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Application of molecular markers in breeding for leaf rust resistance in wheat

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Abstract

The breeding and cultivation of resistant wheat varieties is an effective way of controlling leaf rust. The use of molecular markers facilitates the incorporation of the major leaf rust resistance genes (*Lr* genes) responsible for resistance into new varieties and the pyramiding of these genes. Marker assisted selection was used to incorporate the currently effective *Lr* genes in Hungary (*Lr9*, *Lr24*, *Lr25*, *Lr29*, *Lr35* and *Lr37*) into winter wheat varieties. The *Lr* genes were identified using STS, SCAR and RAPD markers closely linked to them. Investigations were made on how these markers could be utilised in plant breeding, and near-isogenic lines resembling the recurrent variety but each containing a different *Lr* gene were developed to form the initial stock for the pyramiding of resistance genes or creating multiline varieties. Molecular markers are also ideal for the identification of resistance genes in wheat genotypes with unknown genetic background. The presence of *Lr1*, *Lr10*, *Lr26*, *Lr34* and *Lr37* resistance genes has been demonstrated using molecular markers in the Martonvásár gene pool.

Keywords

Marker assisted selection, Puccinia triticina, pyramiding of genes, resistance breeding, *Triticum aestivum*

Introduction

Improving resistance to rust fungi is one of the major tasks facing wheat breeders all over the world. Wheat is attacked by three rust species: leaf rust (*Puccinia triticina* Eriks.), stem rust (*P. graminis* Pers.: Pers. f. sp. *graminis* Eriks. & E. Henn) and stripe (yellow) rust (*P. striiformis* Westend.). All three pathogens are capable of causing substantial economic losses, but their incidence varies due to their diverse ecological requirements. In Hungary the greatest damage is currently caused by leaf rust, which can be expected to infect wheat fields every year. During the first half of the 20th century it was not thought to be of economic importance (HUSZ 1941), but since then it has been shown that under Hungarian conditions leaf rust may cause yield losses of up to 40% (BARABÁS and MATUZ 1983).

The most environmentally sound, low cost method of controlling leaf rust is to breed and grow resistant wheat varieties. So far over 60 leaf rust resistance genes, i.e. *Lr* genes, have been identified and localised on the wheat chromosomes. In addition, a number of temporarily designated resistance genes and quantitative loci (QTLs) are able to provide total or partial protection against various rust pathotypes (MCINTOSH et al. 2008). The effectiveness of resistance genes depends on the composition of the pathogen population. As this changes dynamically, new pathotypes virulent to the given resistance gene multiply from time to time, so the resistance of a variety is not a constant trait. Any variety carrying a single resistance gene may become susceptible within a short time. The postulation of resistance genes is traditionally carried out using rust isolates with known virulence (KNOTT 1989), but this procedure is extremely time-, space- and labour-intensive and cannot be employed if no differential fungal isolate is available. In many cases resistance genes can only be identified using molecular markers (MELCHINGER 1990). Over the last 15 years many efficient markers for leaf rust resistance genes have been described. The molecular markers most closely linked to *Lr* genes are listed in *Table 1*. The table only contains markers based on the PCR technique, as the majority of these can be applied relatively easily in wheat breeding programmes.

Molecular markers are used for two purposes in resistance breeding: (1) to monitor the incorporation of designated resistance genes or QTLs into elite wheat genotypes (i.e. MAS, marker assisted selection), (2) to identify resistance genes in varieties and lines where the genetic background is unknown (i.e. gene detection). The Martonvásár wheat breeding programme makes use of molecular markers linked to leaf rust resistance genes for both of these purposes. Therefore, a backcross programme based on markers has been initiated to incorporate *Lr* genes that are currently effective in Hungary into Martonvásár wheat varieties, while the presence of *Lr* genes in the wheat varieties and lines bred in Martonvásár or used as parental partners in the breeding programme is also investigated.

Materials and methods

Marker assisted selection and identification of designated leaf rust resistance genes

A backcross (BC) programme was started in the Agricultural Research Institute of the Hungarian Academy of Sciences

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Table 1: **Molecular markers used for marker assisted selection of leaf rust resistance genes**

Lr gene	Marker type[1]	Linkage[2]	Name of the marker	Reference
Lr1	RGA	flank	Lr1RGA1	QIU et al. 2007
Lr3	cDNA	func	TaR16	DANNA et al. 2002
Lr9	SCAR	flank	SCS5550	GUPTA et al. 2005
Lr10	Functional	func	T10Rga1	FEUILLET et al. 2003
Lr13	SSR	flank	barc163-2B	BANSAL et al. 2008
Lr14	SSR	dist10	gwm344	HERRERA-FOESSEL et al. 2007
Lr16	SSR	flank	wmc764	MCCARTNEY et al. 2005
Lr19	STS	flank	GBF/GBR	PRINS et al. 2001
Lr20	STS	flank	STS638	KHAN et al. 2005
Lr21	Functional	func	Lr1L/Lr21R	HUANG and GILL 2001
Lr22a	SSR	flank	gwm455	HIEBERT et al. 2007
Lr24	SCAR	flank	SCS73719	PRABHU et al. 2004
Lr25	SCAR	flank	Lr25F20/Lr25R19	PROCUNIER 2009
Lr26	PCR-based	flank	P6M12-P	MAGO et al. 2005
Lr28	SCAR	flank	SCS421570	CHERUKURI et al. 2005
Lr29	SCAR	flank	Lr29F18/Lr29R18	PROCUNIER 2009
Lr34	STS	flank	csLV34	LAGUDAH et al. 2006
Lr35	STS,	flank	SR39 F2/R3,	GOLD et al. 2002
	SCAR		BCD260F1/35R2	SEYFARTH et al. 1999
Lr37	SCAR,	flank	SC-Y15F/SC-Y15R	ROBERT et al. 1999
	CAPS	flank	VENTRIUP/LN2	HELGUERA et al. 2003
Lr38	SSR	flank	wmc773	MEBRATE et al. 2008
Lr39	SSR	dist10	gwm210	RAUPP et al. 2001
Lr46	STS	flank	XSTS1BL2/XSTS1BL9	MATEOS-HERNANDEZ et al. 2006
Lr47	CAPS	flank	PS10R/PS10L	HELGUERA et al. 2000
Lr48	SSR	flank	gwm429b	BANSAL et al. 2008
Lr49	SSR	flank	barc163	BANSAL et al. 2008
Lr50	SSR	flank	gwm382	BROWN-GUERDIRA et al. 2003
Lr51	CAPS	flank	S30-13L/AGA7-759R	HELGUERA et al. 2005
Lr52	STS	flank	txw200	TAR et al. 2008
Lr58	SSR	flank	cfd50	KURAPARTHY et al. 2007
Lr60	SSR	flank	barc149	HIEBERT et al. 2008
Lr63	SSR	flank	barc321	KOLMER 2008
Lr64	SSR	dist10	barc104	KOLMER 2008

[1] CAPS, cleaved amplified polymorphic sequence; RGA, resistance gene analogue; SCAR, sequence characterized amplified region; SSR, simple sequence repeat; STS, sequence-tagged site

[2] dist10, distance between marker and gene >10cM; flank, flanking marker; func, functional marker

aimed at the transfer of effective *Lr* genes. Martonvásár winter wheat varieties with good agronomic and technological quality parameters, but susceptible or moderately resistant to leaf rust (Mv Emma, Mv Madrigál, