ИММУНИТЕТ РАСТЕНИЙ К БОЛЕЗНЯМ
Оригинальное исследование / Original article
УДК 633.521:631.524.86

Вавиловский журнал генетики и селекции. 2019;23(6):650-655
DOI 10.18699/VJ19.537

Localization of rust resistance genes in old local Russian flaxes by methods of classical genetics

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Flax rust, a disease that destroyed a significant portion of the yield before the creation of resistant varieties, is currently defeated, but it can cause new outbreaks as identical resistance genes are used in breeding. Since only one of the allelic genes can be introduced into a variety, the aim of this work is to identify genes for resistance to the disease in lines selected during the evaluation of old Russian flaxes from the VIR collection. The original accessions were added to the collection in 1922, that is, before the release of breeding varieties, so their genes are of natural origin. The analysis was performed on an artificial infectious background by methods of classical genetics, including the test for allelism. Nine monogenic lines with the original *R* genes were crossed to tester varieties for six loci: *K*, *L*, *M*, *N*, *P*, and *Q*. F_2 hybrids in the phase of cotyledon leaves were inoculated with monopustule clones of the fungus, not virulent to any of evaluated genes. Gene allelism was checked by the absence of the segregation. It was exactly proven that *R* genes of the k-716 line from the Pskov kryazh (gc-32) and the k-780 accession from the Minsk oblast (gc-33) were located in the *P* locus, the gene of the k-846 line from the Ivanovo-Voznesensk oblast (gc-39) was in the *M* locus, and the gene of the k-834 line from the Vladimir oblast (gc-38) probably belonged to the *K* locus. The segregation in the crosses of all testers to the k-630 line from the Simbirsk oblast (gc-25) showed that its gene was not allelic to any of the known loci. Probably, there was a formerly unknown locus. The location of the other genes failed to be identified due to the linkage between loci *N* and *P* and the presence of several resistance genes in some lines. The gene in gc-9 was in either *M* or *K* locus; and the genes of gc-34, gc-40, and gc-46 were located in *P* or *K*. Since all the evaluated genes were original, the genes of these lines were different alleles of the identified loci.
Key words: flax rust; resistance genes; localization of genes; linkage; allelism.

For citation: Kutuzova S.N., Porokhovinova E.A., Brutch N.B., Pavlov A.V. Localization of rust resistance genes in old local Russian flaxes by methods of classical genetics. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2019;23(6):650-655. DOI 10.18699/VJ19.537

Локализация генов устойчивости к ржавчине у староместных российских льнов методами классической генетики

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Ржавчина льна – болезнь, которая уносила значительную часть урожая до создания устойчивых сортов, в настоящее время побеждена, однако при использовании в селекции идентичных генов устойчивости могут возникать новые эпифитотии. Поскольку в сорт может быть введен только один из аллельных генов, целью настоящей работы была попытка идентификации генов устойчивости к болезни у линий, выделенных при оценке коллекции ВИР, из староместных российских льнов. Исходные образцы поступили в коллекцию в 1922 г., т.е. до начала распространения селекционных сортов, поэтому их гены имеют естественное происхождение. Анализ проводили на искусственном инфекционном фоне методами классической генетики, используя тест на аллелизм. Девять моногенных линий, обладающих оригинальными *R*-генами, были скрещены с сортами-тестерами шести локусов *K*, *L*, *M*, *N*, *P* и *Q*. Гибриды F_2 в фазу семядольных листьев были инокулированы монопустульным клоном гриба, авирулентным ко всем изучавшимся генам. Об аллельности генов судили по отсутствию расщепления. Точно определено, что *R*-гены у линий из Псковского кряжа к-716 (гк-32) и образца из Минской области к-780 (гк-33) расположены в локусе *P*, ген линии из Иваново-Вознесенской области к-846 (гк-39) – в локусе *M*, а ген линии из Владимирской области к-834 (гк-38), вероятно, относится к локусу *K*. При скрещивании линии из Симбирской области к-630 (гк-25) со всеми тестерами локусов получено расщепление, означающее, что этот ген не аллелен ни одному из известных локусов. Вероятно, существует еще один неизвестный локус. Расположение других генов точно установить не уда-

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лось из-за сцепления между локусами *N* и *P*, а также присутствия в некоторых линиях по нескольку генов устойчивости. Ген устойчивости гк-9 расположен либо в локусе *M*, либо в *K*, гены гк-34, гк-40 и гк-46 – в *P* или *K*. Поскольку все изучаемые гены оригинальны, гены этих линий являются разными аллелями установленных локусов.
Ключевые слова: ржавчина льна; гены устойчивости; локализация генов; сцепление; аллельность.

