PLANT BREEDING AND SEED SCIENCE
Volume 64 2011

DOI: 10.2478/v10129-011-0040-z

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EVALUATION OF OAT GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

ABSTRACT

The objectives of this study were to screen the VIR *Avena* germplasm collection for *Fusarium* head blight (FHB) resistance and to identify the resistant oat genotypes by using the different scoring of the disease. After artificial inoculation harvested grain samples were assays on the combination of three parameters: percentage of *Fusarium* damaged kernels (FDK), DNA of trichothecene-producing *Fusarium* fungi and mycotoxin accumulation. The clear correlation between the parameters for every individual genotype was not detected. The results support the several components of resistance to *Fusarium* head blight in oats (invasion, spreading and mycotoxin accumulation), which are controlled by different genetic systems. The hull-less genotypes considered to be more resistant in the *Avena* germplasm. Seven landraces genotypes and five cultivars originated from Asian region and two cultivars originated from European region seem to be suitable genetic resources for resistance to FHB.

Key words: Avena, disease, *Fusarium*, germplasm, kernel, method, resistance

INTRODUCTION

Oats has become desirable for human consumption due to its high nutritional significance, especially hull-less oats (Redaelli *et al.*, 2009). The main effort of oats breeders is concentrated on breeding of new varieties with high yield and grain quality, adapted to the widely varying climatic conditions (Batalova, 2000). In spite of FHB is a destructive disease in the

Communicated by Edward Arseniuk

cultivation area of this crop, breeders have never brought into oats such the trait as resistance to *Fusarium* fungi. The reason for this is the absence of typical visual symptoms of this disease on affected oats panicles in fields. Therefore the scoring of symptoms is not suitable for evaluation of FHB. It means that the majority of commonly growing oats cultivars do not have a good resistance to FHB (Mielniczuk *et al.*, 2004; Tekauz *et al.*, 2008). Investigation of the genetics of resistance to FHB in oats has not been very extensive, which seems to be due to problems in assessing current status of disease. At present, the information concerning the resistance of oats to *Fusarium* is limited but breeders need the knowledge about resistance, when they are crossing cultivars or lines.

The passive resistance has a role in preventing pathogen invasion (Mesterhazy, 1995; Parry *et al.*, 1995). It was shown that the plant height of wheat and barley genotypes has an influence on *Fusarium* disease and tall genotypes have more natural head blight infection and accumulation of mycotoxins (Mesterhazy, 1995; Hilton *et al.*, 1999; Zinkernagel *et al.*, 1997; Lienemann *et al.*, 2000; Legzdina, Buerstmayr, 2004). In Finland early maturing crops are less susceptible to FHB (Yli-Mattila *et al.*, 2009; Yli-Mattila, 2010) and this is also the case in oats cultivars in Finland (Parikka *et al.*, 2008).

The oats cultivation area in Russia is large and consists of near 30% of the oats production in the whole word. Oats is also an important plant in other Nordic countries, e.g. in Canada, Poland, Sweden and Finland, which together with Russia produce >50% of the oats production (FAOSTAT, 2008). During the last years the surveys have been undertaken to assess FHB in commercial oat crops cultivated in the different parts of Russia, which has shown the great importance of this disease (Kononenko, Burkin, 2002; Ivaschenko *et al.*, 1997; Gagkaeva, Gavrilova, 2009). In 2007-2008 *Fusarium* species were detected in 87–93% of oat grain samples harvested in the north-western region of Russia (Gavrilova, Gagkaeva, 2010). An average 13–17% of the kernels were infected with the range of the infection from 2 to 69%. DON was detected in 47% of grain samples (36 – 2505 ppb), T-2 toxin was found in 46% (4 – 182 ppb). According to our investigations, *Fusarium* infection is significantly stronger in oats grains as compared to wheat and barley.

The collection in the N.I. Vavilov All-Russian Research Institute of Plant Industry (VIR) is the largest gene bank in the world and possesses unique *Avena* germplasm – near 13000 accessions of 26 species (Loskutov, 2007). 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: FDK, amount of DNA of trichothecene-producing *Fusarium* fungi and mycotoxin accumulation.

