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BIOCHEMICAL ASPECTS OF INTERACTIONS BETWEEN FUNGI AND PLANTS: A CASE STUDY OF FUSARIUM IN OATS

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Abstract

Disclosing the mechanisms that build up plant resistance to fungal diseases (pathogenic microorganisms) invariably evokes the need to analyze biochemical factors of resistance. Protective mechanisms are associated with a fairly large number of chemical compounds. *Fusarium* head blight (FHB) affects cereal grains, including wheat, barley and oats. In this work, we report new data on the metabolic profile of oat grains, as influenced by FHB, and a relationship between plant resistance and content of individual metabolites in FHB-susceptible and FHB-resistant varieties. Here, we have challenged the task to assess the connections of FHB-resistance parameters in oat varieties with as many compounds as possible. Such data are required both to understand the mechanisms of resistance and to develop methods for its assessment. Common oat (*Avena sativa* L.) varieties from the collection of the Vavilov Institute (VIR) were studied to evaluate their numerous biochemical characters and their resistance to FHB. The fatty acid composition of oil was analyzed by the method of gas-liquid chromatography with mass spectrometry on an Agilent 6850 chromatographer (USA), and other metabolites were quantitated. Plant resistance was studied under artificial infestation of ears with *F. culmorum* and *F. sporotrichioides* (an experimental field, All-Russian Research Institute for Plant Protection, St. Petersburg—Pushkin, 2015). *Fusarium* Link fungi DNA content and trichothecene mycotoxins were determined in milled grain samples. The amount of DNA of *Fusarium* fungi was measured by real-time PCR techniques using *Tri5* gene-based group-specific primers. Solid-phase competitive enzyme-linked immunosorbent assay (ELISA) was used to measure the content of T-2 toxin and DON in grain. The statistical significance of differences in biochemical parameters, including metabolic profiles, between resistant and susceptible oat varieties was estimated with the Mann-Whitney criterion. For the first time, correlations were found between *Fusarium* resistance and biochemical characteristics of oat. It has been shown that high-protein forms are less affected by FHB, accumulate less toxins, and are more adaptive to biotic stress. Plant resistance to FHB correlates with accumulation of pipecolic acid, monoacylglycegols, tyrosine, galactinol, a number of phytosterols, sugars and adenosine. The values of such correlations and connections between chemical compounds and various parameters of *Fusarium* resistance identified during the study of oat accessions should be regarded as strictly preliminary, since they are the outcome of only one year of field trials. However, the year in consideration was characterized by extremely favorable conditions for the development of parasitic *Fusarium* fungi with all immanent consequences. An assumption can be made that the increased aggressiveness of the latter (kind of a model condition) allowed us to identify with more reliability the connections between a majority of metabolite content and composition parameters and the level of *Fusarium* resistance. Considering the complex polygenic nature of the control over the character "resistance to Fusarium head blight" and, therefore, strong dependence of

its expression on the environments, any future efforts to confirm (or refute) our conclusions will require researching greater intra- and interspecies diversity of this crop's accessions reproduced in various environments and in different years.

Keywords: *Avena sativa* L., oat, varieties, *Fusarium* head blight, *Fusarium*, fungal DNA, PCR, mycotoxins, gas chromatography, mass spectrometry, biochemistry, metabolomics

Fusarium head blight (FHB) is a fungal disease which affects cereals including wheat, barley and oats [1-3]. It leads to yield losses and considered as a global threat to the food security [4]. The most widespread species causing the FHB is *Fusarium graminearum* Schwabe which produces deoxynivalenol (DON). This mycotoxin is the main factor of the fungus aggressiveness, which inhibits, in particular, the translation processes in eukaryotes [5]. Besides the economic damage from yield reduction, the mycotoxins of the *Fusarium* Link genus fungi constitute the threat to the health of people consuming such products [4]. The species of the *Fusarium* genus secrete the toxins having different aggressiveness. Thus, *F. culmorum* (W.G. Sm.) Sacc. is a highly aggressive pathogen producing DON, *F. sporotrichioides* Sherb. is a relatively weak pathogen producing T-2 toxin [6].

The analytical approach based on the chromatography in combination with the mass spectrometry makes it possible to control the changes in many metabolites, including those which synthesis is induced by the pathogen. *F. poae* which produces the nivalenol mycotoxin dominates among the species causing the *Fusarium* disease of oats. This toxin is assumed to be the main factor worsening the nutritional properties of oats. Unfortunately, its control is not carried out for a number of formal reasons [7-9].

The metabolomics approach to the investigation of fungal diseases makes it possible to ensure the reliable identification of components and statistical validity of the quantitative evaluation of their variability in the "plant—pathogen" system [10]. The resistance to the *Fusarium* disease is controlled by the genes of more than a hundred of quantitative trait loci (QTL) and largely depends on the environmental conditions [11, 12]. In the course of evolution, plants have developed the protective reactions to the pathogens' effect. The host plants can produce the chemical compounds which inhibit the pathogen development and have the various strategies of their applying including creating the protective barriers at all stages of the parasite progression [13].

