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Rye inbred line L371 as a genetic source for primary octoploid triticale resistance to wheat leaf rust

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Motivation and Aim: Triticale (×Triticosecale Wittmack) is a synthetic species of the Poaceae family, artificially created more than 100 years ago by crossing of wheat and rye species, mainly *Triticum aestivum* L. and *Secale cereale* L. The greatest value of triticale is extremely wide ecological plasticity inherited from the rye parent. One of the main factors decreasing triticale yield and quality is fungal diseases. Being an allopolyploid, in theory triticale could combine diseases resistance genes of wheat and rye and therefore potentially have a greater variability resistance reaction. In practice triticale of various levels of ploidy is affected by many fungal diseases (stem and leaf rusts, root rots, powdery mildew, etc.) [1].

It has been shown that the gene pool of triticale is extremely poor in terms of effective seedling resistance to leaf rust [2] caused by the fungus *Puccinia triticina* Erikss. Susceptibility of most triticale genotypes to leaf rust could be explained by use in their creation of both (bread wheat and rye) parents susceptible to the disease.

We supposed that breeding of resistant triticale could be in some cases successful when rye resistant to leaf rust is used as one parent component. Previously, line L371 from the Peterhof's Genetic Collection [3] was described as resistant to leaf rust. This line as well as a highly susceptible line L393 were used to produce wheat-rye hybrids (ABDR amphihaploids) and amphidiploids (AABBDDRR = 2n) for their subsequent testing on resistance to leaf rust and genetic analysis to uncover the number of genes controlling leaf rust resistance. Moreover, the analysis of genetic markers linked to leaf rust resistance genes (Lr genes) was performed to understand the novelty of resistance genes from rye line L371.

Methods and Algorithms: To reveal the number of genes involved in conferring resistance to wheat leaf rust genetic analysis was performed on reciprocal hybrids between rye lines L371 and L393 as well as on wheat-rye hybrids (ABDR amphihaploids). Wheat leaf rust resistance of hybrids was evaluated with a complex population of *P. triticina*. For analysis of DNA markers of *Lr* genes PCR with specific primers [4–9], fragment analysis and sequencing of amplified fragments were done. Marker localization in reference genomes of *Aegilops tauschii* (GCF_002575655.2), *S. cereale* (GCA_902687465.1) was determined by *in silico* PCR using ipcress from the exonerate package and searching for previously sequenced sequences in these genomes using the blastn version 2.12.0. Regions of 4 million base pairs upstream of the marker and 4 million base pairs downstream the marker were used for *ab initio* automatic annotations using Augustus (https://bioinf.uni-greifswald.de/augustus/), annotated

sequences were searched in the SwissProt database (version 2024_02), from the final list of genes those related to transposable elements and genes with an e-value worse than 1×10^{-30} were removed.

Results: Evaluation of resistance to wheat leaf rust revealed that rye line L371, as well as amphihaploids and amphidiploids were highly resistant to the complex population of wheat leaf rust after inoculation of intact seedlings. L393 and Chinese Spring were highly susceptible to this inoculum.

Hybridological analysis of rye resistance to wheat leaf rust performed on two reciprocal crosses of rye lines (L371 × L393 and L393 × L371) revealed that F_1 plants from the reciprocal crosses of two rye lines were highly resistant to the rust, so the resistance is a dominant trait. Segregation in F2 plants are in correspondence with the hypotheses of three genes involved in genetic control of resistance: i) two recessive and one dominant genes or ii) one dominant and two complementary dominant genes. To distinguish these two hypotheses, a modified hybridological analysis proposed by Voylokov and Tikhenko [10] was used, and an analysis of the segregation for resistance to *P. triticina* in amphihaploid populations derived from crosses of hybrid F_1 rye plants with susceptible wheat variety was carried.

The segregation ratio for Chinese Spring × F_1 (L371 × L393) and for Chinese Spring × F_1 (L393 × L371) amphihaploid populations did not contradict to the theoretically expected ratio 7:1 for three genes (two recessive and one dominant) involved in genetic control of resistance. Additionally, these three genes control resistance in rye and in amphihaploids, hence bread wheat genome does not inhibit their expression.

Up to date a lot of rust resistance genes (*Lr*) used in wheat breeding programs were genetically mapped. Synthetic population of *P. triticina* used in the study was avirulent to some of *Lr* genes: *Lr9*, *Lr19*, *Lr24*, *Lr41* (=*Lr39*) and *Lr47*. So based on leaf rust resistance test it was not possible to decide whether three rye resistance genes are some of these *Lr* genes or not. To solve the problem specific DNA markers described for *Lr* genes [4–9] were used in PCR on DNA of resistant (L371) and susceptible (L393) rye lines. PCR analysis of DNA markers for *Lr9*, *Lr24* and *Lr47* genes did not lead to the amplification of any products on DNA of both rye lines. Based on the results it is possible to suggest that *Lr9*, *Lr24* and *Lr47* cannot be considered as candidate genes for three resistant rye genes described in the study.

PCR analysis of DNA markers for *Lr19* and *Lr41* genes led to the amplification of PCR products. Fragment analysis of PCR products for *Lr19* marker revealed no polymorphism in length of PCR products between resistant and susceptible rye lines, so *Lr19* also is not a candidate gene.

Fragment analysis of PCR products for *Lr41* marker Barc124 [8] revealed polymorphism in length of PCR products between resistant and susceptible rye lines, so it is possible to consider *Lr41* gene as a gene candidate for one of the three resistant rye genes. *Lr41* gene was introduced into bread wheat cultivars from *Ae. tauschii*. To confirm this suggestion PCR product for *Lr41* markers Barc124 [8] was sequenced and mapped on genomes of *S. cereale* and *Ae. tauschii*. A synteny in a region +/–4 Mb from the localization of Barc124 marker was observed.

Conclusion: Results of genetic analysis of the rye line L371 resistant to *P. triticina* performed on rye and wheat-rye hybrids revealed that this resistance is the dominant trait; cytoplasm has no or minor influence on the phenotypic expression of the resistance; the trait is controlled by three independent genes (two dominant and one recessive); the expression of each gene is not suppressed by bread wheat genome; analysis of DNA

markers of *Lr* genes and synteny of rye and *Aegilops* regions revealed that one of the possible gene candidate is *Lr41* and two other genes can be considered as new rye genes conferring resistance to leaf rust.

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