The main principles of formation of a collection of strains of the causative agents of septoriosis of cereal crops

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Abstract. A collection of strains of the causative agents of septoriosis of cereal crops was established at the All-Russian Scientific Research Institute of Phytopathology (ARRIP) as a result of long-term monitoring of the species and intraspecies structure of pathogen populations in Russia and the CIS countries. It is the basis for immunological, phytopathological and genetic researches both within the Institute and in other institutions working in the field of plant breeding and plant protection. The article describes the main principles of formation of collection, the main criteria of selection of strains, and their significance and evaluation methods. The structure of the collection fund formed on the basis of these principles is presented.

Key words: collection, septoriosis, selection of strains, region, morphotype, sporulation, pathogenicity, virulence.

1. Introduction

On the basis of the All-Russian Scientific Research Institute of Phytopathology (ARRIP), the State Collection of Phytopathogenic Microorganisms (SCPM) has been created. It contains the strains of causative agents of the most harmful diseases of agricultural crops common in Russia, including the strains of three main causative agents of septoriosis of wheat and barley: Stagonospora nodorum [Berk.] Castellani and E. G. Germano, Septoria tritici Rob. et Desm. and Stagonospora avenae Bissett f. sp. triticea T. Johnson.

Septoriosis is a dangerous and economically significant disease for Russian agricultural producers, since the mid-1970s of the last century. Currently, it occupies a dominant position among the harmful fungal diseases in most regions of the Russian Federation. In epiphytotic years, the crop losses from this disease can reach 40% [1, 2]. One of the main causes of great harmfulness of septoriosos is absence of resistant varieties. The presence of a collection of pathogenic strains of the causative agents of septoriosis is an indispensable condition for study of the resistance of the selection material for this disease. The formation of such collection was started in ARRIP in 1981. The main goal was to provide breeding centers and state testing stations located in different agro-climatic zones with the necessary amount of biomaterial to create an artificial infectious background when testing varieties for resistance to septoriosis.

At present, when collections are organized, it is necessary to take into account the broader possibilities of using strains in various fields of science (mycology, immunology, toxicology, genetics,
biotechnology, etc.). In most microbiological laboratories in the world, microorganism collections are a way of preserving biodiversity, providing not only cultures for work, but also significant amounts of information [3]. Modern methods using the sequencing of genomes and proteins make it possible to study populations of biological objects at the molecular level, which is important for selection, since the level of genetic variability within the pathogen population is a reflection of the potential variability of the pathogen by aggressiveness [4]. In this regard, in assessing the stability of germplasm, it is recommended to use a wide variety of pathogen strains to increase the virulence spectrum of natural populations [4, 5, 6]. There is an opinion that chemical companies should also include at least several hundred strains in their tests for assessing resistance to fungicides [5]. The causative agents of septoriosis are known to have a great potential for genetic variability, due to the presence of sexual reproduction. Microsatellite analysis of 251 S. tritici isolates from 4 regions of Russia revealed a high level (89%) of genetic diversity within regional populations [7]. Different genotypes were often found within a single lesion, and most lesions on the same leaf had also different genotypes [8]. The high intraspecific variability of septorial fungi leads to the emergence of new aggressive forms with increased virulence [9, 10]. In this regard, continuous monitoring of the species and intraspecific structure of the Septoria/Stagonospora populations, followed by the isolation of new strains with the aim of replenishment and enriching the collection with new genetic diversity is necessary.

To date, our task, in addition to maintaining and preserving of previously included strains of the causative Septoria agents in a viable state, is the systematic replenishment of collection of ARRIP with new samples. Replenishment of the collection fund is carried out by isolating new strains from the infected plants collected during the annual inspections of grain crops in various agroclimatic zones of the Russian Federation. The selection of strains in the collection is carried out after studying their main properties: cultural-morphological (CM) features, reproductive capacity on nutrient media, pathogenicity, race belongingness and genotype of virulence. The strains that are of the greatest interest for scientific and practical research are selected for the collection.

2. Materials and methods

The isolates of the fungus were isolated from samples of diseased plants with well-defined septoriosis spots. Preliminary microscopic analysis of the samples was carried out to identify the fungal species. For this, small fragments of the diseased tissue were placed on a slide in a drop of water and investigated under a microscope. The species of causative septoriosis agent was determined by the shape and size of the spores emerging from the pycnidia [11, 12].

