Development of wheat genotypes possessing a combination of leaf rust resistance genes *Lr19* and *Lr24*

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ABSTRACT

Endopeptidase allele *Ep-D1c* and DNA marker-assisted selection have been used for the incorporation of *Lr19* + *Lr24* leaf rust resistance genes combination into adapted commercial winter wheat cultivars. The first step was the transfer of the gene *Lr19* from the donor cultivar Agrus into acceptor cultivars Simona and Lívia. The progenies possessing the null allele *Ep-D1c* linked to the gene *Lr19* have been screened for their resistance to leaf rust by isolate 4332 SaBa. The plants homozygous properties at the *Ep-D1c* locus and resistant against leaf rust were used for crossing with NIL Thatcher/*Lr24* – a donor of the gene *Lr24*. Plants possessing both *Lr* genes were selected from F_2 population by STS and isozyme markers linked to the *Lr* genes. Progenies of 18 F_2 plants have been selected by STS marker and tested for resistance against leaf rust. Results obtained with isozyme and STS markers corresponded with resistance testing. Altogether 6 progenies of F_3 generation possessing a resistance gene combination of *Lr19* + *Lr24* in a homozygous condition were developed.

Keywords: *Triticum aestivum* L.; leaf rust; *Lr19* gene; *Lr24* gene; markers; molecular breeding

Leaf rust caused by *Puccinia triticina* (syn. *Puccinia recondita* Rob. ex Desm. f.sp. *tritici*) is one of the most important pathogens of wheat. It causes cardinal yield decreases in susceptible cultivars, mainly in the years with a high infection pressure of the pathogen. Resistance against this fungus is based on the possession of effective leaf rust (*Lr*) resistance genes. Forty-seven different *Lr* genes have been identified until the year 1995 (McIntosh et al. 1995). Other *Lr* genes were included into a catalogue since that time. The commercial wheat cultivars usually exploit only a limited number from them and each cultivar possess usually only one *Lr* gene. The most commonly used *Lr* genes in the western European wheat cultivars are *Lr1*, *Lr3a*, *Lr10*, *Lr13*, *Lr14a*, *Lr17b*, *Lr20*, *Lr26*, *Lr37* (Park et al. 2001). At the present time wheat cultivars cultivated in Slovakia and in the Czech Republic possess mostly genes *Lr3*, *Lr13*, and *Lr26* or their combinations. These genes are effective only against a limited number of pathogen races (Bartoš et al. 2001).

Gene pyramiding is a breeding strategy when two or more genes are combined together within one genotype. The combinations of the genes *Lr16* and *Lr13* (Samborski and Dyk 1982) or *Lr9* and *Lr24* (Long et al. 1994) were reported to provide reliable control against leaf rust. The lines with the pairs of genes *Lr13* and *Lr34*, *Lr13* and *Lr37*, *Lr34* and *Lr37* provided a higher level of resistance than lines with individual genes (Kloppers and Pretorius 1997). The combination of two or more resistance genes is often difficult or impossible due to lack of specific pathogen races necessary for detection and confirmation of specific resistance genes.

Available molecular markers, tightly linked to desired *Lr* genes can help in the selection of individuals with introduced genes, within segregating populations. This approach is used in different crops, also in wheat (Liu et al. 2000). Many specific PCR-based markers, linked to race-specific rust resistance genes, have been already developed. Therefore the STS, SCAR, and CAPS markers for genes *Lr1* (Feuillet et al. 1995), *Lr28* (Naik et al. 1998), *Lr9* and *Lr24* (Schachermayr et al. 1994, 1995), *Lr35* (Seyfarth et al. 1999), *Lr37* (Seah et al. 2000), *Lr47* (Helguera et al. 2000) are available for the molecular breeding approach.

The aim of this work was to transfer a pair of highly effective resistance genes against leaf rust – *Lr19* and *Lr24,* into wheat genotypes with none or limited resistance against leaf rust but adapted to our growing conditions.

