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Diversity of *Avena* Species by Morphological Traits and Resistance to Fusarium Head Blight

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Abstract—Sixty-six genotypes of 19 *Avena* species of different ploidy from VIR collection are analyzed for resistance to Fusarium head blight. The plants were infected in the field with *Fusarium culmorum* strains. For the first time in the world a representative set of the wild *Avena* species is assessed. The DNA content of trichothecene-producing *Fusarium* fungi was identified by a quantitative polymerase chain reaction (qPCR) method. The amount of mycotoxins in the grain was determined by an enzyme-linked immunosorbent assay (ELISA) method. The smallest amounts of *Fusarium* DNA and deoxynivalenol (DON) were detected in seven genotypes of hexaploid species: *A. byzantina*, *A. sterilis*, *A. sativa* and *A. fatua* and one diploid species *A. wiestii*. Statistically significant connections were demonstrated between the morphological traits of the genotypes, the *Fusarium* DNA content and amount of DON.

Keywords: Avena species, ploidy, fungal DNA, *Fusarium*, mycotoxins, quantitative PCR, ELISA
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INTRODUCTION

Oat is one of the most important grain crops cultivated by man. Hence, there is a growing need to study diverse variety of oats in terms of their economically valuable traits and to search for new genotypes that can serve as the base for development of new varieties with high productivity and resistance to diseases.

The *Avena* L. genus is represented by cultivated species with a great practical significance and by wild species that are interesting as objects of taxonomic studies and sources of valuable traits. At present, the *Avena* genus is included by 26 species and three levels of ploidy, most of which refer to wild species. The cultivated species are found in each group of ploidy: *A. strigosa* Schreb. ($2n = 14$), *A. abyssinica* Hochst. ($2n = 28$), *A. byzantina* C. Koch, and *A. sativa* L. ($2n = 42$) (the most important among this group) [1, 2].

In recent years, the *Avena* species have attracted greater interest from breeders; this was promoted by the development of cytological, immunological, biochemical, and other research methods. Wild species that frequently inhabit diverse bioecological conditions are characterised by significant genetic diversity and can carry genes that allow them to survive in adverse conditions. Adaptation of wild species to unfavorable environmental factors, their resistance to pathogenic organisms and a number of traits promoting high productivity and quality suggest the presence of unique sources of the initial material for breeding among them [3]. One of richest collection of the *Avena* species (the samples of which are used as the working material for creating new oat varieties) in the world is housed in N.I. Vavilov All-Russian Institute of Plant Genetics Resources (VIR).

The Fusarium head blight caused by hemibiotrophic fungi from the *Fusarium* Link genus is a deleterious disease leading to loss of the harvest and reduction of grain quality. However, the peculiarities of the infection process of this disease are comparatively less studied. It has a specific etiology: different *Fusarium* species involved in the infection process can produce toxic secondary metabolites (mycotoxins); as a result, the grain becomes unsuitable for use as food or fodder.

High aggressive pathogens such as *F. graminearum* Schwabe and *F. culmorum* (W. G. Sm) are typical causative agents of the Fusarium head blight in the oats

growing regions; they are able to overcome active plant protection and to produce trichothecene mycotoxin deoxynivalenol (DON), as well as zearalenone (ZEN). At the same time, another species, for example, the weak pathogen *F. poae* (Peck) Wollenw. (that are able to populate only weakened plants and/or damaged plant tissues) are also wildly found in the mycobiota of oats grain [4]. The *F. poae* dominance in a complex of *Fusarium* fungi on the oats grain is one of the remarkable peculiarities of the disease of this culture [5, 6]. This fungus produces mycotoxin nivalenol, which as well as DON, refers to a group of trichothecene chemical compounds [7]. Nivalenol does not belong to regulated fusariotoxins in the grain because of the high cost of its identification.

In contrast to wheat, the absence of visible disease symptoms on vegetating plants and on grain is typical of Fusarium head blight of oats even in the presence of significant infection; consequently, it is impossible to assess the degree and severity of the disease in fields. The difficulty in adequately identifying the disease visually largely inhibits breeding work with this culture. It is necessary to use expensive and time-consuming methods (for example, the methods of the quantitative detection of fungal DNA and mycotoxins in grain) for the complete characteristics of the oats genotypes; this significantly complicates screening the genetic material for the resistance to Fusarium head blight.

