Chapter 10

Genetic Improvement of Bread Wheat for Stem Rust Resistance in the Central Federal Region of Russia: Results and Prospects

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http://dx.doi.org/10.5772/intechopen.75379

Abstract

Advanced breeding lines of spring and winter wheat with several effective resistance genes to stem rust, including its aggressive race Ug99, were developed for the first time for the non-Chernozem zone of Russia. Modern wheat varieties cultivated in this region have high productivity and grain quality. However, they are susceptible to fungal diseases and therefore are cultivated using frequent fungicides treatments. The introgression wheat lines with multiple alien translocations ("Arsenal" collection) have been developed in the Moscow Scientific Research Institute of Agriculture "Nemchinovka" by using gamma irradiation of pollen of wild wheat relatives (*Aegilops speltoides, Ae. triuncialis, Triticum kiharae, Secale cereale*). Initial material with several effective Sr resistance genes for wheat breeding was developed using donors from the "Arsenal" and the VIR collections. The created initial material can compete with modern varieties, as it has resistance to leaf rust and powdery mildew, high productivity and numerous other advantages. On this basis, a new direction in breeding of spring and winter wheat is developed for this region, that is, creation of wheat cultivars with resistance to fungal diseases. This allows to reduce the fungicide load during cultivation with the goal of producing ecologically clean grain for healthy diet.

Keywords: bread wheat, stem rust, donors, Sr genes, grain quality

1. Introduction

1.1. Geographical position of the Central Federal District of Russia, achievements in the selection of wheat and the directions of its improvement

The Central Federal District of Russia (CFDR) is an area of more than 650,000 km^2, which includes 18 oblasts with the capital city of Moscow (**Figure 1**). The CFDR lies within the Atlantic-continental climatic region of the north temperate zone. It is characterized by not too cold winter and warm, but not excessively hot summer. The lowest temperatures are observed in January: on the average from −8 to −12°C. Summer temperature ranges from 18 to 20°C. The average duration of the frost-free period is 125–140 days, and the sum of the effective temperatures is 1800–2300, which allows to successfully cultivate most of the cereals, potatoes, vegetables, fodder grasses and flax in the CFDR. The average annual precipitation is 450–600 mm [1] (http://studopedya.ru/2-68711.html). This economic region includes about 16,000,000 ha of arable land, in which winter and spring wheat are the leading crops. The area under these crops is 3,600,000 and 620,000 ha, respectively. Traditionally, until the late 1960s of the twentieth century, rye was grown in this region, which is less demanding for the fertility of sod-podzolic soils. However, rye gave way to wheat due to the efforts of breeders. Breeders P. Lukyanenko (Bezostaya 1, Karlik 1), V. Remeslo (Mironovskaya 808), G. Lapchenko (PPG-1, PPG-186), E. Varenitsa (Zarya), E. Nettevich (Moskovskaya 35, Priokskaya, Lada) and B. Sandukhadze (Inna, Galina, Moskovskaya 39, Nemchinovskaya 17) made a great contribution to the creation of wheat cultivars.

Modern cultivars of bread wheat, derived in the Moscow Scientific Research Institute of Agriculture "Nemchinovka" (Moscow Sc. Res. Inst. of Agr. "Nemchinovka"), are characterized by high winter hardiness, productivity and grain quality. They are cultivated according to intensive technologies with the application of mineral fertilizers up to 150 kg N, 120 kg P_2O_5, 150 kg K_2O, against the background of manure and annual grasses. Seed treatment before sowing, threefold

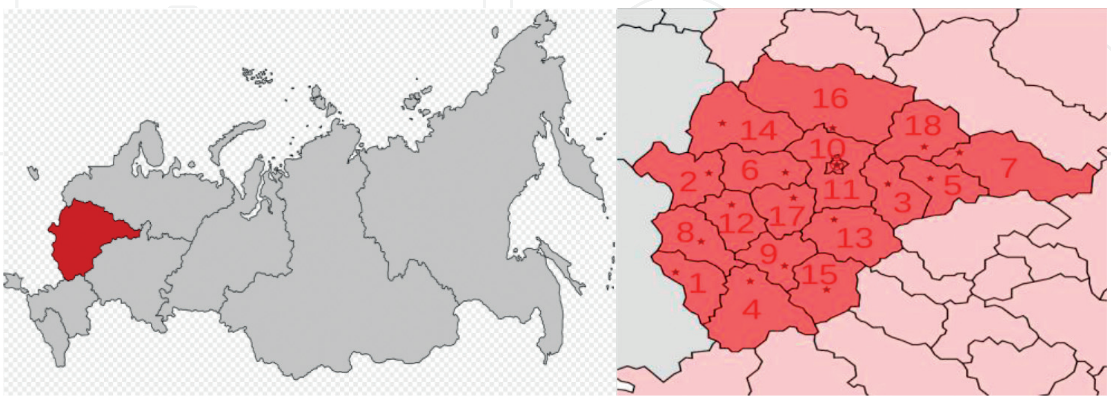


Figure 1. The Central Federal District on the map of Russia (a) and its composition (b): 1—Belgorod Oblast, 2—Bryansk Oblast, 3—Vladimir Oblast, 4—Voronezh Oblast, 5—Ivanovo Oblast, 6—Kaluga Oblast, 7—Kostroma Oblast, 8—Kursk Oblast, 9—Lipetsk Oblast, 10—Moscow, 11—Moscow Oblast, 12—Oryol Oblast, 13—Ryazan Oblast, 14—Smolensk Oblast, 15—Tambov Oblast, 16—Tver Oblast, 17—Tula Oblast and 18—Yaroslavl Oblast.

application of fungicides, herbicides, insecticides and growth regulators for the season ensure the yield of spring wheat up to 7 tons/ha and winter wheat up to 10 tons/ha. However, these cultivars are susceptible to the majority of fungal diseases common in this zone (powdery mildew, leaf rust, stem rust and *Septoria* leaf spot). To date, only one cultivar of winter wheat Nemchinovskaya 24 with two resistance genes *Lr9* and *Lr46* has genetic protection against leaf rust. Therefore, the development of cultivars with increased immunity to fungal diseases is actual for the CFDR. Extensive transport and economic relations in the globalization of the world do not exclude the importation of quarantine diseases into our territory, for example, the aggressive race of stem rust Ug99. The worsening of the phytopathological situation requires increased research in this area, especially in recent years, when the cases of crop damage caused by stem rust occur more frequently (2010, 2013, 2016 and 2017). The aim of this study was to identify the sources and donors of resistance to stem rust, including the race Ug99, from the collection of VIR and "Arsenal" collection, and to create on their basis, the initial material of spring and winter wheat with durable resistance to stem rust.

