

Screening of Parent Material for Breeding Oat Varieties Resistant to *Fusarium* Disease and Accumulation of Mycotoxins in Grain¹

O. P. Gavrilova^a, T. Yu. Gagkaeva^a, and I. G. Loskutov^{b, c}

^aAll-Russian Institute of Plant Protection (VIZR), St. Petersburg–Pushkin, 196608 Russia

^bVavilov All-Russian Institute of Plant Industry, St. Petersburg, 190000 Russia

^cSt. Petersburg State University, St. Petersburg, 199034 Russia

e-mail: olgavriloval@yandex.ru

Received May 23, 2011

Abstract—Oat accessions characterized by multicomponent resistance to *Fusarium* disease of grain from the collection of the Vavilov All-Russian Research Institute of Plant Industry (VIR) are selected. Resistance of the accessions is evaluated on the basis of three parameters: Fusarium damaged grain (FDG): percent of infected grain, quantity of mycotoxins, and DNA content of trichothecene-producing fungi. Five oat landrace accessions and two cultivars Argamak (Russia) and Kuromi (Japan) are the most resistant to infection of grain and accumulation of mycotoxins.

Keywords: oat, fungi, *Fusarium*, disease, mycotoxins, DNA, resistance.

DOI: 10.3103/S1068367412010065

One of the factors determining oat grain quality for food and feed purposes is its level of infection with pathogenic mycobiota. Grain infected with fungi of the genus *Fusarium* has a low germination rate, which worsens the quality of seed material considerably. Furthermore, mycotoxins produced by many species of *Fusarium* fungi accumulate in grain, which, remaining intact in grain and its processing products, on entering the human and animal organism leads to a decrease in immunity and various pathological changes.

The level of infection with *Fusarium* fungi and contamination by mycotoxins in many regions of the Russian Federation are revealed every year by mycotoxicological analysis of oat grain [1]. Our analysis of oats grown in the northwestern territory of the RF in 2008 showed 17.0% infection of grain on average (limits of infection, 2–68.6%); T-2 toxin (T-2) was found in 46% of the accessions (4–182 µg/kg) and the mycotoxin deoxynivalenol (DON) in 47% of them (36–2505 µg/kg [2]).

Breeding oats for resistance to *Fusarium* disease compared with other small grain cereals is complicated by an asymptomatic course of the infection process in the field and by time-consuming of a laboratory

assessment of grain infection. This led to a situation of ubiquitous cultivation of oats without consideration of the resistance of varieties to infection by *Fusarium* fungi and accumulation of mycotoxins.

In a search for potentially useful sources of resistance in VIR *Avena* germplasm collection, we tested FHB reactions of several oat genotypes by using the different scoring of disease: Fusarium damaged grains (FDG), amount of DNA of trichothecene-producing *Fusarium* fungi and mycotoxin accumulation. The VIR collection includes the entire world's botanical and genetic diversity of the genus *Avena* L. [3]. An analysis of such an extensive and genetically diverse collection of oat accessions for resistance to *Fusarium* disease of grain is being carried out for the first time not only in Russia but also worldwide.

MATERIALS AND METHODS

We evaluated 197 oat accessions (120 hulled and 47 naked) from the VIR collection for resistance to *Fusarium* disease of grain. The accessions, represented by species *A. abyssinica*, *A. byzantina*, *A. strigosa* and *A. sativa* (the last included both breeding varieties and lines and landrace accessions of various geographic provenance), were grown under conditions of the Tosno Experiment Station of the All-Russian Institute of Plant Protection (Leningrad oblast) in 2007–2008. In addition to the natural high *Fusarium* infection background existing on the plot, the plants were inoculated with the fungus *F. sporotrichioides*. The inoculum was a grain mixture infected with four strains of

¹ The work was supported by the Russian Foundation for Basic Research (grant no. 08-04-13668-0fi_ts, “To Develop the Technology of Isolating Parent Material for Breeding Oat Varieties Resistant to *Fusarium* Head Blight and Accumulation of Mycotoxins in Grain”); project of the Nordic Research Board no. 090014, “New Emerging Mycotoxins and Toxigenic Fungi in Northern Europe”; and grants of the Finnish Academy of Sciences no. 126917 and no. 131957.

