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Leaf rust resistance of bread wheat (*Triticum aestivum* L.) lines derived from interspecific crosses

Andrii GORASH¹, Alexey GALAEV², Olga BABAYANTS², Lazar BABAYANTS²¹Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry

Instituto 1, Akademija, Kėdainiai distr., Lithuania

E-mail: andrej@lzi.lt

²Plant Breeding and Genetics Institute, National Center of Seed and Cultivar Investigation

Ovidiopol'skaya 3, 65036 Odessa, Ukraine

E-mail: galaev7@rambler.ru

Abstract

Wheat lines which possess resistance to leaf rust were developed from the cultivar crosses between bread wheat (*Triticum aestivum* L.) and wild cereal species: jointed goatgrass (*Aegilops cylindrica* Host), tausch's goatgrass (*Aegilops tauschii* Coss.), wild emmer (*Triticum dicoccoides* Koern.) and synthetic species *Triticum eribuni* Gandil. Resistance of breeding lines was examined at the seedling stage of wheat plants after artificial inoculation. Resistance genes *Lr21*, *Lr22a*, *Lr24* (*T1BL.1BS-3Ae#1L*), *Lr32*, *Lr34*, *Lr39*, *Lr42*, *Lr53* and *TIAL.1RS* were identified by a molecular analysis using sequence-tagged sites (STS) and simple sequence repeats (SSR) markers. Comparison of data of phytopathological evaluation and molecular-genetic analysis of breeding lines revealed that leaf rust resistance was conferred by a combination of several genes. The combination of genes *Lr24+Lr34+TIAL.1RS* conferred resistance, *Lr24+Lr34+Lr21*, *TIAL.1RS+Lr24* and *Lr21+TIAL.1RS+Lr24* conferred resistance and moderate resistance.

Key words: genes of resistance, *Puccinia triticina*, winter wheat.

Introduction

Since ancient times, leaf rust, caused by an obligate parasitic fungus *Puccinia triticina* Erikss., has been a challenge for wheat growing and, which continues to cause annual losses and to be a principal disease of wheat worldwide (Huerta-Espino et al., 2011; Kolmer, 2013). Due to a wide variability of the pathogen, there are continuously emerging new virulent races which overcome resistant genes of a host. Because of the airborne nature of urediniospores, the pathogen can be transferred by wind to adjacent and distant wheat growing areas (Kolmer, 2005).

The greater is the area under the cultivars which have the same mono gene of resistance, the faster pathogen overcomes host resistance. Constriction of diversity of resistance genes creates favourable conditions for development of epiphytoty. In order to prevent it, it is necessary to widen the genetic variability of bread wheat. Wild species which in co-evolution during thousands of years evolved genes of resistance against pathogens are valuable sources of resistance. They contain a broad pool of genetic variation, which carries resistance against the majority of biotic and abiotic stresses. Disease resistance genes, transferred from wild species, support wheat production on a global level (Davoyan et al., 2011).

More than 80 genes and alleles of leaf rust resistance (*Lr*) genes have been identified and described so far. Among them 33 *Lr*-genes were transferred from other species into *Triticum aestivum* L. (Catalogue of gene symbols..., 2012). Most of them are race-specific and follow the gene-for-gene concept. The resistance, based on such single gene, is overcome by pathogen in a short time. Virulent pathotypes emerge in a population via changes in pathogen's genome (sexual recombination or mutation). In contrast, non-specific genes do not confer high level of resistance but due to the slow rusting effect prevent epiphytoty of disease and provide long-time resistance. To date, there are known four loci which contain *Lr*-genes designated as *Lr34*, *Lr46*, *Lr67* and *Lr68* that provide race non-specific, adult plant resistance (APR) (Da-Silva et al., 2012). Combining them in one genotype with race-specific genes ensures more durable resistance than that based on single seedling resistance genes. The most widely and successfully applied gene is *Lr34*, which may enhance resistance of the other seedling genes and has positive pleiotropic effect against other wheat diseases (Dakouri et al., 2013).