The metabolic response induced by DON includes the loss of chlorophyll [14], as the photosynthesis weakens at the infected area [15]. As a result, the primary (carbohydrate) and secondary (nitrogen) metabolism become modified. The blockage of proteins biosynthesis by the mycotoxin leads to accumulation of free amino acids [16].

One of the priorities of metabolomics is the investigation of the reaction to environmental effects. The obtained profiles (fingerprints) reflect metabolic states including the dynamics of the responses [17].

In this work, new data have been obtained on the *Fusarium* head blight effect on the metabolomic profile of oat grains, the relationship of the resistance indices with the biochemical characteristics (protein content, contents of oil and certain metabolites). The statistically valid differences have been shown of the FHB-resistant (RFs) and FHB-susceptible (SFs) oats from the VIR Collection (Vavilov All-Russian Institute of Plant Genetic Resources).

Our objective was to identify the relationship between the biochemical indices of resistance to *Fusarium culmorum* and *F. sporotrichioides* in oat varieties and the accumulation of the mycotoxins produced by these pathogens (DON and T-2 toxin, respectively).

Techniques. The Russian and foreign varieties, as well as breeding lines of hulled and hulless oats (*Avena sativa* L.) (24 samples in total) were received from

the VIR Collection. Oat plants for seed reproduction and biochemical analysis were field-grown (Pushkin branch of the VIR, Leningrad Region, 2015). The phenological observations and field evaluation were performed according to the methodological guidelines [18], the signs manifestation degree was determined according to the International classifier of the *Avena* L. genus [19]. The seeds were sown on the optimum date for the region (1 sq. m plots, sod-podzolic light loamy soil, and seeding rate of 500 seeds per sq. m). The experiment was arranged in one replication. Potato was tThe precursor crop in the cereal crop rotation.

Protein and oil contents in the seeds was evaluated according to the techniques adopted in the VIR with a Kjeltec Auto 1030 Analyzer (FOSS, Sweden) and Kjeltec 2200 (FOSS, Sweden) for protein assay and a Soxhlet apparatus for oil estimates per dry fat-free residue (20). The oil fatty acids composition was analyzed (gas-liquid chromatography, an Agilent 6850 chromatograph, Agilent 5975B VL MSD quadrupole mass selective detector, Agilent, USA) [20]. Privet variety cultivated in the Leningrad Region as a standard for the main economically valuable parameters.

The plant resistance was investigated after inoculation of the spikes with the *F. culmorum* and *F. sporotrichioides* fungi (the experimental field of the All-Russian Research Institute for Plant Protection — VIZR, St. Petersburg—Pushkin, 2015). Each sample was grown on the plots (1 m row length) in 2 replicates for each infectious background. The plots separated by strips where with Borrus standard oat variety was sown were located on the same field. At heading stage, all samples were inoculated with the suspension of the conidia and mycelium of *F. culmorum* (3×10^7 CFU/ml) and F. *sporotrichioides* (1.3×10^7 CFU/ml) at 50 ml/l rates.

After the harvest and threshing, infection degree, fungal DNA and mycotoxin contents in the grain were evaluated. To assess the infection degree, 100 grains were taken from each average sample; the grain surface was sterilized with the 5% sodium hypochlorite solution for 1-3 min. Then the grains were rinsed with sterile water and placed in Petri dishes with standard potato sucrose agar medium (PSA). After 7 day incubation (in the dark at 24 °C) *Fusarium* fungi were counted. DNA was isolated from grain flour (200 mg) as per adapted CTAB method [21-23]. The typical *F. graminearum* and *F. poae* strains from the VIZR Laboratory of mycology and phytopathology collection were grown on PSA, and DNA were isolated from aerial mycelium with Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania). In the milled grain samples, the amount of the fungal DNA was determined by quantitative PCR (polymerase chain reaction) method, T-2 toxin and DON contents — by the enzyme-linked immunosorbent assay (ELISA) as per description [24].

Basing on the assessment of FHB resistance [24, 25], 10 samples were selected for metabolomic analysis. The grains was weighed and homogenized with methanol, 1/10 (w/v). The samples were allowed for 30 days at 5-6 °C. The 100 μl extracts were evaporated (CentriVap® Concentrator, Labconco, USA). The dry residue was silylated for 40 min at 100 °C with bis(trimethyl-silyl)trifluoroacetamide. Samples were analyzed using gas-liquid chromatography with mass spectrometry (gas-liquid chromatography-mass-spectrometry, Agilent, USA) on HP-5MS capillary column (30.0 m, 250.00 μm, 0.25 μm; Agilent, USA) with 5% phenylmethyl polysiloxane and Ome-gawax^{TM250} fused silica capillary column (30.0 m, 250.00 μm, 0.25 μm, Supelco®, Sigma-Aldrich, USA). Other parameters were as follows: 1.5 ml/min helium flow rate; initial and final temperatures of 70 °C and 220 °C, respectively, the heating rate of 4 °C/min; of the mass spectrometer detector temperature of 250 °C, the injector temperature of 300 °C; 1 μl sample aliquotes. The tricosan in pyridine (1 μg/μl) was an inter-

nal standard. The obtained results were processed with UniChrom software (Restek Corporation, USA) [9].

