New prototypes of winter wheat varieties allotted for competitive testing during pre-breeding process

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Abstract. A new original initial material of winter wheat was created by using stem rust resistant donors. The new lines resistant to leaf and stem rust were assessed under the field conditions in the North Caucasus and the Central region of the Russian Federation. Some of the resistant genotypes were assessed in laboratory conditions for abiotic stress resistance to: salinity, acidification, and prolonged flooding of seeds in water. A two-year assessment of the lines productivity in the control nursery under the conditions of the Moscow region 7 genotypes of winter wheat were identified with high and medium resistance to lodging, group resistance to fungal diseases, heading 5-7 days earlier than the standard, the optimal plant height of 90-110 cm, the average development of grain per ear and 1000 grains mass and forming good quality yield. Lines 2-19w, 9-19w, 16-19w, 36-19w, 63-19w, 90-19w, 92-19w will be tested in competitive variety testing and may be presented as prototypes of varieties for the Non-Black Earth Central Region.

1. Introduction
Resistance to biotic and abiotic environmental factors should be an integral characteristic. The soil and climatic conditions diversity in the Russian Federation requires the development of productive varieties adapted to various stress factors [1, 2, 3, 4]. In the Non-Black Earth Region of Russia, these biotic factors include contemporary soft wheat varieties and the development of fungal diseases. Annually sprouts are attacked by snow mold, and adult plants - by powdery mildew, septoriosis, leaf rust, and stem rust damage were observed in the last decade. The most frequently observed factor among others is waterlogging of the soil on the one hand, and a quite frequent spring air-soil drought. The presence of a large number of acidic soils areas is a crucial trait of this area as well. Agricultural lands reclamation and use of protective chemicals help producers to a certain extent to get stable yields of spring and winter soft wheat, but do not cancel the requirements for selection of varieties that are genetically resistant to diseases and changeable factors of cultivation.

Soft wheat relatives are the source of resistance to the listed aggressive environmental factors; however, the process of development of the initial material by the method of remote hybridization is laborious and time-consuming. Therefore, even large plant-breeding centers prefer to use the varieties and accessions that have alien translocations, created in previous years and being stored as collections.
in the Federal Research Center All-Russian Institute of Plant Genetic Resources named after the N. I. Vavilov (Saint Petersburg) and other National Resource Conservation Centers. Federal Research Center "Nemchinovka" has its own collection of introgressive lines of spring and winter wheat "Arsenal" with genetic material of the species Ae. speltoides, Ae. triuncialis, T. kiharae, S. cereale, as well as varieties accessions from other selection centers, which are intensively used for the purposes of soft wheat advancement at the laboratory of genetics and pre-breeding of the Federal Research Center "Nemchinovka". The purpose of this study is to evaluate in the control nursery (CN) complex of economically valuable traits of the winter soft wheat lines, developed by using donors with alien genetic material, and to select genotypes for competitive testing in the Non-Black Earth Region of the Russian Federation.

2. Materials and methods
The lines of winter soft wheat obtained by the method of complex stepwise hybridization of new donors that are resistant to stem rust were the object of the study [5]. These are accessions of winter wheat GT 96/90 (Bulgaria), Donskaya semi-dwarf variety, wheat-Aegilops-rye line 119/4-06rw and spring line 113/00i-4 with genetic material of the species Ae. triuncialis and T. kiharae. By the time of testing in CN, the lines were tested for resistance to powdery mildew, to leaf and stem rust under the Moscow region conditions with a natural background of disease development (2016-2018) and with an artificial background of infection by leaf and stem rust under the Krasnodar Region conditions in 2016 [6]. Diseases severities of plants caused by leaf and stem rust in the field conditions were estimated as a percentage, and the type of reaction - according to Stakman [7]. The septoriosis evolving process was assessed by the intensity of damage to the surface of flag and pre-flag leaves according to the James scale [8]. The recording was carried out at the end of flowering stage.

