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Studying the world collection of millet with a view to select forms immune to lose smut

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Abstract

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The article presents a study of loose smut resistance of 170 millet varieties of the national and international collections in the infectious environment of Akmola region. The inoculation of millet seeds was carried out using the method of artificial contamination with spores of local population of loose smut. The results of the study showed that the genotypes of the national collection did not include immune varieties; the most resistant were the 36 varieties of the international collection as well as the two varieties of the collection of the All-Russian Institute of Plant Genetic Resources (VIR). The results of the immunological assessment indicate that the major part of the working collection used in the study demonstrated a medium level of susceptibility to the local pathotypes of the pathogen.

Keywords: millet; selection; loose smut; resistance

Introduction

The Republic of Kazakhstan is among the leaders in export of agricultural crops (Rsymbetov et al., 2018; Abraliev et al., 2018). Millet is one of the most ancient crops cultivated by man; today it is the one in demand as a valuable food, feed and forage cereal in many countries of the world including Kazakhstan, a crop able to ensure good harvest of grain and green material. The main use of common millet (*Panicum miliaceum* L.) is to receive cereal, which surpasses other crops as well as leguminous crops in terms of its nutritional value (Wang et al., 2016; Dong et al., 2015; Goron & Raizada, 2015; Wang et al., 2014; Minxuan et al., 2016; Hunt et al., 2014; Kobyzeva et al., 2012). Biological value of proteins of the grain is determined by a high level of indispensable amino acids, such as methionine, trypotophan, leucine and isoleucine (Rsymbetov et al., 2018; Agafonov & Kurtseva, 1982; Amadou et al., 2013; Gupta et al., 2010; Habiyaremye et al., 2017; Dyusibaeva & Seitkhozhayev, 2016; Dyusibaeva et al., 2017). High yields are another strength of millet, which is confirmed by the record of Mr. Shyganak Bersiyev with the harvest of 201 centner/ha. Despite the above given merits of this crop, the area planted with millet in the Republic of Kazakhstan is shrinking rapidly, which is linked to the underestimation of its agricultural value and unstable yields over the years (Lu et al., 2005; Martin et al., 2003). One of the factors that limit the yields of millet is the vulnerability of the plant to various diseases. The most widespread among them is the loose smut caused by *Sphacelotheca panici-miliacei*. The damage from the disease manifests itself by lower yields because of the destruction of the panicle of millet and latent losses in harvest as the affected plants are underdeveloped, which can in total amount to 20-30% (Martin et al., 2003; Nagaraja & Mantur, 2008; Abraliev et al., 2018; Santosh et al., 2016; Surkov & Kolyagin, 1988). In cultivation, a chemical method to fight the disease is used – mandatory pre-sowing seed treatment, which substantially raises the cost of the product and has a negative impact on the environment. The most efficient and environmentally friendly way to fight against loose smut is breeding of new resistant millet varieties. The major problem in selection for resistance to phytopathogens is the provision of breeding process with resistance donors. It should be noted that development and improvement of methods to identify loose smut resistance genes, assessment and breeding of viable forms of millet with various resistance types is a topical area for research in selection (Tsygankov et al., 2006).

In connection with the situation described above, our objective was to carry out a screening of the world and national millet collections in terms of resistance to loose smut with a view to select prospective donors for further use in the breeding process.

Materials and Methods

170 samples of millet from the national and world collections were used in the study, of which 100 genotypes were provided courtesy of the Peginal Plant Introduction Station (world collection of USDA), Iowa State University (USA). As a result of a scientific visit to the Pavlodar Agriculture Research Institute, an exchange of millet germplasms that included samples valuable for agriculture carrying resistance genes *Sp1*, *Sp* 2, *Sp* 3 and *Sp4* was carried out. Loose smut spores were provided by the laboratory of plants' immunity to pests and diseases of the A. I. Barayev Grain Cultivation Research and Production Center in 2015 and were reproduced by us annually over 2015 − 2017.

To determine the germination rates of loose smut telio spores, before the contamination spores were placed in Petri dishes with wet filter paper. Then, distilled water was dripped on slides and placed in dampening chambers. Next day, at the temperature of 18 − 20°**C** spores of millet loose smut germinated.