2. The development of the initial material of spring and winter wheat with several Sr genes resistant to *Puccinia graminis* f. sp. *tritici*

2.1. The modern phytopathological stem rust situation in CFDR and possible threats

The situation in the CFDR reflects the general trend observed in the populations of *P. graminis* in all areas of pathogen distribution; the fungus actively evolves. Differences concern only the speed and genes of the pathogen virulence, depending on the geographical location. In the case of Ug99 (TTKSK), the process is very fast (in 18 years, 13 biotypes of the fungus appeared); on the other hand, in the territory of CFDR, the change of the dominant races took place for 57 years. The phytopathological situation is complicated by the proximity of the CFDR to European countries, where aggressive pathogens of *P. graminis* have been identified recently. Six races (TKTTF, TKKTF, TKPTF, TKKTP, PKPTF and MMMTF) were retrieved from 48 isolates, obtained from the *P. graminis* population in 2013 in Germany [2]. The detection of the TKKTP race causes concern because of its virulence to the *Sr24*, *SrTmp* and *Sr1RSAmigo* genes, although it has been determined that none of these races belongs to the race group TTKSK (Ug99), and the German isolates of the TKTTF race are phenotypically different from the TKTTF race that caused plant disease epidemic in Ethiopia in 2013/2014. It is known that 55% of North American and international cultivars and selection lines resistant to the race TTKSK (Ug99) are susceptible to the TKKTP race [2]. On the Italian island of Sicily, a new race of stem rust, the TTTTF, hit several thousand hectares of durum wheat in 2016, leading to the largest outbreak of stem rust in Europe in recent decades. TTTTF is a newly identified race of stem rust that can soon spread over long distances along the Mediterranean basin and the Adriatic coast [3] (http://www.fao.org/news/story/en/item/469467/icode/).

The analysis of the racial composition of *R. graminis* f. sp. *tritici* in CFDR was held annually since 1960. During this time, significant changes occurred in the composition of the dominant races. In the 1960s–1970s, the population of stem rust included physiological races 21, 17 and

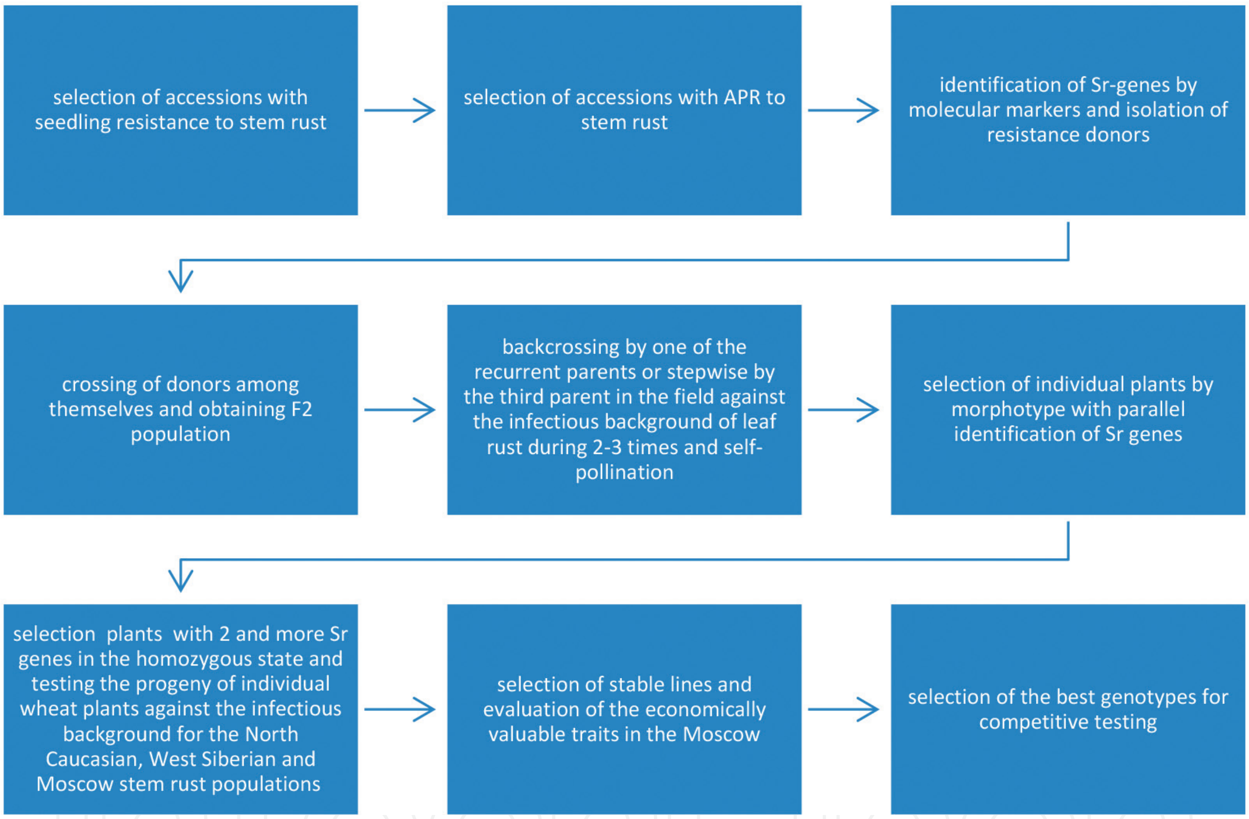
34 according to Stakman's nomenclature [4]. Races 11 and 14 were detected regularly, but were not widely distributed. In the 1960s–1970s, only the resistance genes *Sr7b* and *Sr9g* were completely ineffective. Virulence to the genes *Sr5, Sr21, Sr9e, Sr11, Sr6, Sr8a, Sr36, Sr9b, Sr30* and *Sr17* was low or absent [5]. In subsequent years, the fungal pathotypes, virulent to the resistance genes *Sr5, Sr21, Sr6, Sr8a* and *Sr17*, appeared. Races of the pathogen MKCT, MKCK, MKBK, MKBS, MKBT, RKCT and RKBS dominated in CFDR in 2004 [6]. During this period, the *Sr9e, Sr11, Sr36, Sr9b* and *Sr30* genes were effective. Over the past decade, the structure of the population on the basis of virulence has changed toward the predominance of several aggressive virulent races, including races that are virulent to genes *Sr5, Sr21, Sr9e, Sr7b, Sr6, Sr8a, Sr9g, Sr36, Sr30, Sr9a, Sr9d, Sr10* and *SrTmp*. Among samples from the European part of the Russian Federation, the races of stem rust MKBT and MRLT in 2002 and TKNT, TKST, TTNT in 2005 dominated [7]. The race composition of *P. graminis* f. sp. *tritici* populations in the CFDR in the period 2000–2009 is presented in the work of Skolotneva et al. [8]. They analyzed 387 isolates of the fungus using the North American set of differentiators. Samples were obtained from cereals (wheat and barley), wild herbs and barberry. As a result of the study, 45 races of *P. graminis* f. sp. *tritici* were identified. The predominant races TKNT and TKNTF were isolated. The Ug99 race and its derivatives were not found in the Russian Federation.