MATERIALS AND METHODS

One hundred five genotypes of oats from the VIR collection were represented both hulled and hull-less cultivars, landraces and selections lines. Most of them belong to *A. sativa* (89%) and others to *A. byzantina*. The hulled oats from various origins of Russia and the whole world (Australia, Canada, China, Denmark, Germany, Japan, Korea, Kyrgyzstan, Mongolia, New Zealand, Tajikistan, Turkmenistan, UK, USA) were tested. Analyzed hull-less oats originated from Asian part of Russia and China, Mongolia, Australia.

In the nursery located in Leningrad region (the Tosno Research Station VIZR) each sample was planted in 1 m^2 plots with 15 cm × 3 cm spacing between plants in two repetitions. This nursery is characterized by permanent a high humid air during the vegetation period so it is around by pond and forest with peat marsh. At booting stage of the first oats plants, the inoculum (mixture of four *F. sporotrichioides* strains on autoclaved mixture of barley and wheat grains) was scattered on the soil surface of plots at the rate of 150 g on sq.m. The typical pathogens of cereals in this area (*F. culmorum, F. poae* and *F. avenaceum*) may also infect plants during the growing season (Shipilova, Gagkaeva, 1992; Gagkaeva at al., 2009; Gavrilova *et al.*, 2009). Plant height of genotypes was measured before harvesting as the distance from the base of plant to the top of panicle of the dominating culm for ten plants per plot. According the heading date oats were recorded in the groups as early-, middle- and later-maturing genotypes.

After hand thrashing, 100 kernels from every sample were surface sterilized by 70% ethanol and put into the high-humid plastic boxes. After one week of incubation at 23 ° C the number of kernels covered by fungal mycelium and then the total percentage of FDK were estimated. The grain infection in the hulled genotypes was estimated by two assays: kernels naturally covered by glumes and kernels after manual dehulling.

Every grain samples (10 g) was ground with the laboratory mill and sift through the sieve. For determination of mycotoxins 1 g of meal was extracted with 5 ml of acetonitrile:water (6:1). Mycotoxin contents were determined by indirect ELISA (enzyme linked immunosorbent assay) with detection limits: T-2 and HT-2 toxins (4 ppb), DON (20 ppb) (Kononenko *et al.*, 1999; Kononenko and Burkin, 2002). For characterization of mycotoxin accumulation by genotype has been used the sum of analyzed trichothecenes. DNA was extracted from 200 mg of meal using CTAB method according to the Protocol for GM Food and Feed (European Commission, 2005). The final volume of DNA extract was ca. 50 µl; the total DNA concentration was usually 1-5 ng × μl^{-1}. Quantification of trichothecene producing *Fusarium* species was performed by TaqMan real-time quantitative PCR. The concentrations of TMTRI primers and probe, and the amplification conditions were used as described by Halstensen *et al.* (2006) and Yli-Mattila *et al.* (2008, 2009). Bio-Rad IQ^{TM}5 Real-Time PCR Detection System was used for amplification of DNA. The PCR program con-

sisted of 3 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. Statistical analyses were carried out by using the package STATISTIKA 6.0.

RESULTS AND DISCUSSIONS

In 2007 weather conditions were favorable for *Fusarium* species and the high variation of grain infection was detected. The most common *Fusarium* species isolated from oat kernels were *F. poae, F. sporotrichiodes, F. avenaceum, F. culmorum*, and *F. tricinctum*. In spite of the artificial inoculation by *F. sporotrichioides* isolates, *F. poae* was the most common *Fusarium* species.

Obviously, glumes play an important role in protecting grains against penetration of *Fusarium* infection and accumulation of mycotoxins (Tekauz *et al.*, 2004, Scudamore *et al.*, 2007). In most cases the percentage of *Fusarium* pathogens from peeled kernels of hulled oats was significantly less than in naturally covered by glumes ones. Infection of grain dropped dramatically after manual dehulling — an average from 17.3% to 9.6%.

