrid line. For each cultivar and line, at least 30 leaves were collected for subsequent analysis (Table 1).

Table 1. Origin of infectious material collected in 2022-2023

No. item	Infectious sample	Origin of infectious sample/ host-plant
1	276-22	Almaty region, Karakystak / winter bread wheat Bezostaya 100, 43°7'42"N, 76°6'2"E
2	277-22	Almaty region, Uzynagash / winter bread wheat Brazilskaya elita, 43°13'15" N, 76°18'51" E
3	104-23	Saratov region, experimental field of the Federal Center of Agriculture Research of the South-East Region / spring bread wheat Tulaikovskaya 10, 51°34'28"N, 46°00'20"E
4	118-23	Saratov region, experimental field of the Federal Center of Agriculture Research of the South-East Region / Саратовская область, опытное поле ФАНЦ Юго-Востока / winter bread wheat hybrid line (KSI No.3) 51°34'28"N, 46°00'20"E
5	14-23	Tambov region, experimental field of the Central Russian branch of the FSSI "I.V. Michurin FSC"/ spring bread wheat Ekada 109, 52°40'30"N, 41°9'49"E
6	15-23	Tambov region, experimental field of the Central Russian branch of the FSSI "I.V. Michurin FSC"/ spring bread wheat L 503, 52°40'30"N, 41°9'49"E
7	19-23	Tambov region, experimental field of the Central Russian branch of the FSSI "I.V. Michurin FSC"/ spring bread wheat Kinelskaya yubileynaya, 52°40'30"N, 41°9'49"E
8	23-23	Tambov region, experimental field of the Central Russian branch of the FSSI "I.V. Michurin FSC"/ spring bread wheat Daria, 52°40'30"N, 41°9'49"E

Study of the toxicity of azoxystrobin to Z. tritici

After collecting infectious samples of grain crops, they were subjected to further testing in the laboratory. Monoconidial colonies of *Z. tritici* were isolated in pure culture on potato dextrose agar [27].

Laboratory experiments were conducted to study the toxicity of azoxystrobin to the *Z. tritici* from various agroclimatic zones [28]. The study included 40 fungal isolates from the Almaty region: 276-22-1…20-*Z.t.*, 277-22-1…20-*Z.t.*; 30 from the Saratov region: 104-23-1…15-*Z.t.*, 118-23-1…15-*Z.t.*; 30 from the Tambov region: 14-23-1…10-*Z.t.*, 15-23-1…5-*Z.t.*, 19-23-1…10-*Z.t.*, 23-23-1…5-*Z.t.*); 70 *Z. tritici* isolates were studied.

To study the toxicity of the fungicide against the fungus *Z. tritici*, a sample of the drug was introduced into a molten culture medium (MCM) and the medium was thoroughly mixed. Drug concentrations were expressed in mg/l of the medium in terms of the active

substance (azoxystrobin). The following concentrations of the active substance were studied: 100, 10, 1, 0.1, 0.01 mg/l. The experiment for each active substance concentration was carried out in triplicate [28]. Then, a disk with a diameter of 3 mm, cut from a pure culture of the fungus that was not exposed to the drug, was placed on the surface of the solidified agar with the added fungicide. The fungus was inoculated using asepsis. Petri dishes without drug administration were used as a control. To assess the activity of the active substance, the diameter of the fungal colonies was measured in two mutually perpendicular directions and the average diameter was calculated. Observations were carried out on the 21st day, when, according to methodological recommendations, complete maturation of the colony in the control occurs [27].

2.2 DNA extraction and molecular screening for the G143A mutation

When colonies of *Z. tritici* were detected on a culture medium with a concentration of 100 mg/l of azoxystrobin, having a size close to the control, they were examined for the presence of the G143A mutation, which determines resistance to strobilurins in isolates, using the PCR-RFLP method.

Fungal genomic DNA was extracted from a pure culture of monoconidial isolates obtained on potato dextrose agar using a standard DNA extraction method (CTAB method) [29].

A pair of primers was used [30]: MgcytbF 5'TCGTTACTGGTGTTACACTTGC3' and MgcytbR 5'GCCATAACATAATTCTCGCTGTCACC3' (Beagle, Russia), followed by restriction of the obtained amplified fragment with endonuclease Fsp4H I, 200 e.a. (SibEnzyme, Russia, GC NGC recognition site; GC $\downarrow$NGC; CGN $\uparrow$CG).