The data were statistically processed with Statistica 7.0 (StatSoft. Inc., USA) and Microsoft Excel 2010 software. The mean values (*M*) and standard errors of the mean (±SEM) were calculated. The statistical significance of the differences between the oat forms was estimated with Mann-Whitney and Tukey tests. The differences were recognized statistically significant at $p \leq 0.05$.

Results. The weather conditions during the growing season (2015) were mainly favorable for oat plants, as well as for the development of *Fusarium* infection.

The investigated samples are given in Table 1.

1. Oat (*Avena sativa* L.) from the VIR Collection (Vavilov All-Russian Institute of Plant Genetic Resources) tested for economically valuable biochemical traits and indices of resistance to *Fusarium* infection (St. Petersburg–Pushkin, 2015)

No. according to the VIR catalog	Species, subspecies	Variety, line	Origin
k-14648	*A. sativa* var. *mutica*	Argamak	Kirov Region
k-11840	*A. sativa* var. *aurea*	Borrus	Germany
k-14960	*A. sativa* var. *inermis*	Vyatskii	Kirov Region
k-15068	*A. sativa* var. *mutica*	Konkur	Ul'yanovsk Region
k-14851	*A. sativa* var. *inermis*	Numbat[r]	Australia
k-10841	*A. sativa* var. *aurea*	Bisuandorodu	Sakhalin Region
k-14329	*A. sativa* var. *aristata*	Kouzan Zaizai	Japan
k-13911	*A. sativa* var. *mutica*	Kambulinskii	Leningrad Region
k-14911	*A. sativa* var. *mutica*	Belinda	Sweden
k-15297	*A. sativa* var. *aurea*	Geszti	Hungary
k-15305	*A. sativa* var. *chinensis*	Gehl[r]	Canada
k-15301	*A. sativa-A. byzantina*	CDC Dancer	Canada
k-15442	*A. sativa* var. *mutica*	Zalp	Moscow Region
k-15496	*A. sativa* var. *mutica*	Stipler	Ul'yanovsk Region
k-15444	*A. sativa* var. *mutica*	Sapsan	Kirov Region
k-15494	*A. sativa-A. byzantina*	Medved'	Kirov Region
k-15348	*A. sativa* var. *mutica*	Hurdal	Norway
k-15353	*A. sativa* var. *aurea*	Odal	Norway
k-15611	*A. sativa* var. *aurea*	Bessin	Norway
k-15612	*A. sativa* var. *aurea*	Valer	Norway
k-15347	*A. sativa* var. *mutica*	Gere	Norway
k-15326	*A. sativa* var. *mutica*	KSI 432/08	Ul'yanovsk Region
k-15327	*A. sativa* var. *mutica*	KSI 731/01	Ul'yanovsk Region
k-15506	*A. sativa* var. *mutica*	Fux	Germany
k-14787	*A. sativa* var. *mutica*	Privet	Moscow Region

N o t e. [h] — hulless oats.

The *F. culmorum* and *F. sporotrichioides* DNA concentrations reflect the degree of plants affection by these pathogens [26]. We based the evaluation of resistance to *Fusarium* and mycotoxin accumulation on the combination of the following three indicators: the degree of fungal infection in grain, fungal DNA concentration and the amount of the fungal toxic metabolites in grains (Table 2).

As per the results, the samples were divided into two groups, FHB-resistant (RFs) and FHB-susceptible forms, that was necessary for the further evaluation of the influence of FHB pathogenicity factors on the biochemical parameters including metabolomic analysis (Tables 3, 4). The samples with the *F. culmorum* and *F. sporotrichioide* DNA levels not more than 0.22 and 0.29 pg/kg respectively, and the T-2 toxin and DON values no higher than 10 and 100 μg/kg were classified as FHB-resistant; the rest were constituted as FHB-susceptible ones. The correctness of group formation has been confirmed by the Tukey test for unequal sample sizes. In breeding resistant varieties of high quality it is important to regard the relationship between the FHB-resistance parameters and metabolomics data together with other biochemical characteristics of the grain [27].