Table1

FDK, mycotoxins and DNA of trichothecene producing *Fusarium* species in oats germplasm characterized by height

Plant height, [cm]	No. of samples	FDK* [%]	Mycotoxins [ppb]	*Fusarium* DNA [ng × μl^{-1} of total DNA]
		Mean/ Median (Range)		
< 100	53	10/6 (0 – 98)	1808/ 731 (102 – 14730)	8.7×10^{-1} / 5.3×10^{-1} (0 – 3.4)
> 100	42	5/2 (0 – 20)	498/ 385 (102 – 1578)	4.5×10^{-1} / 4.0×10^{-1} (0 – 1.7)

The height of genotypes varied between 86.4–117.6 cm for hull-less oats and 41.2–140.9 cm for hulled genotypes. It has been speculated that resistance of oats would be related to plant height, because taller oat genotypes having panicles at greater distance from the soil surface with inoculum would be more resistant as compared to those with shorter straw. All oats were grouped according height of plants (less 100 cm and higher than 100 cm). The data indicated the significant differences in the parameters of scoring between groups. Oat genotypes characterized by short straw had significantly more FDK, mycotoxins and fungal DNA (Table 1). In our experiment, the group of early maturing oat genotypes has less FDK, accumulate low amount of mycotoxins and fungal DNA as compared to later maturing ones (Table 2).

Table 2

FDK, mycotoxins and DNA of trichothecene producing *Fusarium* species in oats germplasm characterized by maturing time

Group of maturing (No. of samples)	FDK [%]	Mycotoxins [ppb]	*Fusarium* DNA [ng × µl^{-1} of total DNA]
	Mean/ Median (Range)		
Early-maturing (48)	6/4	718/ 371	4.1×10^{-1} / 3.9×10^{-1}
	(0 – 52)	(102 –5400)	(0 – 1.1)
Middle-maturing (34)	8/3	845/ 669	9.5×10^{-1} / 5.6×10^{-1}
	(0 – 98)	(139 –5580)	(0 – 3.4)
Later-maturing (13)	13/8	4118/ 1416	1.0 / 8.1×10^{-1}
	(0 – 56)	(497 –14730)	(2.4×10^{-1}– 3.4)

The highest variation for all tested parameters was detected in hulled genotypes (Table 3). In spite of the lack of glumes as a barrier to entry of pathogens, hull-less genotypes appeared to be more resistant to FHB than hulled genotypes. All scoring parameters of resistance were in five times less in hull-less oat genotypes as compared to hulled ones. This is in agreement to the previous results demonstrating more resistance to *Fusarium* infection in hull-less oat cultivars than the hulled ones (Techauz *et al.*, 2008; Gavrilova *et al.*, 2009; Šlikova *et al.*, 2010). The hull-less barley genotypes are also significantly more resistant to kernel infection than hulled ones (Gagkaeva *et al.*, 2002; Legzdina, Buerstmayr, 2004). It is known, that some compounds, constitutive in the grain or induced in response to pathogen penetration, can protect plant again the pathogen attack and/or accumulation of mycotoxins (Boutigny *et al.*, 2008). Obviously hull-less germplasm have unique properties to protect kernels from invasion of pathogens and accumulation of mycotoxins.

Table 3

FDK, mycotoxins and DNA of trichothecene producing *Fusarium* species in hulled and hull-less oats germplasm

Oats germplasm	No. of samples	FDK [%]	Mycotoxins, [ppb]	*Fusarium* DNA [ng × µl^{-1} of total DNA]
		Mean/ Median (Range)		
Hulled	91	9.6/ 4.6	1351/ 576	7.8×10^{-1}/ 5.3×10^{-1}
		(0 − 98)	(102 − 14730)	(0 – 3.4)
Hull-less	14	1.9/ 2.0	524/ 418	2.5×10^{-1}/ 2.4×10^{-1}
		(0 − 6)	(124−1422)	(0 – 5.7×10^{-1})

