SZENT ISTVÁN UNIVERSITY

Identification and utilization of PCR-based molecular markers in breeding wheat for leaf rust resistance

Ph.D. Theses

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1. ANTECEDENTS AND OBJECTIVES

One of the most dangerous diseases of winter wheat in Hungary is leaf rust (*Puccinia recondita* Rob. ex Desmaz. f. sp. *tritici* /Erikss./, syn.: *Puccinia triticina* Eriks.), which may cause yield losses of up to 50%, depending on the susceptibility of the cultivars and on environmental conditions. Although at present more than sixty wheat leaf rust resistance genes (*Lr*) are known, only a small fraction of these is present in the cultivated cultivars. As part of continuous breeding activity and in order to enhance the genetic variability of cultivars, it is essential to introduce as many different resistance genes as possible into the cultivars and to keep searching for new sources of resistance.

The traditional method for the introduction of one or more resistance genes into a wheat cultivar is a time-consuming and labour-intensive procedure. The time span of this manipulation can be considerably abbreviated by the utilization of the recently introduced molecular marker techniques. Important areas of the practical utilization of molecular markers are marker-assisted selection (MAS) and the genetic characterization of wheat cultivars and basic breeding materials. It is important for practical utilization that the prospective molecular marker be closely linked with the given resistance gene, that it be reliably and routinely applicable, and that it be co-dominantly inherited in order for the heterozygous and homozygous individuals to be easily differentiated. Although a large number of molecular markers linked to *Lr* genes are known, the majority of these are not inherited in a co-dominant fashion, or are not linked closely enough to the resistance gene; for this reason, there is a continuing demand for the identification of further markers linked as closely as possible to the *Lr* genes.

This work summarized the results of two research projects. In the first part, the identification and genetic mapping of molecular markers linked to *Lr*

genes of medium efficiency (*Lr20* and *Lr52*) are presented. The second part contains a summary of the results of the practical utilization, for diagnostic and breeding purposes, of molecular markers closely linked to the *Lr* genes mentioned above as well as to gene *Lr34*.

The objectives of the individual projects were the following:

1. Identification, characterization and genetic mapping of molecular markers closely linked to leaf rust resistance genes *Lr20* and *Lr52*.

2. Validation of the already known, closely linked STS marker of gene *Lr20* and its utilization in a backcross breeding program using marker-assisted selection.

3. Identification of genes *Lr20*, *Lr52* and *Lr34* using the molecular markers already known as well as those identified in this work, and examination of the frequency of these genes in winter wheat cultivars registered in Hungary in 1970–2005; furthermore, analysis of the efficiency of gene *Lr34* on the basis of natural leaf rust infestation data obtained under field conditions in the course of several years of observation.

2. MATERIALS AND METHODS

2.1 Plant material

Molecular markers were identified in Nearly Isogenic Lines (NILs) RL6092 (Thatcher*6/Timmo) (abbr. NIL *Lr20*) carrying gene *Lr20* and RL6107 (Thatcher*6/V336) (abbr. NIL *Lr52*) carrying *Lr52*, furthermore the susceptible cultivars Thatcher and GK Délibáb. For the purpose of analysing the linkage between molecular markers and resistance genes and for genetic mapping, two F_2 populations (119 and 267 individuals) were generated by NIL *Lr20*/GK Délibáb and NIL *Lr52*/GK Délibáb crosses, respectively. The presence of the resistance genes *Lr20*, *Lr52* and *Lr34* was studied in 222 winter wheat cultivars registered in Hungary between 1970 and 2005 and in Bezosztaja 1, a Russian cultivar registered in Hungary in 1960.

2.2 Inoculation and phenotyping of the plants in F_2 populations

The monospore leaf rust isolate classified as pathotype 02000-04722700, used for the characterisation of genes *Lr20* and *Lr52* in the F_2 populations was prepared and kindly provided for me by Dr. Mária Gyuris (Mrs. László Csősz) (Cereal Research Institute). 7-day-old seedlings of the F_2 populations and the control plants (NIL *Lr20*, NIL *Lr52*, GK Délibáb, Thatcher) were inoculated with this leaf rust race using the method of Csősz (2007). The plants were evaluated on the 10th day after inoculation based on the reaction to inoculation, on a scale of 0–4 (Stakman *et al.,* 1962). Of the infection types (IT), IT≤2 was evaluated as resistant and IT=3 and 4 as susceptible.

2.3 DNA isolation

DNA isolation from the plant material used for the identification of molecular markers was carried out by the CTAB method of Rogers and Bendich (1985) as

modified in our laboratory. DNA isolation from the 222 cultivars and Bezosztaja 1 was done using the method of Purnhauser *et al.* (2011).

2.4 Molecular techniques employed in the experiments

Markers linked with genes *LR20* and *Lr52* were identified using the AFLP (Vos *et al.,* 1995), RGAP (Chen *et al.,* 1998), RAPD (Tar *et al.,* 2008), STS (Neu *et al.,* 2002 and Obert *et al.,* 2005) and SSR (Tar *et al.,* 2008) methods. 82 AFLP, 48 RGAP, 48 SSR primer pairs and 1 STS primer were tested for the identification of markers linked to gene *Lr20*. To identify markers linked to gene *Lr52*, 280 RAPD, 8 STS and 48 SSR primers were tested.

For the purpose of the genetic characterization of the 222 wheat cultivars registered in Hungary, gene *Lr20* was detected with STS marker *Xsts638* (Neu *et al.,* 2002) and with SSR markers *Xgwm344*, *Xwmc809* and *Xwmc274* identified in our laboratory; resistance gene *Lr52* was detected with SSR marker *Xgwm234* and STS marker *Xtxw200* identified in our laboratory, and gene *LR34* with STS marker *XcsLV34* (Lagudah *et al.,* 2006).

2.5 Genetic mapping and statistical calculations

Linkage analysis and genetic mapping of molecular markers for genes *Lr20* and *Lr52* were carried out using the computer program MAPMAKER 3.0 (Lander *et al.,* 1987). The closeness of linkage was calculated on the basis of Kosambi's mapping function (Kosambi, 1944).

Leaf rust resistance data of the cultivars were collected from the yearly publications of the Central Agricultural Office (Mezőgazdasági Szakigazgatási Hivatal, MgSzH). Analysis of the resistance data of cultivars carrying and not carrying gene *Lr34* as compared to the yearly average was done using the t-test (Sváb, 1981).

3. RESULTS

3.1 Identification and characterization of molecular markers linked with gene *Lr20*

In order to identify new molecular markers closely linked with gene *Lr20*, phenotypization of the NIL *Lr20*/GK Délibáb F_2 population (119 plants) was first carried out via artificial inoculation (the ratio of resistant and sensitive individuals was 2:1).

To identify linked markers, 82 Pst/Mse AFLP and 48 RGAP primer combinations were first tested. Numerous polymorphic fragments (candidate markers) were recognized; after the completion of linkage analysis (comparison of phenotypic and marker data), however, no marker linked to gene *Lr20* was found. On the other hand, marker *Xsts638* specific for resistance gene *Lr20*, described by Neu *et al.* (2002) was successfully applied to the characterization of our F_2 population, and was mapped to a distance of 4.6 cM from the resistance gene (Fig. 1).

In the course of testing the 48 SSR primers specific for wheat chromosome 7AL, polymorphic fragments were amplified by 6 primers. Linkage of each of the six SSR markers to gene *Lr20* was confirmed by linkage analysis. The map distance between the markers and the resistance gene ranged from 0.5 to 12.8 cM. Markers *Xwmc809* and *Xgwm344* were present in the repulsive phase, i.e. the polymorphic fragment was detectable only in susceptible and heterozygous individuals. Five SSR markers, namely *Xwmc809* (0.5 cM), *Xgwm344* (0.5 cM), *Xwmc273* (2.8 cM), *Xwmc525* (3.3 cM) and *Xcfa2240* (3.8 cM) mapped to the proximal side of the resistance gene. A single SSR marker, *Xcfa2257* (12.8 cM) mapped to the distal side of the resistance gene (Fig. 1).
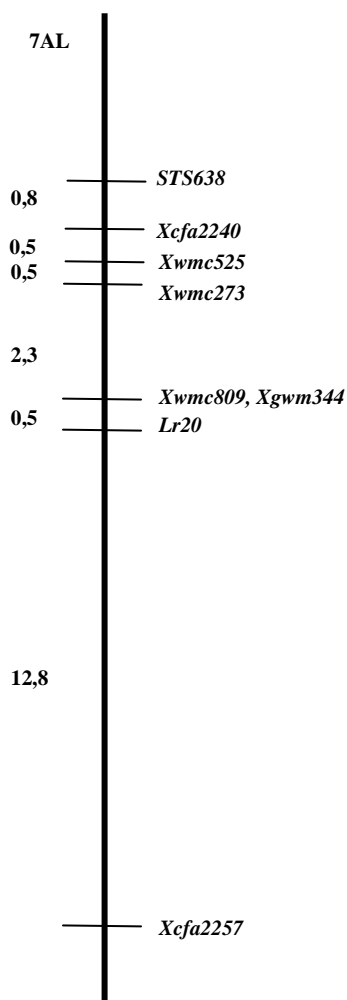
7AL