2. Characterization of the samples of oat (*Avena sativa* L.) from the VIR Collection (Vavilov All-Russian Institute of Plant Genetic Resources) for resistance to the *Fusarium* head blight ($n = 3$, $M \pm$SEM, Pushkin, 2015)

No. according to the VIR catalog	Variety, line	*Fusarium sporotrichioides*				*Fusarium culmorum*			
		infection indicators		T-2 toxin production		infection indicators		T-2 toxin production	
		DNA, pg per kg flour	resistance to infection	μg/kg	resistance to accumulation	DNA, pg per kg flour	resistance to infection	μg/kg	resistance to accumulation
k-14648	Argamak[m]	0.08	R	0	R	0.13	MR	213	S
k-11840	Borrus[m]	0.11	MR	14	S	0.15	MR	64	MR
k-14960	Vyatskii[hm]	0.06	R	5	MR	0.15	MR	128	S
k-15068	Konkur	0.31	S	7	MR	0.42	S	491	HS
k-14851	Numbat[hm]	0.13	MR	59	HS	0.91	HS	285	S
k-10841	Bisuandorodu	0.04	R	5	MR	0.05	R	131	S
k-14329	Kouzan Zaizai	0.05	R	0	R	0.2	MR	148	S
k-13911	Kambulinskii	0.03	R	0	R	0.1	MR	45	R
k-14911	Belinda[m]	0.25	MR	60	HS	0.14	MR	456	HS
k-15297	Geszti	0.03	R	4	MR	0.04	R	25	R
k-15305	Gehl[hm]	0.05	R	23	S	0.03	R	20	R
k-15301	CDC Dancer	1.29	HS	5	MR	0.1	MR	41	R
k-15442	Zalp[m]	0.63	HS	30	S	0.16	MR	163	S
k-15496	Stipler	0.10	MR	0	R	0.03	R	33	R
k-15444	Sapsan[m]	0.12	MR	19	S	0.16	MR	257	B
k-15494	Medved'	0.33	S	13	S	0.69	HS	72	MR
k-15348	Hurdal[m]	0.85	HS	17	S	0.34	S	30	R
k-15353	Odal	0.34	S	7	MR	0.35	S	50	R
k-15611	Bessin	0.39	S	49	S	0.57	HS	331	HS
k-15612	Veler	0.29	MR	0	R	0.5	S	77	MR
k-15347	Gere	0.67	HS	133	HS	0.1	MR	121	S
k-15326	KSI 432/08	0.86	HS	8	MR	3.64	HS	1179	HS
k-15327	KSI 731/01[1]	1.93	HS	45	S	0.22	MR	97	MR
k-15506	Fux	0.40	S	10	MR	0.09	R	331	HS

N o t e. T-2 — T-2 toxin; DON — deoxynivalenol; R — resistant; MR — medium resistant; S — susceptible; HS — highly susceptible; [h] — hulless oats; [m] — samples used for the metabolomic analysis.

3. Characterization of the samples of oat (*Avena sativa* L.) from the VIR Collection (Vavilov All-Russian Institute of Plant Genetic Resources) for biochemical parameters of quality

