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PLANT GROWING, PLANT PROTECTION AND BIOTECHNOLOGY

The Immunological Evaluation of a New Promising Variety of Vegetable Beans (*Vicia faba* L.) Russkaya Belaya for Resistance to the Most Harmful Phytopathogens

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Abstract—The purpose of the research was to evaluate the promising Russkaya Belaya variety that meets the requirements of the modern market for resistance to economically significant diseases. The research was carried out in the conditions of Moscow oblast in 2017–2020 on the basis of the Federal Scientific Vegetable Center (FSBSI FSVC). Agrometeorological conditions during the period of operation were optimal for the development of harmful phytopathogens, which made it possible to objectively assess a promising variety for disease resistance. The pathogenic complex structure analysis of vegetable beans, represented in all years by the dominant pathogens of chocolate spotting (*Botrytis fabae*), ascochytosis (*Ascochyta fabae*), and fusarium wilt (*Fusarium solani*, *F. oxysporum v.oxysporum*, *F.sambucinum*, *F. sporotrichiella*), was carried out. In some years, epiphytotics of bean rust (*Uromyces viciae-fabae*) and stemphyliosis (*Stemphylium* spp.) have been recorded. Due to climate change, there has been an increase in the harmfulness of viral pathogens, in particular the Bean yellow mosaic virus (BYMV) and Cucumber mosaic virus (CMV). The immunological assessment conducted against the background of a tense infectious background in various years showed that the Russkaya Belaya variety has a high resistance to diseases of fungal and viral etiology relative to other zoned and widely grown varieties of vegetable beans. During the epiphytotic years, the degree of lesion with ascochytosis, chocolate spotting, fusarium, and yellow mosaic bean virus remained low and did not exceed 14%. Along with a number of valuable features, the promising Russkaya Belaya variety can be recommended for cultivation in regions with unfavorable epiphytotic conditions.

Keywords: vegetable beans (*Vicia faba* L.), resistance, phytopathogens, epiphytotics, mycoses, viruses

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INTRODUCTION

In recent years, research aimed at solving the problem of providing the population with food protein of plant origin has become of great importance. In this regard, it is economically justified to grow beans (*Vicia faba* L.), which are superior to other vegetables in terms of nutritional qualities, and their cultivation is the least laborious. Beans are the fourth most important leguminous crop in the world after peas, soybeans, and other beans [1, 2]. Today, on an area of 2.5 million hectares, the world production of this important food crop has reached 5.4 million t, including more than 33% in China, 29% in Europe, 27% in North Africa, and approximately 6% in Australia [3].

Despite the fact that beans play an important role in food security programs in many countries of the world, this crop remains narrowly cultivated in Russia. The main centers of its cultivation are concentrated in central Russia and more northern regions on small farms and private households [4], while there is hardly any industrial production of beans in the country. In the State Register of Breeding Achievements, approved for use in 2021, there are only 20 varieties of vegetable beans, of which four varieties are bred by the Federal Scientific Vegetable Center [5].

One of the factors limiting the cultivation of beans is their damage by various phytopathogens. To date, among the pathogenic microorganisms of fungal etiology affecting *Vicia faba* L. are chocolate spotting (*Botrytis fabae*), ascochytosis (*Ascochyta fabae*), bean rust (*Uromyces viciae-fabae*), stemphylliosis (*Stemphylium* spp.), and fusarium wilt (*Fusarium* spp.) [6, 7]. According to the literature, the symptoms, development, and prevalence of chocolate spotting vary greatly depending on the region. Therefore, resistance to the disease is often due to the interaction of the factors "genotype–pathogen–environment," which leads to variation in the phenotypic response of the same varieties in response to *Botrytis fabae* infection: the manifestation of resistance in the conditions of one region and its absolute loss in the conditions of another [8, 9]. According to Sillero et al. [10], it can be

explained by the very narrow virulence of local pathogen populations in different regions, corresponding to specific resistance genes in plant material. According to the results of studies by Terefe et al. [11], temperature change had a significant effect even on the biological characteristics of the fungus species *Botrytis fabae*, leading to a strong decrease in the size of conidia. Resistance to *Botrytis fabae* is polygenic, and all modern varieties and hybrids of beans have incomplete resistance.

To date, there are several methods of artificial infection of bean plants in the breeding program for resistance—artificial infection of seedlings, the method of separated leaves, etc. [12, 13]. However, according to Beyene et al. [14], the screening of breeding material for resistance to chocolate spotting and the creation of varieties based on it must necessarily take place under the conditions of field trials in the region where production crops are planned.

Since the 1980s, the development of ascochitosis was of an epiphytotic nature in many countries. Since then, special attention has been paid to the search for genetic sources of resistance to *Ascochyta fabae Speg.* (*Didymella fabae Jellis & Punithalingam* teleomorph) and their use in breeding programs to create resistant varieties of beans in England, Spain, Syria, and other countries [15, 16].

In the pathogenic complex causing fusarium wilt on vegetable beans, *F. solani*, *F. oxysporum v.oxysporum*, *F. sambucinum*, and *F. sporotrichiella* species are more common. On all legumes, the virulence of previously weakly pathogenic species *F. oxysporum v.artroceras*, *F. semitectum*, and *F. javanicum* increases [17].

The most harmful viral pathogens causing economic damage to the bean culture are Bean yellow mosaic virus, Broad bean mottle virus, Broad bean necrosis virus, Cucumber mosaic virus [18, 19]. Viruses of the true mosaic of Broad bean true mosaic virus and Broad bean wild virus are classified as endemic, the appearance of which is associated with certain conditions of individual countries and regions [20, 21].

In the conditions of the Moscow oblast in recent years, there has been an increasing spread of the Bean yellow mosaic potyvirus (BYMV, VZhMF) and Cucumber mosaic virus (CMV, VOM) [18]. Like most members of the genus *Rotyvirus*, BYMV has a very wide range of plant hosts from different families. All strains of the virus are vectored by more than twenty aphid species in a nonpersistent manner. Horizontal propagation is also possible mechanically, while vertically by seeds. The spread of CMV, a member of the *Cucumovirus* family, is carried out in a nonpersistent way by a large number of aphids. The virus is not transmitted by seeds.

In the current ecological and economic situation, in order to ensure the high quality of domestic products, it is necessary to strengthen research on the creation of highly productive varieties of vegetable beans with high adaptive potential and resistance to economically significant phytopathogens [22]. The variety model in the modern breeding process provides for the presence of such traits as early maturity, determinant type of stem growth, standing power, noncracking of pods, their simultaneous maturation, high attachment of lower pods, high seed productivity and seed quality, suggesting a balanced amino acid composition and high protein content (up to 34–37%), absence or minimum content of antinutritional substances (vicin, convicin, and tannin), and resistance to the most harmful phytopathogens [23].

Therefore, it is necessary to study and use the potential of intraspecific hybridization, which expands the possibilities of combinational variability. To solve this problem, varietal material of vegetable beans from the world collection of Vavilov Institute of Plant Industry and Federal Scientific Center of Vegetable-Growing was studied. As a result, varieties-components of crosses were selected, which are characterized by important selection-valuable traits, and stepped hybridization was carried out.

The appearance of traits representing the greatest value was regulated by the order of involvement of parental breeds in crossings. In the course of studying the F1–F3 generations of various hybrid combinations in terms of quantitative and qualitative traits by repeated individual selection, a new source material with a complex of economically valuable traits was isolated and a new mid-season productive variety of Russian white vegetable beans with bright seeds and a bright seed scar was created [22]. The purpose of the research is to evaluate a new productive variety of Russkaya Belaya beans for resistance to major diseases.