Introduction

Flax rust is a serious disease that destroyed a significant part of the crop in Soviet Union in the last century. Its most destructive epiphytotics occurred on the American continent (Flor, 1964) and gave an impetus to the intensification of its genetic investigation, search for immune forms, and breeding of resistant varieties (Flor, 1946, 1956). By now, it has been discovered that the resistance of cultivated flax *Linum usitatissimum* L. to rust *Melampsora lini* (Pers.) Lev. is controlled by 32 genes located in six loci (*K*, *L*, *M*, *N*, *P*, and *Q*) consisting of closely linked or allelic genes (Islam, Mayo, 1990). Genes of locus *L* in variety Ottawa 770B and *M* in variety Dakota were discovered by W.M. Myers in 1937; genes *N* in Bombay; *P* in Koto, and *K* in variety Klay were found by H.H. Flor (Flor, 1947, 1955). Gene *Q* was discovered by us (Kutuzova, Kulikova, 1989) in Russia, and identified in variety Natasja (Kutuzova, 1994).

Flor found out that resistance genes are characterized by multiple allelism (Flor, 1941, 1947, 1954, 1955; Flor, Comstock, 1972). Among the varieties bred in America, 11 alleles of the *L* gene (locus *L*) have been found: *L1–L8*, *L10*, and *L11*. Allele *L9* was identified in Australia, in the generally susceptible American variety Bison (Kerr, 1960).

Six alleles were found in locus *M*. Flor (1947, 1954) identified five alleles of gene *M* in North America: *M1* through *M5*. Allele *M6* was found in Argentina (Zimmer, Comstock, 1973).

Two alleles were found in the *N* locus: *N1* and *N2* (Flor, 1947, 1955).

Six alleles are known for locus *P*: genes *P1–P3* were identified by H.H. Flor (1955); gene *P4*, by D.E. Zimmer et al. (Zimmer, Compstock, 1973) in North America; genes *P5* and *P6*, by G.B. Kerr (1960) in Australia.

Two alleles are known in locus *K*: gene *K* was identified by H.H. Flor (1955) in North America and allele *K1* was found in Canada (Hoes, Kenaschuk, 1986).

The *Q* gene, which is not identical to genes *L*, *M*, *N*, *P*, or *K*, was mapped to the new *Q* locus. It is effective against all races of the Russian fungus population (Kutuzova, 2014), as well as against the tested races of Australia and North America (Islam, Kutuzova, 1990).

As early as the middle of the 20th century, it was found that all genes in locus *L* were linked rather than allelic (Flor, 1947). In addition, it was shown that loci *K*, *L*, *M* are inherited independently and loci *N* and *P* are linked (Flor, 1962, 1965). Kerr (1960) found that the distance between loci *N* and *P* was 9.5 cM. Flor (1962) determined the distance between genes *N* and *P3* to be 15 cM. However, later it was shown that gene *K1* was located on the same chromosome as the *N* and *P* genes, and the recombination rate was 25 % (Hoes, Kenaschuk, 1986).

Evaluation of the resistance of varieties carrying these genes in different countries showed that some of them had additional genes. In particular, the *P5* gene was found in variety Ottawa 770B in Australia (Kerr, 1960) and India (Misra, Prasada, 1966) in addition to the *L* gene. Gene *P6* was identified in variety Kenya (gene *L4*) in Australia; the same gene was found in variety Bolley Golden (*L10*), and the *L9* gene (*P*) was discovered in variety Koto (Kerr, 1960). In India, an additional *L9* gene was found in already tested varieties: Dakota (*M*), Ward (*M2*), Cass (*M3*),Victory A (*M4*), Polk (*N1*), Koto (*P*) and C. I. 1888-8 (*P4*) (Misra, Prasada, 1966). In Russia, the *Q* gene is allelic to the resistance genes of varieties Kenya (*L4*), Bombay (*N*), Polk (*N1*), and Klaus (*K*) (Kutuzova, Kulikova, 1989).