STS638

0,8

Xcfa2240

0,5

Xwmc525

0,5

Xwmc273

2,3

Xwmc809, Xgwm344

0,5

Lr20

12,8

Xcfa2257

Figure 1. Genetic map of SSR and STS markers linked to gene *Lr20*. The designations of the markers are shown on the right side and the genetic distances on the left side.

3.2 Identification and characterization of molecular markers linked to gene *Lr52*

In the course of the phenotypization of the NIL *Lr52/GK* Délibáb F_2 population (267 individuals), 188 resistant and 79 susceptible plant were identified (the monogenic segregation ratio of 3:1 is statistically confirmed). In order to identify linked markers, 280 RADP primers were first tested. 11 of the primers

studied (OPR-10, OPR-11, OPR-16, OPV-2, OPV-4, OPV-14, OPW-19, OPX-9, OPX-12, OPY-4, OPY-9) proved to amplify polymorphic fragments. Out of these, however, only the 535-bp fragment amplified by primer OPR-10 exhibited linkage with the target gene; it was mapped to a distance of 18.2 cM on the proximal side of the resistance gene (Fig. 2).

8 STS primers specific for chromosome 5BS were also tested. Of these, only primer *Xtxw200* produced a polymorphic pattern upon comparison of NIL *Lr52*, Thatcher and GK Délibáb. In the population studied, this marker was mapped to a location 3.6 cM distal to resistance gene *Lr52* (Fig. 2).

Only 6 of the 44 SSR primers specific for the short arm of chromosome 5B proved to be polymorphic upon comparison of the resistant parent, Thatcher and Délibáb. Interestingly, further tests revealed each of these to be linked to resistance gene *Lr52*: they mapped to distances of 7.2 – 46.5 cM from the target gene (Fig. 2). The SSR marker closest to the gene on the distal side is *Xgwm234 (*7.2 cM) and on the proximal side, *Xwmc149* (11.3 cM). Since the SSR markers identified by us are co-dominant, they are suitable for the identification of both homozygous and heterozygous individuals.
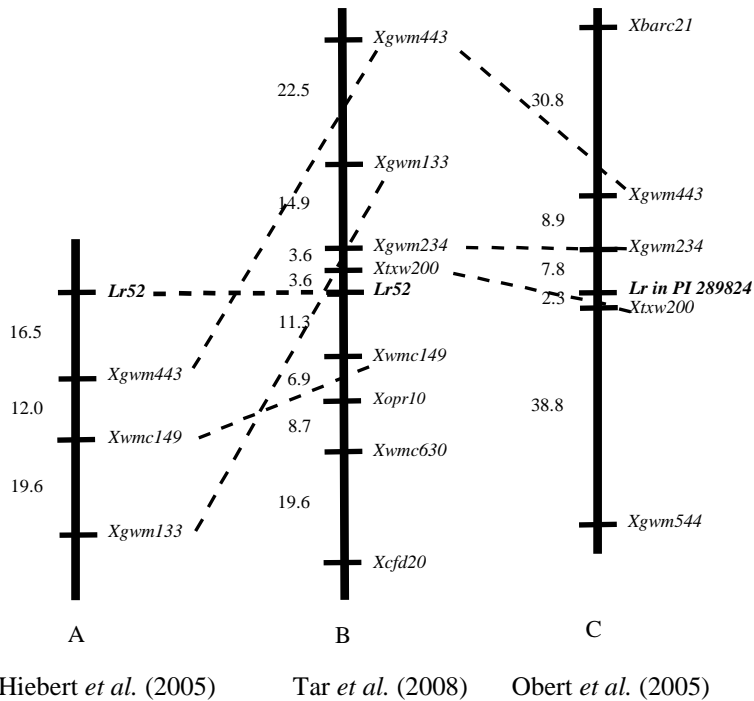
Figure 2. Molecular markers linked to resistance gene *Lr52* on the short arm of chromosome 5B.