No. according to the VIR catalog	Variety, line	Concentration, %		Fatty acids, %					Total amount, %		Ratio	
		protein, N × 5.7	oil	$C_{16:0}$	$C_{18:1}$	$C1_{8:2}$	$C_{18:3}$	$C_{20:1}$	SFAs	UFAs	UFAs/SFAs	18:1/18:2
k-14648	Argamak[m]	11.5±0.02	5.0±0.01	19.3±0.02	31.3±0.00	44.4±0.03	2.0±0.01	0.5±0.01	21.6±0.02	78.4±0.01	3.6	0.7
k-11840	Borrus[m]	12.8±0.01	4.1±0.01	20.0±0.02	30.9±0.02	45.0±0.01	1.4±0.00	0.5±0.00	22.0±0.03	78.0±0.02	3.5	0.7
k-14960	Vyatskii[hm]	14.6±0.01	6.5±0.00	20.0±0.01	40.6±0.03	31.3±0.01	3.5±0.01	0.4±0.01	24.1±0.02	75.9±0.01	3.1	1.3
k-15068	Konkur	12.7±0.01	5.1±0.01	20.9±0.02	31.4±0.05	43.0±0.01	1.0±0.02	0.6±0.02	23.9±0.00	76.1±0.02	3.2	0.7
k-14851	Numbat[hm]	14.9±0.02	7.9±0.00	19.4±0.03	34.3±0.01	42.0±0.02	1.3±0.01	0.7±0.01	21.6±0.02	78.4±0.01	3.6	0.8
k-10841	Bisuandorodu	14.6±0.00	3.9±0.01	16.7±0.01	38.8±0.02	40.2±0.02	1.2±0.01	0.7±0.01	19.1±0.03	80.9±0.01	4.2	1.0
k-14329	Kouzan Zaizai	13.5±0.00	4.3±0.03	18.1±0.00	32.4±0.02	44.6±0.02	1.6±0.00	0.8±0.02	20.4±0.03	79.6±0.01	3.9	0.7
k-13911	Kambulinskii	13.0±0.02	3.8±0.01	20.5±0.00	29.5±0.02	45.4±0.01	1.7±0.03	0.6±0.01	22.7±0.01	77.3±0.02	3.4	0.7
k-14911	Belinda[m]	11.6±0.00	5.2±0.02	19.0±0.02	35.0±0.00	41.2±0.01	1.3±0.02	0.6±0.00	21.7±0.01	78.3±0.01	3.6	0.9
k-15297	Geszti	13.3±0.01	5.8±0.02	16.1±0.01	44.4±0.03	32.7±0.01	2.4±0.04	0.5±0.00	19.9±0.03	80.2±0.01	4.0	1.4
k-15305	Gehl[hm]	15.5±0.01	6.7±0.02	16.8±0.01	44.8±0.01	34.8±0.01	1.6±0.06	1.0±0.02	17.8±0.02	82.2±0.01	4.6	1.3
k-15301	CDC Dancer	10.3±0.01	3.9±0.01	17.6±0.01	40.2±0.01	38.4±0.01	0.7±0.02	0.7±0.01	19.9±0.03	80.1±0.02	4.0	1.0
k-15442	Zalp[m]	11.7±0.01	4.5±0.01	16.6±0.01	43.2±0.02	36.6±0.01	1.0±0.01	0.6±0.01	18.6±0.01	81.4±0.01	4.4	1.2
k-15496	Stipler	11.5±0.01	3.3±0.02	17.7±0.02	38.6±0.02	38.4±0.02	1.9±0.02	0.8±0.01	20.3±0.01	79.7±0.00	3.9	1.0
k-15444	Sapsan[m]	12.5±0.00	4.0±0.01	16.5±0.02	40.3±0.02	39.1±0.02	1.2±0.00	0.7±0.02	18.6±0.01	81.4±0.02	4.4	1.0
k-15494	Medved'	12.7±0.01	4.5±0.01	18.6±0.03	39.1±0.02	37.7±0.02	1.4±0.01	0.6±0.02	21.2±0.01	78.8±0.01	3.7	1.0
k-15348	Hurdal[m]	11.8±0.01	5.1±0.01	19.1±0.02	33.7±0.02	42.6±0.01	1.6±0.01	0.6±0.03	21.4±0.01	78.6±0.01	3.7	0.8
k-15353	Odal	12.2±0.01	5.3±0.01	18.1±0.02	42.1±0.01	35.5±0.01	1.7±0.01	1.0±0.01	19.7±0.02	80.3±0.01	4.1	1.2
k-15611	Bessin	10.6±0.00	4.1±0.02	16.1±0.00	44.0±0.02	35.4±0.01	2.0±0.01	0.8±0.00	17.8±0.01	82.2±0.02	4.6	1.2
k-15612	Veler	10.9±0.03	5.3±0.01	16.1±0.02	45.0±0.02	34.2±0.01	2.4±0.01	1.0±0.01	17.4±0.01	82.6±0.01	4.7	1.3
k-15347	Gere	11.6±0.01	5.6±0.03	17.5±0.03	36.4±0.01	42.6±0.02	1.5±0.01	0.5±0.01	19.0±0.00	81.0±0.02	4.3	0.9
k-15326	KSI 432/08	11.0±0.01	4.6±0.02	15.9±0.05	43.4±0.01	36.6±0.00	1.0±0.01	0.7±0.01	18.2±0.00	81.8±0.02	4.5	1.2
k-15327	KSI 731/01[1]	12.1±0.01	5.0±0.01	15.0±0.03	42.5±0.01	39.3±0.02	0.5±0.02	0.7±0.01	16.9±0.01	83.1±0.00	4.9	1.1
k-15506	Fux	12.5±0.01	3.0±0.01	19.7±0.02	42.8±0.02	31.9±0.01	0.4±0.02	0.4±0.00	24.5±0.00	75.6±0.01	3.1	1.3

N o t e. $C_{16:0}$ — palmitic acid; $C_{18:1}$ — oleic acid; $C_{18:2}$ — linoleic acid; $C_{18:3}$ — linolenic acid; $C_{20:1}$ — eicosenoic acid; SFAs — saturated fatty acids; UFAs — unsaturated fatty acids; [h] — hulless oats; [m] — samples used for the metabolomic analysis.

In our researches, grain proteins and oil in average amounted to 12.5 and 4.9%, respectively (see Table 3). The prevailing saturated fatty acid (SFA) contained in the oat grain was palmitic acid (up to 20.9%), and prevailing unsaturated fatty acids (UFAs) were oleic and linoleic acids (38.5 and 38.9%, respectively). The total amount of UFAs was quite high for cereal crops (up to 83.1%), and the ratio of linoleic and oleic acids was close to 1. Such values testify about high nutritional quality of the investigated oat samples that was confirmed previously [27].

By a nonspecific metabolomic analysis, we have identified over 100 components, i.e. organic acids, free amino acids (including non-protein ones), polyols (including phytosterols), fatty acids, nucleosides, monosaccharides and oligosaccharides, as well as phenol-containing compounds. A pairwise comparison of the resultes for RFs and SFs oats with Mann-Whitney coefficient (at $p < 0.05$) revealed significant differences between the RFs and SFs oats.