MATERIALS AND METHODS

A promising variety of vegetable beans Russkaya Belaya was created as a result of stepped crossing involving geographically distant and morphologically different forms. It passed the test in 2017–2019. In 2020, the Federal State Budgetary Institution “State Commission of the Russian Federation for the Testing and Protection of Breeding Achievements” entered this variety in the State Register of Breeding Achievements Approved for Use. The Russkaya Belaya variety of vegetable beans belongs to the midseason group.

The technical stage of ripeness in the conditions of the Moscow oblast comes on 52–56 days and biological on 85–98 days from mass seedlings. The flowers have white petals. Plant height, depending on the conditions of the year, varies from 65 to 90 cm, averaging 80 cm, which is almost two times higher than that of the standard: the zoned variety Belorusskiye. The linear dimensions of the fruit of the new variety also exceed those of the standard. Straight bean has the

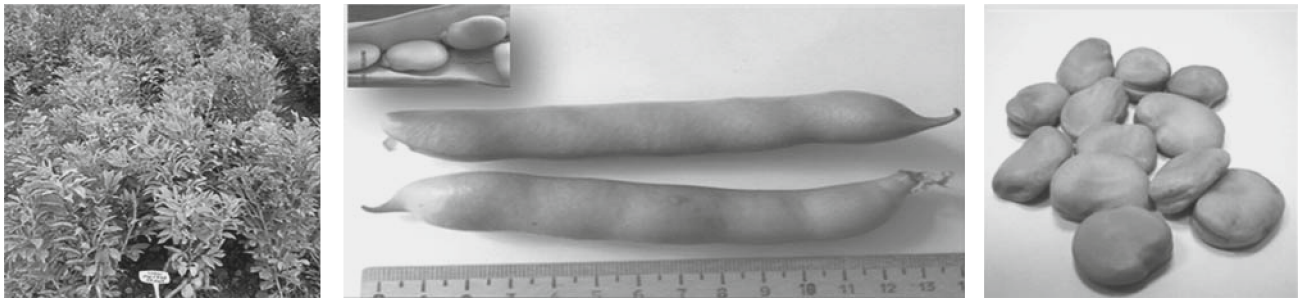

Fig. 1. Vegetable beans, Russkaya Belaya variety.

length varying from 11 to 14 cm and width approximately 2 cm. The surface of the bean is smooth, the color in the technical stage of ripeness is green, and the number of seeds in the bean from 3 to 5 pcs. At biological maturity, the seeds are light brown, ovoid, with a bright scar (Fig. 1).

Plants of the Russkaya Belaya variety are superior to the Belorusskiye variety in all elements of productivity. The average number of beans per plant is approximately 10 pcs.

The mass of beans per plant is higher than that of the standard by 30%, while seeds by 56%. The average number of beans per plant is about 10 pieces. On average, the crop yield at the stage of technical ripeness is 16.8 t/ha, which is 6.0 t/ha more than the standard. The weight of 1000 seeds varies from 1950 to 2060 g, depending on growing conditions.

One of the important requirements for the created varieties is resistance to the most harmful diseases. In view of this, conjugate selection for stability was carried out simultaneously with the selection for the main economically valuable traits. Under conditions of a natural infectious background, the Russkaya Belaya variety was assessed and resistant genotypes were selected for the dominant diseases of fungal etiology: ascochytosis, chocolate spotting, and fusarium wilt, the development of which often has an epiphytotic character. The symptoms of damage to vegetable beans by rust and black spot are noted significantly less often, although the development of rust also reached the scale of an epiphytoty in some years.

Phytosanitary monitoring of the development of fungal and viral infections on crops of the Russkaya Belaya bean variety in the Moscow oblast was carried out in 2017–2020 on the basis of the Federal Scientific Center of Vegetable-Growing. The work on the identification of the isolated virus and mycomycete isolates was carried out on the basis of the Laboratory of Immunity and Plant Protection of the Federal Scientific Center of Vegetable-Growing.

As a standard, the zoned variety Belorusskiye was used as the most common as well as the most similar to the tested variety in terms of the main morphometric parameters (large seeds, seed color).