3.3 Practical utilization of molecular markers

3.3.1 Marker-assisted selection

A breeding program supported by marker-assisted selection was initiated with the purpose of introducing gene *Lr20* into cultivar Jubilejnaja-50 (susceptible to leaf rust). The donor chosen was line NIL *Lr20*. Primer STS638 was used for marker-assisted selection, because according to Neu et al. (2002) the corresponding marker is closely (0.4 cM) linked with gene *Lr20*. The resistance gene was detected in F_2 individuals based on the molecular marker, and the individuals shown to carry the marker as well as cultivar Jubilejnaja-50 were used for back-crosses. The presence of gene *Lr20* was checked in each BC generation using primer STS638. After the second back-cross the lines were sown in the field, and their resistance to natural leaf rust infestation was

evaluated. The evaluation revealed differences between the leaf rust resistances of the lines. At present the resistant lines are included in our field experiments.

3.3.2 Detection of *Lr* genes in wheat cultivars with the help of molecular markers

The presence of gene *Lr34* was detected, using marker X*csLV34* (Lagudah *et al.,* 2006), in 51 of the winter wheat cultivars registered in Hungary between 1970 and 2005 (n=222) and in cultivar Bezosztaja 1 registered in 1960. Comparison of the thirty-year programmes of the two largest Hungarian breeding institutions reveals that gene *Lr34* is identified at nearly identical frequencies: it is detected in 23.9% (n=71) of the Szeged cultivars and in 20% (n=70) of the Martonvásár cultivars. This resistance gene is carried by 31.3% of other cultivars bred in Hungary and by 23.1% of European cultivars registered in Hungary.

The study on the frequency of gene *Lr34* is more relevant if it is analyzed in a yearly distribution compared to all wheat cultivars with valid registration in the given year. The distribution of gene *Lr34* ranged from 11.8% (1980) to 57.1% (1971) in the 222 wheat cultivars studied. Of the cultivars studied, *Lr34* first appeared in the Russian cultivar Bezosztaja 1 registered in 1960. Of the cultivars present in the registry during the time period scrutinized in this work (1970-2005), the first cultivar to carry the gene was Kavkaz, another cultivar of Russian origin registered in 1970. In the nineteen-seventies, further cultivars carrying *Lr34* appeared: 4 cultivars at Szeged [GK 3, GK F2 (1971), GK Tiszatáj (1977) and GK Szeged (1978)], 2 cultivars in Martonvásár [Martonvásári 1 (1971) and Martonvásári 3 (1973)] and one Bulgarian cultivar, Burgas-2 (1972). The frequency of gene *Lr34* was the highest in the period between 1971 and 1974 among the cultivars included in the registry in the given year (41.7-57.1%). The ratio of carrier cultivars transiently decreased, then again increased between 1983 and 1990 (33.3-34.8%). Starting from 1992