Genomic DNA amplification was carried out in 25 µl of a reaction mixture consisting of the following components: 2 µl of genomic DNA (25 ng, acceptable from 2 to 50 ng), 1 µl of each primer (10 pM/µl), 0.5 µl of dNTPs mix (10 mM, aqueous solution of dCTP, dGTP, dTTP and dATP, TransGen, China), 0.5 µl of $MgCl_2$ (100 mM), 0.5 µl of BioTaq DNA polymerase (5U, 5 units/µl, Dialat Ltd., Russia), 2.5 µl 10×PCR buffer and 17 µl of ddH_2O.

The PCR program included an initial denaturation for 2 min at 96°C; then 35 cycles: 1 min – at 96 °C, 1 min – at 50 °C and 1 min – at 72 °C; final elongation for 5 min at 72 °C. The obtained amplified fragments were analyzed by electrophoresis in a 2% agarose gel. Fragment size was evaluated using the GeneRuler 100bp DNA marker (Thermo Fisher Scientific). As a result of amplification, a specific product of about 650 bp in size was obtained. The DNA fragments were then purified from the agarose gel using a purification kit (Cleanup Standard, Evrogen, Russia).

Restriction of the PCR product was carried out in accordance with the following protocol: a reaction mixture with a volume of 15 µl contained 10 µl of the PCR product (purified DNA), 0.4 µl of the Fsp4H I restriction enzyme and 2 µl of SE buffer Y 10× recommended for this restriction enzyme, bringing the volume to final deionized water. The restriction reaction was carried out in a thermostat at 37 °C for 16 hours [31]. The sizes of restriction products were determined by horizontal electrophoresis in a 2% agarose gel at a voltage of 80 V for 1 hour using a Gene Ruler 100bp molecular weight marker (Thermo Fisher scientific).

Two fragments with molecular weights of 442 and 210 bp after restriction of the amplified fragment by endonuclease indicates the sensitivity of the samples to the fungicide action. On the other hand, the presence of three fragments with molecular weights of 298, 210 and 144 bp confirms the presence of the G143A mutation in the genotype of the isolates [30, 31].

2.3 Statistical analysis

For statistical data processing, the computer program “STATISTICA 12” was used. The average values of radial growth of colonies (in millimeters), their percentage relative to the control, standard deviation were determined, and the minimum and maximum values were recorded. During the analysis, post hoc comparisons between groups were used [32]. EC50 was also calculated - half maximal effective concentration of fungicides, at which 50% inhibition of the growth of fungal strains was observed (https://www.aatbio.com/tools/ec50-calculator).

3 Results

The azoxystrobin sensitivity of *Z. tritici* isolates was evaluated by studying the cultures’ growth restriction on a culture medium. Final concentrations of active substance of the drug in the medium were 100, 10, 1, 0.1, 0.01 mg/l.

In the control, on the 21st day of cultivation, the diameter of the colonies of *Z. tritici* strains varied from 14.24±1.43 mm (23-23-1…10-*Z.t.* from the Tambov region) to 19.53±0.66 mm (14-23 -1…10-*Z.t.* from Tambov region). The drug effect on fungal isolates was manifested in inhibition of their growth, which degree depended on the drug concentration (Fig. 1).

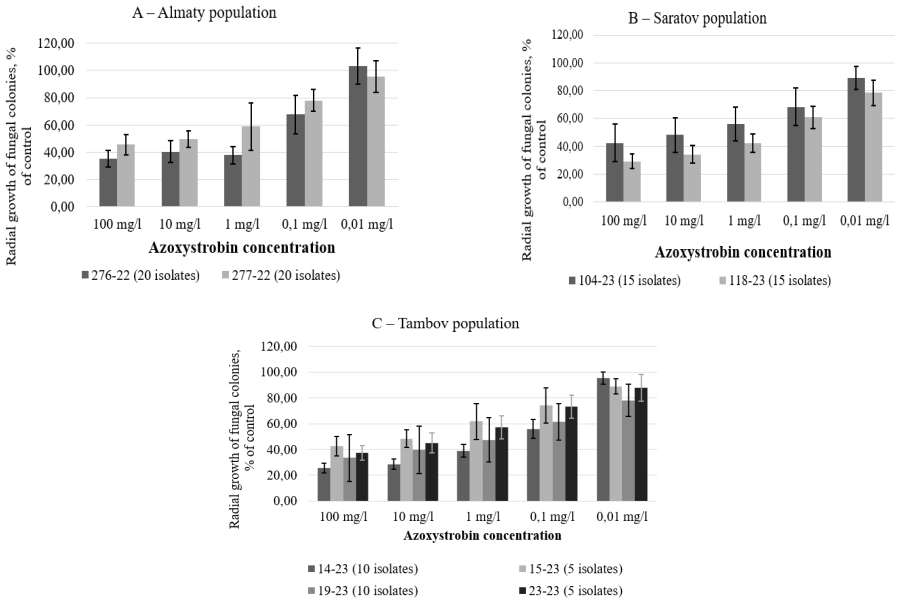

Fig. 1. The effect of azoxystrobin on the growth of strains of *Zymoseptoria tritici* fungus when cultivated on PGA for 21 days at 21°C.