In addition, the previously detected multicomponent character of resistance to Fusarium head blight in wheat is also confirmed during the analysis of different oats varieties: the level of the infected grain frequently does not coincide with the content of mycotoxins [8]. This again confirms the need to use the totality of quantitative parameters for the estimation that more accurately reflect the interaction between the genotypes and toxin-producing fungi.

Due to significance of the problem of oats grain contamination with mycotoxins, breeders have intensified their efforts in searching for sources of resistance to this disease and creating modern varieties that do not accumulate mycotoxins. The characteristic of the oat varietal diversity by Fusarium head blight resistance began to be studied in all countries of the world where this culture is cultivated [9].

The existing diversity of cultivated oat genotypes related to different species is studied for resistance to infection by fungi and accumulation of mycotoxins. It was established that *A. strigosa* and *A. sativa* species are less susceptible to Fusarium head blight than *A. byzantina* and *A. abyssinica* [10–12].

To date, there is no published information about resistance to Fusarium head blight in diverse wild *Avena* species. However, the urgent need for such studies was discussed at an international conference on the genetic resources of the *Avena* genus [11].

The morphological and physiological–biochemical peculiarities of the oats species significantly affect the intensity of flow of the infectious process caused by the fungi. The unique diversity of these characteristics in the *Avena* genotypes allows us to estimate their significance during interaction with *Fusarium* fungi that differ in their pathogenicity.

The aim of the study was to evaluate the genetic diversity of different *Avena* species by the infection with the *Fusarium* fungi (fungal DNA content in the grain) and accumulation of DON and ZEN mycotoxins.

METHODS

In 2015, 57 wild and 9 cultivated *Avena* genotypes were selected for a study on the resistance of grain to Fusarium head blight from the VIR collection: *A. agadiriana* Baum et Fed., *A. atlantica* Baum et Fed., *A. barbata* Pott., *A. canariensis* Baum, *A. clauda* Durieu., *A. damascena* Rajh. et Baum, *A. fatua* L., *A. hirtula* Lag., *A. insularis* Ladiz., *A. longiglumis* Durieu., *A. ludoviciana* Durieu., *A. magna* Murph. et Terr., *A. murphyi* Ladiz., *A. occidentalis* Durieu., *A. sterilis* L., *A. vaviloviana* Mordv., *A. wiestii* Steud., *A. sativa*, and *A. byzantina* (Table 1). Among the studied genotypes, 13.6 referred to diploid, 28.8 to tetraploid, and 57.6% to hexaploid oats forms. The analyzed genotypes originated from different regions: Israel (n = 11); Morocco (n = 9), Russia (n = 5); Spain (n = 4); Azerbaijan, Algeria, and Syria (n = 3 each); Armenia, China, and Norway (n = 2 each); and Bulgaria, Venezuela, Germany, Georgia, Egypt, Italy, Canada, Kenya, and Lebanon (all n = 1). Compared to the wild *Avena* species, the cultivated oats varieties were selected according to the known characteristics of their resistance to the disease.

The assessment was conducted using artificial inoculation in the fields in Pushkin branch of VIR. Each sample was grown on one-meter-plots in double replications according to the methodical guidelines [13]. In addition to the natural background of the *Fusarium* fungi existing in the field, an artificial inoculation by *F. culmorum* fungus was performed. The grain inoculum was distributed by the soil surface (150 g/m^2) when panicle started to emerge in the earliest oat genotypes. To obtain a crumbly inoculum, the grain mixture (wheat and barley) was humidified to 40% moisture, 10 g both chalk and gypsum was added, it was autoclaved under 1 atm pressure for an hour and then it was mixed thoroughly. After cooling, a water suspension of mycelium and conidia of *F. culmorum* strains was introduced to the sterilized grain. For a month, the substrate stored at room temperature was constantly mixed to create favorable conditions for the grain's colonization by the fungi.

Since the spikelets of wild oats slough in the maturation process, therefore harvesting of grain is difficult. In the vegetation process, individual plants were isolated in gauze bags that allowed preservation of crop for subsequent analyses.