Inoculation of millet seeds was carried out using the method of artificial contamination with spores of local population of loose smut. To do this, ripe goiters of loose smuts ruses are gathered when the grain has ripened, dried and put in paper bags; further on, the sources are ground, spores are sifted through a sieve and kept in a glass bulb at the temperature of 18 − 20°C. One month before sowing, millet seeds are contaminated with spores at the rate of 1% of spores per the seeds mass. The process of contamination is carried out using the method of shaking the seeds and spores during 2 − 3 minutes (Zhou et al., 2016).

With the aim to study resistance of starting material of millet to lose smut, artificial infectious environment was created at the pilot site of the A. I. Barayev Grain Cultivation Research and Production Center in Akmola region. A month after the inoculation, millet collection was sown manually. According to the method, 50 samples of contaminated seeds of every variety were sown on two-row plots with 20 cm width between rows. Control over the effectiveness of the infectious environment was carried out relying on the method of sowing the standard Kokchetavskoye 66, a universally susceptible variety, after every 9 plots.

To compare the development of the disease and to objectively assess the resistance of samples to lose smut, a field trial was established in natural conditions with non-contaminated millet seeds. Saratovskoye 6 variety was used as a standard as it is included in the State Register of the Republic of Kazakhstan with the purpose of assessing characteristics of millet gene pool valuable for agriculture.

The assessment of resistance to phytopathogen was carried out on a1to 9 scale of damage (2017): 1 – very slight damage (<10%); 3 − slight damage (10 − 35%); 5 − medium (36 − 60%); 7 – severe (61 − 85%); 9 – very severe (>85%).

Results and Discussion

In the study carried out with an aim to create immune forms, we focused on the assessment of the starting material of millet in terms of its resistance to lose smut. The choice of this object is explained by the fact that this pathogen is the most harmful disease of millet; the pathogen also affects a limited scope of varieties, which ensures the possibility to identify forms immune to it.

Over the period of the study, with a view to identify and select donors of productivity and resistance to diseases in the current year a population of local species of millet loose smut reproduced earlier in the A. I. Barayev Grain Cultivation Research and Production Center was obtained. In the laboratory of plants' immunity to pests and diseases of this research center, survival rate of *Sphacelotheca panici-miliacei* teliospores reproduced in 2012, 2014 and 2016 was identified using a light microscope. Before the contamination, spores were placed in Petri dishes with wet filter paper. Then, distilled water was dripped on slides and placed in dampening chambers. Next day, at the temperature of 18 − 20°**C** spores of millet loose smut germinated (Figure 1).

The results of determining the survival rate of *S.panici-miliacei*spore fund over 2012, 2014, 2016 were 36.5 –

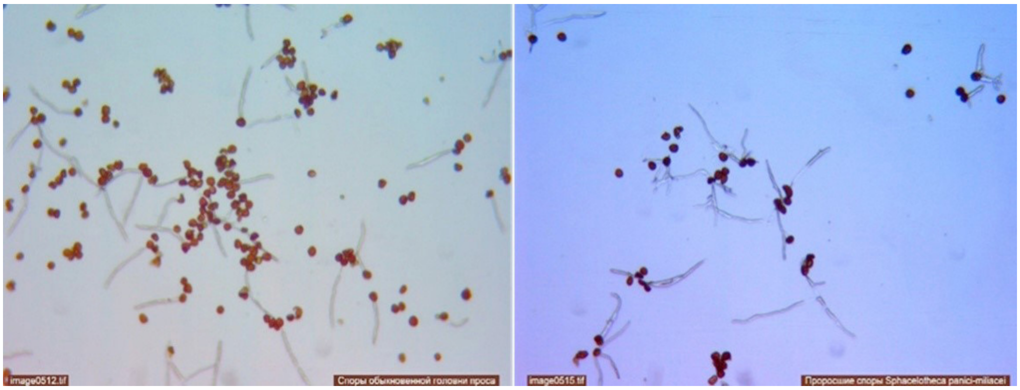

Fig. 1. Germinatingmycelium of loose smut spores

51.8%. The calculation of germination indicated that the 2016 pathotypes were most active. The average survival rate of 2016 telio spores was 51.8%, which exceeds the results of 2012 and 2014 pathotypes for 15.3 and 7.3 % respectively (Table 1).