Plants of vegetable beans (*Vicia faba* L.) of the Russkaya Belaya variety were grown in the field under conditions of natural infectious background in the nursery of competitive variety testing of the laboratory of selection and seed production of legumes in four repetitions [24].

Damage by various phytopathogens was assessed by visual diagnostics of characteristic symptoms on plant leaves according to the following scale: 0—no symptoms; 0.5—weak manifestation of symptoms on single leaves; 1—less than 10% affected; 2—affected 10–30%; 3—affected 30–50%; 4—more than 50% of the entire leaf surface of the plant is affected. The resistance of each sample was evaluated by generally accepted indicators: prevalence (P, %), lesion index (I, score), and degree of disease development (R, %) [25]. The counts were carried out three times during the growing season in the phases of the third pair of true leaves, flowering and technical ripeness of the beans. Based on the totality of all assessments, the variety was assigned to one or another group of resistance.

Analysis of the species composition of micromycetes was performed on the basis of microscopy of affected plants [26, 27], the study of morphological and cultural traits isolated in a pure culture of fungal isolates, and the identification of pathogens using the corresponding determinants [28, 29].

The presence of bean yellow mosaic virus antigens in the leaves of vegetable bean plants was determined using electron micrography methods at the Center for Collective Use “Far Eastern Center for Electron Microscopy” of the National Research Center for Marine Biology by sandwich enzyme-linked immunosorbent assay (ELISA) with Agdia reagents. Evaluation of the results of ELISA was performed using a spectrophotometer at a wavelength of 480 nm by extinction coefficients.

To identify the cucumber mosaic virus, an immunological express method for diagnosing diseases using Agdia immunostrips was used.

The laying of field experiments, phenological observations, crop records, and description of morphological features were carried out according to [30].

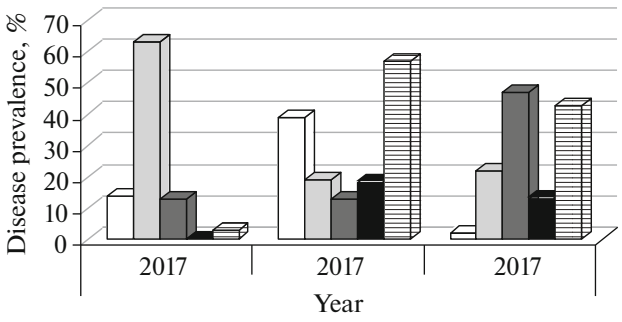

Fig. 2. Prevalence of major diseases in culture of vegetable beans in different years of research: □—ascochitosis, ▨—chocolate spotting, ■—fusarium, ■—alternariosis, and ⊟—viruses.

Mathematical and statistical data processing was carried out according to generally accepted methods [24] using the MS Excel application package.

RESULTS AND DISCUSSION

Due to climate change, there has been an increase in the last decade in the harmfulness of viroses, which were classified in the midland as rare diseases of vegetable bean culture in the last century. Nevertheless, the general structure of the pathocomplex of the natural infectious background in Moscow oblast as a whole remains constant and only the "leader" among pathogens changes every year, which can be seen in the example of 3 years of competitive testing of the Russkaya Belaya variety (Fig. 2). Thus, chocolate spotting epiphytoty was noted in 2017, ascochytosis and viral diseases were most widespread in 2018, along with viroses, a significant development of fusarium was observed in 2019, which manifested itself in the form of tracheomycosis up to the complete death of plants as well as in the form of damage to the leaf apparatus by marginal necrosis and chlorosis until the leaf blades completely dry out and fall off.

A survey of vegetable beans in the KSI nursery revealed a significantly higher disease resistance of the new Russkaya Belaya variety compared to the most widely recognized domestic varieties Belorusskiye and Russkiya chernaya. The degree of development of chocolate spotting in the year of epiphytosis (2017) in Russkaya Belaya variety was 3–5 times lower than in the released varieties, while ascochytosis and black spotting in 2018 were 3–12 and 11–13 times lower, respectively (Table 1). With regard to Fusarium, a new variety showed resistance at the level of the Belorussky variety due to a lower average damage index (0.5 points) of plants, with a higher distribution. It should be noted that the new promising variety was significantly superior during the entire period of research to the Russkiya chernaya variety, which has been noted to have a high degree of plant damage in recent years by diseases of various etiologies in the conditions of Moscow oblast. In 2018, by the end of the growing season, symptoms of rust damage were noted on the plants of a new promising variety, which were absent on the Belorusskiye and Russkiya chernaya varieties.