The lines productivity and the grain quality were evaluated in the CN in 2019-2020. 2019 was moderately humid and favorable for the development of winter wheat plants, while cold springs and heavy rainfalls characterized 2020 during the period of heading and maturation of crops. The control nursery was established on a black fallow on one and a half meter allotments in three-times replications. The winter wheat Moskovskaya 39 was used as a standard variety. The following parameters were assessed: grain yield g/m², ear productivity in grams, mass of 1000 grains. Statistical indicators and reliability of differences were determined in comparison with the standard variety by using the algorithms of statistical analysis "Agros" [9]. For lines that form a yield at the standard level or higher, the protein and gluten content in the grain was determined by using infrared analyzer SpectraStar 2400, the gluten content in the flour was analyzed on Glutomatic Perten device, and the quality of gluten - on an IGD-3M (the measuring instrument of gluten deformation). The baking test of pan bread and hearth bread was conducted. The volumetric yield of bread and the porosity of the crumb were determined. Range stress resistance was studied in laboratory conditions at the early stages of ontogenesis by using NaCl salinization, Al₂(SO₄)₃ acidification, and long-term flooding of seeds in water. The Semushkin, Khazava, Udovenko’s method [10] determined salinity tolerance, and the resistance to anaerobic stress was determined by the method of Beletskaya, Ostaplyuk [11].

3. Results and discussion
In the conditions of 2018-2019, 103 winter wheat lines were evaluated in the CN. The best lines were selected on the base of immunological, phenotypic assessments, as well as the results of one-way analysis of variance for height and productivity elements. When selecting the best genotypes, first of all, attention was paid to the resistance to fungal diseases, the number of days before heading, and the elements of productivity. In total, in 2019, 24 lines with a complex of economically valuable features were selected. The largest group of lines (12 pcs.) was represented by genotypes that produce a yield at the level of a standard variety, have a shorter stem and headed 5-8 days earlier than the standard (ranges 9-19°, 16-19°, 31-19°, 32-19°, 36-19°, 40-19°, 49-19°, 62-19°, 63-19°, 87-19°, 92-19°, 96-19°). Reliably several genotypes exceeded the standard variety in yield (91-19°, 93-19°, 97-19°, 98-19°), but they were tall and headed only 2-4 days earlier than the standard one. And, finally, the third
group of lines was represented by genotypes, which quantitative traits did not differ from the Moskovskaya 39 variety, but demonstrated high resistance to powdery mildew, as well as leaf and stem rust in two environmental zones: under the conditions of the Central Non-Black Earth Region and the North Caucasus (lines 2-19*, 20-19*, 37-19*, 58-19*, 72-19*, 73-19*, 74-19*, 90-19*).

To assess the adaptive qualities of new lines to abiotic stresses (flooding of seeds, salinization with sodium chloride, acidification with aluminum sulfate), 14 of these best genotypes were selected and tested. The lines with the highest level of resistance were selected: to sodium chloride 9-19*, 20-19*, 37-19*, to aluminum sulfate: 36-19*, 35-19*, 2-19* and to flooding - 37-19*, 32-19* and 16-19*. Three genotypes with high complex adaptability to all stress factors - 9-19*, 37-19* and 32-19* were picked up. Twenty-four lines were retested under 2020 conditions. The results of quantitative traits development in 2019 and 2020 are shown in the Table 1.

Table 1. Development of quantitative characteristics of the best winter wheat lines in CN under the Moscow region in 2019 and 2020.