Checking of effectiveness of known *Lr*-genes in single use and in different combinations, continuous

virulence analysis of wheat leaf rust and transferring new genes from wild species to wheat pool are important strategies for wheat improvement. In PBGI-NC SCI, original initial material was developed through interspecific crossing with *Aegilops tauschii* Coss., *Aegilops cylindrica* Host, *Triticum erebuni* Gandil. and *Triticum dicoccoides* Koern. It has been established that wild species *A. tauschii* and *A. cylindrica* carry in their D-genome genes of resistance – *Lr21*, *Lr22a*, *Lr32*, *Lr39* and *Lr42*, *T. dicoccoides* – gene *Lr53* (B genome) (Singh, 2004; Catalogue of gene symbols..., 2012). Cultivar ‘Amigo’ which carries wheat-rye translocation *T1AL.1RS* and wheat-*Agropyron elongatum* translocation chromosome *T1BL.1BS-3Ae#1L (Lr24)* is used to enhance resistance to leaf rust (McIntosh et al., 2013). Gene *Lr34* is present in greater part of bread wheat cultivars in PBGI-NC SCI (Galaev, Sivolap, 2012). The developed breeding lines have high resistance against the majority of bread wheat diseases, including leaf rust, widely distributed in the south of Ukraine (Babayants, 2011; Babayants et al., 2012).

Table 1. Pedigree of breeding material, and lines resistant to leaf rust developed in Plant Breeding and Genetics Institute, Ukraine

| Breeding line | Pedigree |
|---------------|---|
| CN 3/12 | <i>Triticum erebuni</i> /Obriy/Odesskay 162/2/Ukrainka odesskaya/Amigo |
| CN 15/16 | <i>Triticum dicoccoides</i> / <i>Triticum tauschii</i> //2/Albatros odesskiy//Odesskaya polukarlikovaya/ <i>Aegilops cylindrica</i> /Odesskaya polukarlikovaya/Tira/Amigo |
| CN 16/12 | <i>Triticum dicoccoides</i> / <i>Triticum tauschii</i> //2/Albatros odesskiy//Odesskaya polukarlikovaya/ <i>Aegilops cylindrica</i> /Odesskaya polukarlikovaya/Tira/Amigo |
| CN 28/12 | <i>Triticum erebuni</i> /Obriy/Odesskay 162/2/Ukrainka odesskaya/Amigo |
| CN 42/12 | <i>Triticum erebuni</i> /Obriy/Odesskaya 162/2/Ukrainka odesskaya/Selaynka/Amigo |
| CN 64/12 | <i>Triticum erebuni</i> /Obriy/Odesskaya 162/2/Ukrainka odesskaya/Skarbnitsa/Amigo |
| CN 82/12 | Odesskaya polukarlikovaya/ <i>Aegilops cylindrica</i> /Odesskaya polukarlikovaya/ Kuyalnik odesskiy//Donskaya polukarlikovaya/ <i>Aegilops variabilis</i> /Ukrainka odesskaya/Nikonika/Amigo |
| CN 83/12 | <i>Triticum dicoccoides</i> / <i>Triticum tauschii</i> //2/Albatros odesskiy//Odesskaya polukarlikovaya/ <i>Aegilops cylindrica</i> /Odesskaya polukarlikovaya/Tira/Amigo |
| CN 84/12 | <i>Triticum erebuni</i> /Obriy/Odesskay 162/2/Ukrainka odesskaya/Vdala/Amigo |
| CN 153/12 | <i>Triticum erebuni</i> /Obriy/Odesskay 162/2/Ukrainka odesskaya/Antonovka/Amigo |
| CN 519/12 | <i>Triticum dicoccoides</i> / <i>Triticum tauschii</i> //2/Albatros odesskiy//Odesskaya polukarlikovaya/ <i>Aegilops cylindrica</i> /Odesskaya polukarlikovaya/Tira/Amigo |
| CN 520/12 | <i>Triticum dicoccoides</i> / <i>Triticum tauschii</i> //2/Albatros odesskiy//Odesskaya polukarlikovaya/ <i>Aegilops cylindrica</i> /Odesskaya polukarlikovaya/Tira/Amigo |

a result of hybridization between *Aegilops tauschii* Coss. and *Triticum urartu* Thum. ex Gandil. (Gandilyan, 1984).

Seeds of the Thatcher near-isogenic lines (NILs) with different *Lr*-genes: *Lr21*, *Lr22a*, *Lr24*, *Lr32*, *Lr34* and cultivars/lines with additional known genes: *Lr39*, *Lr42*, *Lr53* were obtained via USDA, Germplasm Resources Information Network (<http://www.ars-grin.gov>). Seeds of *Triticum erebuni* Gandil. were kindly provided by Dr. Olexandr Rybalka. Uredospores of the local population of leaf rust collected in the fields of PBGI-NC SCI served as inoculums. The uredospores were collected and preserved according to the procedure of Babayants (1988).

The principal aim in this research is to conduct phytopathological evaluation of resistance of wheat breeding lines developed from interspecific crosses and to identify genes that confer resistance to leaf rust using molecular markers.

Materials and methods

The study was carried out in 2013 at the Plant Breeding and Genetics Institute, National Center of Seed and Cultivar Investigation (PBGI-NC SCI), Ukraine. Initial breeding material resistant to leaf rust was developed from the crossing of bread wheat (*Triticum aestivum* L.) cultivars with wild cereal species. The pedigree of breeding material from interspecific crossing contained the following species: *Triticum erebuni* Gandil. (genome: AⁿAⁿDD), jointed goatgrass (*Aegilops cylindrica* Host) (genome: CCDD),tausch's goatgrass (*Aegilops tauschii* Coss.) (genome: DD), wild emmer (*Triticum dicoccoides* Koern.) (genome: AABB) (Table 1). *Triticum erebuni* Gandil. is the synthetic allotetraploid species formed as

Effectiveness of leaf rust resistance genes was studied at the seedling stage in a greenhouse and on adult plants in the field.

Evaluation of resistance at seedling stage was carried out in a greenhouse with controlled conditions (temperature – 21 ± 2°C, illuminance – 10000 lux, day length – 16 hours, night length – 8 hours). Ten-day-old seedlings of the studied material were inoculated with a mixture of uredospores and talcum powder. After inoculation the dew was created via putting plants in plastic bag, dark conditions for 16 hours at a temperature of 21°C. On the 12th day after inoculation the plants were evaluated for rust scoring, infection type and severity.

Evaluation of resistance in the seedling stage was scored according to the scale: VR – very resistant, R – resistant, MR – moderately resistant, MS – moderately susceptible, S – susceptible, VS – very susceptible.

Evaluation of resistance in the field was made after artificial inoculation. Artificial inoculation by pathogens of wheat rusts was performed in the mixture of uredospores with talcum powder. The quantity of uredospores recalculated on the basis of 100 percent of alive spores was 10 mg per 1 m². Adult resistance in the field was scored according to a 9-points scale: 1–2 – very susceptible, 3 – highly susceptible, 4 – susceptible, 5 – moderately susceptible, 6 – moderately resistant, 7 – resistant, 8 – highly resistant, 9 – immune (Babayants, 1988). Infection types were scored by the modified Mains and Jackson scale, rust intensity – by Peterson of Babayants (1988). Standard of susceptibility to leaf rust was cv. ‘Odesskaya polukarlikovaya’.

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR) amplification. DNA was extracted from green leaves and 3 to 5 day old seedlings with the help of CTAB (cetyltrimethyl ammonium bromide) buffer (Sivolap, 1998). The reaction mixture (25 µl) contained: 10 × PCR buffer (SibEnzyme Ltd., Russia), 0.2 mM of each deoxynucleotide (dNTP) (SibEnzyme Ltd.), 0.25 µM of the primer and 20 nanograms of DNA, and 1 unit of *Taq* polymerase (SibEnzyme Ltd.). Mineral oil (30 µl) was added into the reaction solution. The reaction conditions were as follows: denaturation at 94°C for 30 s (original for 2 min), annealing at 55°C and 60°C (depending on the primers) for 30 s, and elongation at 72°C for 1 min (final elongation for 4 min). The primer sequences used in the work for the detection of resistance genes are presented in Table 2.