Fusarium damaged grains (FDG), amount of mycotoxins, and DNA content of fungi of trichothecene-producing *Fusarium* species in grain of naked and hulled oat forms

Form	Year	Number of accessions	FDG, %	Mycotoxins, µg/kg		DNA of fungi, ng/µl of total DNA
				T-2	DON	
on average (limits of variation)						
Hulled in hull	2007	91	17.3 (0–100)	–	–	–
			9.6 (0–98)	543 (0–5620)	728 (53–11190)	0.78(0–3.40)
dehulled in hull	2008	59	8.0 (0–24)	–	–	–
			4.0 (0–12)	225 (0–2000)	454 (44–4840)	0.77 (0–3.70)
Naked	2007	14	1.9 (0–6)	178 (0–774)	346 (57–1256)	0.25 (0–0.57)
	2008	33	3.0 (0–9)	178 (0–1145)	580 (72–3300)	0.38 (0.04–1.70)

this pathogen, which was scattered over the soil surface at a rate of 150 g/m².

Fusarium resistance of oat was evaluated on the basis of the following parameters: FDG (%), DNA content of trichothecene-producing species of *Fusarium* fungi (ng/µl of total DNA), and amount of accumulated mycotoxins DON and T-2 (µg/kg).

FDG level was determined on potato–sucrose agar after preliminary surface sterilization of grain. This parameter was determined after incubation for a week at 23°C. The grain infection in the hulled genotypes was estimated by two assays: grain naturally covered by hull and grain after manual dehulling.

The total DNA content in grain of the set of trichothecene-producing species of fungi having the *Tri5* gene in the genome (primers TMTri,f/r) was measured by the quantitative PCR technique (TaqMan PCR with fluorescent probe). The trichothecene-producing fungi include the species *F. sporotrichioides*, *F. poae*, *F. langsethiae*, *F. graminearum*, and *F. culmorum*. We extracted DNA from each accession, using the CTAB method according to the protocol proposed by the European Commission [4].

The content of T-2 and DON in grain was analyzed by solid-phase competitive ELISA by means of the test systems [5, 6]. The method consists in extracting toxins from a ground grain sample (10 g) screened through a fine-mesh sieve to separate the hulls by a mixture of acetonitrile and water with subsequent measurement of the optical density of the solution on a spectrophotometer (wavelength 492 nm) and calculation of the amount of mycotoxin content.

RESULTS AND DISCUSSION

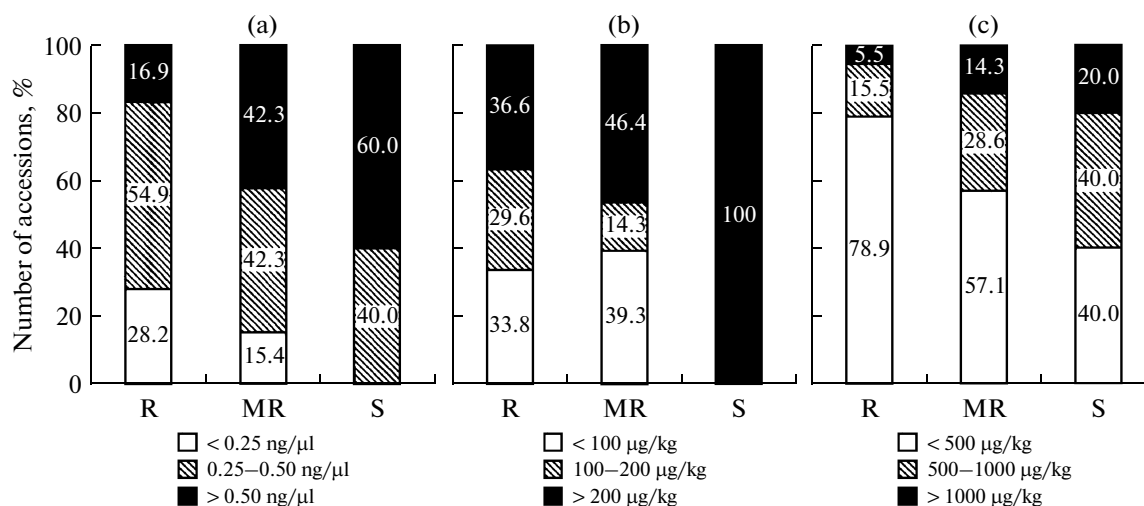
In 2007, under favorable conditions for *Fusarium* fungi, FDG varied considerably, from 0 to 100%; in 2008, it was low, from 0 to 24%. The oat hull prevents penetration of the pathogen into the grain. Therefore, infection of grain of hulled oats in the hull and after its