| Line, variety | Quantity of days from germination to heading | Plants height, cm | Grains on ear, g | 1000-grains mass, g | Yield of 1.5 m² |
|---------------|---------------------------------------------|------------------|-----------------|---------------------|-----------------|
| 2-19*         | 248                                         | 261              | 117             | 103                 | 1.2             | 1.3             | 44.0 | 47.5 | 882  | 817.0 |
| 9-19*         | 249                                         | 263              | 122             | 120                 | 1.6             | 1.5             | 41.0 | 46.3 | 847  | 1277 |
| 16-19*        | 246                                         | 257              | 103*            | 100.3               | 1.6             | 1.3             | 44.3 | 44.0 | 851  | 906   |
| 20-19*        | 252                                         | 263              | 135             | 126.8               | 2.2*            | 1.53            | 46.7 | 40.4 | 947.1 | 1043.4 |
| 31-19*        | 247                                         | 263              | 110*            | 88.8*               | 1.6             | 1.34            | 49.6* | 48.8* | 765.3 | 915   |
| 32-19*        | 247                                         | 263              | 108.3*          | 90.0*               | 1.6             | 1.0             | 50.2* | 49.8* | 850.8 | 866.4 |
| 36-19*        | 248                                         | 261              | 111.6*          | 107.0*              | 1.9             | 1.5             | 49.5* | 47.3* | 974.4 | 997.0 |
| 37-19*        | 252                                         | 264              | 128.3*          | 135*                | 2.2*            | 1.6             | 51.5* | 45.0* | 845.1 | 954   |
| 40-19*        | 250                                         | 267              | 107.5*          | 117.5               | 2.2*            | 1.2             | 48.4* | 44.2* | 1035 | 754.3 |
| 49-19*        | 250                                         | 265              | 110*            | 100.0               | 1.7             | 1.8             | 47.7 | 49.7 | 853 | 866.1 |
| 58-19*        | 251                                         | 267              | 116.7           | 108.8               | 1.5             | 1.2             | 41.0 | 39.0 | 888.3 | 774.3 |
| 62-19*        | 247                                         | 266              | 115             | 101.3               | 1.8             | 1.1             | 45.3 | 43.1 | 910.2 | 839.4 |
| 63-19*        | 247                                         | 263              | 111.7*          | 100                 | 1.5             | 1.1             | 42.5 | 45.0 | 931.5 | 948.8 |
| 72-19*        | 248                                         | 261              | 126.7           | 123.3               | 1.7             | 1.3             | 41.0 | 46.7 | 890.4 | 790.0 |
| 73-19*        | 249                                         | 266              | 118.3           | 103.4               | 2.0             | 1.3             | 43.0 | 41.0 | 946.2 | 693.3 |
| 74-19*        | 249                                         | 266              | 120             | 113.8               | 2.0             | 1.4             | 43.3 | 42.1 | 881 | 927.8 |
| 87-19*        | 250                                         | 266              | 113.3*          | 107.5               | 1.6             | 1.3             | 51.0* | 47.1 | 777.3 | 761.7 |
| 90-19*        | 251                                         | 267              | 88.3*           | 110.0               | 2.9*            | 2.0             | 51.0* | 40.4 | 300 | 852.3 |
| 91-19*        | 250                                         | 262              | 130*            | 119.4               | 2.0             | 1.34            | 48.0* | 47.2 | 120.0 | 998.2 |
| 92-19*        | 249                                         | 267              | 115*            | 112.8               | 1.9             | 1.5             | 43.4 | 43.1 | 958.3 | 850.6 |
| 93-19*        | 252                                         | 269              | 136.7           | 111.3               | 1.8             | 1.44            | 46.0 | 43.6 | 1100* | 770 |
| 96-19*        | 253                                         | 266              | 113.3*          | 112.8               | 1.9             | 1.4             | 49.3* | 42.3* | 876 | 1138.4 |
| 97-19*        | 250                                         | 266              | 116.7           | 103.1               | 2.2*            | 1.2             | 43.6 | 41.6 | 1201* | 758.3 |
| 98-19*        | 250                                         | 267              | 131.7*          | 107.8               | 2.2*            | 1.7             | 45.7 | 43.0 | 1053* | 660 |
| M             | 254                                         | 270              | 121.7           | 110                 | 1.6             | 1.7             | 45.2 | 39.1 | 813 | 953.1 |

LSD P<0.05  5.8  18.5  0.4  F_act<F_teor  2.8  F_act<F_teor  228.6  F_act<F_teor
In general, the conditions of 2019 were favorable for the plants growth and development. It was characterized by warm April and May and did not differ significantly from the average long-term values in the amount of precipitation during the growing season. The tested lines demonstrated a significant differentiation in stem length (from 89 to 135 cm) and a variation of traits in the values of ear productivity (from 1 to 2.9 g), 1000-grain mass (from 41 to 51 g). 4 lines which formed the yield reliably higher than the standard one (91-19*, 93-19*, 97-19*, 98-19*) were defined. These lines were tall, but did not lodge down even during the heavy rainfall before harvesting.

The growing conditions in 2020 were generally characterized as warm and humid, but with the lack of active temperatures in April and May. In that testing year the differences between the lines were negated, and the indicators of the productivity elements were reduced. Differences in ear productivity, 1000-grain mass and yield capacity were not significant comparing to the standard variety. The cold spring and the lack of the summed active temperatures above 10 degrees led to a delay in winter crops heading. In 2020, the Moskovskaya 39 variety headed out 16 days later, and the lines 11-16 days later than in 2019. After heading winter crops, the leaves were infected with Septoria. The disease evolving began in the lower tiers of leaves, and within two weeks the disease had spread to flag leaves. The development of leaf and stem rust in 2020 during the flowering of winter wheat was not observed. Decreased photosynthesis and rapid yellowing and drying of leaves during grain filling led to the decrease in yield of most part of ranges. For a number of lines, the situation was aggravated by crop lodging during heavy rainfall in July in the phase of grain filling and ripening. Lodging resistance proved to be a critical factor in the selection of ranges for competitive variety testing.