all the way to the turn of the millennium, the frequency of gene *Lr34* is seen to decrease continuously (Fig. 3). It must be noted that in spite of the decrease in the ratio of cultivars carrying the gene, the number of cultivars containing this gene is generally on the rise, since the number of cultivars included in the registry in the given year increased tenfold between the beginning and the end of the period studied. Evaluation of the data on year 2005 reveals that out of the registered cultivars cultivated at the time (n=105), cultivars carrying *Lr34* occur most frequently among cultivars bred in Szeged and Martonvásár (25% and 25%, respectively) (Figs. 4-5).
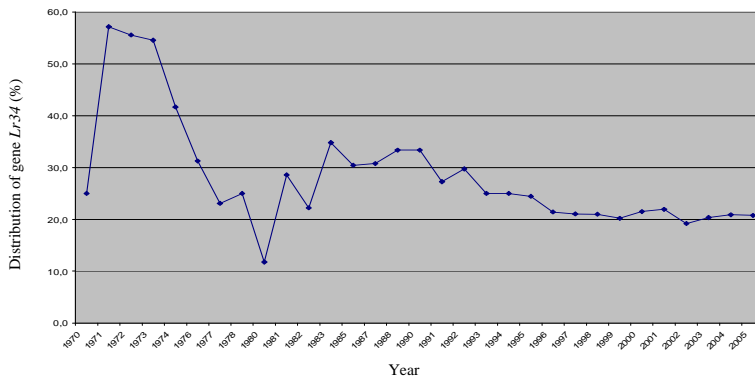
Figure 3. Distribution of gene *Lr34* in 1970–2005 in wheat cultivars included in the registry in the given year
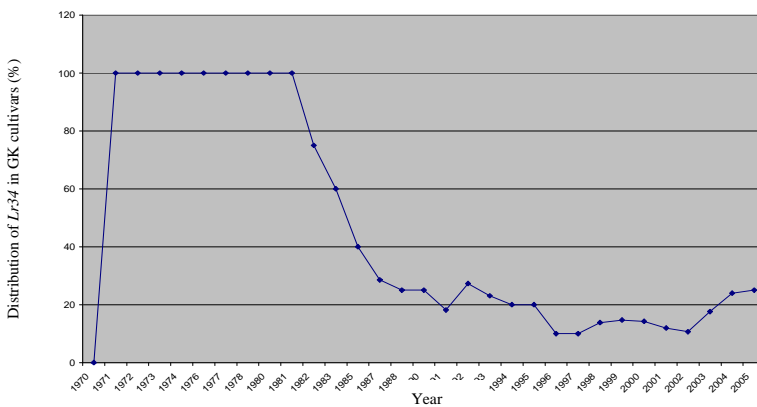
Figure 4. Distribution of gene *Lr34* in 1970–2005 in GK wheat cultivars included in the registry in the given year
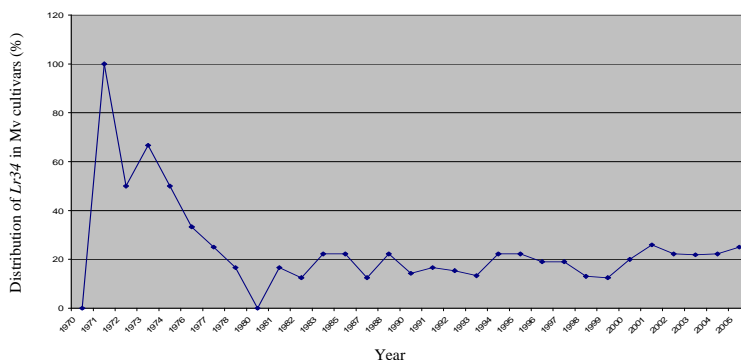
Figure 5. Distribution of gene *Lr34* in 1970–2005 in Martonvásár wheat cultivars included in the registry in the given year

Natural leaf rust infestation data for the period between 1985 and 2003 were available for 72.1% of the cultivars studied. Natural leaf rust infestation data were evaluated on a yearly basis (for 9 years). Average infestation was calculated for the given year, and the value of the most heavily infested cultivar was determined (maximum infestation). Defining the latter as 100%, four infestation groups were formed: cultivars exhibiting 0–25% infestation relative to maximal infestation were classified as resistant, and those with 25.1–50, 50.1–75 and 75.1–100% infestation were termed moderately resistant, moderately susceptible and highly susceptible, respectively. The t-tests performed on the data of four years (1985, 1988, 1997 and 2002) showed significant differences between the averages of the infestation percentages of cultivars carrying and of those not carrying gene *Lr34*.

Using molecular markers, gene *Lr20* was detected in cultivars Partizánka, Mv Szigma and Boszanova, which represents a frequency of 1.3% among the 222 cultivars studied. Gene Lr20 was detected in these 3 wheat cultivars by each of the 4 markers used (*Xsts638*, *Xwmc273*, *Xwmc809* and *Xgwm344*). Gene *Lr52* was also identified in three cultivars (GK Olt, GK Szálka, Dunai) using SSR marker *Xgwm234* and STS marker *Xtxw200*. In addition to the marker of gene *Lr52*, the Szeged cultivar GK Olt was also shown to contain that of *Lr34*.

