Resistance to stem rust and a molecular marker analysis of *Sr* genes in spring bread wheat introgression lines

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DOI 10.18699/ICG-PlantGen2019-15

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Abstract: 128 introgression lines of spring bread wheat developed by Agricultural Research Institute of the South-East Region and 11 cultivars cultivated in the Volga Region were analyzed for resistance to the Ug99 race group in Kenya (KARI). 10 immune lines (infection type 0) and 16 lines with moderate resistance (infection type MR) were identified. Cultivars and part of the lines (58 introgression lines) were assessed for resistance to Lysogorsk and Omsk stem rust pathogen populations and to stem rust isolates PgtZ1(TKSTF) and PgtF18.6 (TKSTF+*Sr33*) and also analyzed for the presence of the known Sr resistance genes (*Sr22, Sr25, Sr26, Sr31, Sr35, Sr36, Sr38, Sr39*) using molecular markers. The *Sr31/Lr26, Sr25/Lr19, Sr22, Sr35* and *Sr38/Lr37* genes were identified in the introgression lines. All lines carrying *Sr31/Lr26* and *Sr22* were resistant to all local pathogen populations taken into analysis. The *Sr26, Sr36* and *Sr39* genes were not detected in the wheat lines analyzed.
Key words: spring bread wheat; introgression lines; *Puccinia graminis* f. sp. *tritici*; Ug99; *Sr* genes.

1. Introduction

Stem rust of wheat, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn., is one of the most devastating diseases of wheat worldwide. The emergence of a new highly aggressive stem rust race in Uganda in 1999, Ug99 (TTKSK), infecting wheat cultivars which contain the *Sr31* gene (Pretorius et al., 2000) caused great concern in world wheat producers, because the epiphytotic development of this pathogen on susceptible cultivars can result in yield losses reaching 100 % (Hailu et al., 2015). On the other hand, new aggressive races of stem rust that differ from Ug99 have appeared in the world including Europe and the Russian Federation. In the Russian Federation, the epiphytotic development of the disease was noted in 2015, 2016, 2017 and 2018 in Western Siberia.

In the Lower Volga Region the strong epiphytotics of stem rust was observed in 2004 and 2006 (the severity of disease development was 50–60 %), in 2016 in the Saratov Region the degree of disease development reached 80 %, the yield losses were more than 50 %, and the weight of 1000 seeds was at the level of 18–19 grams (Sibikeev et al., 2017; Sibikeev, unpublished data). Furthermore, in 2016, the spread of stem rust on spring bread wheat cultivars during earing was noted throughout the Republic of Tatarstan (Vasilova et al., 2017).

It is a fact that as a result of breeding aimed at the productivity and quality of grain, there was a strong reduction in wheat genetic diversity in many aspects, including resistance to fungal diseases (Wulff, Moscou, 2014).

Thus, the expansion of the genetic basis of varieties, obtaining breeding material, diverse in resistance genes is extremely important. The problem of searching for new resistance genes is successfully solved by application in the breeding alien wheat species from such genera as *Aegilops*, *Secale* and *Agropyron* (Rahmatov et al., 2016; Lapochkina et al., 2017; Kishii, 2019).

In the framework of our project, these are 128 introgression lines with genetic material from *Tritcum durum*, *T. dicoccum*, *T. kihara*, *T. persicum*, *T. timopheevii*, *T. compactum*, *Aegilops squarrosa*, *Ae. speltoides*, *Agropyron elongatum*, *Secale cereale*, Satu triticale, which gives a wide opportunity to identify known and search for new resistance genes. The Lower Volga region is one of the main bread wheat production zones.

Rust pathogen inoculum, in addition to the local population, is spread into the Lower Volga region from Western Europe, the North Caucasus, Central Asia, and North Africa through the Middle East (Iran) and the Caspian Sea, which greatly increases the likelihood of the spread of race Ug99 into the Russian Federation.Our study is devoted to evaluation of the genetic potential of wheat breeding material and the identification of resistance genes that are effective not only against local populations of the pathogen, but also against Ug99 and its biotype, as well as against the aggressive races of *P. graminis* f. sp. *tritici* outside the Ug99 lineage.

Therefore, the aims of this study were to (i) evaluate the resistance of introgression lines (128) and spring bread wheat cultivars to the stem rust race Ug99 lineage; (ii) evaluate resistance of introgression lines (58) and spring bread wheat cultivars (11) to the local population of stem rust and to identify *Sr* genes using available molecular markers.

2. Materials and methods

The breeding material from the FSGFSI ARI of the South-East Region (128 introgression lines) and 11 cultivars of spring bread wheat cultivated in the Volga Region and related to the analyzed lines were used in the work. The lines were obtained with the participation of CIMMYT synthetics, durum wheat cultivars, direct crossing with alien species such as *Agropyron elongatum, Ag. intermedium*, different species of the genus *Triticum* L., *Secale cereale* and Satu triticale.

The Omsk and Lysogorsk populations of the pathogen, collected in 2018 from the wheat cultivar Favorit, which carries the 6Agi (6D) substitution, were used for laboratory assessment of resistance at the seedling stage. Also in analy-

ses we used two stem rust isolates. PgtZ1 (TKSTF) from the Zernograd pathogen population and F18.6 (TKSTF+*Sr33*) from Lysogorsk populations of *P. graminis* f. sp. *tritici*. The virulence of the pathogen isolates was evaluated using a set of 20 differentiators (North American differential set) (Cereal Disease Laboratory) and near isogenic lines (24 lines). The cultivars Avrora (*Sr31*) and Khakasskaya (susceptible control) were also used in the analysis. Inoculation of plants was carried out in accordance with the methods adopted in the world practice (Jin et al., 2007).

The reaction of seedlings to inoculation (IT) with the spore suspension of the stem rust pathogen was taken into account on the 12-14th day after infection on a standard scale (Stackman et al., 1962). Evaluation of the resistance of introgression lines to race Ug99 was conducted on the base of the Kenya Agricultural Research Institute (KARI) in 2018. The criteria for evaluation of adult plants' resistance were the ITs and the degree of plant damage on the scale recommended by CIMMYT (Roelfs et al., 1992).

DNA was isolated from five-day wheat seedlings using cetyltrimethylammonium bromide (CTAB) (Murray, Thompson, 1980). DNA markers recommended for marker-assisted selection (MAS https://maswheat.ucdavis.edu/) were used to identify resistance genes (*Sr22*, *Sr25*, *Sr26*, *Sr31*, *Sr35*, *Sr36*, *Sr38*, *Sr39*), separation of PCR products was carried out in 2 % agarose gels. Near isogenic lines and cultivars with *Sr* genes served as the positive control, the susceptible cultivar Khakasskaya served as the negative control.

3. Results and discussion

3.1 Phytopathological analysis of resistance to Ug99 in Kenya

The prospect of the analyzed introgression wheat lines for resistance breeding was confirmed by the results of the analysis conducted on the base of the Kenya Agricultural Research Institute (KARI). The analysis was conducted against the background of a strong disease development (up to 90S on susceptible varieties). The racial composition of the pathogen population included races such as TTKSK (Ug99), TTKST (Ug99 + *Sr24*), TTTSK (Ug99 + *Sr36*), TTKTK (virulent to *SrTmp*), TTKTT (*Sr24* and *SrTmp*). In the result, from the 128 introgression lines studied in Kenya, 10 lines were immune (reaction type 0).

Also, 16 lines showed reaction type 5MR, all the ten varieties tested (Saratovskaya 55, Saratovskaya 68, Saratovskaya 73, Albidum 32, Prokhorovka, Yugo-Vostochnaya 2, Dobrynya, Favorit, Voyevoda and Lebedushka) were not resistant to the Kenyan population of *P. graminis*. The lowest reaction types were 10M with 'Favorit' (*Sr6Agi*) and 20M with 'Dobrynya' (*Sr25/Lr19*).

3.2 Phytopathological analysis of resistance to stem rust at the seedling stage

At the next stage of work, 58 lines and 11 wheat cultivars were evaluated for resistance to the Saratov population of stem rust under field conditions and for resistance to the Omsk and Lysogorsk pathogen populations and to the two monopustule isolates (TKSTF, TKSTF+*Sr33*) at the seedling stage under laboratory conditions. All introgression lines were resistant to the Saratov population of stem rust in the field in 2018.