Isolation of the fungus pure culture was carried out under sterile conditions, using the method of "strokes" [13]. The potato-glucose agar (PDA) was used as a most universal nutrient medium for all septoria species. After 4-5 (S. nodorum) or 7-8 (S. tritici) days of incubation, single-spore colonies were transferred into individual Petri dishes. The colonies of S. nodorum were incubated under additional light with erythematous LE-30 lamps stimulating sporulation.

Description of the culture-morphological (CM) signs of isolates was carried out on the 20th (S. nodorum and S. avenae triticea) or the 30th (S. tritici) day, noting the size, structure and color of colony. 7 morphotypes of colonies of S. nodorum isolates, 10 morphotypes - S. tritici, 4 morphotypes - S. avenae triticea were distinguished [11, 14, 15]. The stability of CM features is important term for the selection of strains in the collection. That was monitored by three consecutive subcultures of 10-day colonies.

The reproductive capacity of the culture on a nutrient medium (in vitro) was determined according to the developed method [11]. The intensity of sporulation is more than 1 mln. spores / cm² of the colony area for S. nodorum isolates and more than 10 mln. spores / cm² of the colony area for S. tritici isolates is considered to be quite high.

Assessment of the pathogenicity of isolates was carried out on test-cultivars at seedling stage in artificial climate chambers or in a greenhouse according to the method developed by ARRIP [16]. The pathogenicity level of S. nodorum and S. avenae triticea isolates was determined by the average lesion degree of two leaves of plants of the test-cultivars set: I – low pathogenic (degree of
lesion less than 20%), II - medium pathogenic (21-50%), III - highly pathogenic (more than 50%). The pathogenicity of \(S. tritici\) isolates was determined by two parameters: the degree of plants lesion and the sporulation activity of the fungus in vivo (table 1). Sporulation was determined by the number of spores/leaf using the hemocytometer.

| Pathogenicity groups of \(S. tritici\) isolates. |
|-----------------------------------------------|
| Infection degree, % | Sporulation intensity (thousand spores/leaf) |
|---------------------|---------------------------------------------|
| Low (<20)           | I                                           |
| Middle (21-50)      | II                                          |
| High (51-100)       | III                                         |

As is known, resistance to Septoria tritici blotch pathogen is isolate-specific [17, 18]. Moreover, gene-for-gene relationship in pathosystem “wheat - Mycosphaerella graminicola (anamorph Septoria tritici)” has been demonstrated by genetic analysis of the host-pathogen interaction [19]. Therefore, to \(S. tritici\) strains, such additional indexes as race and genotype of virulence were assessed.

3. Finding and discussion
As a result of a long-term study of the species’ structure of populations of septoriosis of cereal crops, three types of septoriosis pathogen have been identified on the territory of the Russian Federation. \(Stagonospora nodorum\) and \(Septoria tritici\) are the most harmful pathogen species and they present in all regions of the country, dominating in different zones. \(S. nodorum\) is most common in the central, northern and eastern zones (Central, North, North-West, West Siberian regions). \(S. tritici\) prevails in the southern zone (North Caucasus, Central-Chernozem regions). In addition, \(S. tritici\) along with \(S. nodorum\) is found in the Central region, and is widely distributed in the Volga region. In recent years, representation of \(S. tritici\) has increased in the North-West region. The third type of species, \(S. avenae triticea\), is also noted in all regions of the Russian Federation, but its share in the septoria complex is much smaller [16, 23, 24]. This one is less harmful, since it occurs after the appearance of the ear [25].

The economic importance of the species, their distribution areas and frequency of occurrence in different regions of Russia were taken into account when forming a collection of cultures of Septoria causative agents. First of all, the collection included strains of the most harmful and widespread types of Septoria pathogens, which are the most-used for immunological tests. The presence in Collection of strains from all agroclimatic zones and grain producing regions is great importance since strains used in field trials must meet requirements of their origin correspondence to the region where the tests are conducted [11]. In addition, it was essential to take the cultivar of the host plant into account when
isolating the fungus into a pure culture, since cultivars with different resistance can select different pathogen genotypes with specific virulence.

Currently, the State Collection of Phytopathogenic Microorganisms of ARRIP contains 342 strains of septoriosis pathogens from 10 regions of the Russian Federation, and from Ukraine, Belarus, Moldova, Kazakhstan, the Baltic republics. There are 116 S. nodorum strains isolated from wheat and barley, 224 S. tritici strains isolated from wheat, and 2 S. avenae f. sp. triticea isolated from wheat.