Negative selection of lines with regard to tall-growing and low resistant to lodging (3 points) plants, even if they formed a high yield, was executed. For further testing, 7 genotypes were selected. They were characterized by: high and medium resistance to lodging, high resistance to fungal diseases (Table 2), the heading date 5-7 days earlier than the standard, an optimal plant height of 90-110 cm, average development of grain mass per ear and 1000-grain mass and forming the grain yield at the level of Moskovskaya 39. These are the ranges: 2-19*, 9-19*, 16-19*, 36-19*, 63-19*, 90-19*, 92-19*.

Table 2. Characteristics of winter wheat lines for resistance to lodging and diseases under the field conditions (2019-2020) and abiotic stress in laboratory experiments.

| Line, variety, point | Lodging resistance | Disease severities in Krasnodar 2019 | Disease severities in Moscow region in 2019 | Disease severities in Moscow region in 2020 | Abiotic stress resistance revealed |
|----------------------|---------------------|---------------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------------|
|                      | Pgt* | Pt** | Pt | B. g. *** | Septoria sp. |  |
| 2019                 | 2019 | 2020 |    |            |               |  |
| 2-19*                | 9/20 | 1/5  | 3/5 | 1           | 33             | Al (SO₄)₃                      |
| 9-19*                | 12/20| 1/5  | 3/5 | 3           | 28             | NaCl                           |
| 16-19*               | 12/10| 1/10 | 5   | 5           | 35             | flooding                       |
| 20-19*               | 5/20 | 2/20 | 5/20| 5           | 24             | NaCl                           |
| 31-19*               | 5/10 | 1/10 | 2/5 | 1           | 30             | -                              |
| 32-19*               | 5/10 | 1/10 | 2/5 | 1           | 40             | flooding                       |
| 36-19*               | 5/20 | 1/10 | 1/1 | 5           | 28             | Al₂ (SO₄)₃                     |
| 37-19*               | 5/20 | 1/1  | 0   | 5           | 50             | NaCl, flooding                 |
| 40-19*               | 3/20 | 0/1  | 1/1 | 5           | -              | flooding                       |
A full technological analysis of grain and baking test were carried out for these lines (Figure 1, Table 3). Almost all the selected lines accumulated high protein and gluten content in the grain, regardless of the year of cultivation. Despite the high content of protein and gluten, the decrease in the quality of baked goods to 3-4 points was observed due to low gluten (high IGD), which can be explained by the lack of higher temperatures during grain filling.

|   | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| 49-19* | 9 | 5 | /5 | 0 | 0 | 5 | 13 | - |
| 58-19* | 9 | 3 | 0 | /1 | 0 | 1 | 45 | - |
| 62-19* | 9 | 5 | /5 | /2/5 | 0 | 5 | 17 | - |
| 63-19* | 9 | 5 | /5 | /2/5 | 0 | 7 | 38 | - |
| 72-19* | 9 | 7 | 2/20 | /1/5 | /1 | 0 | 11 | - |
| 73-19* | 9 | 5 | /50 | /5 | 0 | 3 | 36 | - |
| 74-19* | 9 | 5 | 0 | 0 | /1 | 5 | 50 | - |
| 87-19* | 9 | 3 | 0 | /2/5 | 0 | 0 | 40 | - |
| 90-19* | 9 | 5 | /5 | /1 | 0 | 5 | 17 | - |
| 91-19* | 9 | 7 | 0 | 2.3/10 | /15 | 0 | 33 | - |
| 92-19* | 9 | 5 | /10 | 2.3/10 | /5 | 0 | 30 | - |
| 93-19* | 9 | 3 | /5 | /1 | 0 | 3 | - | - |
| 96-19* | 9 | 9 | /40 | /1 | /1 | 0 | 28 | - |
| 97-19* | 9 | 3 | /50 | 2.3/10 | 2.3/10 | 3 | 53 | - |
| 98-19* | 9 | 3 | /30 | 0 | 0 | 5 | 41 | - |

**M 39**

|   | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| (St) | 5 | 2.3/10 | 2.3/10 | /20 | 1 | 32 | - | - |

* - Puccinia graminis f.sp. tritici (stem rust).
** - Puccinia tritici (leaf rust).
*** - Blumeria graminisf.sp.tritici (powdery mildew).