When comparing two groups of isolates isolated from the same cultivar and grown on potato dextrose agar without the application of a fungicide and with an active substance concentration of 0.01 mg/l, using Student's t-test with Bonferroni correction, statistically significant differences were detected between groups ($p < 0.05$) for isolates obtained from cultivars from the Saratov and Tambov regions. At the same time, no statistically significant differences were detected between the control group and isolates from Kazakhstan at the lowest evaluated concentration of 0.01 mg/l ($p > 0.05$).

At a concentration of the active substance of 0.01 mg/l, azoxystrobin was the most effective in limiting the growth of *Z. tritici* on potato dextrose agar from the Saratov region (the average diameter of the colonies was 83.79 ± 7.45% of the control). For isolates from the Tambov region, the diameter of fungal colonies at this concentration averaged 87.55±7.17% compared to the control. At the same time, the fungicide had the least effectiveness against strains from Kazakhstan, where the average diameter of the colonies was 99.45±5.48% compared to the control.

A comparison of the fungicide sensitivity of *Z. tritici* was based on EC50 values (Table 2). Azoxystrobin had a significant inhibitory effect on the growth of fungal isolates from the Tambov region, manifesting itself with an EC50 of 1.72 μg/l, from the Almaty and Saratov regions its values were 2.36 and 2.63 μg/l, respectively.

Table 2. Concentrations of azoxystrobin in the culture medium leading to half-maximal inhibition of the growth of *Zymoseptoria tritici* colonies

Fungus isolates / origin	Number of isolates studied	Isolates with low azoxystrobin sensitivity	Mean EC50 for *Z. tritici* (range of values for strains), μg/l of nutrient medium	
			Valid N	Mean±Std.Dev. (min – max)
276-22-1…20-*Z.t.* / Almaty region	20*	0	20	0,15±0,04 (0,24 – 1,11)
277-22-1…20-*Z.t.* / Almaty region	20	277-22-5 277-22-13	18**	4,56±3,96 (1,14 – 16,47)
Mean / Almaty region			2.36±3.12	
104-23-1…15-*Z.t.* / Saratov region	15	104-23-9 104-23-10	13**	4,82±7,22 (0,11 – 22,5)
118-23-1…15-*Z.t.* / Saratov region	15	0	15	0,43±0,38 (0,16 – 1,59)
Mean / Saratov region			2.63±3.1	
14-23-1…10-*Z.t.* / Tambov region	10	0	10	0,2±0,11 (0,11 – 0,47)
15-23-1…5-*Z.t.* / Tambov region	5	0	5	3,21±1,95 (1,13 – 5,61)
19-23-1…10-*Z.t.* / Tambov region	10	19-23-5 19-23-8	8**	0,23±0,09 (0,11 – 0,36)
23-23-1…5-*Z.t.* / Tambov region	5	0	5	3,24±1,47 (1,54 – 5,6)
Mean / Tambov region			1.72±1.74	

* Fungal isolates were studied at 3-fold repetition of each azoxystrobin concentration (100; 10; 1; 0.1; 0.01 μg/l).

** When calculating EC50, the traits of isolates with low azoxystrobin sensitivity were not taken into account, because at all studied fungicide concentrations, no suppression of the growth of fungal colonies ≤ 50% relative to the control was observed.

To establish the significance of differences between isolates of different geographical origins, ranked by sensitivity to azoxystrobin, Student's t-test with Bonferroni correction was used (Table 3). Statistically significant differences were

detected between the mean values of colony diameter at different active concentrations. between isolates from the Tambov region and from the Saratov region, as well as between isolates from the Tambov region and from the Almaty region (Table 3). No differences were detected between isolates from the Saratov region and the Almaty region.