Around that time, the complicated structure of rust resistance loci was revealed. It was discovered that in locus *L* genes *L*, *L2*, *L5*, and *L6* were closely linked or allelic (Flor, 1962). In the opinion of M.R. Islam and K.W. Shepherd, genes in locus *L* are allelic and their products may differ by one amino acid. Also, in their experiments the relationship between genes and expression of many of them depended on temperature and the presence of inhibitor genes in some lines (Islam, Shepherd, 1991). Linkages were also found in the *M* locus between alleles *M1* and *M4* (Lawrence et al., 1981) and between *M* and *M3* (Hausner et al., 1999b). According to M.R. Islam, all genes in this locus are closely linked, and gene *M* is influenced by inhibitor genes *I-1* and *I-2* (Islam et al., 1989).

Modern methods of molecular genetics allowed discovering the structure and features of *R* gene expression. It was found that the *L* locus is a single gene with 13 allelic variants (*L*, *L1–L11*, and *LH*), which can be distinguished by the response to different races of the pathogen. Loci *N*, *M*, and *P* have a more complicated structure and consist of 4–15 or 6–8 tandem paralogs (Ellis et al., 1999; Dodds et al., 2001a, b; Lawrence et al., 2010). Unique DNA fragments marking genes *L2*, *L6*, *L9*, *L11* (Hausner et al., 1999a); *P* and *P2* (Dodds et al., 2001b); gene *M3*, effective in Canadian environment (Hausner et al., 1999b), and gene *M4*, effective against the majority of rust pathogen races in China (Bo et al., 2008) were discovered. Currently, 19 genes have been sequenced (11 in locus *L*, 3 in *M*, 3 in *N*, and 2 in *P*), and a partial homology between sequenced genes located in different loci has been proven (Ravensdale et al., 2011). The size of these genes is about 4500 bp, and their products are about 1200 aa (Lawrence et al., 1995). Each gene consists of four exons and three introns (Lawrence et al., 1995; Anderson et al., 1997; Dodds et al., 2001a, b). Thus, despite of the extensive study of the genes responsible for flax resistance to rust, there is no clear view of their structure and location in the genome.

The proteins encoded by rust resistance genes, which control the signal transmission about the infection within the cell, belong to the class of TIR-NBS-LRR proteins. Their amino terminal regions contain the LZ domain, leucine zipper, in most cases belonging to the TIR family (Toll/Interleukin-1 Resistance), and the nucleotide-binding site (NBS). The carboxy terminal region is enriched with imperfect leucine-rich repeats (LRRs) (Hammond-Kosack, Jones, 1997). The LRR

domain of a protein molecule is horseshoe-shaped. It consists of leucine-enriched repeats (xxLxLxx motifs, where L is leucine and x is any amino acid) of 24–30 aa each, and is responsible for R-Avr interaction (Kobe, Deisenhofer, 1995; cit. ex: Ravensdale et al., 2011). The differences between alleles of one gene relate mostly to leucine-rich domains. In particular, the products of genes *P2* and *P* differ in the replacement of 10 amino acids in four xxLxLxx motifs of the LRR domain. Differences among products *P*, *P1*, *P2*, *P3*, and *P4* are also related to the LRR domain (Dodds et al., 2001b). Protein products of genes *L6* and *L11* differ in 32 amino acids in the LRR domain (Ellis et al., 2007; cit. ex: Ravensdale et al., 2011).