Table 2. PCR primers used to identify resistance genes in breeding material and breeding lines

| Gene, arm of wheat and rye chromosomes | Pairs of markers | Sequence of primers 5'-3' | Annealing temperature | Fragment size, bp | Reference |
|--|--|---|-----------------------|-------------------|---|
| <i>Lr21</i> | Xgdm33F Xgdm33R | ggc tca aat tca acc gtt ctt tac gtt ctg gtg gct gct c | 60 | 128 | Spielmeier et al., 2000 |
| <i>Lr22a</i> | Xwmc296F Xwmc296R | gaa tct cat ctt ccc ttg cca c atg gag ggg tat aaa gac agc g | 55 | 378 | Hiebert et al., 2007 |
| <i>Lr24</i> | Sr24/ <i>Lr24.1</i> Sr24/ <i>Lr24.2</i> | cac ccg tga cat gct cgt a aac agg aaa tga gca acg atg t | 60 | 500 | Mago et al., 2005 |
| <i>Lr32</i> | Xbarc135F Xbarc135F | atc gcc atc tcc tct acc a gcg aac cca tgt gct aag t | 60 | 240 | Thomas et al., 2010 |
| <i>Lr34</i> | csLV34F csLV34R | ggt ggt taa gac tgg tga tgg ggt ggt taa gac tgg tga tgg | 55 | 150 | Lagudah et al., 2006 |
| <i>Lr39</i> | Xgdm35F Xgdm35R | cct gct ctg ccc tag ata cg atg tga atg tga tgc atg ca | 55 | 170 | Singh et al., 2004 |
| <i>Lr42</i> | Xcfd15F Xcfd15R | ctc ccg tat tga gca gga ag ggc agg tgt ggt gat gat ct | 60 | 179 | Sun et al., 2010 |
| <i>Lr53</i> | Xcfd1F Xcfd1R | acc aaa gaa ctt gcc tgg tg aag cct gac cta gcc caa at | 60 | 222 | Dadkhodaie et al., 2011 |
| <i>IRS</i> | Xscm9F Xscm9R | tga caa ccc cct ttc cct cgt tca teg acg cta agg agg accc | 60 | 220 | |
| <i>IAL</i> | Xbarc263F Xbarc263R | gga agc gcg tca gca cta ggc aac ggc ttc tag gtg ctg cgg ctt ttg tc | 60 | 230 | http://wheat.pw.usda.gov |
| <i>IBL</i> | Xgwm18F Xgwm18R | tgg cgc cat gat tgc att atc ttc ggt tgc tga aga acc tta ttt agg | 55 | 186 | |

Detection of product amplification. The amplification products (10 ml aliquot of the PCR mixture) were fractionated in 9% polyacrylamide gels in 1 × TBE (Tris-borate-EDTA). Electrophoresis was carried out at a constant voltage of 500 V in a scientific instrument ‘‘Hofer’’ (USA) for vertical gel electrophoresis. Visualization of the products of electrophoretic division was performed by impregnation of gels with silver nitrate

following the procedure of Budowle et al. (1991). Video images and the sizes of the amplified fragments were obtained using a video system ‘‘ImageMaster VDS’’ (Amersham Pharmacia Biotech, USA) according to the recommendations of the manufacturer. The *pUC 19/MspI* (SibEnzyme Ltd.) and 100 bp DNA ladder (SibEnzyme Ltd.) standards were used to calibrate the molecular mass of the obtained amplicons.

Results

Comparison of field and seedling test evaluation results indicated that breeding lines CN 3/12, CN 16/12, CN 64/12, CN 82/12 and BN 519/12 were resistant and CN 15/12 was moderately resistant to leaf rust at seedling and highly resistant and resistant at adult plant stage. Six breeding lines CN 28/12, CN 42/12, CN 83/12, CN 84/12, CN 153/12 and BN 520/12 were heterogeneous in this trait (Tables 3).