The groups formed by the characters of germplasm have included both susceptible and resistant genotypes. There was no clear correlation between FDK,

amount of fungal DNA and accumulation of mycotoxins for any particular genotype. This shows that percentage of FDK, which is most often used for estimation of resistance, is not enough to predict a potential level of toxin and DNA accumulation and should not be used alone in the screening of genotypes. In spite of the absence of any clear correlation between different parameters of resistance in an individual genotype, the group of oats with lower FDK values accumulated less fungal DNA and mycotoxins. The lack of clear correlation between these parameters suggest there are the different components of resistance (invasion of pathogens, accumulation of *Fusarium* biomass and mycotoxins) as it was found in wheat germplasm (Schroeder, Christensen, 1963; Snijders, 1990; Mesterházy, 2002; Boutigni *et al.*, 2008).

Moreover, a complex of *Fusarium* species has been detected in grains. Several of them (*F. avenaceun, F. arthrosporioides, F. tricinctum*) are not able to produce trichothecenes (Langseth *et al.*, 1999; Jestoi *et al.*, 2004; Uhlig *et al.*, 2007). The kernels infected by these pathogens increase the FDK value, but not the amount of measured DNA and mycotoxins. In addition, the most common occurred *F. poae* is producer of trichothecenes mycotoxin – nivalenol, which was not been analyzed in our study. *F. poae* is characterized as a weak pathogen (Parry *et al.*, 1995; Jestoi *et al.*, 2008; Stenglein, 2009; Yli-Mattila *et al.*, 2009) and there is an assumption that this fungus cannot penetrate a plant defense and become localized in glumes and surface layers of kernel. Mycological analyses detected the number of infected kernels but did not quantify the fungal biomass in the grain samples. Regardless of the degree of fungal invasion (and accumulation of the biomass of the *Fusarium* fungus in kernel) every diseased kernel will produce on the agar media one colony of *Fusarium* and later it will be calculated as a FDK value.

The most promising FHB resistant genotypes should have a low and moderate FDK values and accumulate minimal amount of *Fusarium* DNA and mycotoxins. In summary, all 14 tested hull-less cultivars and landraces were relatively resistant according to the tested parameters of resistance (8 landraces genotypes and 6 cultivars) — VIR–1926, 1927, 1928, 1929, 2471, 2472, 4075, 4076, 10099, 11014, 14784, 14851, 14960, 15014. Seven of the hulled landraces genotypes (VIR–2513, 6899, 6934, 6963, 7766, 7934, 8479) and five cultivars (VIR–10841, 11632, 11693, 14415, 14435) originating from Asian region and two cultivars originating from Europe VIR–11501 (Denmark) and VIR–14648 (Russia) appeared to have superior levels of resistance to all tested components of disease and these should be used in the breeding programs for increasing the resistance to FHB. All relatively resistant genotypes belong to *A. sativa* except of one landrace genotype *A. byzantina* from China (VIR–7934).

But even after having provided sources of resistance to FHB for breeders, quick and reliable estimation of resistance in breeding lines and varieties to *Fusarium* is needed. The breeding strategy must combine both resistances to pathogen invasion and to toxin accumulation. Detection of FDK in the labora-

tory analyses is tedious and time-consuming, and percentage of diseased kernels is not always reflecting back the depth of invasion pathogen and accumulation of mycotoxins (Sarlin *et al.*, 2006; Fredlund *et al.*, 2008). According to the results, the amount of fungal DNA should be more realistic assessment of genotype resistance to pathogen invasion. Thus the currently available PCR-based detection and quantification methods of toxigenic *Fusarium* species should be used in the breeding of resistant to FHB cultivars.

The investigations were supported by project No. 08-04-13668 of the Russian Foundation for Basic Research "Development of technology for selecting initial material for oats breeding of resistance to *Fusarium* Head Blight and accumulation of mycotoxins". The visits of Dr. T. Yli-Mattila to the All-Russian Plant Protection Institute and the visits of Dr. T. Gagkaeva and Ph.D. student O. Gavrilova to the University of Turku were supported financially by the Academy of Finland (no. 126917 and 131957) and the Nordic network project New Emerging Mycotoxins and Secondary Metabolites in Toxigenic Fungi of Northern Europe (project 090014), which was funded by the Nordic Research Board. Special thanks are due to G. P. Kononenko and A. A. Burkin (Moscow) for analysis of the mycotoxins.