Table 1. Wild and cultivated *Avena* genotypes studied in this work

No.	*Avena* species	Ploidy (2*n*)	Genome	Number of samples	VIR catalog number (origin)
1	*A. atlantica* Baum et Fedak	14	As	1	k-2109 (Morocco)
2	*A. canariensis* Baum	14	Cv	1	k-1914 (Spain, Canary Islands)
3	*A. clauda* Durieu.	14	Cp	1	k-200 (Azerbaijan)
4	*A. damascena* Rajh. et Baum	14	Ad	1	k-1862 (Syria)
5	*A. hirtula* Lag.	14	As	1	k-2 (Israel)
6	*A. longiglumis* Durieu.	14	Al	2	k-87 (Israel), k-1881 (United States)
7	*A. wiestii* Steud.	14	As	2	k-94 (Egypt), k-95 (Israel)
8	*A. agadiriana* Baum et Fedak	28	AB?	1	k-2122 (Morocco)
9	*A. barbata* Pott.	28	AB	7	k-9 (Ethiopia), k-230 and k-316 (Azerbaijan), k-1848 (France, Corsica), k-1925 (Israel), k-2043 (Iran), k-2070 (Syria)
10	*A. vaviloviana* Mordv.	28	AB	3	k-10, k-11, k-755 (Ethiopia)
11	*A. insularis* Ladiz.	28	CD?	1	k-2067 (Italy, Sicily)
12	*A. magna* Murph. et Terr.	28	AC	5	k-145, k-1786, k-1851, k-1896, k-2100 (Morocco)
13	*A. murphyi* Ladiz.	28	AC	2	k-1986, k-2088 (Spain)
14	*A. fatua* L.	42	ACD	8	k-30 (Russia, Tyva), k-46 (Georgia), k-49 (Armenia), k-65 (Bulgaria), k-80 (China), k-97 (Czechoslovakia), k-116 (Armenia), k-743 (Ethiopia)
15	*A. ludoviciana* Durieu.	42	ACD	4	k-389 (Venezuela), k-461 (Syria), k-547 (Israel), k-701 (Ethiopia)
16	*A. occidentalis* Durieu.	42	ACD	1	k-1966 (Spain, Canary Islands)
17	*A. sativa* L.	42	ACD	6	k-11840 (Borrus, Germany), k-14911 (Belinda, Sweden), k-15068 (Konkur, Russia, Ulyanovsk oblast), k-15353 (Odal, Norway), k-15611 (Bessin, Germany), k-14787 (Privet, Russia, Moscow oblast), k-15301 (CDC Dancer, Canada)
18	*A. byzantina* K. Koch	42	ACD	3	k-15494 (Medved', Russia, Kirov oblast), k-15524 (Bai Yan 7, China)
19	*A. sterilis* L.	42	ACD	16	k-140 and k-146 (Morocco), k-473 (Lebanon), k-655, k-931, k-980 (Algeria), k-846 (Kenya), k-866 (Tunisia), k-1425 and k-1429 (Turkey), k-511, k-555, k-2049, k-2052, k-2059, k-2063 (Israel)

All samples were estimated by the plant height and weight of 1000 grain, as well as by the grain's hairiness and percentage of husk according to the methodical guidelines [13].

After collection of the crop, the grain of each sample (10 g) were homogenized in sterile grinding jars at the Tube Mill Control (IKA, Germany) mill. Grinding speed was 25000 rpm for 25 s for *A. sativa* and *A. byzantina* grain and 60 s for the grains of other *Avena* species. The milled flour was stored at −20°C till the subsequent DNA and mycotoxin extraction.

DNA was isolated from 200 mg of the obtained grain flour using cetyl trimethylammonium bromide (CTAB) method [14]. Typical *F. graminearum* and *F. poae* strains from the microorganism collection of the Laboratory of Mycology and Phytopathology of

the All-Russian Institute of Plant Protection were cultivated on potato–sucrose agar, and then the DNA was extracted from the air fungal mycelium using Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania).

Concentrations of the obtained DNA from the flour and the *Fusarium* strains were estimated using the Qubit 2.0 fluorimeter with the Quant-iT dsDNA HS Assay Lit reagent kit (Thermo Fisher Scientific, Unites States). DNA solution of the *Fusarium* strains were diluted to 10 ng/μL and used to construct calibration curves in subsequent dilutions of factors of 10 from 10^{-1} to 10^{-6} ng/μL. The concentration of the DNA isolated from the grain was adjusted to working values in the range 2–50 ng/μL.