Table 3. Results of phytopathological evaluation of breeding lines resistance to *Puccinia tritici*

| Line | Seedling ¹ | Adult plant stage ² |
|-----------|-----------------------|--------------------------------|
| CN 3/12 | R | 8 |
| CN 16/12 | R | 8 |
| CN 64/12 | R | 8 |
| CN 82/12 | R | 8 |
| BN 519/12 | R | 8 |
| CN 15/12 | MR | 7 |
| CN 28/12 | R (S) | 8 (5) |
| CN 42/12 | R (S) | 8 (5) |
| CN 83/12 | MR-R (MS-S) | 7–8 (4) |
| CN 84/12 | MR-R (S) | 7–8 |
| CN 153/12 | R (S) | 8 (5) |
| BN 520/12 | R (MS-S) | 7–8 |

¹ – type of reaction: VR – very resistant, R – resistant, MR – moderately resistant, MS – moderately susceptible, S – susceptible, VS – very susceptible; ² – 1 to 9 point scale: 1–2 – very susceptible, 3 – highly susceptible, 4 – susceptible, 5 – moderately susceptible, 6 – moderately resistant, 7 – resistant, 8 – highly resistant, 9 – immune

Table 4. Results of identification of *Lr*-genes using molecular genetic markers

| Lines / species | <i>Lr21</i> | <i>Lr22a</i> | <i>Lr24</i> | <i>1AL.1RS</i> | <i>Lr32</i> | <i>Lr34</i> | <i>Lr39</i> | <i>Lr42</i> | <i>Lr53</i> |
|---------------------------------|-------------|--------------|-------------|----------------|-------------|-------------|-------------|-------------|-------------|
| CN 3/12 | – | – | + | + | – | + | – | – | – |
| CN 15/12 | – | – | + | + | – | – | – | – | – |
| CN 16/12 | – | – | + | + | – | + | – | – | – |
| CN 64/12 | – | – | + | + | – | + | – | – | – |
| CN 82/12 | + | – | + | + | – | + | – | – | – |
| BN 519/12 | – | – | + | + | – | – | – | – | – |
| <i>Triticum erebuni</i> Gandil. | + | + | – | – | – | – | – | – | – |

Discussion

One of the ways to improve the gene pool of bread wheat is transfer of new genes, which confer resistance to biotic and abiotic stresses, from wild relatives of the *T. aestivum*. Experiments for the development of initial material and donors of resistance against the main wheat diseases in the south of Ukraine were conducted in PBGI-NC SCI. The species used as sources of resistance were: *Triticum erebuni* Gandil., *Triticum dicoccoides* Koern., *Aegilops tauschii* Coss. and biotypes of the local population of *Aegilops cylindrica* Host. After artificial inoculation by a population of wheat leaf rust, interspecific hybrids possessing resistance to leaf rust

Detection of *Lr*-genes (*Lr21*, *Lr22a*, *Lr24*, *Lr32*, *Lr34*, *Lr39*, *Lr42* and *Lr53*) and translocation *T1AL.1RS* was performed using molecular markers. DNA of 3 plants was taken for PCR analysis of breeding lines CN 3/12, CN 15/12, CN 16/12, CN 64/12, CN 82/12 and BN 519/12. In heterogeneous breeding lines CN 84/12, CN 28/12, CN 42/12, CN 83/12, CN 153/12 and BN 520/12 DNA for PCR analysis was taken from 5 to 11 plants. The marker for the resistance gene *Lr21* was found in the breeding line CN 82/12. The markers for the gene *Lr24* and translocation *T1AL.1RS* were detected in breeding lines: CN 3/12, CN 15/12, CN 16/12, CN 64/12, CN 82/12 and BN 519/12. The marker for the gene *Lr34* was found in the breeding lines CN 3/12, CN 16/12, CN 64/12 and CN 82/12. Markers for genes *Lr21* and *Lr22a* were found in *Triticum erebuni* (Table 4).