Products of the flax *R* genes can be divided into two subclasses, differing in the presence of a domain at the C-end of CNL, which is not enriched with leucine, and the homology of the LZ domain to TIR. The first subclass includes the *L*, *M*, and, possibly, *N* loci, which have the TIR-NBS-CNL structure (Dodds et al., 2001a). Products of genes *L* and *M* also have an N-terminal hydrophobic site, probably responsible for anchoring to the membrane, which supposes their location inside the cell (Ellis et al., 2007; cit. ex: Ravensdale et al., 2011).

Another feature of genes *L6*, *M*, *N*, but not *P*, is alternative splicing, which results in the formation of two products: full-size and truncated (Dodds et al., 2001a, b). For example, the most part of the LRR domain is missing from the truncated product of the *L6* gene, and there is a short C-terminal end. This situation is explained by the translation of only part of the third intron and the termination of exon 3 and 4 translation (Lawrence et al., 1995; Dodds et al., 2001a, b).

Thus, the specificity of rust resistance genes may be caused by changes in different parts of their sequences and the accumulation of various mutations. The accumulation of both "neutral" mutations and ineffective alleles of these genes is indicative of long coevolution of flax and rust. The high polymorphism of the *R* genes at sites of "specificity" may point to a high frequency of mutations, which holds the promise of finding new genes (alleles) not described in the literature thus far.

Methods of molecular genetics allowed description of the structural features of *R* genes, but the effectiveness of genes against the pathogenic fungus and their breeding value can be determined only by genetic and phytopathological methods. That is why another aspect of the work with disease resistance in plants is the analysis of the diversity of the pathogen races. It was previously found that the standard set of differentiator lines developed by Flor (1955), ambiguously distinguished local races of the pathogen from different regions of Russia. For instance, with this set T.V. Krylova identified 5 races among 45 monopustule isolates of the fungus collected in 18 flax-cultivating regions of Russia. Addition of four extra differentiators from local varieties allowed identification of 13 more races (Krylova, 1981). In our experiments with Flor's differentiators we managed to identify 6 races among 50 clones. Addition of three monogenic lines isolated from local Russian flax to the standard set allowed identification of five more races (Kutuzova, Kulikova, 1985).

The existence of six loci for resistance to flax rust allows the inclusion of six or more resistance genes in the variety due to the complex structure of some loci and the linkage of genes *N* and *P*. This result can be achieved by step hybridization between monogenic lines with resistance genes located at different loci or by crossing of two lines having two or three resistance genes. For example, the resistance of variety Rio C. I. 280 is controlled by four genes: *L6*, *M*, *N1*, and *P* (Flor, Comstock, 1972).

With regard to the fact that in the course of breeding only one of the allelic rust resistance genes can be introduced to a variety, the mapping of the genes is necessary to avoid waste of time and money and eliminate the risk of new epiphytotic provocation.

The objectives of the current experiment included an attempt to apply classical genetic methods to map rust resistance genes found in old Russian hill kryazhes and local accessions, which are part of the oldest group of flaxes in the VIR world collection gathered in 1922–1923, before the release of breeding varieties. Lines isolated from them have genes controlling not complete but satisfactorily high resistance and can be successfully used in the creation of convergent and multilinear varieties more resistant than monogenic ones. Currently, genes *L1*, *L3*, *L4*, *L6*, *L8*, *L10*, *M2–M6*, *N*, *N1*, *P*, *P1*, *P3*, *P4*, *P6*, and *K* (identified in oilseed flax on the American continent) and gene *Q* (identified in Russia) are highly effective against rust in Russia (Kutuzova, 2014). Genes of old local Russian varieties are likely to be the primary sources of flax resistance to rust throughout the world, because Europe and America purchased the seed material from Russia for centuries.

Materials and methods

Experiments were conducted with 19 relatively rust-resistant inbred lines from the VIR flax genetic collection. The lines had been selected from heterogeneous and somewhat susceptible accessions of old Russian flaxes that included few resistant plants. They were raised and maintained at artificial isolation. Test crosses of the selected lines showed that each of them had one dominant gene of resistance with rather high efficiency: resistance against 70–100 % of virulent clones isolated from local populations of the fungus (see the Table). This was inferred from the results of infection with 50 monopustule fungus clones. Tests of the genes for their response to infection with five clones of *M. lini* showed that they all were unrelated (Kutuzova, 1981).