According to the data obtained in 2013 [9], during assessing the collection of lines with known Sr genes, the effective genes of resistance to stem rust in CFDR were the genes *Sr2, Sr9e, Sr13, Sr25, Sr26, Sr31, Sr32, Sr36, Sr44, SrWld* and the combination of genes *Sr17* + *Sr13*, *Sr31* + *Sr38*. According to the data of Skolotneva et al. [8], the resistance genes *Sr9e* and *Sr36* in 2009 were ineffective in the Central region of Russia. Differences in the data are probably due to a change in the composition of the pathogen population. Thus, in the same work of Skolotneva, a change in the percentage of fungal isolates virulent to the *Sr17* gene was noted from 92.5% in 2000 to 0% in 2008, and the genes of *Sr31* and *Sr24* remained effective against all local races of stem rust. Observations of the pathogen development in the period 2013–2017, conducted in the All-Russian Research Institute of Phytopathology, showed the annual development of stem rust. The development of the disease on the susceptible genotype of Khakasskaya in 2017 reached 100% [10].

2.2. Development of bread wheat lines with several resistance Sr genes

The process of creating the initial material of bread wheat with several resistance genes passed in several stages. *First stage:* Isolation of resistance sources in the seedling stage; evaluation of the spring bread wheat line against the background of Ug99 natural infection in Ethiopia. *Second stage:* Identification of resistance Sr genes using specific molecular markers and isolation of resistance donors. *Third stage:* Selection of pairs for crossing and hybridization of donors among themselves, obtaining of the segregative population of hybrids F_2. *Fourth stage:* Performing backcrossing by one of the recurrent parents or stepwise hybridization of individual plants by the third parent in the field against the infectious background of leaf rust; subsequent self-pollination or repeated backcrossing with test of progeny against infectious background of leaf rust. At this stage, work with spring and winter plants was carried out in parallel at different plots and with different planting times. *Fifth stage:* Selection of individual plants by morphotype with parallel identification of Sr genes. *Sixth stage:* Testing the progeny of individual spring wheat plants against the infectious background for the North Caucasian and West Siberian populations of stem rust, and plants of winter wheat for the North Caucasian population of stem rust (infectious background) and natural epidemic development of stem rust in the Moscow Oblast. *Seventh stage:* Evaluation of the economically valuable traits of selected stable lines in the Moscow Oblast conditions in comparison with standard cultivars, selection of the best genotypes for competitive testing.

Schematically, the process of breeding lines with several effective Sr genes can be represented in the following sections.



2.2.1. First stage

The identification of the resistance sources to the race Ug99 of stem rust was started by an employee of FSBSI VIZR Anna Anisimova together with the scientists from Minnesota University (USA) in 2010. At the seedling stage, 386 accessions of bread wheat from the collection of VIR and the "Arsenal" collection from Moscow Sc. Res. Inst. of Agr. "Nemchinovka" were evaluated, six accessions of winter wheat and one accession of spring wheat with resistance to this disease were selected (type of reaction during pathogen penetration from 0 to 2) [11]. It is the selection line GT 96/90 (hereinafter referred to as line 96) from Bulgaria with genetic material of the species *T. timopheevii*, a cultivar of winter wheat Donskaya polukarlikovaya (hereinafter referred to as D) in the pedigree of which *Aegilops tauschii* was present (accessions from the collection of VIR). From the collection, "Arsenal" lines with translocations from *Ae. speltoides* were selected: 9/00w ($2n = 42$), disomic addition lines of *Ae. speltoides:* 19/95w and 141/97w ($2n = 44$); wheat-*Ae. speltoides*-rye line 119/4-06rw ($2n = 42$), hereinafter referred to as line 119. The only stable accession of spring wheat 113/00i-4 ($2n = 42$) (in the text accession 113), obtained from crossing the spring cultivar Rodina with irradiated pollen of the species *Ae. triuncialis* [12] and then crossed with the line with the genetic material of *T. kiharae*, showed immunity to the natural infection of stem rust race Ug99 in Ethiopia at the stage of an adult plant [13].

2.2.2. Second stage

Identification of resistance Sr genes was carried out using molecular markers both to effective genes against the Ug99 race (*Sr2*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr32*, *Sr35*, *Sr36*, *Sr39*, *Sr40*, *Sr44*, *Sr47*) and to ineffective ones: *Sr9a*, *Sr15*, *Sr17*, *Sr19* and *Sr31*, but providing resistance to local populations of the pathogen. The list of molecular markers is given in **Table 1**. For each primer, the most optimal PCR conditions were selected; the conditions given in the original studies are taken as basis. Separation of amplification products was performed by electrophoresis in 2% agarose and 8% polyacrylamide gels stained with ethidium bromide at a voltage of 100 V for

Sr gene	Chromosome	Marker	References
Sr2	3B	Xgwm533	[14]
Sr9a	2BL	Xgwm47	[15, 16]
Sr15	7AL	STS638	[17]
Sr17	7BL	Wpt5343	[18]
Sr19	2BS	Wpt9402	[18, 19]
Sr22	7AL	Xbarc121, cfa2123	[20, 21]
Sr24/Lr24	3DL/1BS	Sr24#12, Sr24#50	[22]
Sr25/Lr19	7DL	Gb	[23]
Sr26	6AL	Sr26#43	[22]
Sr31	1R/1B	Scm9	[24]
Sr32	2AS,2B	Xbarc55, Xstm773	[19, 25, 26]
Sr35	3AL	Xcfa2170, BE485004	[27]
Sr36	2BS	Xwmc477, Xstm773-2	[28]
Sr39	2BS	Sr39#22	[29]
Sr40	2BS	Xgwm344	[30]
Sr44	7DS	Wpt2565	[18]
Sr47	T2BL-2SL·2SS	Xgwm501	[31]

Table 1. Specific primers used to identify Sr genes.

3 h in 0.5× TBE buffer. As markers of molecular weights, 50 bp, 100 bp and 1 kb GeneRulerTM DNA Ladder from "Fementas" were used. The results of gene identification in new sources are given in **Table 2** [32].

We explain a wide range of identified genes in donors from the "Arsenal" collection by multiple alien translocations of the genetic material of species *Aegilops speltoides*, *Ae. triuncialis*, *Triticum kiharae*, *Secale cereale*, arising during the irradiation of pollen, and in the selection line GT 96/90 by the presence of translocations from the species *T. timopheevii*. The use of such donors, even in paired crosses, can lead to the creation of plant forms with an unusual combination of resistance Sr genes due to the recombination of genes in meiosis. However, since we were faced with the task of obtaining the initial material for the selection process, we had to take into account not only the level of donors ploidy but also the presence of economically valuable traits. It should be noted that despite the positive identification of the *Sr22* gene in the wheat-*Aegilops* lines (9/00w, 141/97w and 119/4-06rw) using the *Xbarc121* and *Xcfa2123* markers, the absence in the pedigree of these lines of the genetic material *T. monococcum* leaves doubt in the presence of this gene.