Meteorological conditions and mass bioflows of insect vectors significantly influenced the development of viral diseases, which made it possible to assess the resistance of the variety to the two most dangerous pathogens of viroses for the culture (Table 2). In 2018, the cucumber mosaic virus represented the greatest danger, the prevalence of which on Russkiya chernaya variety exceeded 90% with a lesion index of 3–4 points; the variety poorly set beans and seeds. The promising variety Russkaya Belaya showed tolerance to this pathogen. In its variety population, only half of the plants showed signs of VOM leaf damage at the level of 1–2 points, in contrast to the Belorusskiya variety with a wide range of variation in the plant damage index (1–4 points). As a result, the degree of VOM development in the new variety was two times lower than in the standard.

The conditions of 2019 contributed to a wider spread of the Bean yellow mosaic virus on bean crops, to which the Belorusskiya variety turned out to be more susceptible, on which the highest proportion of affected plants and the highest average infection score were noted. The promising variety Russkaya Belaya showed high resistance to this pathogen: symptoms of damage were noted in only 3% of plants, which is

Table 1. Mycosis infestation of vegetable bean varieties in the phase of technical ripeness of beans

Variety	Disease progression rate (in year of highest prevalence)											
	chocolate spotting (2017)			ascochytosis (2018)			fusarium (2019)			black spot (2018)		
	P, %*	*I*, score	*R*, %	*P*, %	*I*, score	*R*, %	*P*, %	*I*, score	*R*, %	*P*, %	*I*, score	*R*, %
Belorusskaya (standard)	65	2.3	37.4	26	1.5	11.4	5	1.5	1.8	35	2.9	25.5
Russkaya Chernaya	92	3.2	73.6	70	2.5	43.8	20	2.5	12.5	30	3.0	22.5
Russkaya Belaya	41	1.3	14.1	12	0.9	3.4	12	0.5	1.5	4	2.0	2.0
HCP05	18	0.9	14.1	13	0.3	8.9	8	0.5	4.7	17	0.5	13.2

**P*—prevalence; *I*—lesion index; *R*—the degree of disease development.

Table 2. Infection by viroses of vegetable bean varieties in the phase of technical ripeness of beans

Variety	Disease progression rate (in year of highest prevalence)					
	CMV (2018)			BYMV (2019)		
	P, %*	*I*, score	*R*, %	*P*, %	*I*, score	*R*, %
Belorusskaya (standard)	62	2.7	42.9	44	2.2	22.2
Russkaya Chernaya	91	3.5	78.8	32	1.5	12.0
Russkaya Belaya	50	1.6	20.0	3	2.0	1.5
HCP05	22	0.8	24.6	15	0.5	10.4

**P*—prevalence; *I*—lesion index; *R*—the degree of disease development.

more than ten times lower compared to the other two varieties.

It should also be noted that at a sufficiently high intensity of the natural infectious background in the studied cultivar populations (with the exception of the Russkiya chernaya cultivar), visually "healthy" asymptomatic plants were present. Compared to the standard, their share in the crops of the new variety was more than ten times higher and averaged from 8 to 15%, depending on the year. This indicates that the Russkaya Belaya variety is of interest for further breeding work on resistance as a source of genes for high group resistance to the main harmful diseases of vegetable beans in the conditions of the Nonchernozem Zone of the Russian Federation.

Thus, the new Russkaya Belaya bean variety surpasses the old varieties in terms of group resistance to diseases. Its distribution in production will expand the range of vegetable legumes grown in our country.

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