In the heterogeneous breeding lines plants showed different types of reaction to leaf rust – from susceptible to resistant. Comparison between the results of molecular analysis and phenotypic reaction to leaf rust revealed that resistance was conferred by different combinations of *Lr*-genes. The plants with a combination *Lr24+Lr34+T1AL.1RS* were resistant. Among the plants with a combination *Lr21+Lr24+Lr34* three plants were resistant and two moderately resistant. The plants with a combination *T1AL.1RS+Lr24* were resistant, with combinations *Lr21+T1AL.1RS+Lr24* and *Lr21+Lr24* – moderately resistant. Among the plants with a combination *T1AL.1RS+Lr34* two plants were resistant and one plant susceptible. The plants with a combination *Lr21+T1AL.1RS*, and also with one *Lr24*-gene were – moderately susceptible. The plants with a combination *Lr21+Lr34*, and also with one *T1AL.1RS* and one *Lr34*-gene were – susceptible (Table 5).

were selected (Babayants, Babayants, 2007). Applying backcross and selection methods on artificially infected nurseries a range of wheat breeding lines possessing resistance to the main wheat diseases, including leaf rust, was developed (Babayants, 2011). Cultivar ‘Amigo’, which is resistant to the pathogen in the south of Ukraine, was used to enhance the resistance to leaf rust. The resistance of cv. ‘Amigo’ was conferred by two translocations – *T1BL.1BS-3Ae#1L* from tall wheatgrass (*Agropyron elongatum* (Host). Beauv.) and *T1AL.1RS* from rye (*Secale cereale* L.) (MacIntosh et al., 2013).

Table 5. A comparison between the results of molecular analysis and phenotypic reaction to leaf rust of heterogeneous breeding lines

| Line | Plant No. | Type of infection | Genes | | | | Plant No. | Type of infection | Genes | | | |
|-----------|-----------|-------------------|-------------|------------|-------------|-------------|-----------|-------------------|-------------|------------|-------------|-------------|
| | | | <i>Lr21</i> | <i>IRS</i> | <i>Lr24</i> | <i>Lr34</i> | | | <i>Lr21</i> | <i>IRS</i> | <i>Lr24</i> | <i>Lr34</i> |
| CN 28/12 | 1 | R | - | + | + | + | 4 | S | + | - | - | + |
| | 2 | R | - | + | + | + | 5 | R | - | + | + | + |
| | 3 | R | - | + | + | + | 6 | R | - | + | + | + |
| CN 42/12 | 1 | R | - | + | + | + | 4 | R | - | + | + | + |
| | 2 | S | + | - | - | + | 5 | R | - | + | + | + |
| | 3 | R | - | + | + | + | | | | | | |
| CN 83/12 | 1 | MR | + | + | + | - | 6 | S | - | + | - | - |
| | 2 | MS | + | - | + | - | 7 | MS | + | + | - | - |
| | 3 | MS | + | + | - | - | 8 | MR | + | + | + | - |
| | 4 | MR | + | + | + | - | 9 | MS | + | + | - | - |
| | 5 | MR | + | + | + | - | 10 | MR | + | + | + | - |
| CN 84/12 | 1 | R | + | - | + | + | 7 | R | - | + | + | + |
| | 2 | R | - | + | + | + | 8 | R | + | - | + | + |
| | 3 | MR | + | - | + | + | 9 | R | - | + | + | + |
| | 4 | S | - | + | - | + | 10 | S | - | + | - | + |
| | 5 | MR | + | - | + | + | 11 | R | + | - | + | + |
| | 6 | S | - | - | - | + | | | | | | |
| CN 153/12 | 1 | R | - | + | - | + | 5 | R | - | + | + | + |
| | 2 | R | - | + | + | + | 6 | S | + | - | - | + |
| | 3 | R | - | + | + | + | 7 | R | - | + | + | + |
| | 4 | R | - | + | + | + | 8 | R | - | + | + | + |
| BN 520/12 | 1 | MS | - | - | + | - | 4 | R | - | + | + | - |
| | 2 | R | - | + | + | - | 5 | MS | - | - | + | - |
| | 3 | MS | - | - | + | - | 6 | R | - | + | + | - |