2.2.3. Third stage

We rejected the use of disomic addition lines with chromosomes of *Aegilops speltoides* in the selection of pairs for crossing, since the supplemented alien chromosome with which we bind resistance was rarely conjugated to wheat chromosomes and was lost in the process of division in meiosis. The remaining donors had an euploid number of chromosomes, but were different according to the morphophysiological and agronomic characteristics (terms of ear formation, height, susceptibility to powdery mildew). The D cultivar and the GT 96/90 line had a very short stem (60–70 cm), early ear formation (late May to early June) and were affected by powdery mildew to a high degree (severity 30–50%), remaining resistant to leaf rust. For donors from the "Arsenal" collection, on the contrary, later ear formation, long stem, but the high resistance to powdery mildew and leaf rust were characteristic. Parent pairs for crossing were selected with alternative development of traits (short stem, early ear formation,

Line, cultivar	Identified Sr genes	Severity, %		Height, cm	Grain weight per ear, g	1000 grains weight, g
		Powdery mildew	Leaf rust			
9/00w	*Sr22, Sr32, Sr44, Sr15*	0–5	0	70–80	1.5	40.0
141/97w	*Sr22, Sr44*	0–10	0	90–110	1.4	36.0
113/00i-4	*Sr2, Sr36, Sr39, Sr40, Sr44, Sr47, Sr15*	0–1	0	90–110	1.4	41.0
119/4-06rw	*Sr22, Sr32, Sr44, Sr9a, Sr17, Sr19*	0–1	$10/_1$	80–100	1.5	42.0
GT 96/90	*Sr24,Sr36, Sr40, Sr47, Sr15, Sr17, Sr31*	30	0	60–70	1.5	42.0
Donskaya polukarlikovaya	*Sr32, Sr44, Sr9a, Sr17, Sr19*	50	$5/_2$	60–70	1.3	40.0

Table 2. Results of the identification of Sr genes in resistance donors to stem rust [32] and their economically useful traits.

susceptibility to powdery mildew) x (long stem, later ear formation, resistance to powdery mildew). The first crossings were conducted in 2010 in the greenhouse. The following pairs of direct crossing and backcrossing were successful: (GT 96/90 × 113/00i-4), (119/96rw × GT 96/90), (113/00i-4 × 119/96rw). In the conditions of the greenhouse, the D cultivar was found to be the earliest ripening, and it was not possible to hybridize with it because of the mismatch of the flowering periods. Later, this cultivar was used in stepwise hybridization. F_1 plants were also grown in the greenhouse. The fact of the segregation of future F_2 populations into winter and spring genotypes from the crossing of winter lines with the spring line 113/00i-4 was taken into account when planning crossings. Crossing with the productive wheat-*Ae. speltoides* line 145/05i (grain weight from ear is 1.9 g, weight of 1000 grains is 49.0 g), which had group resistance to powdery mildew and leaf rust, but was susceptible to Ug99, was additionally planned in order to shift the formative process toward the isolation of productive spring forms of plants.

2.2.4. Fourth stage

Beginning with F_2, the work with spring and winter forms of plants was carried out against the infectious background of leaf rust at different seeding times. Half of the seeds were sown in February in the heated plot after snow melting. After the emergence of shoots, the heating of the soil was switched off, and the plants passed vernalization at natural low temperatures and natural snow cover. In this case, spring plants perished, and winter plants formed the ear. The second half of the seeds were sown in the field in spring. Under these conditions, spring plants formed the ear, and winter crops remained in the tillering phase. Backcrossing of plants resistant to leaf rust, beginning with F_2, was conducted by recurrent spring parent or line 145/05i (when working with spring genotypes) and winter recurrent parent or D cultivar (when creating winter wheat lines). The infectious background of leaf rust was created using all races characteristic for the Moscow Oblast. For further hybridization, only plants resistant to leaf rust were selected. The second backcrossing or self-pollination was carried out under the conditions of a greenhouse, the progeny was sown on the appropriate soil background, and the process of backcrossing on the infectious background of leaf rust was repeated again. Then self-pollination of plants was carried out. The scheme of the selection process for obtaining spring and winter lines with several resistance Sr genes is shown in **Figure 2**.

2.2.5. Fifth stage

In the progeny of self-pollinated plants, which were sown as lines of different generations $BC1F_3$, $BC2F_2$, $BC3F_2$, F_4, F_5, individual plants were selected by morphotype with parallel identification of Sr genes by PCR analysis. During the selection, attention was drawn to the habitus of the plant (bush form, the number of productive shoots), the location of the leaves, the shape of the ear, the presence of marker morphological features (the presence of awns and anthocyanin on different parts of the plant such as stem, ear, anther), the date of ear formation and the degree of severity of affection by powdery mildew and leaf rust were taken into account. Preference was given to plants with group resistance to diseases, with optimal plant height (80–110 cm), early ripening and an ear with 19–21 developed spikelets, that is, the

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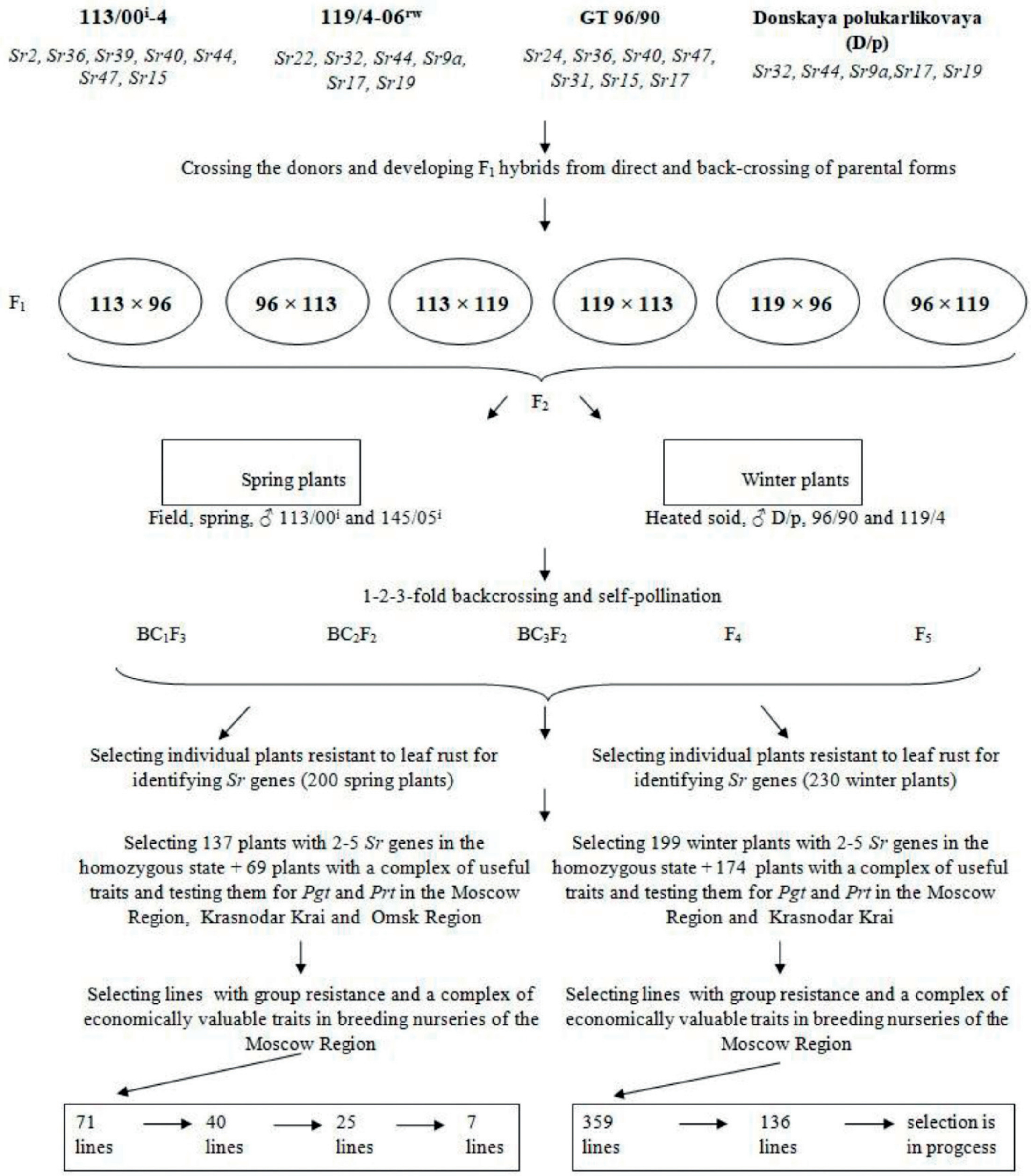