REFERENCES:

Batalova G.A. 2000. The oats. The technology of cultivation and breeding. Kirov: The research agriculture. 126–128 (in Russian).

Boutigni A-L., Richard-Forget F., Barreau C. 2008. Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. Eur J. Plant Pathol. 121: 411–423.

European Commission. 2005. Community Reference Laboratory for GM Food and Feed. Event-specific for the quantitation of maize line NK603 using real-time PCR.

FAOSTAT. 2008. http://faostat.fao.org/

Gagkaeva T.Yu., Gavrilova O.P. 2009. *Fusarium* head blight — actual problem of obtaining of qualitative cereal crops. Plant Protection and Quarantine. 12: 13–15 (in Russian).

Gagkaeva T.Yu., Levitin M.M., Nazarova L.N., Sanin S.S. 2009. Infected grain and complex of *Fusarium* fungi on the territory of Russia Federation for 2004-2006. Agro XXI. 4/6: 4–6 (in Russian).

Gavrilova O.P., Gagkaeva T. Yu. 2010. *Fusarium* head blight of small grain cereals harvested in the north of Nechernozem region and in Kaliningrad region in 2007-2008. Plant Protection and Quarantine. 2: 23–25 (in Russian).

Gavrilova O.P., Gagkaeva T.Yu., Burkin A.A., Kononenko G.P. 2009. Mycological infection by *Fusarium* strains and mycotoxins contamination of oats and barley grain in the north of Nonchernozem'e. Agrobiology. 6: 89–93 (in Russian).

Halstensen A.S., Nordby K.C., Eduard W., Klemsdal S.S. 2006. Real-time PCR detection of toxigenic *Fusarium* in airborne and settled grain dust and associations with trichothecene mycotoxins. J. Environm. Monitoring. 8: 1235–1241.

Hilton A.J., Jenkinson P., Hollins T.W., Parry D.W. 1999.Relationship between cultivar height and severity of *Fusarium* ear blight in wheat. Plant Pathol. 48: 202–208.

Ivaschenko, V.G., Shipilova, N.P., Nefedova L.I., Gagkaeva T.Ty., Nazarovskay L.A., Chlopunova L.B. 1997. Biology and sanitary surveillance of *Fusarium* head blight. Mycology and Phytopathology. 31/2: 58–63 (in Russian).

Jestoi M., Rokka M., Yli-Mattila T., Parikka P., Rizzo A. , Peltonen K. 2004. The presence and concentrations of the *Fusarium*-related mycotoxins Beauvericin, Enniatins and Moniliformin in Finnish grain samples. Food Additives and Contaminants. 21: 794–802.

Kononenko G.P., Burkin A.A. 2002. Fusariotoxins in grain feedstuff. Veterinary pathology. 2: 128–132 (in Russian).

Kononenko G.P., Burkin A.A., Soboleva N.A., Zotova E.V. 1999. Enzyme immunoassay of T-2 toxin in contaminated grain. Applied biochemistry and microbiology. 35/4: 457–462 (in Russian).

Langseth W., Bernhoft A., Rundberget T., Kosiak B. and Gareis M. 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia. 144: 103–113.

Legzdina L., Buerstmayr H. 2004. Comparison of infection with *Fusarium* head blight and accumulation of mycotoxins in grain of hull-less and covered barley. J. Cereal Sci. 40: 61–67.

Lienemann K., Oerke E-C., Dehne H-W. 2000. Effect of differences amoung wheat genotypes on the occurrence and damage caused by *Fusarium* head blight. In Proceedings of the 6th European *Fusarium* seminar, Berlin, Germany. 26.

Loskutov I.G. 2007. The oats (Avena L.) — distribution, classification, evolution and breeding value. Sankt-Petersburg: VIR. (in Russian).