VR – very resistant, R – resistant, MR – moderately resistant, MS – moderately susceptible, S – susceptible, VS – very susceptible

The investigated breeding lines carried genes *Lr21*, *Lr24*, *Lr34* and *TIAL.IRS*. As was reported, the gene *Lr21* was derived from *A. tauschii* (Catalogue of gene symbols..., 2012). The results indicate that *T. erebuni* also carried *Lr21*. We suppose that *Lr21* could be transferred in breeding lines from species which have D-genome (*A. tauschii*, *A. cylindrica* or *T. erebuni*), translocations *T1BL.1BS-3Ae#1L* (*Lr24*) and *TIAL.IRS* were transferred from cv. 'Amigo', non-specific gene *Lr34* was transferred from recurrent cultivars.

All the studied breeding lines carry translocation *TIAL.IRS*. It has been reported that rye arm *IRS* transferred into wheat genotypes provides heterotic effects causing biomass accumulation, grain yield increase and adaptation to stressful environmental conditions (Tabibzadeh et al., 2013). We can suggest that the presence of *TIAL.IRS* in all breeding lines is connected not only with resistance to leaf rust but also with visual selection of more attractive heterotic plants during the breeding process. According to the pedigree of the studied breeding lines they may carry *Lr34*, which is the adult plant resistance gene that enhances the action of some seedling genes (Dakouri et al., 2013). In this study, we chose to investigate the influence and possible synergy between *Lr34* and other *Lr*-genes at seedling stage.

Comparison of data of phytopathological evaluation and molecular-genetic analysis of breeding lines showed that resistance of breeding line CN 15/12, BN 519/12 was conferred by a combination *TIAL.IRS+Lr24*, in breeding lines CN 3/12, CN 16/12, CN 64/12 by *Lr24+TIAL.IRS+Lr34*, and in breeding line CN 82/12 by *Lr21+Lr24+TIAL.IRS+Lr34*.

In the heterogeneous breeding lines, plants with different types of resistance to leaf rust were noticed. The plants with combinations *Lr24+Lr34+TIAL.IRS* were resistant. Among the plants with a combination *Lr21+Lr24+Lr34* three plants were resistant and two plants were moderately resistant. The plants with a combination *TIAL.IRS+Lr24* were resistant and with combinations *Lr21+TIAL.IRS+Lr24* and *Lr21+Lr24* were moderately resistant. Among the plants with a combination *TIAL.IRS+Lr34* two plants were resistant and one plant susceptible. The plants with a combination *Lr21+TIAL.IRS*, and also with one *Lr24*-gene were moderately susceptible. The plants with a combination *Lr21+Lr34*, and also with one *TIAL.IRS* and one *Lr34*-gene were susceptible. The combination *Lr24+TIAL.IRS* was detected in the resistant breeding lines CN 15/16 and CB 519/12, the plants of the former were moderately resistant and those of the latter resistant.

In the heterogeneous breeding line CN 83/12, the plants with a combination *Lr21+Lr24+TIAL.IRS* were moderately resistant. The different types of resistance in the genotypes with the same combination of genes may indicate the presence of other not detected minor genes which alongside the main genes enhance the resistance of plants to leaf rust. In this context, more detailed investigation for deeper elucidation of resistance in the breeding material from interspecific crossing is needed.