Identification of *R* genes was carried out by classical genetics methods including the allelism test. Evaluated lines were crossed to tester lines for each of the six known loci (genes) of resistance (*L*, *M*, *N*, *P*, *K*, and *Q*). The F_1 plants were grown in a greenhouse in winter. For segregation account, F_2 seeds of each hybrid combination, its parental lines, and the universally susceptible variety were sown in rows in boxes. The numbers of required F_2 seeds were calculated with regard to the expected number of *R* genes and possible allelism of *N* and *K* to *Q*. Plants were inoculated in the cotyledon leaf phase with a fungus clone avirulent to all tested genes except *L*, which is practically ineffective against the local Russian population of the fungus. Plants were sprayed with water from a spray gun, spores of the fungus mixed with talc were applied to each plant with a brush, and a wet chamber was arranged for a day. The results of infection were assessed after 8–10 days in case of well-developed mycelium on the susceptible standard variety. The absence of segregation from the hybrid meant allelism of genes between the analyzed and tester lines.

F_2 segregation for resistance against *Melampsora lini* in hybrids of analyzed monogenic lines with tester varieties for loci *K*, *L*, *M*, *N*, *P*, and *Q*

Line, putative locus	Percentage of non-virulent clones	Varieties differentiating rust races, genes					
		Ottawa 770B, *L*	Bombay, *N*, *Q*	Dakota, *M*	Koto, *P*	Clay, *K*, *Q*	Natasja, *Q*
gc-9 line 1-1-1 from k-467 Vologda oblast, *M* or *K*	100 ± 2	35 : 6 $\chi^2 = 2.35$ 1 gene	325 : 5 $\chi^2 = 0.00$ 3 genes	268 : 0	73 : 2 $\chi^2 = 0.59$ 3 genes	359 : 0	74 : 0
gc-25 line 4-1 from k-630 Simbirsk oblast, *X* or *L*	98 ± 2	40 : 6 $\chi^2 = 3.51$ 1 gene	302 : 7 $\chi^2 = 0.99$ 3 genes	97 : 2 $\chi^2 = 3.02$ 2 genes	185 : 2 $\chi^2 = 0.3$ 3 genes	319 : 2 $\chi^2 = 1.84$ 3 genes	103 : 6 $\chi^2 = 2.28$ 2 genes
gc-32 line 2-1 from k-716 Pskov kryazh, *P*	78 ± 5.9	45 : 7 $\chi^2 = 3.69$ 1 gene	346 : 7 $\chi^2 = 0.41$ 3 genes	77 : 7 $\chi^2 = 0.62$ 2 genes	110 : 0	236 : 6 $\chi^2 = 1.32$ 3 genes	76 : 5 $\chi^2 = 0.00$ 2 genes
gc-33 line 2-1 from k-780 Minsk oblast, *P*	84 ± 5.2	41 : 7 $\chi^2 = 2.78$ 1 gene	331 : 4 $\chi^2 = 0.30$ 3 genes	118 : 4 $\chi^2 = 1.84$ 2 genes	159 : 1 $\chi^2 = 0.91$ 3 genes linkage	340 : 7 $\chi^2 = 0.47$ 3 genes	75 : 3 $\chi^2 = 0.77$ 2 genes
gc-34 line 2-1 from k-791 Gomel oblast, *P* or *K*	98 ± 2.0	35 : 8 $\chi^2 = 0.94$ 1 gene	279 : 13 $\chi^2 = 1.61$ 2 genes	74 : 8 $\chi^2 = 1.72$ 2 genes	103 : 0	350 : 0	83 : 8 $\chi^2 = 1.00$ 2 genes
gc-38 line 2-1-1 from k-834 Vladimir oblast, *K*	96 ± 2.7	35 : 5 $\chi^2 = 3.33$ 1 gene	279 : 6 $\chi^2 = 0.55$ 3 genes	80 : 9 $\chi^2 = 2.27$ 2 genes	79 : 6 $\chi^2 = 0.09$ 2 genes	359 : 0	92 : 0
gc-39 line 2-1-1 from k-846 Ivanovo-Voznesensk oblast, *M*	94 ± 3.4	43 : 8 $\chi^2 = 2.36$ 1 gene	355 : 9 $\chi^2 = 1.96$ 3 genes	124 : 0	114 : 4 $\chi^2 = 1.65$ 2 genes	335 : 8 $\chi^2 = 1.32$ 3 genes	83 : 5 $\chi^2 = 0.05$ 2 genes
gc-40 line 1-1 from k-867 Votskiy kryazh, *P* or *K*	86 ± 4.9	35 : 6 $\chi^2 = 2.35$ 1 gene	312 : 5 $\chi^2 = 0.00$ 3 genes	86 : 3 $\chi^2 = 1.26$ $\chi^2 = 1.83$ 2 or 3 genes	206 : 0	321 : 0	86 : 0
gc-46 line 5-1-3 from k-944 Tyumen, *P* or *K*	96 ± 2.7	37 : 8 $\chi^2 = 1.25$ 1 gene	371 : 2 $\chi^2 = 1.87$ 3 genes	77 : 4 $\chi^2 = 0.24$ 2 genes	106 : 0	270 : 0	101 : 0