Mesterhasy A. 1995. Types and components resistance to *Fusarium* head blight of wheat. Plant Breed. 114: 377–386.

Mesterházy Á. 2002. Theory and practice of the breeding for *Fusarium* head blight in wheat. J. Appl. Genetics, 43A: 289–302.

Mielniczuk E., Kiecana I., Perkowski J. 2004. Susceptibility of oat genotypes to *Fusarium* crookwellense Burgess, Nelson and Toussoun infection and mycotoxin accumulation in kernels. Biologia. 59/6: 809–816.

Parikka P., Hietaniemi V., Rämö S., Jalli H. 2008. *Fusarium* infection and mycotoxins contents of oats under different tillage treatments. 8th International oat Conference. Minneapolis, USA. Session V – Effective Pest.

Parry D. W., Jenkins P., McLeod L. 1995. *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol. 44: 207–238.

Redaelli R., Sgrulletta D., Scalfati G., De Stefanis E., Cacciatori P. 2009. Naked Oats for Improving Human Nutrition: Genetic and Agronomic Variability of Grain Bioactive Components. Crop Sci. 49: 1431–1437.

Sarlin T., Yli-Mattila T., Jestoi M., Rizzo A., Paavanen-Huhtala S., Haikara A. 2006. Real-time PCR for quantification of toxigenic *Fusarium* species in barley and malt. European Journal of Plant Pathology, 114: 371–380.

Scudamore K.A., Baillie H., Patel S., Edwards S.G**.** 2007. The occurrence and fate of Fusarium mycotoxins during the industrial processing of oats in the UK. Food Additives and Contaminants. 24/12: 1374-1385.

Shipilova N.P., Gagkaeva T.Yu. 1992. *Fusarium* head blight in north-western region of Russia. Plant Protection. 11: 7–8 (in Russian).

Šlikova S., Šrobarova A., Šudyova V., Polišenska I., Gregova E., and Mihalik D. 2010. Response of oat cultivars to *Fusarium* infection with a view to their suitability for food use. Biologia. 65/4: 609–614

Snijders C.H.A. 1990. The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphitica. 50: 9–17.

Stenglein S.A. 2009. *Fusarium poae*: a pathogen that needs more attention. J. of Plant Pathol. 91: 25–36.

Tekauz A., B. McCallum N. Ames., and Mitchell Fetch J.W. 2004. *Fusarium* head blight of oat: Current status in western Canada. Can. J. Plant Pathol. 26:473–479

Tekauz A., Fetch M. J., Rossnagel B.G., Savard M.E. 2008. Progress in assessing the impacy of *Fusarium* head blight on oat in western Canada and screening of *Avena* germplasm for resistance. Cereal Res. Comm. 36: 49–56.

Uhlig S., Jestoi M. and Parikka P. 2007. *Fusarium avenaceum* – the North European situation. Int. J. Food Microbiol. 119: 17–24.

Yli-Mattila T., Paavanen-Huhtala S., Parikka P., Hietaniemi V., Jestoi M., Gagkaeva T., Sarlin T., Haikara A., Laaksonen S., Rizzo A. 2008. Real-time PCR detection and quantification of *Fusarium poae, F. graminearum, F. sporotrichioides* and *F. langsethiae* as compared to mycotoxin production in grains in Finland and Russia. Archives of Phytopathol. and Plant Protection. 41: 243–260.

Yli-Mattila T., Parikka P, Lahtinen T., Ramo S., Kokkonen M., Rizzo A., Jestoi M., Hietaniemi V. 2009. *Fusarium* DNA levels in Finnish cereal grains In: Current Advances in Molecular Mycology. Gherbawy Y., Mach L., Rai M. (Eds.). Nova Science Publishers. Inc., New York: 107–138

Yli-Mattila, T. 2010. Ecology and evolution of toxigenic *Fusarium* species in cereals in northern Europe and Asia. J. Plant Pathol. 92: 7–18.

Zinkernagel V., Adolf B., Habermeyer J., 1997. The spread of *Fusarium* spp. from the above ground level to the ears of wheat. Cereal Res. Comm. 25: 677–679.