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4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Evaluation of the molecular markering of gene *Lr20*; conclusions

In the work presented here, 6 new SSR markers closely linked with gene *Lr20* were identified. Interestingly, although SSR markers are usually inherited in a co-dominant fashion, we identified as many as two SSR markets that are dominantly inherited (*Xwmc809* and *Xgwm344*), and, what is more, are in repulsion, but these two markers exhibited the closest linkage (0.5 cM) to gene *Lr20*. Although the first two traits mentioned represent a certain disadvantage for breeding, it is more important that, according to our data, these two markers have the closest linkage with gene *Lr20*. 3 [*Xwmc273* (2.8 cM), *Xwmc525* (3.3 cM) and *Xcfa2240* (3.8 cM)] out of the 6 SSR markers mapped in the course of our experiments were localized within 10 cM from the gene and were inherited in a co-dominant pattern, thus these are easier to use for the purpose of MAS, and at the same time their distances from the resistance gene are not too large to interfere with the efficiency of selection.

Marker *Xsts638*, described by Neu *et al.* (2002) as a diagnostic STS marker was also included in our work. According to our observations, however, in the F_2 mapping population generated by crossing the NIL *Lr20* (resistant) and GK Délibáb (susceptible) cultivars this marker mapped to a larger distance from *Lr20* than reported by Neu *et al.* (2002) (4.9 cM vs. 0.4 cM). Our results confirm the data published by Khan *et al.* (2005), who also measured a larger genetic distance between *Lr20* and *Xsts638* (7.1 cM). In addition to the STS marker mentioned above, Neu *et al.* (2002) also identified several RFLP and two SSR markers co-inherited with the resistance gene. The two latter (*Xgwm282* and *Xgwm332*), however, mapped too far (32.8 cM) from the resistance gene. Five (*Xcfa2240*, *Xgwm344*, *Xwmc273*, *Xwmc525* and *Xwmc809*) out of the six SSR markers identified in our experiments proved to be linked to *Lr20* even closer (4.9 cM) than marker *Xsts638*.

Neu *et al.* (2002) identified 12 markers co-inherited with resistance gene *Lr20*; however, none of these markers was situated on the distal side of the resistance gene. In the work presented here we succeeded in identifying an SSR marker (*Xcfa2257*) in a distance of 12.8 cM on the distal side of the resistance gene.

4.2 Evaluation of the molecular markering of gene *Lr52*; conclusions

We determined the map positions of 6 SSR (*Xcfd20*, *Xgwm133*, *Xgwm234*, *Xgwm443*, *Xwmc149* and *Xwmc630*), 1 STS (*Xtxw200*) and 1 RAPD (*Xopr10*) linked markers relative to gene *Lr52*. SSR marker *Xgwm234* mapped to a distance of 7.2 cM from the resistance gene, and is thus suitable for use in MAS. Hiebert *et al.* (2005) determined the location of gene *Lr52* in the genome using microsatellite markers. They mapped *Xgwm133* to 48.1 cM, *Xwmc149* to 28.5 cM and *Xgwm344* to 16.5 cM from the resistance gene. Thus, the SSR marker reported by these authors to be closest to the resistance gene was *Xgwm344*, positioned on the proximal side of the resistance gene, in contradiction with our results. Also, our experiments revealed that in our mapping population marker *Xwmc149* mapped considerably closer to resistance gene *Lr52* than reported by Hiebert *et al.* (2005) (11.3 cM vs. 28.5 cM). According to our results, SSR marker *Xwmc149* is also suitable for utilization in marker-assisted selection.