According to other authors, fungal pathogens affect almost all stages of primary and secondary metabolism that has an impact on changing of the main biochemical parameters including the metabolomic profile [28].

We noted the inverse relationship between the protein content in the RFs oats and the amount of DNA of *F. sporotrichioides* ($r = –0.5$), *F. culmorum* ($r = –0.6$) and DON toxin ($r = –0.5$). In the SFs, only the direct relationship was found with DNA concentration of *F. sporotrichioides* ($r = 1$). The oil level in the RFs correlated negatively with the concentrations of T-2 toxins ($r = –0.6$) and DON ($r = –0.6$), in the SFs it had the direct relationship with the amount of DNA of *F. sporotrichioides* ($r = 0.5$) and *F. culmorum* ($r = 0.5$). The ratios we determined in the RFs oat allow us to assume that high-protein forms are less affected and accumulate less toxins. At the same time, in the SFs, the high oil content in the grains contributed to the fungal development. Our assumptions are confirmed by the paper [29] which established the nature of the FHB toxin influence on the grain protein and oil levels in cereals.

It was reported [29] that FHB exerts a significant influence on the total content of free amino acids. In our researches, the *F. sporotrichioides* DNA levels in SRs oat directly depended on the free amino acids content ($r = 0.5$) that is most likely related to the damage to ribosomes and destruction of the plant tissues due to the affection by FHB [26]. The inverse correlation between the DON content and the total amount of amino acids in the RFs oat ($r = –0.8$) has been found that may reflect the activation of the plant protection mechanisms. In the SFs oat we have not found such relationships. The DON content in the RFs had the direct dependence on the concentration of asparagine ($r = 0.7$) and glutamine ($r = 0.6$), and in the SFs it had the inverse dependence (the r values were –0.4 and –0.8, respectively). In addition, in the RFs, there was an inverse relationship between *F. sporotrichioides* DNA concentration and the amount of asparagine ($r = –0.5$), T-2 toxin and glutamine ($r = –0.5$). An increase in asparagine and glutamine in the RFs cereals in response to the toxin inflow into the plant tissues was confirmed by other researchers [26] who suppose that such changes are related to plant protection from FHB. It was discussed [29] that the proline content is expected to be increased proportionally to the toxin concentration because proline participates in the inactivation of free radicals which accumulate in the plant tissues affected by FHB. However, in our researches we revealed inverse correlation between the proline accumulation and T-2 toxin production ($r = –0.6$) in the SFs group.

The tyrosine and phenylalanine amino acids are involved in the shikimate pathway the products of which, e.g. phenylpropanoids, actively participate in plant protection. As per available data [26], the content of tyrosine, phenylal-

anine and secondary metabolites, which are the products of the shikimate pathway, directly depended on the indices of affection by FHB. In the SFs, we have found such relationship between the content of tyrosine and *F. sporotrichioides* DNA of (r = 1); in other cases, the inverse relationship between the tyrosine and DON concentrations, as well as between the phenylalanine and DON concentrations has been found (the r values were –0.5 and –0.7, respectively). The negative correlation between alanine and DNA amounts and the FHD toxins both in RFs and SFs oat was confirmed by other authors [29]. The experimental data [16] testify about the increase in serine concentration in response to the effect of FHB toxins that has not been confirmed in our research. On the contrary, the serine indices were decreasing, and the correlation coefficients between their values and T-2 and DON production amounted to –0.6 and –0.5, respectively.

In the discussing of the obtained data, the special attention should be paid to a pipecolic acid. The role of this non-protein amino acid is considered to be related to the mechanisms of plant protection from stressful environmental factors including FHB [26, 29]. We have found the positive correlation (r = 0.5) between the content of this acid and *F. sporotrichioides* and *F. culmorum* DNA concentrations, as well as DON content in SFs; the DON accumulation in the RFs oat inversely correlated with the pipecolic acid level (r = –0.8). The different directions and values of these correlations may be explained by the different immune response in the RFs and SFs oats.

In the samples of FHB-susceptible forms, we have found the negative relationship between the amount of DNA of *F. sporotrichioides* and linolenic acid (up to r = –0.7) and the positive relationship with the total amount of unsaturated and saturated fatty acids (the r values were up to 0.7 and 0.7, respectively). The positive relationship has also been found between *F. sporotrichioides* DNA and MAG2-18:2 (monoacylglycerol) (r = 0.6), between *F. culmorum* DNA and MAG-2-18:2 (r = 0.6), as well as between *F. culmorum* DNA and MAG-16:0 (r = 0.5) (Table 4). In the previous paper [9] we assumed that monoacylglycerols may be related to the plant adaptability, in particular, to resistance to biotic and abiotic factors that was confirmed by other researchers [29]. The content of DON toxin positively correlated with the amounts of linoleic (r = 0.6), eicosanoic (r = 0.5) and eicosanic (r = 0.5) acids and negatively — with the oleic (r = –0.5) and linolenic (r = –0.5) acids. Perhaps, the positive and negative values of the correlation coefficients are related to the different roles of certain fatty acids in the fungal vital activity.