The fungal DNA content was determined by the quantitative PCR (qPCR) method, which simultaneously allowed the amplification and measurement of the amount of the target DNA copy in the sample relative to the introduced specific DNA sequence.

The amount of fungal DNA measured in percentage of the total isolated DNA was estimated in each sample. In spite of the fact that the plant was inoculated by *F. culmorum* species, in addition other *Fusarium* fungi were also detected in this field [15]. In this regard, the grain infection was estimated according to the total DNA content for all fungi having the *Tri5* gene in the genome and producing trichothecene mycotoxins (Tri-*Fusarium*). Additionally, the DNA of only one species (the *F. poae*, usually the dominant species in *Fusarium* complex in the oats grain) was detected.

The content of the Tri-*Fusarium* fungi group's DNA in the grain was estimated by the primers TMTri,f (CAGCAGMTRCTCAAGGTAGACCC) and TMTri,r (AACTGTAYACRACCATGCCAAC), and fluorescent probe (Cy5-AGCTTGGTGTTGGGATCTGTCCTTACCGBHQ2) [16, 17]. The content of *F. poae* DNA in the grain was estimated by the primers TMpoae,f (GCTGAGGGTAAGCCGTCCTT) and TMpoae,r (TCTGTCCCCCCTACCAAGCT) and the fluorescent probe (TET-ATTTCCCCAACTTCGACTCTCCGAGGA-BHQ1) [18]. The PCR was amplified using the CFX 96 real-time PCR detection system (Bio-Rad, United States). The amplification protocol included 1 cycle [95°C, 15 s] and 40 cycles [95°C, 15 s; 60°C, 1 min].

The mycotoxins were extracted by adding 5 mL water acetonitrile solution (84 : 16) to 1 g of flour and leaving it for 14–16 h under constant mixing on a rocker (ELMI S-3M orbital shaker, Latvia) at 300 rpm. The amount of DON and ZEN mycotoxins in the obtained extracts were determined by the enzyme-linked immunosorbent assay method using the Deoxynivalenol-ELISA and Zearalenone-ELISA test systems (Pharmatex, Russia) with a lower limit of mycotoxin determination 20 μg/kg. The analysis was conducted in kit polystyrene plates (Medpolymer) and the optical density of the solutions was measured on an LEDETECT 96 spectrophotometer (Biomed, Austria) at a wavelength of 492 nm.

Each laboratory experiment was repeated at least twice. Statistical treatment of the results was conducted through Microsoft Office Excel 2007 and Statistica 10.0 (ANOVA) programs. Differences were considered significant at the level $p < 0.05$.

RESULTS

A significant diversity of the morphological traits of the studied genotypes was detected. The plant height varied from 50 cm (*A. canariensis*, k-1914, Spain, Canary Islands) to 175 cm (*A. sterilis*, k-1425, Turkey).

The thousand-grain weight of the 66 analyzed genotypes varied within 8.2–76.8 g. The smallest grain (up to 10 g) was detected in the tetraploid *A. barbata* (k-207, k-316, k-1848) and *A. atlantica* (k-2109) and the diploid *A. clauda* (k-200) species. The largest grain (more than 50 g) is typical for the tetraploid *A. murphyi* (k-1986 and k-2088, Spain), *A. magna* (k-145, k-1786, k-1896, and k-2100, Morocco), and *A. insularis* (k-2067, Italy, Sicily) species and hexaploid *A. sterilis* (k-511, Israel).

Percentage of husk in the analyzed genotypes was on average 15–87%. Proportion of husk less than 30% of the whole grain was detected in the diploid *A. vaviloviana* (k-755, Ethiopia) and in all hexaploid cultivated oat genotypes (*A. sativa* and *A. byzantina*), as well as in the wild species *A. fatua* (k-49, Armenia). The *A. canariensis* genotype (k-1914, Spain, Canary Islands) was characterised by the highest proportion of the husk.

According to visual estimation, the hairiness varied significantly. The most pubescent lemma was typical for the genotypes of *A. insularis*, *A. magna*, and *A. agadiriana* species, while the genotypes of the *A. sativa*, *A. vaviloviana* and *A. wiestii* species had glabrous lemma of grain.

The amount of Tri-*Fusarium* DNA varied from 0.65×10^{-3} to 0.12% of the total DNA. The *F. poae* DNA was detected in the samples in the range 0.3×10^{-3}–23.8×10^{-3}% of the total DNA (Fig. 1). It was established that diploid and tetraploid species (2.2×10^{-2}–2.4×10^{-2}% total DNA) contained on the average 2.6–2.8 times more Tri-*Fusarium* DNA than the hexaploid species (0.8×10^{-2}% of the total DNA). The oats genotypes belonging to diploid *A. canariensis* (k-1914, Spain, Canary Islands), tetraploid *A. magna* (k-1786 and k-2100, Morocco) and hexaploid *A. ludoviciana* (k-701, Ethiopia) were the most infected. The genotypes containing low amount of Tri-*Fusarium* DNA in the grain were hexaploid *A. sativa* (k-15611, Norway; k-15068, Russia), *A. ludoviciana* (k-461, Syria), *A. fatua* (k-116, Armenia), and tetraploid *A. barbata* (k-2070, Syria).

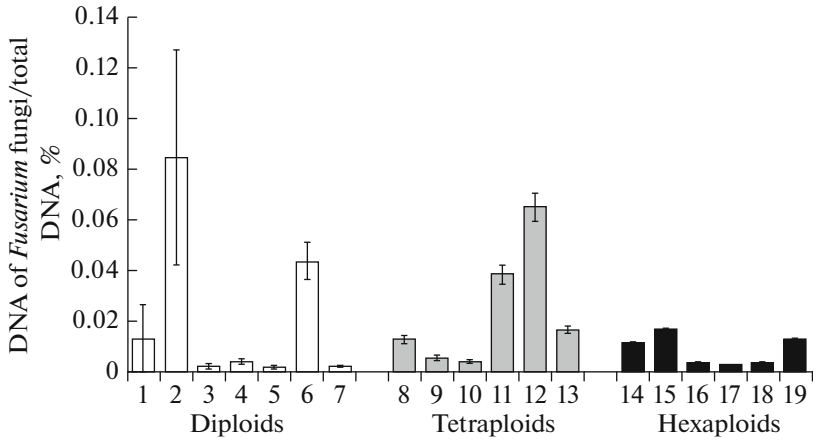

Fig. 1. DNA content of *Fusarium* trichothecene-producing fungi (mean ± standard error) in grain of *Avena* genotypes of different ploidy. Diploid species: (1) *A. atlantica* ($n = 1$), (2) *A. canariensis* ($n = 1$), (3) *A. clauda* ($n = 1$), (4) *A. damascena* ($n = 1$), (5) *A. hirtula* ($n = 1$), (6) *A. longiglumis* ($n = 2$), (7) *A. wiestii* ($n = 2$); tetraploid species: (8) *A. agadiriana* ($n = 1$), (9) *A. barbata* ($n = 7$), (10) *A. vaviloviana* ($n = 3$), (11) *A. insularis* ($n = 1$), (12) *A. magna* ($n = 5$), (13) *A. murphyi* ($n = 2$); hexaploid species: (14) *A. fatua* ($n = 8$), (15) *A. ludoviciana* ($n = 4$), (16) *A. occidentalis* ($n = 1$), (17) *A. sativa* ($n = 6$), (18) *A. byzantina* ($n = 3$), (19) *A. sterilis* ($n = 16$).