Figure 1. Bread of Moskovskaya 39 standard grain (above) and the line 92-19*.
Table 3. Grain quality indicators for the best winter wheat lines under the CN of Moscow region conditions in 2019 and 2020 and the baking test results in 2020.

| Range, variety | Density of grain, g/l | Protein in grain, % | Gluten in grain, % | GDI, the appliance scale | Volume of bread output, cm³ | Sponginess, score | General assessment of hearth bread |
|----------------|-----------------------|---------------------|-------------------|--------------------------|----------------------------|-------------------|-------------------------------|
|                | 2019  | 2020  | 2019  | 2020  | 2019  | 2020  | 2019  | 2020  | 2019  | 2020  |
| 2-19w          | 781   | 782   | 21.1  | 18.8  | 47.8  | 35.6  | 93    | 870   | 3.5   | 4.4   |
| 9-19w          | 765   | 811   | 14.5  | 14.1  | 33.9  | 23.0  | 88    | 830   | 3.0   | 3.5   |
| 16-19w         | 783   | 760   | 16.4  | 16.1  | 44.4  | 34.1  | 91    | 800   | 3.3   | 3.8   |
| 31-19w         | 740   | 17.9  | 18.6  | 42.7  | 41.2  | 32-19w | 761   | 726   | 19.9  | 19.5  | 56.8  | 41.3  |
| 36-19w         | 808   | 797   | 16.4  | 14.8  | 35.8  | 30.5  | 78    | 940   | 3.5   | 4.0   |
| 40-19w         | 739   | 16.5  | 16.5  | 34.8  | 34.8  | 49-19w | 796   | 732   | 17.2  | 18.1  | 42.8  | 38.5  |
| 58-19w         | 813   | 768   | 17.5  | 17.6  | 44.2  | 38.1  | 62-19w | 746   | 724   | 18.1  | 18.3  | 40.1  | 40.1  |
| 63-19w         | 790   | 754   | 16.4  | 16.4  | 38.1  | 35.1  | 91    | 880   | 3.3   | 3.8   |
| 73-19w         | 760   | 18.1  | 17.2  | 37.2  | 37.2  | 74-19w | 746   | 17.1  | 17.4  | 39.5  | 36.9  | 90-19w | 748   | 16.0  | 34.0  | 94    | 690   | 2.8   | 3.0   |
| 92-19w         | 742   | 18.1  | 38.3  | 87    | 1030  | 96-19w | 787   | 757   | 15.9  | 14.4  | 38.4  | 28.3  | M39   | 819   | 777   | 18.7  | 16.5  | 40.2  | 32.5  | 51    | 1170  | 4.0   | 4.5   |

However, in practical baking, this feature may be easily eliminated by adding grain with tighter gluten when forming grinding batches. By the volumetric bread output samples 92-19w, 36-19w, 63-19w and 2-19w were distinguished. Lines 63-19w and 2-19w were submitted to the Institute of Seed Production and Agrotechnology - (formerly Ryazan Research Institute of Agriculture) for competitive variety testing; line 9-19w to the Omsk State Agrarian University, and the rest of the genotypes were sown in the competitive variety testing in the Moscow region.

4. Conclusion
The result of pre-breeding studies (2010-2020) include:
- evaluation of the original collection material and the selection of donors resistant to stem and leaf rust;
- development of the initial material by the method of complex stepwise crossing of donors;
- the subsequent assessment of this initial material within different ecological-geographical locations (North Caucasus and Central Regions) for disease resistance check, and laboratory assessment of the adaptive properties of new breeding lines to abiotic stresses (flooding of seeds, salinization with sodium chloride, acidification with aluminum sulfate);
- a two-year assessment of the lines productivity in the CN under the conditions of the Moscow region 7 genotypes of winter wheat were identified with high and medium resistance to lodging, group resistance to fungal diseases, heading 5-7 days earlier than the standard, the optimal plant height of 90-110 cm, the average development of grain per ear and 1000 grains mass and forming good quality
yield. Lines 2-19°, 9-19°, 16-19°, 36-19°, 63-19°, 90-19°, 92-19° will be tested in competitive variety testing and may be presented as prototypes of varieties for the Non-Black Earth Central Region.

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