Figure 2. Scheme of development of spring and winter wheat lines with several resistance Sr genes.

selection of individual plants was not accidental, but aimed to combining economically valuable traits. In such plants, a piece of leaf was taken to isolate DNA and to identify Sr genes. In total 200 spring plants and more than 200 winter plants were selected for PCR analysis. The spectrums of identified effective Sr genes in spring and winter plants differed.

In spring plants, the genes *Sr2*, *Sr44*, *Sr36* and *Sr40* were found most often in the homozygous state (71, 89, 78 and 26%, respectively, of the number of plants tested). The *Sr22* gene, which was originally identified in the winter donor 119/4-06rw using two markers Xbark 121 and cfa 2123, was detected in the progeny of selected spring plants at a frequency of 20%, when the donor was used for backcrossing and the resulting progeny was self-pollinated. The *Sr39* and

Sr47 genes were rare, with a frequency of 4.4 and 1.4%, respectively. After PCR analysis, 137 individual plants with several Sr genes in the homozygous state were selected from 200 spring plants, namely: with two resistance genes—54 plants, with three—64 plants, with four—15 plants and with five genes—4 plants.

In individual winter plants, selected from the hybrid population represented by the families F3, BC1F2, BC1F3, BC2F3, BC3F2 of different origin, eight genes were identified, which form a row: *Sr2* > *Sr44* > *Sr32* > *Sr36* > *Sr22* > *Sr31* > *Sr47* > *Sr39* and *Sr40* by the frequency of occurrence in progeny. The combination spectrum of the identified genes in winter wheat plants differed from the spectrum of genes identified in spring wheat lines. This is connected with the orientation of backcrossings conducted in winter and spring wheat. The combination of Sr genes compound in the genotypes of winter wheat is more diverse. The plants with the combination of the *Sr22, Sr32* and *Sr44* genes in the homozygous state were most often encountered. Plants with a unique combination of genes characteristic only of winter plants have been found: *Sr2 + Sr22, Sr2 + Sr32, Sr2 + Sr36, Sr36 + Sr44, Sr36 + Sr47, Sr32 + Sr44, Sr22 + Sr44, Sr31 + Sr36, Sr31 + Sr47, Sr31 + Sr44, Sr22 + Sr44 + Sr47, Sr22 + Sr31 + Sr32, Sr22 + Sr31 + Sr44, Sr22 + Sr36 + Sr44, Sr32 + Sr44 + Sr47, Sr31 + Sr36 + Sr47, Sr36 + Sr39 + Sr47, Sr2 + Sr22 + Sr36 + Sr44,Sr2 + Sr31 + Sr36 + Sr44, Sr22 + Sr32 + Sr40 + Sr44, Sr22 + Sr31 + Sr36 + Sr44, Sr2 + Sr22 + Sr32 + Sr44, Sr2 + Sr22 + Sr32 + Sr40 + Sr44.* Specific features in transmission of some resistance genes are noted. In particular, no plants with the *Sr24* gene were detected. The second feature is associated with the *Sr2* gene (the gene was originally identified only in spring wheat 113/00i-4). The *Sr2* gene was in a heterozygous state in more than 70% of winter plants in which it was identified (**Figure 3**).

The presence of *Sr32, Sr39, Sr40, Sr44* genes, which are poorly studied in relation to other *Pgt* races and rarely used in selection programs, with the resistance *Sr2* gene of an adult plant showing "slow rusting" effect, gives particular value to the selected winter plants. However, the presence of the recessive *Sr2* gene of resistance in the heterozygous state in most winter wheat plants will require additional efforts to transfer it to a homozygous state. In particular, we have planned experiments on the production of digaploid lines using androgenesis method. Individual plants with the identified genotype of resistance to stem rust differed greatly in height (75–145 cm), ear productivity (1.0–2.7 g), weight of 1000 grains (36–60 g) and morphological features. For further testing in infectious nurseries of stem and leaf rust, 373 individual winter wheat plants were selected: 199 plants with the identified Sr genes and 174 plants selected for a set of other economically valuable traits. From the populations of spring

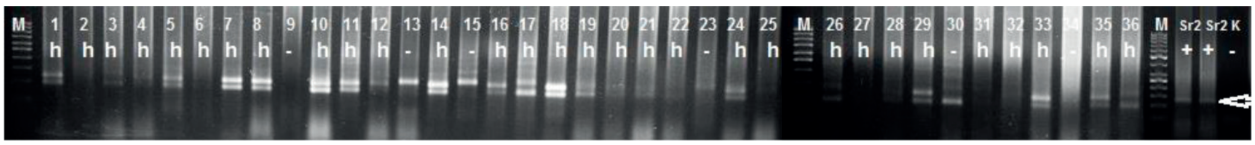

Figure 3. Identification of the *Sr2* gene using the molecular marker *Xgwm533* in winter plants 1–36: M—molecular weight marker of 50 bp "Fermentas", *Sr2*—positive control Pavon76, K—negative control Saratovskaya 29 cultivar. The arrow indicates a diagnostic fragment with a molecular weight of 120 bp. The amplification products were separated in 2% agarose gel. "+"—presence of the diagnostic fragment; "−"—absence of a diagnostic fragment; h—heterozygote.

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wheat, only 198 spring plants were selected for further testing: 129 plants with identified Sr genes and 69 plants with a set of valuable traits.

2.2.6. Sixth stage

2.2.6.1. Spring wheat

Progeny testing of individual spring wheat plants was carried out against the infectious background for the North Caucasian and West Siberian populations of stem rust and leaf rust, and against the natural background of the disease course in the Moscow Oblast. It should be clarified that in the south of Russia (Krasnodar Krai), most of the known resistance genes are ineffective against the causative agent of stem rust. Genes *Sr1, Sr5, Sr6, Sr9a, Sr9e, Sr13, Sr24, Sr27, Sr31, Sr32, Sr35, Sr36* remain effective [33]. One hundred and fifty-eight lines of spring wheat (or 81% of the number of studied lines) showed high resistance to infection (0R) by the North Caucasian population of stem rust, and 160 lines were resistant to leaf rust.