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MATERIAL AND METHODS

Wheat cultivars Simona (without *Lr* genes) was highly susceptible to leaf rust in 1996 (Bartoš et al. 1999). Cultivar Lívia possessing gene *Lr26* (Bartoš et al. 1994) expressed a highly susceptible reaction in the years 1995 and 1996. Both cultivars have been used as recipients of *Lr19* + *Lr24* gene pair. The cultivar Agrus has been used as a donor of the *Lr19* gene, which was introgressed into wheat genome from *Agropyron elongatum* (Host.) Beauv., characterized and included into the catalogue of wheat genes (McIntosh et al. 1995). Near isogenic line (NIL) based on cultivar Thatcher possessing the gene *Lr24* has been used as a donor of this gene. The gene *Lr24* originated from *Agropyron elongatum* (Host.) Beauv. was incorporated into the wheat chromosome 3D by spontaneous translocation (Smith et al. 1968). The *Lr19* + *Lr24* gene pair was transferred into adapted cultivars by crosses (Simona × Agrus) × Thatcher/*Lr24* and (Lívia × Agrus) × Thatcher/*Lr24*.

Protein extracts for endopeptidase analyses were isolated either from young leaves or from embryos. Isoelectrofocusing was performed in prefocused polyacrylamide gels contained ampholyte (Pharmalyte pH 4.2–4.9) according to Koebner et al. (1988) and Winzeler et al. (1995). The catolyte was 0.5 mol/l NaOH, the anolyte 0.5 mol/l acetic acid. Fast Black K salt was used for specific staining of endopeptidases. Endopeptidase alleles encoded by the *Ep-D1* locus were classified according to Koebner et al. (1988).

DNA was isolated from young leaves and purified by the method of Dellaporta et al. (1993). A PCR-based DNA-STS marker, linked to the gene *Lr24*, developed by Schachermayr et al. (1995), has been used for the screening of plants possessing this gene. The sequences of primers (TCTAGTCTGTACATGGGGGC – forward primer, TGGCACATGAACTCCATACG – reverse primer) and amplification conditions were according to Schachermayr et al. (1995).

Plants of F_3 generations were tested by inoculation with leaf rust pathotype 4332 SaBa virulent to *Lr26* and avirulent to *Lr24* in greenhouse conditions by rubbing of the first leaf with urediospore water suspension and then plants were kept 24 hours at high air humidity in closed glass cylinders. Infection types were scored 14 days after inoculation using the scale developed by Stakman et al. (1962).

RESULTS AND DISSCUSSION

Altogether 7 plants from the cross Simona × Agrus and 10 plants from Lívia × Agrus, respectively, possessing null allele *Ep-D1c* have been selected from segregating F_2 populations. All 17 plants were tested for leaf rust resistance by phytopathological test. Seven individuals from the cross Simona × Agrus and 8 from Lívia × Agrus were resistant to leaf rust in the seedling stage. Plants possessing null endopeptidase marker allele and resistant against leaf rust at the same time, have been used for a second cross to combine gene *Lr19* and *Lr24*. Altogether 168 F_2 plants from the cross (Simona × Agrus) × Thatcher/*Lr24* and 172 from the cross (Lívia × Agrus) × Thatcher/*Lr24* were obtained and screened by DNA-STS specific marker linked to *Lr24* gene (Figure 1). Altogether 118 plants carrying *Lr24* DNA-STS dominant marker linked to the desired gene were selected from the cross (Simona × Agrus) × Thatcher/*Lr24* and 120 from the cross (Lívia × Agrus) × Thatcher/*Lr24*. Segregation of the marker in the F_2 generation in both types of crosses fitted in with 3:1 ($\chi^2 = 2.02$, $\chi^2 = 2.51$; $P > 0.05$). This agreed with Schachermayr et al. (1995) who confirmed that DNA-STS is a dominant marker and all resistant F_2 plants expressing an amplified DNA fragment of 350 bp, that is completely linked with the *Lr24* gene, while none of the susceptible plants showed this amplification product. The codominant DNA markers are preferred but dominant markers, as the one used in our study, has been used also successfully for marker assisted selection in wheat for *Lr* genes transfer (Naik et al. 1998, Seyfarth et al. 1999).