During evaluation at the seedling stage, 42 lines were resistant to the Omsk pathogen population and 32 lines were resistant to the Lysogorsk pathogen population. When evaluating resistance to isolates PgtZ1 (TKSTF avirulent to *Sr11*, *Sr17*, *Sr24*, *Sr31*) and PgtF18.6 (TKSTF+ virulent to *Sr33*), it was found that 15 lines were resistant and 7 were heterogeneous to PgtZ1, 15 lines were resistant and 5 were heterogeneous to PgtF18.6.

Only 11 lines were resistant to the Omsk and Lysogorsk pathogen populations and to both isolates of *P. graminis* used in analysis. During laboratory analysis of data on 11 cultivars, the following results were obtained: 'Prokhorovka', 'Yugo-Vostochnaya 2' and 'Dobrynya' were resistant to the Lysogorsk population of stem rust; 'Prokhorovka' and 'Yugo-Vostochnaya 2' were resistant to the Omsk pathogen population. Also, only 'Prokhorovka' (*Sr31*), 'Yugo-Vostochnaya 2' (*Sr31*) and 'Lebyodushka' (*Sr25+Sr6Agi*) are resistant to PgtF18.6, while 'Prokhorovka' and 'Yugo-Vostochnaya 2' are resistant to PgtZ1.

3.3 Identification of resistance genes

From the genes known to be ineffective against Ug99, but effective against local stem rust populations, the *Sr31* gene in the introgression lines was identified. The *Xscm9* marker, developed for the 1BL.1RS rye translocation carrying the resistant genes to stem (*Sr31*), leaf (*Lr26*) and yellow (*Yr9*) rust and powdery mildew (*Pm8*), was used to identify it.

The 1BL.1RS translocation (gene *Sr31*) was identified in 15 lines out of 58. All samples carrying the 1BL.1RS translocation were resistant to the Saratov population of stem rust during the field evaluation and to all the rest of the analyzed pathogen populations during resistance evaluation at the seedling stage.

It was also concluded that the *Sr31* gene still retains its effectiveness toward local stem rust populations.

The *Sr25/Lr19* gene was identified in 40 lines (69 %) using the Gb marker recommended for the marker-assisted selection. The combination of the *Sr31/Lr26* and *Sr25/Lr19* genes was identified in 12 wheat lines. The *Sr38* gene was detected in two lines using the VENTRIUP-LN2 primers; at this stage, the combination of the *Sr25/Lr19+Sr38/Lr37* genes was identified in these lines. The *Sr22* gene was introgressed from *T. monococcum* L. ssp. *Aegilopoide*s to wheat.

Among the analyzed set of introgression lines there were three in the pedigree of which was line W3435 carrying *Sr22*. For the identification of *Sr22*, three closely linked molecular markers, *Xcfa2019*, *Xcfa2123* and *Xwmc633*, were used with the size of diagnostic fragments of 238, 234, and 117 bp, respectively.

In the analysis, line SWSR22TB containing the *Sr22* gene and the parent line W3435 (*Sr22*) was used as a positive control. According to the results of PCR analysis, *Sr22* can be postulated in two lines (L503 / W3534 // L503 / 3 / L503; L503 / W3534 // L503).

In both lines, a combination of the *Sr25 / Lr19 + Sr22* genes was identified and both of these lines were resistant to the Omsk and Lysogorsk pathogen populations and to *P. graminis* f. sp. *tritici* isolates PgtZ1 and PgtF18.6. The *Sr35* gene using

the *Xcfa2170* marker was identified in one line, 1230/2 (L503 / Sr 35 // L503 / 3 / L503), obtained from crossing lines L503 (*Sr25*) and *Sr35*. As the control, the parent line *Sr35* and the line Marquis * 5 / G2919 (*Sr35*) were used. The *Sr25/Lr19* and *Sr35* genes were indentified in this line. Previously, line 1230-1 (L503 / Sr 35 // L503 / 3 / L503) with a possible presence of the *Sr35* gene was immune to Ug99 in Kenya.