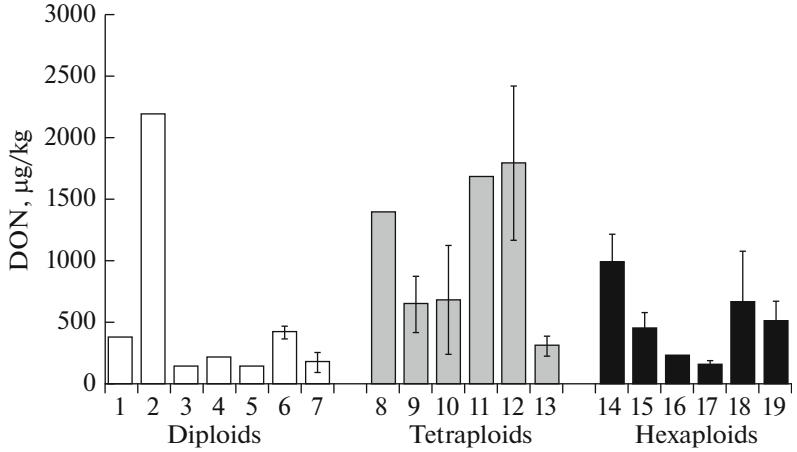

Fig. 2. Amount of deoxynivalenol (mean ± standard error) in grain of *Avena* genotypes of different ploidies. Diploid species: (1) *A. atlantica* ($n = 1$), (2) *A. canariensis* ($n = 1$), (3) *A. clauda* ($n = 1$), (4) *A. damascena* ($n = 1$), (5) *A. hirtula* ($n = 1$), (6) *A. longiglumis* ($n = 2$), (7) *A. wiestii* ($n = 2$); tetraploid species: (8) *A. agadiriana* ($n = 1$), (9) *A. barbata* ($n = 7$), (10) *A. vaviloviana* ($n = 3$), (11) *A. insularis* ($n = 1$), (12) *A. magna* ($n = 5$), (13) *A. murphyi* ($n = 2$); hexaploid species: (14) *A. fatua* ($n = 8$), (15) *A. ludoviciana* ($n = 4$), (16) *A. occidentalis* ($n = 1$), (17) *A. sativa* ($n = 6$), (18) *A. byzantina* ($n = 3$), (19) *A. sterilis* ($n = 16$).

All the analyzed genotypes accumulated mycotoxins in the grain; the DON frequency was 100% with range of values 57–3862 μg/kg (Fig. 2). On an average, the amount of DON in the group of tetraploid *Avena* species was 2.0–2.2 times larger (on average 1088 ± 262 μg/kg) than in the groups of di- and hexaploid species (518 ± 196 and 457 ± 67 μg/kg on average, respectively).

Less than 100 μg/kg of DON was detected in the grain of seven hexaploid genotypes: *A. sterilis* (k-1425 and k-1429, Turkey; k-2052, Israel), *A. sativa* (k-15301, Canada; k-15353, Norway), *A. byzantina* (k-15524, China), and *A. fatua* (k-30, Tyva, Russia), and in one sample of the diploid *A. wiestii* species (k-94, Egypt).

High amounts of DON (>1500 μg/kg) were detected in the *A. barbata* (k-9, Ethiopia), *A. canariensis* (k-1914, Spain, Canary Islands), *A. fatua* (k-65, Bulgaria; k-743, Ethiopia; k-80, China), *A. insularis* (k-2067, Italy, Sicily), *A. magna* (k-2100 and k-1786, Morocco), and *A. sterilis* (k-511, Israel; k-140, Morocco) genotypes. The Bessin variety (k-15611, Norway) contained the largest amount of DON (321 μg/kg) among the analyzed *A. sativa* genotype. The late-ripening Belinda variety (k-14911, Sweden), previously characterised as susceptible [10], contained average amount of DON (147 μg/kg). The lowest amount of DON (57 μg/kg) was detected in the Odal variety (k-15353, Norway) grain. The two analyzed *A. byzantina* genotypes demonstrated contrasting reactions. Low DON amounts

Table 2. The connection of the identified traits in different *Avena* genotypes

Parameters	Ploidy	Weight of 1000 grain	Height	Proportion of the husk	Hairiness	Tri-*Fusarium* DNA	*F. poae* DNA
Weight of 1000 grain	0.22						
Height	0.00	–0.24					
Proportion of the husk	–0.12	0.05	0.20				
Hairiness	–0.49**	0.15	0.15	0.48*			
Tri-*Fusarium* DNA	–0.28*	0.38*	–0.26*	0.39*	0.53**		
F. poae DNA	–0.24	0.02	0.10	0.47**	0.46**	0.33*	
DON	–0.26*	0.22	–0.22	0.15	0.37*	0.67**	0.13

* Significant at $p < 0.05$ and ** significant at $p < 0.01$.

were detected in the Bai Yan 7 variety (k-15524, China) grain, while the Medved' variety (k-15494, Russia, Kirov oblast) was characterised by a very high content of this mycotoxin (1238 μg/kg).