Table 1. Determining survival rate of teliospores of millet loose smut in the laboratory

Spores of inoculum *Sphacelotheca panici-miliacei*	Years		
	2012	2014	2016
Total number of spores, pcs	159	146	170
Number of spores that germinated, pcs	58	65	88
Survival rate, %	36.5	44.5	51.8

Consequently, the 2016 pathotypes of the phytopathogen were used for the inoculation of millet collection seeds.

During the period of observation over the intensity of damage from the phytopathogen from August 20th to 30th, the variety with the best indicators of resistance in the national collection was Kormovoyeproso *(fodder millet)* variety (36%), and the variety with the most severe damage among the varieties studied was Aktiubinskoyekormovoye *(Aktiubinsk fodder millet)* (67%) (Table 2).

Saratovskoye 3 and Uralskoye 109 varieties appeared to be susceptible to lose smut, as well. Figure 2 includes photos of the pathogen – causative agent of *Sphacelotheca panici-miliacei.*

Shortandinskoye 10, Shortandinskoye 7, Yarkoye 3 on average had better indicators than the standard variety Kokchtavskoye 66 (64%), the intensity of damage to these varieties was 37, 38 and 39%, respectively.

As a result of assessment in severe infectious environment, 70% of the 20 varieties showed medium resistance levels to local loose smut pathotypes, 20% appeared to be highly susceptible. We did not find absolutely immune varieties among all the genotypes of the national collection used in the study. Thus, millet varieties used in cultivation in our republic are not resistant to local widespread species of the pathogen. The screening of millet collection of VIR (All-Russian Institute of Plant Genetic Resources) to identify resistance to the harmful phytopathogen revealed sources of resistance that showed immunity to the disease (0): samples K-803 and K-1437. High vulnerability to the disease (7 points) was observed for the samples K-2468, K-148, K-8873, K-9655, K-9658, their intensity of damage

Table 2. The level of resistance of the national collection varieties to loose smut (2018, infectious environment)

Variety	Vulnerability		Degree of damage
	%	points	
Kokchetavskoye 66 St	64	7	severe
Aktiubinskoyekormovoye	67	7	severe
Bersiyev Memorial	47	5	medium
Yarkoye 3	39	5	medium
Yarkoye 5	51	5	medium
Yarkoye6	–	–	–
Yarkoye7	48	5	medium
Omskoye11	56	5	medium
Kormovoye89	53	5	medium
Pavlodarskoye	52	5	medium
Zolotistoyekormovoye	42	5	medium
Barnaulskoyekormovoye	42	5	medium
Saratovskoye6	52	5	medium
Kormovoyeproso	36	5	medium
Shortandinskoye 7	38	5	medium
Saratovskoye3	61	7	severe
Uralskoye109	66	7	severe
Shortandinskoye10	37	5	medium
Shortandinskoye 11	54	5	medium
Abakanskoyekormovoye	–	–	–

Fig. 2. Symptoms of millet loose smut on the plants of standard variety "Kokchtavskoye 66"

