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IMMUNOLOGIC EVALUATION OF MAIZE COLLECTION SAMPLES

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Summary. Seven inbred maize lines from the active collection of Laboratory of plant genetic resources were tested for their susceptibility to fungal pathogens using visual evaluation of disease symptoms and molecular diagnostics. The highest infection rate was registered in MAN2492 and was 35%, therefore the respective genotype can be classified as susceptible. MAN2466 lacked symptoms of diseases and for MAN2488 minimum value of the respective parameter was registered (5%) – resistant genotype group. Quantitative analysis showed that the overall value of DNA sequences specific for the fungal genome was the lowest in the grain of MAN2488, MAN2491, MAN2470 genotypes.

Keywords: *maize, pathogenic fungi, PCR, disease resistance*

Introduction. The damage that agriculture suffers due to diseases are enormous. Diseases and pests are the cause of 10-15% of crop losses [1,9]. In addition, affected is the yield quality; some diseases make crop production inedible. A wide range of fungal pathogens cause serious losses of maize yield both causing devastating field infections (e.g. *Fusarium spp*.) and inducing grain and silage spoilage during storage (*Aspergillus spp., Penicillium spp.*) [2-5]. These fungi have the ability to infect corn at every stage of ontogenesis, plants being most prone to infestation at the generative phase [6, 7]. Cultivating resistant maize genotypes presents the most prominent strategy in terms of sustainable agriculture and environment protection. It allows reducing soil and water pollution because of the excessive usage of pesticides and conserving the biodiversity of ecosystems. This approach requires testing corn plants in field trial for selecting the promising donors of resistance traits to be used in maize breeding programs.

The aim of the current study is evaluation of maize collection sample by the characters of resistance to causal agents of main corn diseases under exposure of climatic conditions of 2022.

Materials and methods. Self-pollinated maize lines from active collection of the Laboratory of plant genetic resources served as plant material for the study: MAN2488, MAN2493, MAN2491, MAN2483, MAN2470, MAN2466, and MAN2463. Genotypes' trial was conducted on the experimental fields of the Institute of Genetics, Physiology and Plant Protection (year 2022). Disease accounting was performed by the criteria of infection dissemination or number of infected plants on the plot. For registration of the phytopathogens' symptoms, the system of visual valuation was applied: maize common smut (causal agent - *Ustilago zeae* Beckm.); maize head smut (causal agent - *Sporisorium reilianum* McAlp); Northern corn leaf blight (causal agent - *Helminthosporium turcicum* Pass); ear rot (causal agent - *Fusarium spp.*).

Samples of mature kernels were used for pathogens' identification. Total DNA was extracted using CTAB protocol with modifications [8]. Molecular diagnostics was performed using PCR with a set of primers specific for different fungi of the *Fusarium, Aspergillus* and *Penicillium* genera.

Table 1

Particularities of the primers for PCR analysis

Primer	Primer sequence	Taxon	Target-sequence (FASTA)
fqtri8gr2	CTCGTCACTTCCTTGATGACACA	*Fusarium graminearum*	MH514940.1
fqtri8gr3	GGGGGCCGACATTCACTTC		
fqprfum62	ATCGCCCTCTGCACGATAGA	*Fusarium proliferatum*	KF889207.1
fqprfum63	TGGGAGGTTGCTCTGAGTGA		
fqfum6ve2	TCGCCCTTTGCACCATTGAC	*Fusarium verticillioides*	KF889190.1
fqfum6ve3	AGCCTGCCGCTTGAACTTTG		
aqfap2	GTGTCGGGTGTGCCTATTTAACC	*Aspergillus flavus*	FJ877830.1
aqfap3	AAGGCTTTCGGTCGGTTGATG		
apap2	CCGCGAAAGAACAAACAGAGA	*Aspergillus parasiticus*	DQ390914.1
apap3	AACACATATTCTCCAACTTTCTTG		

	CT		
pchbt1	GTTGCTAACTGGATTACAGGCAAAC	*Penicillium chrysogenum*	GQ498281.1
pchbt4	CACCGCTGGCCTAGATTGTC		
pex2	CTCTCTCCGTTCACCAGCTT	*Penicillium expansum*	FJ012871.1
pex3	TCAACTCGGGGACGTTGAC		

The PCR was performed in a 25µl mix containing 66 mM Tris-HCl (pH 8.4), 16 mM $(NH_4)_2SO_4$, 2,5 mM $MgCl_2$, 0,1 % Tween 20, 7 % glycerol, 100 $\mu g/ml^{-1}$ BSA, 0,2 mM of each dNTPs, 1,25 U Taq DNA polymerase (Thermo Fisher Scientific), 5 pM of each primer and 10 ng of DNA. The amplification protocol included 1 cycle at 95°C for 3 min, followed by 45 cycles: 95°C –1 min, 60°C – 1 min, 72°C – 1 min. The synthesis finished with terminal elongation at 72°C for 7 minutes. Quantification of target-sequences in the samples was performed using conventional PCR with Poisson distribution. The products of amplification were separated in 1,5% agarose gel at the 6V/cm in 1xTBE migration buffer (pH 8.0) with ethidium bromide, viewed in the UV (302 nm) and photographed.

Results and discussion. Symptoms of maize common smut and head smut were registered before and after the flowering phase. Generally, there was observed a low genotype infestation rate (<1%) with different pathogens (Tab.2), wherein for MAN2463 the value of this parameter was 10%. Ten percent of MAN2483 plants were affected by maize head smut. No visual symptoms of Northern were identified on the tested genotypes. Mean rate of ears affected by fusariosis was 17,1%. The highest rate of infection was registered in MAN2493 and was 35%, therefore the respective genotype can be classified as susceptible. MAN2470 lacked symptoms of diseases and for MAN2488 minimum value of the respective parameter was registered (5%) – resistant genotype group.