The number of strains in the collection from different regions varies considerably (table 2). This is due both to the frequency of occurrence of species in region, and to the possibility of collecting samples of infected plants. The largest set of S. tritici strains is represented from 4 regions of Russia: Central, North Caucasian, Centra-Chernozem, North-West. A lot of strains from the Volga and Vyatka regions are included in the collection. S. nodorum strains from the Central region were brought more often to the collection; in recent years a set of strains of this species from the Volga and West Siberian regions has been expanded.

| Septoria/Stagonospora species | Origin (region of RF, country) | The number of strains |
|------------------------------|-------------------------------|----------------------|
| S. tritici                   | Central                       | 37                   |
|                              | North Caucasian               | 53                   |
|                              | North-West                    | 34                   |
|                              | Centra-Chernozem              | 50                   |
|                              | Volga                         | 16                   |
|                              | Vyatka                        | 14                   |
|                              | Kaliningrad                   | 7                    |
|                              | West Siberian                 | 7                    |
|                              | Ukraine                       | 3                    |
|                              | Kazakhstan                    | 3                    |
| S. nodorum (wheat)           | Central                       | 34                   |
|                              | North Caucasian               | 7                    |
|                              | North-West                    | 5                    |
|                              | North                        | 7                    |
|                              | Volga                         | 10                   |
|                              | West Siberian                 | 17                   |
|                              | East Siberian                 | 3                    |
|                              | Far Eastern                   | 2                    |
|                              | Ukraine                       | 5                    |
|                              | Belarus                       | 1                    |
|                              | Moldova                       | 1                    |
|                              | Baltic                        | 1                    |
|                              | Kazakhstan                    | 7                    |
| S. nodorum (barley)          | Central                       | 4                    |
|                              | North-West                    | 1                    |
|                              | North                        | 1                    |
|                              | Baltic                        | 1                    |
|                              | Belarus                       | 2                    |
| S. avenae triticea           | North Caucasian               | 1                    |
|                              | East Siberian                 | 1                    |

One of the main ways of intraspecific pathogen identification is to evaluate the culture-morphological (CM) features of strains. Collection of strains from a certain geographical area must have different CM signs to reflect the intraspecific diversity of the pathogenic population as much as possible. This is not only an important condition for carrying out phytopathological and
immunological tests, but it can also serve as a base for fundamental studies of the intraspecific structure of the populations using various molecular-genetic methods.

The sporulating ability of the strain on a nutrient medium is of great importance in the production of biomaterial for inoculation of plants in various tests, where strains with high reproductive capacity are usually used.

To date, the collection presents all the morphotypes of S. nodorum and 9 of the 10 known morphotypes of S. tritici (table 3-4). The number of strains with a particular morphotype was selected by taking into account the frequency of occurrence of morphotypes in different populations, and also the connection between the morphotype and sporulation in vitro. In general, strains with good sporulation capacity prevail in the Collection. There are 99 strains of S. nodorum (27 - with the light colonies (morphotypes Ia and Ib), and 72 – with dark colonies (morphotypes IIa and IIb)), and 162 strains of S. tritici (47 - with yeast-like type of colonies (morphotypes Ia, Ib, Ic) and 115 - with mixed type of colonies (IIa, IIb, IIc, IId)).

Table 3. The representation of morphological types of colonies of S. nodorum strains in the Collection.

| Morphological types of colonies | Characteristics of morphological type                                                                 | The number of strains |
|--------------------------------|-------------------------------------------------------------------------------------------------------|-----------------------|
| Light (I)                      | a Pink, granulated, rare air mycelium, many pycnidia.                                                 | 9                     |
|                                | b Grey, woolly or woolly-powdery, many pycnidia.                                                      | 18                    |
|                                | c White or light-grey, cotton-like, the surface is rough, wrinkled or bumpy, few or no pycnidia.     | 5                     |
| Dark (II)                      | a Dark brown, granulated, rare air mycelium, many pycnidia.                                         | 30                    |
|                                | b Dark-brown, woolly or woolly-powdery, many pycnidia.                                               | 42                    |
|                                | c Black or brown, surface smooth, few pycnidia.                                                      | 6                     |
| Zonal (III)                    | - Brown in the middle, light on the periphery, woolly, many pycnidia.                               | 6                     |

Table 4. The representation of morphological types of colonies of S. tritici strains in the Collection.

| Morphological types of colonies | Characteristics of morphological type                                                                 | The number of strains |
|--------------------------------|-------------------------------------------------------------------------------------------------------|-----------------------|
| Yeast-like (I)                 | a Pink, surface is crimped                                                                          | 15                    |
|                                | b Black, crimped                                                                                     | 24                    |
|                                | c Black, crimped, pink border                                                                        | 8                     |
| Mixed (II)                     | a Black, yeast-like centre, black; border is mycelial, black                                        | 79                    |
|                                | b Yeast-like centre, pink, border is mycelial, black                                                | 13                    |
|                                | c Grey, yeast-like centre, pink                                                                      | 18                    |
|                                | d Mycelial centre; yeast-like border, crimped, black                                                 | 5                     |
| Mycelial (III)                 | a White or grey                                                                                     | 38                    |

Determination of pathogenic properties of collection strains is one of the main tasks of researchers when selecting them for collection. Highly pathogenic strains are the most valuable, as they have wide practical application in creating a severe infectious background. More than half of the collection strains of S. nodorum and S. tritici are characterized by high and medium pathogenicity level.