In the group of FHB-resistant samples, the negative correlation between DON toxin and linolenic acid concentrations increased up to r = –0.9 compared to the FHB-susceptible ones. The relationship between the linolenic acid content and FHB-resistance has been confirmed [28]. The negative correlation was observed between the T-2 toxin and eicosenoic acid contents (r = –0.5). The negative correlation between the amounts of *F. sporotrichioides* DNA and linolenic acid decreased to r = –0.5 as compared to the values in the FHB-susceptible samples.

We have not found any relationship between the total content of organic acids and FHB-resistance, which has been noted by other authors. The direct correlation between the content of nicotinic acid and the FHB characteristics (up to r = 0.7), and the inverse relationship between the content of erythronic and methylmalonic acids (up to r = –0.9) were found. The concentration of lactic acid positively correlated with DON (r = 1) and T-2 toxin (r = 1) in RSs oats, and with *F. sporotrichioides* DNA (r = 0.7) in SFs. The negative correlation between the accumulation of lactic acid and amount of the *F. sporotrichioides*

DNA (r = –0.7) has been found in RSs, and between the DNA of *F. culmorum* (r = –0.5) and DON (r = –0.7) in SFs. The changes in the content of the main organic acids of primary metabolism including the acids of the Krebs cycle under the impact of FHB are confirmed by other authors [26, 29].

4. Характеристика устойчивых и неустойчивых к фузариозу форм овса (*Avena sativa* L.) из коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова по средним показателям накопления основных метаболитов (n = 25, M±SEM, г. Пушкин, 2015 год)

Main metabolites, mg/100 g	Forms	
	resistant	susceptible
Lactic acid	2.28±0.76	1.89±0.51
Methylmalonic acid	2.85±0.82	2.98±0.69
Glyceric acid	0.58±0.21	0.61±0.18
Erythronic acid	0.828±0.31	0.92±0.36
Nicotinic acid	0.198±0.08	0.20±0.10
Glycine	14.42±1.97	12.67±2.33
α-Alaniin	4.88±1.63	5.16±1.80
Valine	1.95±0.77	2.28±1.16
Leucine	0.59±0.14	0.61±0.14
Proline	5.02±2.63	4.87±2.65
Serine	2.42±1.12	3.00±1.38
Threonine	1.64±0.95	1.84±1.11
Oxyproline	0.80±0.63	0.62±0.48
Phenylalanine	1.72±1.04	1.66±0.81
Tyrosine	32.51±17.58	27.37±16.33
Glutamine	2.25±1.15	2.12±1.06
Asparagine	7.79±2.24	9.62±3.82
Total amount of free amino acids	78.15±17.66	74.01±16.52
Ethanolamine	2.16±0.56	2.17±0.67
Pipecolic acid	1.56±0.89	1.78±1.13
Adenosine	3.22±0.75	3.29±0.78
MAG-16:0	14.4±2.81	14.23±2.69
MAG-2-18:2	31.75±6.73	30.13±5.97
Dulcitol	18.38±3.72	16.33±2.41
Galactinol	38.41±13.43	36.87±11.5
Chiroinositol	0.37±0.12	0.74±0.57
Total amount of polyols	192.11±38.84	188.17±24.61
Cholesterol	0.64±0.19	0.86±0.39
Campesterol	0.69±0.23	0.68±0.17
Stigmasterol	0.47±0.17	0.55±0.20
β-Sitosterol	8.22±2.41	9.51±2.41
Total phytosterols	14.32±3.53	17.95±5.24
Glyceroaldehyde-3-phosphate	10.72±3.85	11.67±3.67
Total monosugars	974.22±303.73	843.34±175.47
Total disaccharides	2123.87±480.62	2543.03±477.91

The total content of polyols inversely correlated with the amount of DON toxin, at r = –0.8 in SFs and r = –0.5 in RFs. The dulcitol content in SFs positively correlated with the accumulation of DON and T-2 toxins (the r values were 0.6 and 0.7, respectively) and with the amount of *F. culmorum* DNA (r = 0.7). In the RFs group, we have found the inverse relationship between the galactinol and DON toxin, as well as with the *F. sporotrichioides* DNA concentration (r = –0.7). The inverse relationship between the DON and T-2 toxins concentrations and the total content of polyols confirmed by other authors [29] is due to the FHB influence on the primary metabolism. At the same time, the increase in the galactinol content [28] is related to the plant defense mechanisms under the influence of abiotic environmental factors; however, in this case, the change in the galactinol concentration is obviously due to the FHB influence.

In SFs, phytosterols, cholesterol and β-sitosterol have the inverse relationship with the amount of the *F. sporotrichioides* DNA (the r values were –0.7; –0.7 and –0.5, respectively). In the RFs group, we have also found the inverse relationship between the DON content and phytosterols, the campes-

terol (r = –0.6) and stigmasterol (r = –0.6). The dependence of the amount of *F. sporotrichioides* DNA on the β-sitosterol level (r = 0.5) turned out to be direct, in contrast to that in the FHB-resistant samples. The increase in the ergosterol amount under the *Fusarium* affection of tobacco leaves [29] is due to phytosterols participation in plant protection from biotic stresses, which is confirmed by our data.