The assumption of the presence of the *Sr35* gene in line 1230-1 was confirmed with the *Xcfa2170* marker in the present work. Thus, a combination of the *Sr35*+*Sr25/Lr19* genes was identified in this line. No known stem rust resistance genes from *Ae. speltoides* were identified in the introgression lines, although the presence of introgressions from this species in the lines suggested that genes such as *Sr39/Lr35* could be present.

Thus, the *Sr31/Lr26* and *Sr25/Lr19* genes were mainly identified in the introgression lines. The combination of these genes (*Sr31*+*Sr25*) was found in 15 lines (26.3 %). The *Sr26*, *Sr36* and *Sr39* genes were not detected in the analyzed wheat lines.

The strategy of anticipatory breeding for immunity is based, on the one hand, on studying the pathogen population, identifying dangerous pathotypes capable of overcoming resistance genes used in cultivated varieties, and, on the other hand, on finding effective resistance genes for them and then introducing these genes into the local, well-adapted germplasm. Such a strategy will make it possible to obtain a source material for breeding that is resistant to leaf and stem rust, including Ug99, and adapted for the zone of the Lower Volga region.

This work is an ongoing study, the goal of which is to obtain initial material for stem rust resistant breeding, including Ug99, and adapted for the zone of the Lower Volga region. Previously, we identified *Sr* genes in 57 introgression lines (Baranova et al., 2019), and now in 58 lines.

On the whole, in the lines studied, we identified mainly the *Sr31* and *Sr25* genes and their combination. In two lines, we identified *Sr22*. Its presence in them is indirectly confirmed by the fact that these lines were resistant to the local populations of the pathogen and to fungal isolates PgtZ1 and PgtF18.6, which are avirulent to *Sr22*.

These lines were not evaluated for resistance to Ug99, which will be done later. In some lines resistant to Ug99 in Kenya, resistance genes were identified. Thus, the new high-performance combinations are *Sr25 / Lr19* + *LrKuk / SrKuk*, *Sr25/ Lr19* + *Sr31 / Lr26* + *Sr28*, *Sr25 / Lr19* + *Sr38/Lr37*, and *Sr25/Lr19* + *Sr35*. Of the 128 lines studied, 16 showed infection type 5MR.

Thus, the effectiveness of the above combinations has been confirmed, but line 11, apparently, still has unidentified *Sr* genes, since ‘Lebedushka’ with the identified *Sr25/ Lr19* + *Lr6Agi* combination was at the level of 20MS. In addition, if the combinations coincide with infection types 0 and 5MR, it appears that the lines with infection type 0 have additional resistance genes, which will be tested in further studies.

4. Conclusions

From the 128 introgression lines studied in Kenya, 10 lines were found immune (infection type 0) and 16 lines showed reaction type 5MR. Gene combinations identified in these lines are *Sr25 / Lr19* + *LrKuk / SrKuk*, *Sr25/ Lr19* + *Sr31 / Lr26* + *Sr28*, *Sr25 / Lr19* + *Sr38 / Lr37* and *Sr25/Lr19* +*Sr35*. The *Sr31/Lr26* and *Sr25/Lr19* genes were mainly identified in the analyzed 58 introgression lines. The combination of these genes – *Sr31/Lr26* + *Sr25/Lr19* – was identified in 15 lines (26.3 %) from 58. The combination of the *Sr38/Lr37* + *Sr25/Lr19* genes was identified in two lines; *Sr25/Lr19* + *Sr35*, in two lines. A promising combination of genes, *Sr22* + *Sr25/Lr19*, was identified in two lines.

These lines were resistant to all analyzed populations of the pathogen, which makes them promising for further use in breeding. All lines carrying *Sr31/Lr26* and *Sr22* were resistant to all local pathogen populations taken into analysis. The *Sr26*, *Sr36* and *Sr39* genes were not detected in the analyzed wheat lines.

It is also necessary to note promise for the use of the triticale cultivar Satu, which carries the linkage genes *LrSatu/SrSatu*, in protection against stem rust pathogen (McIntosh et al., 1995). Two lines (Satu/C70//C74/3/C70/4/C70) and (Satu/C70//C74/3/C74) were immune to Ug99 and its biotypes in Kenya.

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Acknowledgements. The work is supported by the RFBR grant No. 18-016-00170 a.

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