Testing of the same set of spring lines in Western Siberia (Omsk), which were sown with a special late spring sowing (late crops are more affected than those sown in the optimal time) led to the death of some lines, but from the 167 surviving lines, 111 lines (66.5%) with resistance to stem rust were selected. In the year of testing (2015), strong epidemic of stem rust was observed in the region. Under these conditions, only a small group of genes, according to the observations of the researchers, was effective *(Sr2, Sr9e, Sr11, Sr12, Sr13, Sr19, Sr24, Sr25, Sr26, Sr27, Sr30, Sr31, Sr35 Sr37)*, but none of these genes provided full protection against the disease. The severity of lines with known resistance genes varied from 5 to 30% in comparison with 50–60% severity of cultivars without effective genes [34]. Selected in such harsh conditions, stable lines with group resistance to stem and leaf rust are valuable initial material for the selection of spring wheat in this region. Structural analysis performed in comparison with the standard cultivar Omskaya 37 allowed to select 20 lines with the least decrease in productivity in the unfavorable dry conditions of Western Siberia. In 2016, these lines were involved in crosses with the best adapted varieties cultivated in this region (Shamanin, personal communication).

In the Moscow Oblast, in 2015, no development of stem and leaf rust was observed even on the highly susceptible line Khakasskaya because of unfavorable weather conditions for the development of these pathogens (low air humidity, lack of dew, strong wind). However, in the Moscow suburbs, the spring lines were evaluated for resistance to powdery mildew. After that, the results of lines estimates at three geographic locations were combined, and genotypes that showed resistance simultaneously to leaf and stem rust in Krasnodar and Omsk and resistance to powdery mildew in the Moscow Oblast (71 genotypes) were selected. In 2016, under the conditions of epidemic development of stem rust in the Moscow Oblast, after negative selection for resistance to diseases, the timing of the ear formation, height and the presence of segregation by morphological features, 40 genotypes were left for further tests. After the statistical evaluation of the productivity elements (yield of grain from 0.3 m^2, productivity of the ear, weight of 1000 grains), 25 best genotypes with a set of economically valuable traits were selected (see Stage 7).

2.2.6.2. Winter wheat

The progeny of winter wheat plants was tested in two geographical locations: the Moscow Oblast against the natural but epidemic course of stem rust in 2016 and Krasnodar for the North Caucasian population of stem rust against the infectious background. In the Moscow Oblast in 2016, favorable conditions for the epidemic development of stem rust arose on wheat crops. The focus of the disease arose on winter wheat in the phase of milk ripeness of grain and then switched to spring wheat. The disease affected the standard winter wheat cultivar Moskovskaya 39 by 40% with the type of response to infection 3–4, and allowed a clear differentiation of the genotypes among the sown source material on the basis of resistance, and also the evaluation of the spring wheat lines collection with known resistance Sr genes for the effectiveness of individual genes in the Moscow Oblast.

During assessing the collection of lines with known Sr genes in 2016, it was found that compared to 2013, the spectrum of effective resistance genes to this disease narrowed, which indicates possible mutational processes in the fungus population or various sources of plant disease epidemic. If in 2013, the following genes: *Sr2*, *Sr9e*, *Sr13*, *Sr22*, *Sr25*, *Sr26*, *Sr28kt*, *Sr30*, *Sr31*, *Sr32*, *Sr36*, *Sr44*, *SrWld* and combinations *Sr13* + *Sr17* and *Sr31* + *Sr38* were effective, then in 2016, only lines with the following Sr genes showed high resistance (severity 0) or resistance (up to 1% severity with the reaction type of 1 point): *Sr28kt*, *Sr30*, *Sr31*, *Sr32* and *SrWld*, and lines with the *Sr9e*, *Sr17*, *Sr25*, *Sr26*, *Sr33* and *Sr40* genes showed moderate resistance (from 5 to 20% severity with the reaction type of 2 points).

The evaluation of the created winter wheat lines for fungal diseases showed high resistance of most genotypes to local populations of leaf rust and stem rust and to powdery mildew. Only 14 out of 373 sown lines (about 4% of the genotypes) were susceptible to the *P. graminis* of the Moscow population and segregating along this trait. Even more lines (98.7%) were resistant to *P. triticina*. In the test material, there were 147 lines resistant to powdery mildew with severity up to 10% (**Table 3**). One hundred and thirty-six lines with group resistance to the three diseases were selected.

The evaluation of 367 winter wheat lines in the Krasnodar Krai made it possible to isolate 146 immune lines (severity 0) and 22 resistant lines (up to 5% severity, reaction type of 1, 2 points), that is, 46% of the genotypes which showed resistance to the North Caucasian population of stem rust. By comparing the results obtained in the Moscow Oblast and in the Krasnodar Krai, 50 genotypes that showed stability in both geographically remote points were selected.

Pathogen	Total number of lines, pcs.	Immune and resistant lines allocated, pcs.	Susceptible and segregating lines allocated, pcs.
Pucinia triticina	373	368	5
Pucinia graminis	373	359	14
Blumeria graminis	373	147	226

Table 3. The results of estimations of winter wheat lines for fungal diseases against the natural background of leaf, stem rust and powdery mildew development in the Moscow Oblast (2016).

2.2.7. Seventh stage

Evaluation of the economically valuable traits of selected stable lines in the Moscow Oblast conditions in comparison with standard cultivars, selection of the best genotypes for competitive testing.

2.2.7.1. Spring wheat

During the selection of spring genotypes, we were guided by such characteristics as the earlier (43–46 days) or simultaneous ear formation with the standard spring cultivar Lada, the optimum height of the plant (up to 110 cm), the grain mass from the ear (1.6–2.6 g) and weight of 1000 grains (45–50 g). During the selection of winter lines, the wintering of the lines was taken into account, and we also oriented toward the listed characteristics and compared them to the standard winter cultivar Moskovskaya 39. The reliability of the differences in the indices (the productivity of the ear, the mass of 1000 grains, the height) was estimated upon the results of a single-factor disperse analysis using the "Agros" statistical analysis algorithms [35]. Protein and gluten content in the grain was determined on an infrared analyzer SpectraStar 2400 in the productive lines with large grain. The content of gluten in the flour was analyzed on Glutamatik Perten device, and the quality of gluten on an IGD-3 M (the measuring instrument of gluten deformation). Other indicators of flour quality (strength and dilution) were determined on alveograph and farinograph. The main physiological trait of the selected lines is the group resistance to fungal diseases (leaf and stem rust, powdery mildew) and the presence of several identified genes of resistance to stem rust that must provide durable resistance to the *Pgt* population in the Central Federal District of the Russian Federation and on the territory of Western Siberia. The distinctive morphological sign of the majority of the lines is the presence of anthocyanin on the pericarp of the grain, which causes the grain to acquire a different degree of coloration (from dark red to dark purple). As a rule, lines with purple grain have the manifestation of anthocyanins on other organs too (stems, ears, anthers). As stated earlier, the 25 best genotypes with a set of economically valuable traits were selected among the spring progeny, which were estimated in 2017 in the control nursery for resistance to diseases, grain harvest from the plot, grain nature and its quality. The control nursery was laid in triple replication in the conditions of the Moscow Oblast (area of the registration plot 1.5 m^2). All tested lines of spring wheat confirmed their high resistance to stem and leaf rust, but none of the lines exceeded the standard cultivar Lada by the yield of grain from the plot. Only three accessions (11-17, 21-17 and 23-17) out of 25 produced a crop that is not inferior to this standard. The second standard cultivar Zlata was strongly affected by stem and leaf rust (up to 70%) and formed a yield significantly lower than Lada and some tested lines (**Table 4**). Some of the selected lines, when compared with standard cultivars, look attractive in terms of the number of days before ear formation, which was reduced by 1–2 days and height (lines 1-17, 12-17, 23-17), group resistance to diseases (lines 1-17, 4-17, 7-17, 8-17, 9-17, 12-17, 15-17, 16-17, 20-17, 23-17), the grain size (lines 12-17, 21-17, 23-17) and grain nature (lines 11-17, 15-17).