Table 3. Results of analysis of variance of post hoc comparisons of mean values of radial growth of *Zymoseptoria tritici* colonies cultivated on potato dextrose agar, from different geographical zones (by azoxystrobin sensitivity), obtained using Student's t-test with Bonferroni correction

Z.tritici colonies, obtained from wheat cultivars	Almaty region	Saratov region	Tambov region
Almaty region	-	0.45	*0.03**
Saratov region	0.45	-	*0.04**
Tambov region ь	*0.03**	*0.04**	-

* Differences are statistically significant at $p < 0.05$ level

Differences were identified between isolates obtained from a hybrid line from the Saratov region and the Bezostaya 100 cultivar from Kazakhstan; from the variety Bezostaya 100 and from the cultivars L 503, Kinelskaya yubileynaya, Daria from the Tambov region; from the Brazilskaya elita cultivar from the Almaty region and from the Daria variety from the Tambov region; from the Tulaikovskaya 10 cultivar from the Saratov region and from the Kinelskaya yubileynaya and Daria cultivars from the Tambov region; as well as between isolates isolated from a hybrid line from the Saratov region and from the Daria cultivar from the Tambov region (Table 4).

Thus, the influence of the host cultivar and geographic origin of *Z. tritici* on the azoxystrobin sensitivity of fungal isolates can be traced.

Table 4. Results of the analysis of variance of post hoc comparisons of mean values of radial growth of *Zymoseptoria tritici* colonies cultivated on potato dextrose agar, from 8 wheat cultivars (according to azoxystrobin sensitivity), obtained using Student's t-test with Bonferroni correction

Zymoseptoria tritici colonies, obtained from wheat cultivars	276-22-1…20-*Z.t.* / Bezostaya 100	277-22-1…20-*Z.t.* / Brazilskaya elita	104-23-1…15-*Z.t.* / Tulaikovskaya 10	118-23-1…15-*Z.t.* / Hybrid line	14-23-1…10-*Z.t.* / Ekada 109	15-23-1…5-*Z.t.* / L 503	19-23-1…10-*Z.t.* / Kinelskaya yubileynaya	23-23-1…5-*Z.t.* / Daria
276-22-1…20-*Z.t.* / Bezostaya 100	-	0.08	0.61	*0.03**	0.27	*0.04**	*0.04**	*0.01**
277-22-1…20-*Z.t.* / Brazilskaya elita	0.08	-	0.28	0.58	0.75	0.59	0.52	*0.04**
104-23-1…15-*Z.t.* / Tulaikovskaya 10	0.61	0.28	-	0.12	0.54	0.22	*0.04**	*0.02**
118-23-1…15-*Z.t.* / Hybrid line	*0.03**	0.58	0.12	-	0.45	0.89	0.88	*0.04**
14-23-1…10-*Z.t.* / Ekada 109	0.27	0.75	0.54	0.45	-	0.48	0.41	0.09
15-23-1…5-*Z.t.* / L 503	*0.04**	0.6	0.22	0.89	0.48	-	0.98	0.39
19-23-1…10-*Z.t.* / Kinelskaya yubileynaya	*0.04**	0.52	*0.04**	0.89	0.41	0.98	-	0.31
23-23-1…5-*Z.t.* / Daria	*0.01**	*0.04**	*0.02**	*0.04**	0.09	0.39	0.314	-

* Differences are statistically significant at $p < 0.05$ level

The results of biotesting made it possible to identify isolates of *Z. tritici* resistant to azoxystrobin: 277-22-5, 277-22-13 (from Kazakhstan); 104-23-9, 104-23-10 (from the Saratov region); 19-23-5, 19-23-8 (from the Tambov region). It is assumed that the genotype of these strains contains the G143A mutation.

To identify the mitochondrial fungicide resistance mutation G143A, we used a pair of primers MgcytbF/R followed by restriction of the amplified fragment with endonuclease Fsp4H I, 200 e.a. (Fig. 2.).

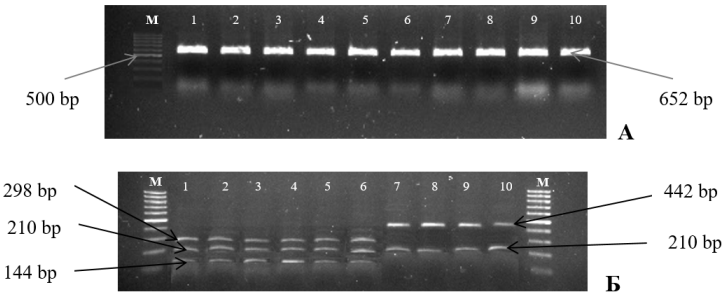

Fig. 2. Results of PCR-RFLP analysis performed for *Zymoseptoria tritici* isolates, showing the presence/absence of the G143A mitochondrial mutation. A – results of PCR analysis; B – results of restriction of the PCR product. The G143A resistance mutation is determined by the presence of three DNA restriction fragments: 298, 210 and 144 bp and is present in isolates 1) 277-22-5-*Z.t.*, 2) 277-22-13-*Z.t.*, 3) 104-23-9-*Z.t.*, 4) 104-23-10-*Z.t.*, 5) 19-23- 5-*Z.t.*, 6) 19-23-8-*Z.t.* The absence of a mutation is characterized by the presence of two fragments of 442, 210 bp in size: 7)15-23-1-*Z.t.* 8)15-23-2-*Z.t.* 9)19-23-6-*Z.t.* 10)19-23-10-*Z.t.* M – DNA marker Gene Ruler 100bp (Thermo Fisher scientific).