Obert *et al.* (2005) identified a new *Lr* gene in an Iranian cultivar (PI289824). Somers *et al.* (2004) localized this gene on wheat chromosome 5BS with the help of SSR markers mapped on the 42 wheat chromosomes, thus they considered it possible to be identical with gene *Lr52*. In their experiments, the new resistance gene exhibited linkage with the following SSR markers: *Xbarc21*-5BS (47.4 cM), *Xgwm443*-5BS (16.7 cM), *Xgwm234*-5BS (7.8 cM) and *Xgwm544*-5BS (41.1 cM). In agreement with our results, Obert *et al.* (2005) also mapped marker *Xgwm443* to the distal side of the new resistance

gene. In the course of our work we also tested the other 3 SSR markers shown to be linked by Obert *et al.* (2005), but only *gwm234* amplified a polymorphic fragment in our population. After the linkage test we established that out of the 6 SSR markers identified in our laboratory, the one closest to resistance gene *Lr52* was *Xgwm234* (7.2 cM). Obert *et al.* (2005) also reported an AFLP marker closely linked to the new resistance gene identified by them; they converted this AFLP marker to an STS marker and gave it the name of *Xtxw200*. Having found similarities in the results of SSR studies, we also examined the linkage of STS marker *Xtxw200* in our F_2 population. We found that in our population both *Xgwm234* and *Xtxw200* are situated on the distal side of the new resistance gene, contrary to the results of Obert *et al.* (2005), who localized *Xgwm234* on the distal side of the new resistance gene. These results leave open the question whether or not the resistance gene identified by Obert *et al.* (2005) is identical with gene *Lr52* studied by us, or is only situated in its close proximity. In order to find the answer, it would be worth running the allelic test between the two genes.

4.3 Marker-assisted selection and identification of *Lr* genes with the help of molecular markers in wheat cultivars

We used the dominant marker *Xsts638* (Neu *et al.,* 2002) for selection in our back-crossing program aiming at the introduction of resistance gene *Lr20*. SSR markers of co-dominant inheritance, however, are more advantageous to use in marker-assisted selection, because they allow the identification of homozygous individuals already in the F_2 generation. It is therefore expedient to use the SSR markers identified by us for further marker selection for gene *Lr20*. Although gene *Lr20* in itself is of medium efficiency against leaf rust infestation (Vida *et al.,* 2009), it is still worth including in breeding programs, in itself or in combination with other leaf rust resistance genes, in order to increase genetic diversity.

One possibility for the practical utilization of molecular markers linked to *Lr* genes is identification of resistance genes in the individual winter wheat cultivars. In the course of our work we examined 222 winter wheat cultivars registered in Hungary between 1970 and 2005, with the purpose of detecting the presence of resistance genes *Lr34, Lr20* and *Lr52* based on the molecular markers they are closely linked with. Gene *Lr34* was identified in winter wheat cultivars included in the Hungarian registry between 1970 and 2005 using the co-dominant STS marker described by Lagudah *et al.* (2006) (*csLV34* 0.4 cM). Marker *XcsLV34* was also successfully applied in Hungary by Wang *et al.* (2009) and Vida *et al.* (2009) for the identification of gene *Lr34* in breeding lines as well as in cultivars from Martonvásár and abroad. In the course of their experiments, these authors detected the presence of the marker in 12 registered Martonvásár wheat cultivars, namely: Mv3, Mv13, Mv17, Mv Emese, Mv Garmada, Mv Gorsium, Mv Laura, Mv Mambó, Mv Pálma, Mv Palotás, Mv Táltos and Mv Vilma. We also identified the molecular marker of gene *Lr34* in the cultivars listed, with the exception of two cultivars not studied by us, Mv Gorsium and Mv Laura.

Gene *Lr34* is carried by 23.0% of the 222 cultivars studied in our laboratory. The frequency of gene *Lr34* has been studied on several occasions all over the world. For example, Winzeler *et al.* (2000) observed adult resistance in 55% of the European cultivars tested, which they supposed to be due to gene *Lr13*, *Lr34* or their combination. Our studies confirm that the spread of gene *Lr34* in Hungary is mostly due to Bezosztaja 1. Yearly evaluation of the available natural leaf rust infestation data (published by the Central Agricultural Office) revealed that the majority of cultivars carrying gene *Lr34* are classified as resistant. Since this resistance gene in itself is of medium efficiency (Mesterházy *et al.,* 2000), it is presumably present in the cultivars in combination with another gene leading to adult resistance (e.g. *Lr13*) (Singh *et al.,* 2000), or with a race-specific resistance gene classified as

efficient or of medium efficiency (Kolmer and Oelke, 2006). Although, after the turn of the millennium, gene *Lr34* is present in only a quarter of the cultivars included in the Hungarian registry, the utilization of this gene in resistance breeding continues to be an important task.