According to the results of complex assessments, seven genotypes were selected for the evaluation of grain quality (**Table 5**) and ecological testing in CFDR (Moscow Oblast, Vladimir Oblast, Tula Oblast). After the results of the environmental test, which is planned in 2018, the best prototype of the cultivar will be sent to the State Test and determination of the cultivation regions.

Line, cv	*Sr* genes	Days to heading	Height, cm	Disease severities in Moscow Region, %			Yield, g/1.5 m^2	Nature of the grain, g/l	1000 grain mass, g
				Pgt	*Pt*	*Bgt*			
1-17	*2,36,40h,44*	41	119	0	0	0	780	774	41.2
2-17	*2,36,40,44*	43	128	0	0	15	794	778	47.4
4-17	*2,36,40h*	45	132	0	0	10	735	758	47.8
7-17	*2,32,40,44*	43	140	0	0	1	664	732	40.4
8-17	*2,36,40h,44*	45	147	0	$10/_1$	10	750	782	45.0
9-17	*2,36,40h,44*	42	128	0	0	7	574	764	45.3
11-17	—	43	142	0	0	15	866	794	44.2
12-17	*2,36,40,44*	42	120	0	$1/_1$	5	630	758	48.0
15-17	—	42	135	0	0	10	790	790	46.0
16-17	—	44	123	0	0	10	650	778	47.5
20-17	*2,36,39,40,47,44*	44	123	0	0	0	661	758	41.4
21-17	*2,36,40h,44*	43	120	0	0	15	814	752	50.5
23-17	—	42	120	0	0	3	925	754	49.0
Zlata (St)	—	43	125	70	70	15	625	782	41.0
Lada (St)	—	44	131	40	40	15	1012	792	44.0
LSD < p 0.05			6	—	—	—	198	6	4.4

Table 4. Variety of spring wheat lines from the control nursery for some qualitative and quantitative traits (2017).

The analysis of grain and flour samples in 2017 is presented in **Table 5**. Grain has a good nature, corresponding to the first class and the high weight of 1000 grains (see **Table 4**). Almost all the lines have an increased grain hardness, according to the protein content in the grain, they correspond to the first class (>14.5%), and to the gluten content in grain to the second class (> 28%). This allows us to attribute them to a group of strong wheat and use them in mill grist to improve lower quality grain. The high content of gluten in flour characterizes it as a premium product. However, the quality of gluten is characterized as satisfactorily weak in the indications of the measuring instrument of gluten deformation (third group). Only one sample (8-17) for gluten quality corresponds to second group. The strength of the flour (245), determined on the alveograph, allows it to be attributed to a good filler group, and according to the dilution factor of the dough (80), the flour is at the standard level and corresponds to valuable wheat. The results of the baking test show that the volume yield of tin bread of this line exceeds the standard, and the color and porosity of the crumb are not inferior to it. Due to the high content of protein and gluten in the flour of other lines, one should also consider their other purpose, for example, making flour confectionery products, where satisfactorily weak gluten is required (GDI > 85).

According to the data available in the literature, grain cereals with anthocyanin coloration have increased antioxidant activity [36]. However, during obtaining the premium flour, the

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Line	Color of grain*	Grain hardness, %	protein in grain, %	Gluten in flour, %	GDI, units the scale of the instrument	Alveograph, W	Dough dilution, units the scale of the instrument	Volume yield of tin bread cm^3	Crumb color	Crumb porosity
4-17	3	74	15.0	37.3	97	145	130	660	2.8	3.0
7-17	2	80	13.6	29.7	98	130	110	610	3.0	3.0
8-17	3	63	14.4	32.3	87	245	80	970	4.3	4.5
9-17	4	72	15.5	33.7	97	150	100	790	2.8	3.3
12-17	3	57	16.0	36.3	97	93	150	640	2.8	2.5
16-17	3	55	15.1	40.1	102	93	175	460	2.8	2.0
17-17	4	54	15.5	34.6	105	71	125	580	2.0	1.5
St cv. Lada	2	46	13.7	29.3	71	306	80	950	4.5	4.8

*1—light red, 2—red, 3—dark red, 4—purple.

Table 5. Indicators for the quality of grain, gluten and test baking of bread in spring wheat lines with different intensity of grain coloring (harvest of 2017).

colored shells of the grain go into the bran. An attempt was made to use whole-wheat flour of bread wheat purple grain lines with a high content of antioxidants (up to 70 mg/100 g) in confectionery technologies. Whole-grain flour had an increased water holding capacity, far exceeding control. But according to their technological properties, the samples were inferior to the standard—the dough formed worse, crumbled, less amenable to laminating. The best technological properties, closest to the standard, were shown by the samples of lines 8-17, 9-17 and 17-17. Sample 17-17 was distinguished by the presence of large bran, which prevented the formation of the dough. The baked sugar cookies were better on the indicators of swelling in water (up to 78%) and specific volume (up to 0.76 g/cm^3) compared to the standard (58% and 0.62 g/cm^3, respectively). The structure of the cookies from all the samples was more crumbly and fragile than that of the standard, and the organoleptical properties (taste, smell, appearance, cross-sectional texture) were at the standard level. Using flour from whole grains in industrial conditions will allow to obtain pastry with a high yield of products suitable for healthy eating.

2.2.7.2. Winter wheat

From the 373 winter wheat lines created during the experiment, 137 were selected for further testing in breeding nurseries of the Moscow Oblast. This group also included 49 stable genotypes, which were selected during the study in the Krasnodar Krai. **Table 6** shows the diversity of the best winter wheat lines by the identified resistance Sr genes and some economically valuable traits in comparison with the standard cv. Moskovskaya 39 in the Moscow Oblast.