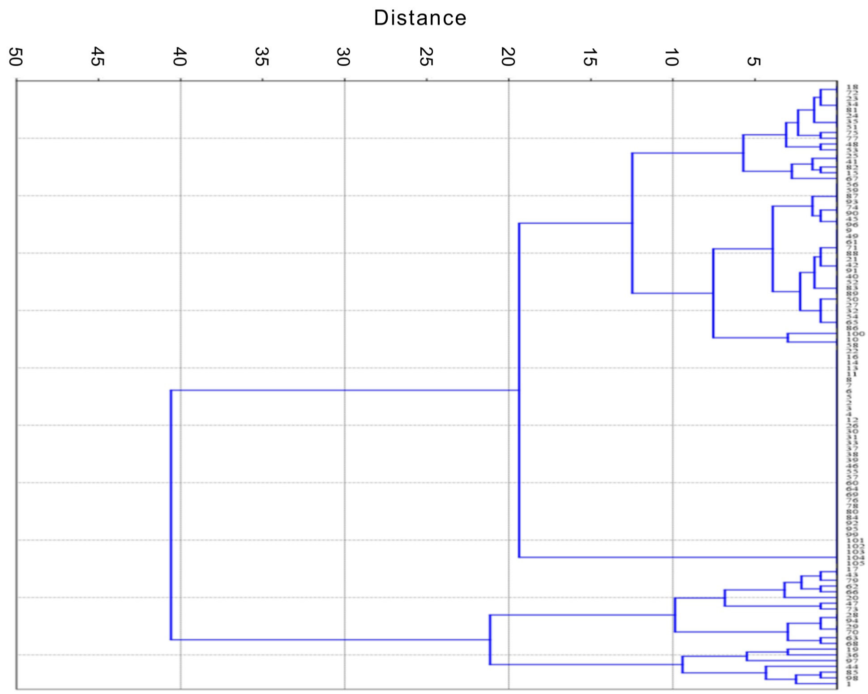

Fig. 3. Level of loose smut resistance of the VIR collection samples

being on the level of the universally susceptible variety Kokchetavskoye 66 (64%).

The results of immunological assessment indicated that the major part (64.3%) of the VIR catalogue samples were in the group with medium resistance to millet loose smut (30 – 60%) (Figure 3).

As a result of the trial of 100 samples received from NCRPI (USDA), 36 varieties were distinguished as fully immune (0) to this disease. According to the results of phytopathological testing, high resistance to the disease (<10%) was observed for the genotypes K-2 (Bai Li Shu, China) – 6%; PI 170589 (Turkey) – 9% and PI 253955 (Afghanistan) – 9%.

The group with low vulnerability included 43 samples, their damage from the disease ranged from 10 to 35%. 16 samples of the international collection (PI 173752, PI 170604, PI 209790, PI 211058, PI 212862, PI 219931, PI 269953, PI 346945, PI 220670, PI 2963761, PI 289324,

PI 289324, PI 296376, PI 222811, PI 365847, PI 649373) had a medium level of damage by the causative agent of *S. panici-miliacei,* their intensity of damage being 36 − 60%. Most vulnerable to lose smut were the 4 samples: PI 173750 (Turkey) – 71%; PI 182258 (Turkey) – 74%; PI 654403, PI 463247 (India) – 61%; Ma zha Yan (China) – 62%, which amounted to 4% of the total international collection.

Cluster analysis in terms of intensity of damage by the pathogen enabled us to divide the samples of the international millet collection into the first and second groups (Figure 4). As a result of clustering of the studied material, the first group included immune, highly resistible and comparatively resistible samples. Vulnerable samples that were close to the universally susceptible (*Sp* 0) standard variety Kokchetavskoye 66 were included in the second group of the cluster.

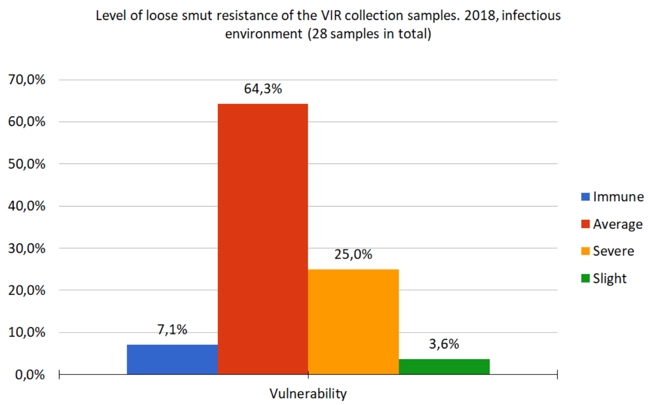

Figure 4. Cluster analysis of the international millet collection in terms of vulnerability to loose smut, %

Conclusions

An immunological assessment in the weather patterns and climate of 2018 in Akmola region indicated that 41 collection samples showed high resistance to the causative agent of loose smut (<10%). It was also established that the millet varieties and breeding samples approved for cultivation in the Republic of Kazakhstan are not resistant to the local widespread species of the phytopathogen. Therefore, this highlights the need to breed millet to achieve higher resistance to lose smut in our republic.

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