F_2 plants were at the same time screened with isozyme marker linked to *Lr19* gene. Thirteen

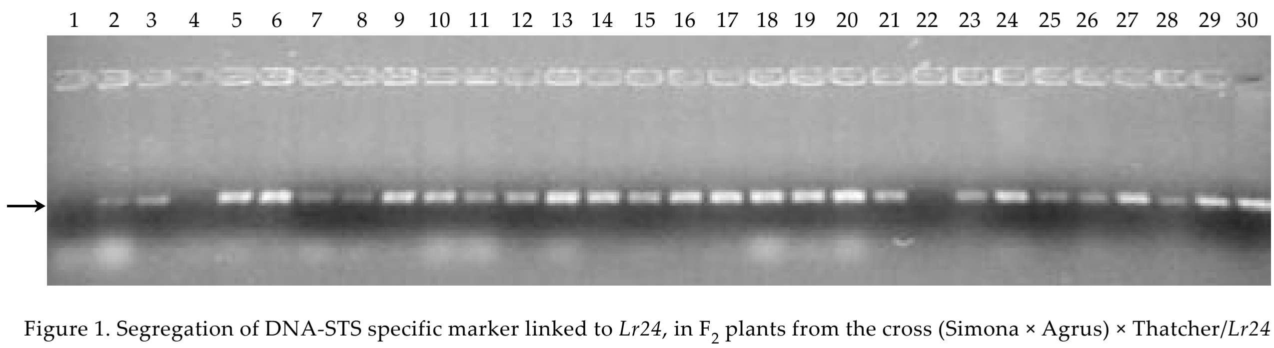

Figure 1. Segregation of DNA-STS specific marker linked to *Lr24*, in F_2 plants from the cross (Simona × Agrus) × Thatcher/*Lr24* (line 1 = negative control, lines 2–30 = individual plants, length of amplified fragment is 350 bp)

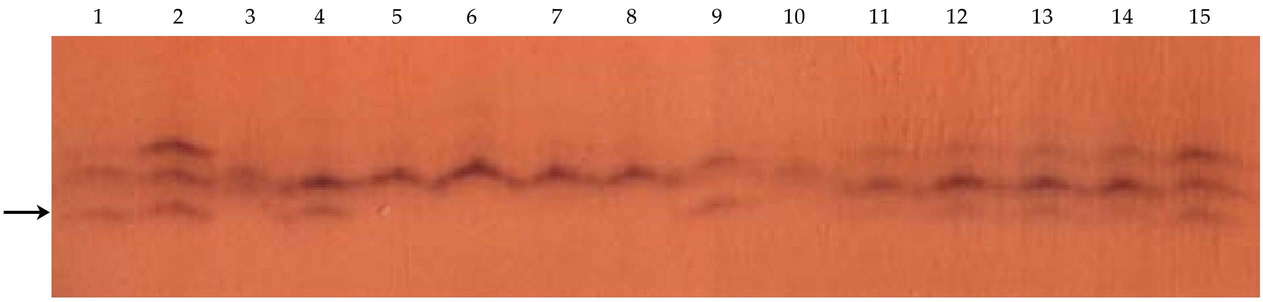

Figure 2. Endopeptidase zymograms of parental cultivars, progenies of F_2 generation with *Ep-D1c* allele and the susceptible progenies with *Ep-D1a* allele – embryo extract (arrow indicates band encoded by *Ep-D1a* allele or lacked band corresponding to null allele *Ep-D1c*)