The key gene in the studied breeding lines was *Lr24* (*T1BL.1BS-3Ae#1L*), which in combination with other genes provides resistance against leaf rust. The plants which had *Lr24* in combination with *Lr21*, *Lr34* and translocation *TIAL.IRS* were resistant and moderately resistant. In single use, genes *Lr21*, *Lr34* and translocation *TIAL.IRS* confer susceptibility (Babayants, 2011). As was documented by Huerta-Espino et al. (2011), *Lr21* remains effective in Canada, North America, South Africa, Middle East, North Africa and Central Asia, local virulence was found in Europe, but the gene still continues to be effective in most European countries. Virulence to *Lr24* was very rare in Germany, Spain, Hungary, Slovak Republic, Czech Republic (Huerta-Espino et al., 2011), Lithuania (Liatukas, 2003) and China (Liu, Chen, 2012). In Ukraine, *Lr24* confers adult plant resistance (Babayants, 2011). The adult plant resistance gene *Lr34* remains effective in North America, virulence is absent in South Africa, Middle East, North Africa and Central Asia, the gene is effective in Canada. Moreover, many researchers have reported that *Lr34* in combination with other genes may enhance the resistance to leaf rust. In the last decades, phytopathologists throughout the world have chosen the strategy for durable resistance. According to it resistance to leaf rust may be prolonged using combinations of several race-specific genes or several race-specific genes with non-race specific genes like *Lr34* and *Lr46*. It was established that a combination of genes with partial resistance or of several ineffective/effective genes with adult plant resistance genes can provide a sufficient level of durable resistance (Singh et al., 2011; Keller et al., 2012).

Conclusions

1. Bread wheat (*Triticum aestivum* L.) breeding lines CN 3/12, CN 15/12, CN 16/12, CN 64/12, CN 82/12 and BN 519/12 possess resistance to leaf rust. Breeding lines CN 28/12, CN 42/12, CN 83/12, CN 84/12, CN 153/12 and BN 520/12 are heterogeneous by this trait.

2. Leaf rust resistance in the breeding lines CN 3/12, CN 16/12, CN 64/12 and CN 82/12 is conferred by a combination of *Lr24+Lr34+TIAL.IRS*, in the breeding line CN 82/12 by *Lr21+Lr24+Lr34+TIAL.IRS*, in the breeding lines CN 15/12 and BN 519/12 by *TIAL.IRS+Lr24*.

3. The combination *Lr24+Lr34+TIAL.IRS* conferred resistance, *Lr24+Lr34+Lr21*, *TIAL.IRS+Lr24* and *Lr21+TIAL.IRS+Lr24* – resistance and moderate resistance.

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Paprastojo kviečio (*Triticum aestivum* L.) introgresinių linijų, gautų taikant tarprūšinę hibridizaciją, atsparumas rudosioms rūdimis

A. Gorash¹, A. Galaev², O. Babayants², L. Babayants²

¹Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas

²Ukrainos augalų selekcijos ir genetikos instituto Nacionalinis sėklų ir veislių tyrimo centras

Santrauka

Kviečių introgresinės linijos buvo sukurtos paprastojo kviečio (*Triticum aestivum* L.) veisles kryžminant su laukinių rūšių javais: *Aegilops cylindrica* Host., *Aegilops tauschii* Coss., *Triticum dicoccoides* Koern. ir *Triticum erebuni* Gandil. Selekcinių linijų atsparumas tirtas kontroliuojamomis sąlygomis šiltnamyje, kviečių augalus daigų tarpsniu užkrėtus rudosiomis rūdimis. Atsparumo genai *Lr21*, *Lr22a*, *Lr24* (*T1BL.1BS-3Ae#1L*), *Lr32*, *Lr34*, *Lr39*, *Lr42*, *Lr53* ir *T1AL.1RS* buvo identifikuoti taikant molekulinis metodus – specifinių sekų atkarpų ir paprastų pasikartojančių sekų žymeklius. Palyginus selekcinių linijų fitopatologinio vertinimo ir molekulinės genetinės analizės duomenis nustatyta, kad atsparumą rudosioms rūdimis suteikė kelių genų kombinacijos: kombinacija *Lr24+Lr34+T1AL.1RS* suteikė atsparumą, o kombinacija *Lr24+Lr34+Lr21*, *T1AL.1RS+Lr24* bei *Lr21+T1AL.1RS+Lr24* – atsparumą ir vidutinį atsparumą.

Reikšminiai žodžiai: atsparumo genai, *Puccinia triticina*, žieminiai kviečiai.