In our experiments the presence of gene *Lr20* in the cultivars was detected with the help of three SSR markers identified in our laboratory (*Xwmc809*, *Xgwm344*, *Xwmc273*). The gene was shown to be present in only 3 out of the 222 cultivars tested (1.3%). Our results are in agreement with those reported by Winzeler *et al.* (2000) and Singh *et al.* (2001), who detected the presence of gene *Lr20* in only 4% of the European and English cultivars tested using special leaf rust isolates. The occurrence of gene *Lr52* in wheat cultivars has only been mentioned in a few cases (McIntosh *et al.,* 1995). Out of the 222 cultivars tested, we detected the presence of this resistance gene in 2 Szeged-bred and 1 Austrian-bred wheat cultivars using the SSR marker closely linked to the gene (7.2 cM), identified in our laboratory. In Hungary, seedling and adult resistance due to gene *Lr52* was studied by Manninger (2002) and Csősz (2007), and it was established that the gene was of medium efficiency at both developmental stages. Although gene *Lr52* is not frequent, on the basis of adult resistance data it is still commendable to introduce into new cultivars, in combination with other *Lr* genes providing efficient race-specific or non-race-specific resistance. Since linkage of the markers used with the given *Lr* genes is not 100%, further tests (pathophysiological tests using special leaf rust isolates) are also needed for the confirmation of the presence of the genes.

It is to be hoped that the elucidation of the so far unknown genetic background of resistant cultivars (identification of resistance genes) and the application of MAS, a method with ever increasing popularity will soon accelerate the purposeful introduction of resistance genes into breeding lines and cultivars.

NEW SCIENTIFIC RESULTS

We performed our experiments in the field of resistance breeding. The molecular markers described in the first part of these theses can be utilized for the characterisation and mapping of resistance genes as well as for MAS. Practical utilization of molecular markers described in the second part of this work may provide useful information for breeders in the course of the detection of resistance genes present in the various cultivars.

In the course of our work, the following new results were obtained:

1. Six new SSR markers (*Xcfa2240, 3.8 cM*, *Xwmc525, 3.3 cM*, *Xwmc273, 2.8 cM*, *Xwmc809, 0.5 cM*, *Xgwm344, 0.5 cM*, *Xcfa2257, 12.8 cM*) linked to gene *Lr20* were identified and mapped.

2. An SSR marker localized on the distal side of gene *Lr20* was successfully identified (*Xcfa2257*, 12.8 cM).

3. We mapped the position of the dominant marker *Xsts638* (0.4 cM; Neu *et al.,* 2002) to a considerably larger distance (4.9 cM) from gene *Lr20*.

4. We identified a new RAPD (*Xopr10*, 18.2 cM), a new STS (*Xtxw200,* 3.6 cM) and 6 new SSR (*Xgwm133*, 22.1 cM, *Xgwm234*, 7.2 cM, *Xgwm443*, 44.6 cM, *Xwmc149*, 11.3 cM, *Xwmc630*, 26.9 cM and *Xcfd20*, 46.5 cM) markers and mapped their positions relative to gene *Lr52*.

5. From the markers linked to gene *Lr52* identified by us, the dominant STS marker *Xtxw200* (3.6 cM) and the co-dominant SSR markers *Xgwm234* (7.2 cM) and *Xwmc149* (11.3 cM) mapped closer than marker *Xgwm443* described in the literature (16.5 cM; Hiebert *et al.,* 2005).

6. Using molecular markers, we identified genes *Lr52*, *Lr20* and *Lr34* in 222 winter wheat cultivars included in the Hungarian registry in the years 1970–2005 and determined their frequencies. We established that both *Lr52* and *Lr20* occurred at very low frequencies (1.3%). Gene *Lr34*, however, was shown to have spread extensively in the cultivars grown in Hungary (23.0%).

7. Based on the natural infestation data observed under field conditions between 1985 and 2003 we established that, in several of the years studied, the cultivars carrying gene *Lr34* are more significantly resistant than those lacking this gene.

ACKNOWLEDGEMENTS