The genotype of virulence was determined in 196 strains of S. tritici on 6-8 cultivars with known resistance genes. Of these, 155 strains were virulent to 1-6 monogenic varieties. The virulence spectrum of 107 strains was 1-3 genes, 48 strains has 4-6 genes of virulence. Isolates with a wide range of virulence can be used further to assess the resistance of varieties and the effectiveness of Stb-
genes. This information is of great importance for practical selection for immunity to septoriosis, since the inclusion of an effective Stb-gene in the selection process will provide long-term resistance of a new variety.

At present, a collection of strains of the Septoria causative agents is primarily a basis for providing immunological studies both in our institute and in other institutions. Pathogenic strains of the most harmful species are used to create an artificial infectious background in assessing the resistance of varieties. The composition of infectious backgrounds for different regions of Russia is determined, taking into account the area of distribution and the frequency of *Septoria/Stagonospora* species. For testing using strains of the fungus species dominant in this region is recommended. For example, for the Central region, it is necessary to use strains of both *S. nodorum* and *S. tritici*, since these two species are distributed here almost identically. In the Central-Chernozem and North Caucasian regions, it is recommended to use strains of *S. tritici*, and in the North - *S. nodorum* strains, since these species are dominant here. On the other hand, in the Volga, North-Western, Volga-Vyatka and West Siberian regions, in addition to isolates of the predominant fungus species, it is also expedient to use strains of the second most frequent occurrence of a species that has a significant share in the species structure of the population (table 5).

**Table 5. Septoria/Stagonospora species, recommended for various regions of the Russian Federation to create artificial infectious background.**

| Region of RF | The frequency, % | Septoria/Stagonospora species recommended to create artificial infectious background |
|--------------|------------------|-----------------------------------------------------------------------------------|
|              | *S. nodorum*     | *S. tritici*                       | *S. avenae triticea*                      |
| Central      | 40.6             | 42.4                               | 17.0                                     |
| Central-Chernozem | 14.3          | 74.7                               | 17.0                                     |
| North-Caucasian | 17.9            | 65.9                               | 16.2                                     |
| Volga        | 28.3             | 52.5                               | 19.2                                     |
| North-West   | 64.4             | 28.7                               | 6.9                                      |
| North        | 81.3             | 7.1                                | 11.6                                     |
| Vyatka       | 59.7             | 24.1                               | 16.2                                     |
| West Siberian | 61.8             | 24.0                               | 14.2                                     |

When testing varieties for resistance to septoriosis in the field, the selected strains must meet the following requirements:

- according to their geographic origin, they must correspond to the region in which the tests are carried out;
- to have high reproductive capacity on nutrient medium;
- to have a sufficiently high level of pathogenicity (II or III group);
- to have a wide range of virulence (for *S. tritici*).

5-7 (but not less than 3) strains from different morphological groups reflecting the intraspecies diversity of the local fungus population are used to reliably estimate the resistance of the breeding material.

In addition to immunological studies, pathogenic strains of the most harmful Septoria pathogens are used in the development of chemical methods for plant protection and evaluation of the effectiveness of fungicides. The selection of strains for the creation of an artificial infectious background in such tests is carried out on the same principle as described above.
In addition to applied use, the existing collection of cultures of the causative agents of Septoria can serve as a source of genetically diverse pathotypes in fundamental scientific molecular-genetic studies for solving problems of population genetics, taxonomy, identification, etc. For these purposes, it is necessary to select strains of phytopathogens, different both in morphological features, and in the degree of pathogenicity, race belonging and virulence genotype.

4. Conclusion
Phytopathogenic microorganisms studied by phytopathological and genetic methods and stored in the State Collection of Phytopathogenic Microorganisms can be used in various areas: immunological, phytopathological, molecular-genetic studies, registration tests, etc. Only a scientifically grounded approach to the selection of stable and examined strains for the planned studies can lead to the desired and qualitative results.

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