ZEN mycotoxin was detected in the grain of only three genotypes, including that of *A. barbata* (k-316, Azerbaijan), *A. ludoviciana* (k-547, Israel), and *A. wiestii* (k-95, Israel), in amounts of 38 to 237 μg/kg.

DISCUSSION

Wild genotypes of cultivated plants are valuable material that can be used for breeding new varieties with specified properties and are adapted to changing environmental conditions. In order to compare the infection with fungi and contamination with mycotoxins of diverse genetic material such as the *Avena* species, they were artificially inoculated with the *F. culmorum* (which is a typical pathogen in the region of the experiment). Due to morphological differences and biological peculiarities of the oats genotype, the method of inoculum distribution on the surface of the soil under vegetating plants rather than direct forced panicle sprinkling was selected. In our opinion, this method better imitates the natural environmental conditions.

The tetraploid *A. insularis* and *A. magna* species with genotype C (having the largest percentage of the husk) were characterised by high content of Tri-*Fusarium* DNA and DON in the grain. Previously, it was demonstrated that the genotypes of these species were also not resistant to other biotic and abiotic factors [1, 2, 19].

A significant positive correlation ($r = +0.67$, $p < 0.01$) between the amount of Tri-*Fusarium* DNA and DON was established in the grain of all the analyzed genotypes (Table 2). As expected, no association between the amount of *F. poae* DNA and DON was found. Detected connection between the amounts of Tri-*Fusarium* DNA and *F. poae* DNA is quite logical, since the latter species is also able to synthesize the trichothecene mycotoxins, which however ends with the production of nivalenol but not DON.

A representative set of the genotypes of 17 *Avena* wild species by resistance to grain colonization with the *Fusarium* fungi and accumulation of mycotoxins in the grain was estimated for the first time. The lowest amount of fungal DNA and mycotoxins were detected in seven genotypes of the hexaploid oat species: *A. byzantina* (k-15301, CDC Dancer, Canada; k-15524, Bai Yan 7, China); *A. sativa* (k-15353, Odal, Norway); and *A. sterilis* (k-1425, CAV 1568 and k-1429, CAV 1577, Turkey; k-2052, Israel). The lowest content of fungi and mycotoxins was also established for the hexaploid *A. fatua* (k-30, Tyva) and one diploid *A. wiestii* (k-94, An 109, Egypt).

Recently, researchers have shown an increased interest in the genotypes relating to *A. sterilis* and *A. fatua*; as the members of these species have many economically valuable traits and can be easily used in introgressive hybridization for extending the genetic pool and improving the existing varieties.

Wild oats species were for the first time used for practical breeding purposes in the early 1920s, although the first commercial varieties with their involvement did not appear until the 1960s. As a result of the gene allele transfer from the *A. sterilis* species in the cultivated oats, the productivity of the latter increased by 15–20%. At present, the use of *A. fatua* and *A. sterilis* species in crossings for breeding purposes is a routine matter. Based on these species, many oat varieties were created; they occupy significant cultivated areas in the United States, Canada, Brazil, and Australia [1, 2].

Studies done by Turkish scientists who demonstrated that the protein extract obtained from *A. fatua* grain had a significant inhibitory effect on the growth of *Aspergillus* and *Fusarium* fungi as compared to the extracts from the grain of other cereals are of interest [20]. The *A. fatua* and *A. ludoviciana* are dangerous weeds that clog the sown agricultural cultures. In a few publications concerning the Fusarium head blight of wild oats, the authors mainly demonstrate an infection of the grain of these cereals with the *Fusarium* fungi and the possibility of using the strains of fungi to reduce the weediness of the crops [21–23].

The multicomponent characterization of the plant resistance to Fusarium head blight is largely due to the

morphological and physiological–biochemical plant peculiarities (passive immunity) affecting both the penetration and distribution of hemibiotrophic pathogens of *Fusarium* fungi and the production of mycotoxins by them.

