Breeding of perennial forage crops for disease resistance

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Abstract. The creation of the initial breeding material of forage crops, promising in terms of disease resistance, is a difficult task that requires the solution of many related issues. Such studies should be carried out on artificial field infectious backgrounds. In FSC "VIK named after V.R. Williams" such backgrounds were created on the basis of data of long-term phytosanitary monitoring to identify the main diseases of forage crops. Techniques for creating field backgrounds and methods for laboratory assessment of resistance have been developed, which make it possible to reduce the number of samples evaluated against backgrounds, due to the rejection of susceptible to the disease.

When evaluating 20 collection samples of meadow clover and awnless rump, 10 samples with increased disease resistance were identified. As a result of further breeding study of this material by methods of selection, self-pollination, cloning, promising biotypes in terms of disease resistance were obtained. On the basis of these biotypes, polycross populations of meadow clover and awnless rump were created. An assessment of the stability of these populations against artificial infectious backgrounds was carried out. In terms of resistance to fusarium, the polycross population of meadow clover exceeded the standard variety VIC 77 by 24%. The population of awnless rump exceeded the standard Fakelny cultivar by 21% on average over 2 years. A breeding program has been developed to create the initial material for meadow clover and awnless rump with increased disease resistance.

Keywords: perennial grasses, diseases, infectious backgrounds, breeding programs, sources of resistance.

Domestic and world experience shows that the variety is the main biological means of increasing yields. The successful solution of the fundamental problems of breeding to create fundamentally new, resistant to environmental stress, high-yielding varieties of forage crops that meet the objectives of sustainable development of modern animal husbandry and ecological agriculture, can be realized in the presence of an appropriate diversity of genetic resources and an identified gene pool [1].

Breeding varieties of intensive type with high potential productivity is the main direction of breeding for perennial forage crops. However, experience shows that the high stability of the yields obtained largely depends on the resistance of varieties to diseases.
The creation of resistant varieties is one of the difficult areas of breeding. The high reproduction rate and, as a consequence, the wide potential of pathogen variability provide them with significant adaptive capabilities. A complex issue in the study of immunity genetics is the genetics of the relationship between the host plant and the pathogen. Plants with the most favorable combination of resistance genes are rare and difficult to identify. Moreover, resistance often negatively correlates with economically valuable traits of plants [2].

In this regard, the search and creation of breeding material promising for disease resistance is of paramount importance. NI Vavilov noted that the creation of the source material is as relevant as the selection methods themselves [3].

The most important tool in the study and creation of promising breeding material is a field artificial infectious background with an optimal infectious load. Artificial infectious backgrounds for assessing the resistance of perennial forage crops are created for those diseases, the causative agents of which persist in the soil and on plant debris (clover cancer, fusarium, helminthosporium of cereal grasses).

In FSC "V.R. Williams VIK" field artificial infectious backgrounds have been used to assess the resistance of forage crops to diseases for more than 20 years. A technique for creating artificial backgrounds and methods for assessing the disease resistance of meadow clover, awnless rump, perennial ryegrass, etc. have been developed [4].

On the basis of many years of research on the creation of an initial breeding material with increased resistance to diseases, when an assessment on infectious backgrounds was combined with a breeding study of a promising source material, a breeding scheme for varieties with increased disease resistance was developed [5].

A promising direction in breeding for immunity is the creation of disease-tolerant varieties. The creation of a promising breeding material based on the selection of hardy and resistant biotypes has a number of advantages. The main one is that the natural process of stabilizing selection in the "pathogen – plant – host" system and the ecological niche for the existence of mildly aggressive races of the parasite are preserved.

The method of creating varieties-populations with high plasticity and stable yield is promising for obtaining varieties of meadow clover tolerant to Fusarium and cereal grasses to helminthosporium.

Root rot (fusarium) is one of the most harmful diseases of meadow clover. The disease is caused by more than 10 species of fungi of the genus Fusarium Lk: Fr. The ratio of species in the pathogen population depends on the age of the host plant, climatic factors, soil type, agricultural technology, etc. Pathogens infect seedlings and adult plants, causing root and stem rot and wilting. The death of plants in some years reaches 30% or more.

Wild plants and varieties created in different climatic zones are a promising source for obtaining starting material with increased resistance to fusarium.

In previous years (2011-2013), on an artificial fusarium field background, the resistance of 25 wild and varietal accessions of meadow clover from the collection of the gene pool department of the Federal Research Center "V.R. Williams VIK" was assessed. Were identified 4 varieties (Orpheus, Atlant, Carmine and Saba) with increased resistance to fusarium (table 1).

| Varieties | The prevalence of the disease, % |
|-----------|---------------------------------|
|           | 2 year of life | 3 year of life | 2 year of life | 3 year of life |
| Orpheus   | 48             | 40             | 40             |
| Atlant    | 40             | 42             | 42             |
| Niva      | 42             | 48             | 48             |
| Carmine   | 49             | 53             | 53             |
| VIC 77st  | 62             | 68             | 68             |
| HCP0.5    | 14             | 14             | 14             |
In 2016, against an infectious background in isolation, an experiment was laid to create a polycross population of meadow clover with increased resistance to Fusarium. When forming a polycross population, the varieties Orfey, Atlant, Niva, Karmin and Saba were used. The latter was included in the program, as it stood out against the cancerous background for its resistance to clover cancer.

The surveys carried out in 2017-2018 confirmed their increased resistance to the disease (Table 2).