After restriction of the amplified fragment with endonuclease, two fragments with a molecular weight of 442 and 210 bp were noted. These fragments were sensitive to the fungicide action (15-23-1-*Z.t.*, 15-23-2-*Z.t.*, 19-23-6-*Z.t.*, 19-23-10-*Z.t.*). In isolates with the G143A mutation (277-22-5-*Z.t.,* 277-22-13-*Z.t.*, 104-23-9-*Z.t.*, 104-23-10-*Z.t.*, 19-23-5-*Z.t.*, 19-23- 8-*Z.t.*) three fragments with a molecular weight of 298 bp, 210 bp and 144 bp were detected.

Thus, the use of the PCR-RFLP method made it possible to identify mitochondrial G143A mutations, which are associated with the development of fungicide resistance in the causative agent of Septoria in populations from the Almaty, Tambov and Saratov regions.

4 Discussions

The literature indicates a decrease in field effectiveness against STB QoI fungicides in many countries, especially in European regions [33 – 35], where there is a high fungicidal load in crop fields [36]. We studied fungal isolates obtained from fields that had not been treated with fungicides for several years. Also, no fungicide treatment was carried out on neighboring fields within a 10 km radius. However, it is worth noting that pycnospores and ascospores of the fungus can spread over long distances due to wind and raindrops.

The results of our research confirmed the effectiveness of the strobilurins application in the fields of three different areas. We compared the sensitivity of *Z. tritici* to fungicides using the EC50 index. Azoxystrobin showed a significant inhibitory effect on the growth of fungal strains cultivated on potato dextrose agar on isolates from the Tambov region, with an EC50 of 1.72 μg/l. In general, isolates from the Almaty and Saratov regions showed sensitivity, with mean EC50 values of 2.36 and 2.63 μg/l, respectively. To prevent the

emergence and spread of resistance, it is recommended to use modern mixtures of strobilurins with other classes of fungicides [37].

We used Student's t test with Bonferroni correction to analyze the effect of host cultivar and geographic origin of *Z. tritici* isolates on their azoxystrobin sensitivity. The results showed significant differences between isolates from the Tambov region and fungal strains obtained from plant material from the Saratov and Almaty regions.

Previously, it was discussed that the Saratov region is located on the border with a large grain-growing region - the Republic of Kazakhstan. E.I. Gultyaeva et al. [38] in their studies show a high similarity *of P. triticina* populations in the Western Asian regions of Russia and Northern Kazakhstan, which confirms the assumption of the existence of a single fungus population in the studied regions. Based on this, wheat crops in the Saratov region can be infected with inoculum of the most diverse virulence, which requires constant monitoring by phytopathologists, breeders and geneticists [8, 39].

The main mechanism for the development of resistance to strobilurin fungicides is the G143A mutation in the cytb gene, which quickly spread throughout Europe [18, 30]. As a result of our biotests, azoxystrobin-resistant *Z. tritici* isolates were identified: 277-22-5, 277-22-13 (from Kazakhstan); 104-23-9, 104-23-10 (from the Saratov region); 19-23-5, 19-23-8 (from the Tambov region). Using the PCR-RFLP method, we confirmed the presence of the G143A mutation in their genotype. This indicates the presence of mutants in plant pathogen populations; besides, unjustified use of any strobilurin may contribute to the further spread of *Z. tritici* strains carrying G143A.

This work begins a more in-depth study of the sensitivity of the economically significant phytopathogen *Z. tritici* to fungicides.

5 Conclusion

The results obtained in this study indicate that fungicides containing the active substance azoxystrobin remain effective against the causative agent of Septoria leaf spot of wheat in the conditions of the Tambov, Saratov and Almaty regions. However, we identified *Z. tritici* isolates from these three regions whose genotypes contained the G143A mutation. This highlights the need to promote and implement resistance management strategies to maintain the effectiveness of QoI fungicides applied in the field to control Septoria leaf spot in wheat.

Acknowledgments

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