1, 9 = Chinese Spring – allele *Ep-D1a*
2 = Simona – allele *Ep-D1a*
3, 10 = Agrus – allele *Ep-D1c*
4 = Thatcher/*Lr24* – allele *Ep-D1a*
5, 6, 7, 8 = progenies from cross (Simona × Agrus) × Thatcher/*Lr24* – allele *Ep-D1c* (linked to *Lr19*)
11, 12, 13, 14 = progenies from cross – (Lívia × Agrus) × Thatcher/*Lr24* – allele *Ep-D1a*
15 = Lívia – allele *Ep-D1a*

plants possessing a marker linked to *Lr19* gene were selected from the cross (Simona × Agrus) × Thatcher/*Lr24* and 5 from the cross (Lívia × Agrus) × Thatcher/*Lr24*. All 18 F_2 selected plants were self-pollinated to create F_3 progenies. Consequently homozygous from heterozygous plants were distinguished by simultaneous comparison and analysis of DNA-STS marker in F_3 progenies. Differentiation of homozygous and heterozygous individuals has been performed by the analysis of ten plants from each of the F_3 progenies by DNA-STS the marker linked to *Lr24*. If F_3 individuals in all ten-desired STS marker was present, and then selected F_2 plant was homozygous in marker locus. Six of the 13 progenies of (Simona × Agrus) × Thatcher/*Lr24* and two from the 5 progenies of (Lívia × Agrus) × Thatcher/*Lr24* were found as homozygous in DNA-STS linked *Lr24* marker. The last step in the

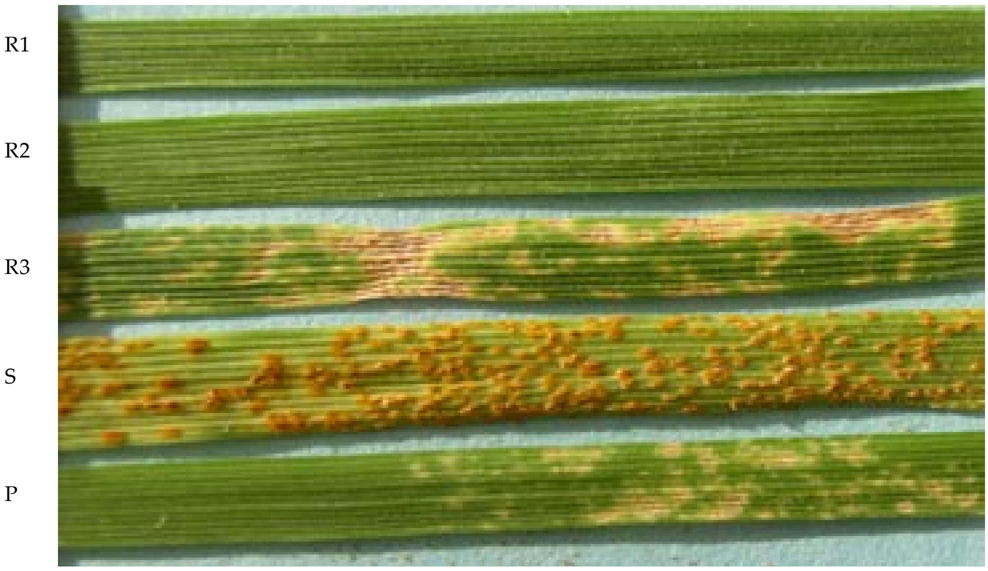

Figure 3. The first leaves of plants from the progenies of the F_2 generation with *Lr19* + *Lr24* and the first leaves of parents 14 days after inoculation with leaf rust pathotype 4332 SaBa