Results

Since variety Ottawa 770B, which tests locus *L*, is susceptible to almost all races of the Russian rust populations, the fungus clone that we used in this study revealed one dominant gene in the tested flax line in each hybrid combination. None of the identified genes is an allele of locus *L* (see the Table).

In crosses of the line selected from accession k-467, Vologda oblast (gc-9), to variety Bombay (genes *N* and *Q*), segregation of three genes was noted. This indicates the absence of allelism between the gene of the tested line and loci *N* and *Q* and the presence of gene *P*, linked to *N*. The segregation of three genes in the hybrid of the same line to variety Koto confirms the presence of gene *P*, linked to *N* gene, and another gene specific for the line itself. The absence of segregation in F_2 hybrid populations of this line with varieties Dakota (locus *M*) and Clay (loci *K*, *Q*) can be explained only by the fact that the resistance gene present in line gc-9 is located either in locus *M* or in locus *K*, or both of them are present. The absence of segregation in hybrid populations from crosses of line gc-9 to varieties Clay (loci *K*, *Q*) and Natasja (gene *Q*) suggests the probability of the location of the tested line gene in locus *Q*, but the segregation in its hybrid to Bombay (loci *N*, *Q*) refutes this suggestion.

Segregation was observed in crosses of gc-25, from k-630, originating from the Simbirsk oblast, to the testers of all known loci. With all this, hybrids to varieties Koto (locus *P*) and Bombay (*N*, *Q*) showed a linkage between genes *N* and *P*. This result can be explained by assumptions that the gene of this line is an effective allele of locus *L* or there is a previously unknown locus. According to M.M. Levitin and I.V. Fedorova (1972), flax has at least eight loci responsible for resistance to rust agent races. Experiments conducted in the Soviet Union (Krylova, 1981; Kutuzova, Kulikova, 1985) showed that the set of differentiator lines created 35 years ago could not convincingly discriminate the races of the fungus. Fiber flax is a crop that since ancient times is widespread in Russia from the western border to the Pacific coast. It was brought from Russia to Europe and America later, and it is reasonable to expect that in Russia there should be the greatest diversity of resistance genes to this pathogen, more than the known set of loci identified in linseed in other countries can house. Flax rust is known in Russia since 1885. It became widespread in the early twentieth century (Yachevsky, 1911), which boosted the search for highly efficient resistance genes.

The F_2 hybrid of gc-32 selected from k-716 (Pskov oblast) to variety Koto (locus *P*) showed no segregation. It means that

the *R* gene of this line is located in locus *P*. This suggestion was supported by the expected segregation in crosses to testers for loci *N*, *M*, *K*, and *Q*.

In the hybrid population obtained by crossing the gc-33 line selected from k-780 (Minsk oblast) to variety Koto (gene *P*), only one rust-susceptible plant was found. This fact, most likely caused by a crossing over, indicates that the gene of the tested line is linked to locus *P* (16 cM), and, correspondingly, is also linked to gene *N*. This was proven by one-gene segregation in the hybrid to variety Ottawa 770B (locus *L*). In F_2 hybrids to tester varieties for loci *NQ*, *M*, *KQ*, and *Q*, the expected segregation was noted.