In the SFs and RFs, we have found the inverse relationship between the FHB indices (accumulation of DNA of *F. sporotrichioides*, *F. culmorum* and DON) and the total amount of monosaccharides (up to r = –0.9). The total amount of disaccharides in the RFs group showed a direct dependence on the DON content (r = 0.9) and the inverse dependence on the amount of *F. sporotrichioides* DNA and glyceroaldehyde-3-phosphate (r = –0.5). In several researches, the changing in saccharides content under the influence of *Fusarium* causing agents has been noted. The inverse relationship between the DON and saccharides contents may be related to the inactivation of this toxin owing to the synthesis of the form conjugated with saccharides and the increase in the activity of glycolysis and the pentose-phosphate cycle [26].

The correlations between the contents of adenosine and the *F. sporotrichioides* DNA (r = –0.5), as well as DON (r = – 0.5) have been found only in SFs oat. It is obvious that this dependence is conditioned by the fungal influence on the synthesis of nucleic acids and protein as a whole in the affected plant tissues; there are no any information about detecting such relationships in other authors' papers.

All the r values obtained by us were at $p < 0.05$.

So, FHB exerts the influence practically on the all stages of primary metabolism including the synthesis of protein, oil, sugars, polyols, and activates the synthesis of the compounds related to the plants' protection against FHB, which, in particular, include pipecolic acids, acyl glycerols and phytosterols. The correlation between the protein content and amount of DNA of the main FHB infection agents, which have been found by us in the RFs oat, gives us the reason to suggest that in high-protein forms, the damage caused by pathogens is lower and, as a result, the toxins accumulate in a less amount, while in the SFs the high oil content in the grains contributes to the infection development. The unequal changes in the content of certain metabolites and their groups, which has been noted by us, may be related both to the synthesis of the compounds necessary for the fungal vital activity and to the peculiarities of the immune response in plant tissues. The comparing of the composition and content of the most important chemical groups of compounds including minor ones in the grains with the FHB-resistance indices is the stage necessary when investigating the biochemical processes occurring when the plants became infected by pathogenic microorganisms.

The physiological and biochemical factors of a passive immunity include the metabolic particularities, presence and content in the plant tissues of chemical compounds playing the protective role, physicochemical particularities of the tissues and plants themselves. The resistance related to the plants' physiological and biochemical properties may be explained by the absence in their tissues of the nutrients and physiologically active substances necessary for the pathogen as well as by the pathogen inhibition by toxic matters or other factors which are unfavorable. In the optimal case, the plants may contain the components which are harmful for the pathogenic fungus, for example, the glycosides, i.e. phenolic, cyanogenic and other compounds, as well as synthesize phytoalexins in response to the microbial infection [13, 30, 31].

The metabolome of a plant (grain) may be at different extent unfavorable

for the pathogen development, as it has been shown above in the analysis of the oat varieties with different resistance to FHB. The levels of certain substances have different extents and patterns of correlation with plant resistance characterized by the content of the fungal mycotoxins and DNA in grain. It is appropriate to recall the adaptive role of prolamins (avenins) in cereal resistance to biotic and abiotic factors [32-34]. The biochemical approach combined with the genetic investigations can provide the researchers with the valuable information about the mechanisms of plant protection against *Fusarium* fungi [35]. We have noted the relationship between the FHB-resistance indices and the accumulation of pipecolic acid, monoacylglycegols, tyrosine, galactinol, a number of phytosterols, sugars and adenosine in the plants.

So, we have obtained the data on the relationship of a wide range of compounds with different indices of FHB resistance in oat varieties, that is important not only for understanding the nature of this trait, but also for developing the diagnostic methods. These results of one-year field tests should be considered as preliminary, however, it should be noted that 2015 was extremely favorable for *Fusarium* infection. It can be assumed that the increased aggressiveness of *Fusarium* fungi made it possible for us to more reliably identify the dependences of the metabolite composition and content on the indices of plant resistance to FHB. Considering the complex polygenic nature of the control of FHB resistance and strong dependence of this trait manifestation on environmental conditions, for the confirmation or refutation of our conclusions, it is necessary to investigate the greater intraspecific and interspecific diversity of the oat samples reproduced under different conditions and in different years. Nevertheless, we have succeed in the identification of a number of dependencies between the resistance indices and the accumulation of certain metabolites, that makes it possible to select directly, at the initial stage, the samples with high content of such compounds for breeding oat varieties which combine resistance to *Fusarium* and high nutritional quality. Further researches will make it possible for us to expand the list of the metabolomic profile components which are promising when selecting the oat samples for resistance not only to the *Fusarium*, but also to other pathogens.

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