Table 2

Number of infected plants (nr, %)

N	Inbred line	Infected plants							
		Ustilago maydis		*Sorosporium reiliana*		*Helminthosporium turcicum*		*Fusarium spp.*	
		nr	%	nr	%	nr	%	nr	%

1	MAN2463	2	10	-	-	-	-	5	25
2	MAN2470	-	-	-	-	-	-	-	-
3	MAN2466	-	-	-	-	-	-	4	20
4	MAN2483	-	-	1	5	-	-	5	25
5	MAN2491	-	-	-	-	-	-	2	10
6	MAN2493	-	-	-	-	-	-	7	35
7	MAN2488	-	-	-	-	-	-	1	5
	Mean value	0,3	1,4	0,1	0,7	-	-	3,4	17,1

According to the results of PCR tests, the following pathogenic fungi were found in samples of mature corn seeds: *F. graminearum, F. proliferatum, P. chrysogenum* and *P. expansum* (Fig. 1). At the same time, the absence of such pathogens as *A. flavus, A. parasiticus, F. verticillioides* was determined. The presence of one causal agent in the grain of three lines - MAN2488, MAN2491, MAN2470 (*F. graminearum, F. proliferatum* and *F. graminearum,* respectively) was revealed. Two types of infection were found in the grain of two inbred lines. Thus, *P. expansum* and *F. proliferatum* were identified in the MAN2483 line, and *F. proliferatum* and *F. graminearum* were identified in the grain of MAN2466 line. The highest species diversity of pathogens (*F. proliferatum, F. graminearum, P. chrysogenum*) was identified in the grain of MAN2493 genotype.

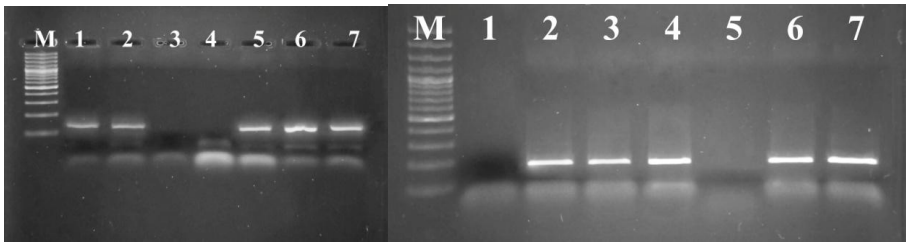


Fig.1. Electrophoregrams of PCR products of DNA isolated from maize with primers for *F. graminearum* (left) and *F. proliferatum* (right):
1 - MAN2488; 2 - MAN2493; 3 - MAN2491; 4 - MAN2483; 5 - MAN2470;
6 - MAN2466; 7 - MAN2463; M –GeneRuler 100 bp Plus DNA ladder
(Thermo Fisher Scientific)

Quantitative analysis showed that the overall value of DNA sequences specific for the fungal genome was the highest in the grain of MAN2483 genotype (Fig. 2). On the contrary, the lowest values of this indicator were observed in MAN2488, MAN2491, MAN2470 genotypes.

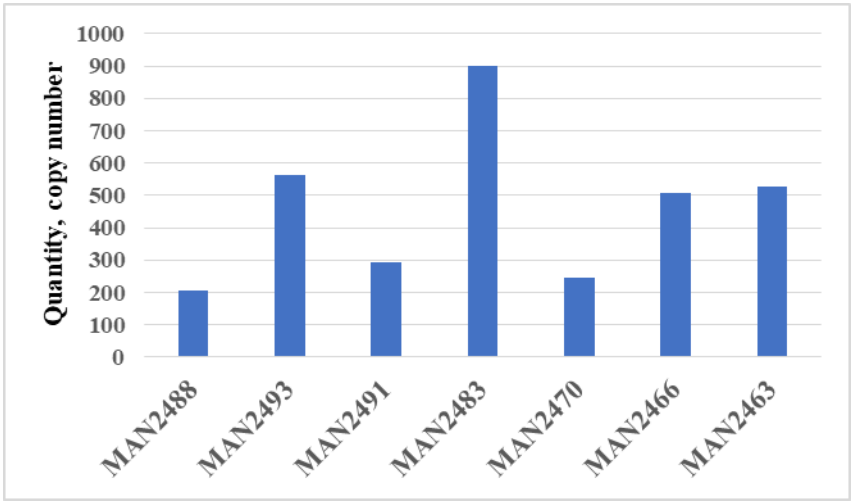


Fig.2. Histogram of overall quantity of fungal genome sequences in maize grain samples

Taking into account the results, the inbred lines were divided into two groups based on their resistance to major pathogens. Four lines were classified as susceptible genotypes: MAN2483, MAN2493, MAN2463 and MAN2466. The second group comprised genotypes that showed resistance to main corn pathogens: MAN2488, MAN2491 and MAN2470.

Conclusions. As a result of immunological assessments of seven inbred maize lines for resistance to major diseases under natural conditions, genetic sources of complex resistance to major diseases were identified.

Molecular analysis of grain samples of corn collection samples revealed the presence of toxigenic strains of *F. graminearum, F. proliferatum, P. chrysogenum* and *P. expansum*. At the same time, such pathogens as *A. flavus, A. parasiticus, F. verticillioides* were not identified. Comparative analysis of quantitative components revealed genotypic differences in the susceptibility to fungal infestation.

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