dehulling differed substantially: in 2007, on average, respectively 17.3 and 9.6% and in 2008, 8 and 4% (table).

High resistance to FDG was noted in the group of naked forms. Despite the absence of a mechanical barrier in the form of a hull covering the grain, infection of all naked accessions was low (on average, 1.9% in 2007 and 3% in 2008); the maximum was 9% (k-2299, cv. Polard, Canada, 2008).

The DNA content of trichothecene-producing *Fusarium* species in the accessions varied considerably. In the naked forms in 2007 it was 0–0.57 ng/µl and in hulled forms, 0–3.4 ng/µl. On the whole, naked accessions also accumulated less DNA of the pathogens and analyzed mycotoxins than the hulled.

A clear-cut relation was not found between FDG of a particular accession and the content in it of DNA of *Fusarium* species and two analyzed mycotoxins. The reason for this was the presence in the grain mycobiota of *Fusarium* species not able to produce this group of chemical compounds (for example, *F. avenaceum* and *F. tricinctum*). Furthermore, the percentage infected grain reflects more the spread of disease and not the degree of infection of the caryopsis (depth of penetration of the pathogen). The DNA content of fungi in the accession is a more accurate index of infection level, adequately describing the interaction of the plant's genotype with pathogens. The absence of a relation between percentage infection, fungal DNA content, and total content of toxins T-2 and DON in individual accessions possibly confirms the opinion that the resistance of cereals to the penetration and spread of *Fusarium* fungi as well as to the accumulation of mycotoxins is controlled by different genes and is inherited independently [7–9]. Despite the absence of a clear-cut relation between the analyzed resistance indices, on the whole the group of plant genotypes more resistant to grain infection contained significantly smaller concentrations of DNA and mycotoxins.



Distribution (%) of oat accessions according to DNA content of fungi of genus *Fusarium* (a) and accumulation of mycotoxins T-2 and DON (c) in different groups of grain infection resistance: resistant (R), moderately resistant (MR), and susceptible (S), in 2007.

In 2007, the accessions were distributed into three groups on the basis of FDG: resistant, grain infection level not more than 6%; moderately resistant, 7–40%; susceptible, more than 40%. The DNA content in the group of resistant genotypes was substantially less than in the susceptible (Fig. 1a). Accessions grouped according to the grain infection level also differed in amount of accumulated mycotoxins (Fig. 1b, c). In 2008, no clear-cut positive relation between indices was found against the background of the low grain infection level.

As the result of the 2-year investigations, seven accessions with multicomponent resistance to *Fusarium* grain infection were isolated. Most of them belonged to the species *A. sativa* and were landrace accessions of Asian provenance (k-2513, k-6963, k-7766, k-7934, and k-8479). Cultivars Argamak (k-14648) and Kuromi (k-11632) were the most resistant among the analyzed breeding varieties.

The existence of different types of resistance to the penetration and spread of the pathogen and accumulation of mycotoxins requires the use of a set of quantitative assessment parameters. Breeders need information about *Fusarium* disease resistance in oat accessions when creating new cultivars with high grain quality.

ACKNOWLEDGEMENTS

We are grateful to Dr. T. Yli-Mattila (University of Turku, Finland) for help in conducting molecular genetic research and Candidate of Medical Sciences A.A. Burkin for help in determining mycotoxins.

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