### Table 2. Evaluation of resistance to fusarium disease of samples in the polycross nursery (sowing 2016, counts 2017-2018)

| Varieties | The prevalence of the disease, % |
|-----------|---------------------------------|
|           | 2 year of life                  | 3 year of life                  |
| Orpheus   | 36                              | 40                              |
| Atlant    | 30                              | 39                              |
| Niva      | 30                              | 38                              |
| Saba      | 50                              | 56                              |
| Carmine   | 40                              | 43                              |
| VIC 77 st | 69                              | 72                              |
| HCP 0.5   | 10                              | 12                              |

In the spring of 2018, the final assessment of the resistance of the samples to root rot and the culling of diseased and weakened plants was carried out.

From the seeds collected in the fall, a promising polycross population of meadow clover, promising in terms of resistance, was subsequently formed. The final assessment of the resistance to fusarium of the created population against a field infectious background (sowing 2019) showed that in the fall of 2 years of plant life, the prevalence of the disease was 24% lower than that of the VIK 77 standard variety.

Awnless rump is a valuable forage crop characterized by longevity and stable yield. This perennial cereal grass is characterized by high winter hardiness and frost resistance, rather drought-resistant, but at the same time very responsive to moisture.

In our country, more than 40 varieties have been created that are suitable for use for hay, for grazing, for growing in a pure form and a mixture with legumes. The selection of awnless rump is carried out on the basis of selection of biotypes from wild-growing populations, varieties of domestic and foreign selection. Methods of chemical mutagenesis are widely used [6].

More than 30 pathogens have been registered on the boneless rump in our country [7]. A widespread and harmful disease is helminthosporiosis or dark brown spotting. The causative agent of the disease is the mushroom Helminthosporium bromi Died. = Drechslera bromi Ito with the marsupial stage Pyrenophora bromi Drechs.

According to our long-term data, with the development of the disease over 50%, mass drying and leaf fall begins, which leads to a deterioration in the quality of feed, a decrease in the yield of green mass and seeds. In some years, the seed yield may decrease by 20%, and the seeds obtained from diseased plants will be puny and have a reduced germination capacity.

Fungicides recommended for use on seed crops of cereal grasses on the territory of the Russian Federation have not been registered. Selection work on the creation of rump varieties with increased disease resistance remains an urgent and priority direction in the fight against spotting. To solve this problem, not only new genetic sources are required, but also a wide study of the evolutionary potential of a phytopathogen to predict the duration of action of resistance genes [8].

The introduction of new high-yielding and disease-resistant varieties of awnless rump is one of the main reserves for increasing the productivity of hayfields and pastures by at least 25-30% [9].

Against the artificial infectious background of the FSC "VIK named after V.R. Williams", active work is underway to create an initial breeding material that is slightly affected by dark brown spotting.
The main object of the work is the collection of perennial cereal grasses of the Federal Research Center "VIK named after V.R. Williams", consisting of wild samples of rump and zoned varieties collected in various regions of Russia.

With the help of the benzimidazole method, developed in the laboratory of immunity, a primary assessment of the infestation of the starting material was carried out [10]. In 2013, out of 15 analyzed samples, 5 forms were isolated, the incidence of which did not exceed 2 points. The selected material was sown on a field artificial helminthosporium background. Based on the results of assessments of the prevalence of dark brown spot, carried out according to the phenophases of the development of rump plants in 2014, 10 biotypes were selected. Plants were selected in which only small spots of the pathogen of helminthosporiosis developed on the flag leaf at the end of the flowering phase. Seeds were obtained from the isolated plants after self-pollination of flowers in isolation conditions, which in 2015 were sown in a wide-row method against a field infectious background. In 2016, as a result of individual selection on the basis of disease resistance, 3 weakly affected plants were identified (damage score - 3). In autumn, an isolated polycross nursery was formed from these plants by cloning to carry out limited directional cross-pollination. The seeds received the next year were sown against an infectious background in 2018 to conduct a comparative assessment of the resistance to helminthosporiosis of the new U-1 sample and the standard Fakelny variety (Table 3).

Table 3. Evaluation of the resistance of a promising sample U-1 to the causative agent of helminthosporiosis (sowing 2018)

| Sample name | Disease prevalence, %, seed ripening phase | Average |
|-------------|------------------------------------------|---------|
|             | 2 year of life | 3 year of life |       |
| Torch st    | 75 | 95 | 85 |
| U-1         | 51 | 77 | 64 |
| HCP_{0.5}   | 23 | 16 |     |

Thus, as a result of research work (2013-2020) in laboratory conditions and against a field infectious background, sample U-1 was created, the resistance of which to helminthosporiosis, on average for two years, exceeded the standard Fakelny variety by 21%. The obtained sample should be included in the breeding process to create highly productive varieties of awnless rump with increased disease resistance.

On the basis of the conducted studies, a breeding program was developed to create the initial material of meadow clover and awnless rump with increased resistance to fusarium and helminthosporium (Figure 1). A distinctive feature of the program is that all its stages take place in conditions of artificial field infectious backgrounds.
Figure 1. Breeding program for the creation of the initial material of meadow clover and awnless rump with increased resistance to fusarium and helminthosporium.

This program was based on the following stages:
- search for samples characterized by increased resistance to diseases when evaluated by laboratory methods;
- assessment of the stability of promising samples against backgrounds and selection of the best biotypes;
- selection study of the selected biotypes and the formation of a polycross population;
- assessment of the stability of the created polycross populations;
- reproduction in isolation;
- creation of polycross populations of clover and awnless rump with increased resistance to fusarium and helminthosporiosis.

Conclusion
10 samples of meadow clover and awnless rump with increased resistance to fusarium and helminthosporium were isolated from the collection samples of the gene pool department and the laboratory for breeding cereal grasses of the Federal Research Center "V.R. After the selection study of this material (selection, self-pollination, cloning, polycross), polycross populations with increased disease resistance were obtained from the best resistance biotypes.

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