The estimation of a correlation between the morphological traits of *Avena* genotypes and fungal DNA in the grain demonstrated a significant increase in the Tri-*Fusarium* fungi DNA in the oat genotypes with larger grains ($p < 0.05$) having high hairiness. These parameters had no effect on the amount of DON in the grain. The percentage of husk increased not only the content of the fungal DNA but also the DON content in the grain. An increase in the plant height had a significant negative effect on the quantitative presence of Tri-*Fusarium* DNA; however, it had no effect on the amount of the *F. poae* DNA. Indeed, the plant residues located on the surface or in the upper layers are a place of preservation for many fungi. In our experiment, the *F. culmorum* inoculum distributed on the soil surface promoted an increase in this association.

On the average, the content of the Tri-*Fusarium* fungi DNA was 2.8 times larger than that of the *F. poae* DNA for all the analyzed *Avena* genotypes. However, the *F. poae* on a natural background is always isolated from the oat grain with a larger frequency than other *Fusarium* species [4, 6, 24, 25]. The *F. poae* is a relatively weak pathogen, and its permanent presence in the oats grain mycobiota still cannot be precisely explained. Apparently, the high frequency of its occurrence (detected by the microbiological method) did not allow the estimation of the depth of the invasion of this fungus in the grain.

At the same time, the method of quantitative fungal DNA detection also has its limitations and does not always allow to correctly compare the amount of biomass of different target microorganisms in the same host. Thus, in our case, the primer pair (TMTrif/r) on the group of the *Fusarium* species (capable of producing trichothecene mycotoxins) was created based on the *Tri5* gene sequence, which encodes the initial stage of the biosynthesis of these toxic metabolites [16]. The TMpoae,f/r primers for the quantitative detection of the *F. poae* were created based on the IGS nucleotide sequence of the ribosomal DNA region [18]. The original differences between the initial number of molecules containing the amplified fragment do not allow comparison of the abundance of the DNA of these two objects in the same sample, but allow to compare the genotypes between themselves by infection with a target object.

The grain's hairiness and the proportion of husk in oats resulted in a significant increase in the amount of the *F. poae* DNA in the grain ($p < 0.01$). It is possible that the saprotrophic fungi grow in the period of the physiological aging of the spikelet and husk that are not tightly adjacent to the grain. Thus, these morphological structures of plant play an important role in protecting the grain, they are an additional barrier under the pathogen penetration, and they are a passive resistance factor.

No significant relationship between the plant height of the oats and the amount of the *F. poae* DNA in the grain was detected. Unlike the *F. culmorum*, this species is not capable of abundantly producing macroconidia, but produce only microconidia (approximately 5 μm in diameter) that is easily spread by the wind from the surrounding cultivated and weed cereal grasses. The presence of quite strong fruity-sweet flavor is also typical for most *F. poae* strains. According to some researchers, the *F. poae* flavor is attractive for insects and ticks that promote the distribution of conidia and the occupancy of the plant tissues (damaged by them) by the fungus [26–28].

The study of the relationships between the *Avena* and *Fusarium* is of undoubted scientific and practical interest due to the wide intragroup diversity of these groups of organisms and the significant demand for the oat quality grain as food and fodder. The involvement of diverse genetic material, including the wild *Avena* species of different origins, in the breeding process is a modern requirement for improving the oat culture and reducing the genetic erosion.

CONCLUSIONS

The comparative estimation of the *Avena* genus was for the first time conducted on a sample of 66 genotypes related to 2 cultivalted and 17 wild species by resistance to Fusarium head blight (infection with the *Fusarium* fungi and accumulation of the DON mycotoxin).

The characteristics of the genotypes of both the cultivated *Avena* species and the wild *Avena* species not subjected to breeding were given using the artificial inoculation by *F. culmorum* species.

Statistically significant associations were demonstrated between the morphological traits and the amount of *Fusarium* fungi DNA (characterised by the different pathogenic properties), as well as the content of the DON mycotoxin.

The lowest amount of fungal DNA and DON was detected in seven hexaploid genotypes related to the *A. byzantina*, *A. sterilis*, *A. sativa* and *A. fatua* species and in one diploid *A. wiestii*. The detected relatively resistant genotypes of the cultivated and wild *Avena* species should be used in the breeding of new oats varieties.

Estimating the diversity of the existing genetic oat species is of scientific interest; for this, further studies including samples of the global diversity of the *Avena* genus are required.

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