R1 and R2 = resistant progenies of the F_2 generation from cross (Simona × Agrus) × Thatcher/*Lr24* with *Lr19* + *Lr24*
R3 = resistant progeny of the F_2 generation from cross (Simona × Agrus) × Thatcher/*Lr24* with *Lr24*
S = susceptible parental cultivar Simona without *Lr* genes
P = resistant parental NIL – Thatcher/*Lr24* with gene *Lr24*

marker-assisted selection was the detection of the presence or absence of *Ep-D1c* null allele, respectively, in embryos of 8 progenies of F_2 generation. This marker allele was confirmed in 4 of them, others possessed *Ep-D1a* allele (Figure 2). It is probably caused by the ambiguity of evaluation of leaf endopeptidase patterns of plants from the F_2 generation. Winzeler et al. (1995) calculated a genetic distance between *Lr19* gene and *Ep-D1c* allele to 0.33 ± 0.33 cM but a recombination between the *Agropyron elongatum* segment and the wheat 7DL chromosome occurred. The parental cultivars and 8 progenies of F_2 generation were tested for leaf rust resistance with six leaf rust isolates. Six progenies were resistant to leaf rust. Their reselection and reaction to leaf rust confirmed a resistance to leaf rust and the presence of the combination of genes *Lr19* and *Lr24*. One progeny that responded as the parent Thatcher/*Lr24*, i.e. reaction indicated the presence of only *Lr24* gene and the absence of *Lr19* gene (Figure 3). Another progeny segregated for infection type as shown by Thatcher/*Lr24* and by genotypes with *Lr19* and *Lr24* genes (the reselection confirmed presence of allele *Ep-D1a*) and the response to leaf rust showed that plants are not homozygous at the *Lr19* locus.

Two effective leaf rust resistance genes *Lr19* and *Lr24* were successfully transferred into six wheat genotypes with the assistance of molecular markers. Plants carrying two leaf rust resistance genes *Lr19* and *Lr24* were identified simultaneously in F_2 generation by protein and DNA marker, respectively. To our knowledge, no pyramiding leaf rust resistance genes by molecular markers has been reported. The gene pyramiding in wheat has been published e.g. Liu et al. (2000) who selected double homozygotes possessing powdery mildew resistance gene combinations *Pm2* and *Pm4a, Pm2* and *Pm21, Pm4a* and *Pm21* by molecular markers. Molecular markers have been used also in development of advanced breeding rice lines by cumulated three resistance genes against bacterial blight pathogen (Singh et al. 2001). Hittalmani et al. (2000) used markers to combine three blast resistance genes into a single rice genotype. Indirect selection using DNA markers would facilitate the combination of these closely linked resistance genes into cultivars. It is shown in our study that molecular markers can effectively help to pyramid important genes in wheat and generate advanced breeding lines.

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ABSTRAKT

Tvorba genotypů pšenice s kombinací genů rezistence ke rzi pšeničné *Lr19* a *Lr24*

Pro přenos genů rezistence ke rzi pšeničné *Lr19* + *Lr24* do komerčních odrůd ozimé pšenice byl užit výběr na základě alely *Ep-D1c* endopeptidázy a markeru DNA. Nejdříve byl přenesen gen *Lr19* z donorové odrůdy Agrus do odrůd-akceptorů Simona a Lívia. Potomstva mající nulovou alelu *Ep-D1c*, která je ve vazbě s genem *Lr19*, byla vyselektována na odolnost ke rzi pšeničné infekcí izolátem 4332 SaBa rzi pšeničné. Rostliny homozygotní v lokusu *Ep-D1c* a rezistentní ke rzi pšeničné se křížily s NIL Thatcher/*Lr24* – donorem genu *Lr24*. Rostliny mající oba *Lr* geny byly vybrány z F_2 populace pomocí STS a izozymových markerů, které jsou ve vazbě se zmíněnými *Lr* geny. Potomstva 18 F_2 rostlin byla vybrána STS markerem a testována na odolnost ke rzi pšeničné. Výsledky získané izozymovým a STS markerem odpovídaly testům rezistence. Celkem bylo získáno 6 potomstev F_3 generace s kombinací genů rezistence *Lr19* + *Lr24* v homozygotní sestavě.

Klíčová slova: *Triticum aestivum* L.; rez pšeničná; gen *Lr19*; gen *Lr24*; markery; molekulární šlechtění

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