Line gc-34, selected from accession k-791 (Gomel oblast), gave an expected segregation for two genes when crossed to tester varieties for loci *NQ*, *M*, and *Q*. With testers for loci *P* and *KQ*, no segregation was found. This can be explained by the fact that the *K1* gene is linked to genes *N* and *P*, as found by Hoes and Kenaschuk (1986). Perhaps the gene of this line is located in the *P* or *K* loci and is allelic to *K1*.

In crosses of line gc-38, selected from k-834 (Vladimir oblast), to the testers for loci *NQ*, *M*, and *P*, the expected segregation was obtained. There was no segregation in hybrids to *KQ* and *Q* testers. But since the hybrid with Bombay, having genes *N* and *Q*, demonstrated the absence of allelism between the resistance gene of the evaluated line and locus *Q*, we have but to suppose that the size of the hybrid population with variety Natasja was insufficient for analysis, and the gene of gc-38 was located in locus *K* and was an allele other than that in gc-34.

The segregation of hybrids between line gc-39, selected from k-846 (Ivanovo-Voznesensk oblast), and testers for all resistance loci, except for variety Dakota, indicated that the gene of this line is located in the *M* locus.

As shown by previous analysis, the gene of line gc-40, selected from k-867 (Votskiy kryazh), is not located in loci *N* or *M*. There was no segregation in F_2 hybrids between this line and testers for loci *P*, *KQ* and *Q*. As already shown, the resistance gene of this line cannot be located in locus *Q*. The possibility of the location of *N* and *P* on the same chromosome as locus *K* (Hoes, Kenaschuk, 1986), may explain the absence of segregation in crosses to varieties Koto and Clay. It suggests that the resistance gene of gc-40 is located either in locus *K* or *P*. The rust resistance gene of this line was repeatedly used by breeders to create rust-resistant varieties. As our research shows, varieties Belorusskiy 1 (k-6601), Uspekh (k-6818), and some other modern varieties are protected by an identical gene (Kutuzova, 2012). A significant part of rust resistance donors created at VNIIL and available for breeding are also protected by an identical gene, inherited from variety Uspekh (Rozhmina, 1988), and this fact should be taken into consideration when using them.

The segregation in the cross of line gc-46, selected from k-944 (Tyumen), is very similar to the results of the previous line. Probably, the gene of k-944 belongs to another allele of the same locus: *K* or *P*.

Thus, classical genetic methods are insufficient for unambiguous mapping of rust resistance genes in all old Russian flaxes. The work is also hindered by the linkage between loci *N* and *P*.

Conclusion

In this work, we discovered that most of the resistance genes in evaluated lines are located in loci *P* and/or *K*. It was exactly determined that the *R* gene of line gc-32, selected from k-716 (Pskov oblast), is located in locus *P*, linked to *N*. The gene for rust resistance in line gc-33, selected from k-780 (Minsk oblast), also belongs to locus *P* (linkage was confirmed in our experiment), and the resistance gene in line gc-39 from k-846 (Ivanovo-Voznesensk oblast), being effective against 94 % of the fungus races, belongs to locus *M*. The resistance gene of line gc-38 from k-834 (Vladimir oblast), is probably located in locus *K*. The positions of other genes could not be clearly identified. Perhaps, the use of molecular methods would clarify their identity.

The lines with genes *P* and *K* should be used in breeding with caution, because it is unknown which of these genes is already quite widespread in the varieties bred in Russia. However, with regard to the linkage of genes *N* and *P*, as well as the association of gene *Q* with loci *N* and *K*, it is difficult to predict which genes (or gene) may be inherited by the hybrid.

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Acknowledgements. This work was supported by State Budgeted Project No. 0662-2019-0001 for VIR.

Conflict of interest. The authors declare no conflict of interest.

Received May 6, 2019. Revised June 3, 2019. Accepted June 3, 2019.