Lines	Pedigree	genes	Days to heading	Height, cm	Grain per ear, g	1000 grain mass, g
1-16	(113/119)/D/D	*Sr2h**,22,44,47*	257	90	2.1	50.0
149-16	(113/119)/D/D/D	*Sr2h,22,32,44*	262	95	2.2	48.0
198-16	(113/119)	*Sr31,36*	268	130	1.9	52.0
167-16	(113/119)/D/D	*Sr2h,22,36*	260	83	1.7	51.0
165-16	(96/113)/ D/96	*Sr2h,36,44*	263	97	2.0	47.0
30-16	(96/113)/ D/D/D	*Sr2h,31,47*	260	86	2.0	48.0
16-16	(96/113)/ D/D	*Sr32h,36 h,22*	259	106	1.7	46.0
43-16	(113/96)/ D/D/D	*Sr2h,22,32,40*	259	78	1.5	48.0
45-16	(113/96)/ D/D/D	*Sr22,32,44*	259	87	2.0	54.0
48-16	(113/96)/ D/96	*Sr2h,32 h,36 h*	260	93	2.4	51.0
54-16	(113/96)/ D/D	*Sr22,31,32 h,36 h*	259	99	1.9	49.0
85-16	(113/96)/96/96	*Sr2h,36,47*	262	99	2.0	45.0
86-16	(113/96)/96/96	*Sr2h,36*	263	98	2.7	49.0
99-16	(113/96)/119/96	*Sr2h,36,39,47*	264	97	1.9	54.0
326-16	(113/96)/119	*Sr2,32*	264	135	2.1	44.0
103-16	[(119/96) × (113 × 96)]/96	*Sr22,36*	264	95	1.8	46.0
128-16	(119/96)/ D/ D	*Sr2h,22,32*	261	91	1.9	61.0
138-16	(119/96)/ D/ D	*Sr2h,22,32*	259	98	2.2	57.0
129-16	(119/96)/ D/ D	*Sr22,32,44*	261	84	2.1	63.0
124-16	(119/96)/ D/ 96	*Sr2h,22,31,32*	262	115	2.3	61.0
131-16	(119/96)/D/96/96	*Sr2h,31,36,47*	263	90	1.8	49.0
St cv. Moskovskaya 39			265	115	1.9	49.0
LSD < p 0.05				25	0.50	$F_{actual} < F\ t_{theoretical}$

***h*—heterozygous state of gene.

Table 6. Some economically valuable traits of the winter wheat lines with identified genotype of resistance to *Pgt*.

Among the winter genotypes, it was possible to select the lines that formed ear 2–8 days earlier than the standard and had a shorter stem than the standard cv. Moskovskaya 39. Both attributes are of selective importance for the Central Federal District of the Russian Federation, and breeders tend to create early ripening short-stemmed analogues of productive cultivars. This is due to the climatic conditions of the CFDR: abundant rainfall with the wind during the ripening of cereals lead to lodging of cereals and crop losses. Thick stiff short stem provides resistance to lodging. Most of the created lines form a large grain with the mass of 46–60 g and the productive ear at the standard level. Several lines (86-16, 48-16) were selected, which are superior to the standard cultivar according to the productivity of the ear (grain mass from ear of 2.7 and 2.4 g, respectively). Preliminary evaluation of lines by grain quality (protein and gluten content in grain) on an infrared analyzer showed an increased value of these parameters in comparison with the Moskovskaya 39 cultivar, which is a quality standard in the non-Chernozem zone of the Russian Federation. The fluctuation of the protein content in the grain

http://dx.doi.org/10.5772/intechopen.75379

from the isolated lines was in the range of 15.2–20.2%, and the gluten content was 29.7–41.5% (cv. Moskovskaya 39 had 17.6% of protein and 31.4% of gluten in the grain). An additional assessment of the gluten content in flour, carried out on the Glutomatik device, confirmed such a high gluten content in the selected lines (37–61.3%), but the quality of gluten of most lines corresponded to the third class (GDI unit of the instrument 92–114). Such gluten is characterized as satisfactorily weak. Flour with such indicators is used in the confectionery industry for baking biscuits and cookies.

Selected winter wheat lines will have to undergo tests at the control nursery in the Moscow Oblast, and then environmental testing at three geographical locations, before they receive the status of the prototype of a new cultivar.

3. Conclusion

During the period 2010–2017, the initial material of spring and winter wheat was developed in the Moscow Scientific Research Institute of Agriculture "Nemchinovka," which differs fundamentally from the varieties of wheat that have been obtained to date. Namely, for the first time, prototypes of cultivars with group resistance to the most widespread fungal diseases in the Central Federal District of Russia (leaf and stem rust and powdery mildew) were developed. Resistance to stem rust is determined by the presence of 2–4 effective resistance genes not only to the European but to the North Caucasian and West Siberian *Pgt* pathogen populations. Taking into account the presence of the APR gene *Sr2* with other effective genes *Sr22*, *Sr32*, *Sr39*, *Sr40*, *Sr44* and *Sr47*, lines can also have a selection value for regions where the rust race Ug99 is common. The genetic diversity of lines, as far as the spectrum of resistance genes is concerned, differs from that obtained earlier in the world practice. The possibility of creating such genotypes in a short time is explained by the presence of original resistance donors having in their genealogy an alien genetic material of species relatives (*Aegilops speltoides, Ae. triuncialis, Triticum kiharae, Secale cereale, T. timopheevii, Ae. tauschii*) and the presence of several effective Sr genes in donors identified using specific molecular markers. The advantage of the used donors was the presence of other selection valuable traits such as resistance to leaf rust and powdery mildew, early ripeness and the presence of a short stem. As a result of simple, stepped and backcross crossings with subsequent self-pollination, hybrid populations were obtained from which the individual plants were initially selected, and then on their basis, lines were obtained that were tested for resistance to stem rust at three geographical locations: Moscow, Krasnodar and Omsk. According to the results of testing, the progeny in breeding nurseries of the Moscow Oblast and the results of genotypes resistance to stem rust, the lines of spring and winter wheat are selected in three geographical locations, which form the crop at the level of standard cultivars without the use of chemical protection agents during cultivation. This technology allows to get environmentally friendly products for a healthy diet. In fact, these are new prototypes of spring and bread wheat cultivars for the Central Federal District of Russia, which can be used as donors of resistance to stem rust while improving wheat in other regions. These lines have some morphophysiological features such as the presence of anthocyanin on the stem, anthers and grain. The presence of anthocyanins gives the grain an increased content of antioxidants and increased resistance to unfavorable environmental factors, according to the literature. Technological evaluation of the grain from the created lines of spring and winter wheat showed an increased content of protein and gluten in flour, which allows them to be classified as a group of strong wheat and used in mill grist to improve the lower quality grain in baking. However, the quality of gluten in new lines is characterized as satisfactorily weak. An attempt to define a different direction for the use of such grains in the food industry, taking into account grain coloring by anthocyanins, namely, in the production of flour confectionery products (sugar cookies) has been undertaken. Product from whole-wheat flour exceeded the standard baking on the basis of the features of swelling in water, volume, crumbliness and fragility, but in organoleptical indicators was not inferior to the standard. It is concluded that the use of whole-grain flour with increased antioxidant activity for baking confectionery products determines the use of this grain for healthy food (not only because of the lack of residual chemical protection agents not used in cultivating such varieties, but also due to the presence of anthocyanins in the grain and its antioxidant properties). Taking into account the conducted researches, a new direction in selection for the Central Federal District of Russia is defined: development of spring and winter wheat varieties with group resistance to fungus diseases and with grain suitable for healthy nutrition.

Acknowledgements

This study was partially supported by the Russian Foundation for Basic Research in 2013–2015.

Conflict of interest

The authors declare that they have no conflict of interest.

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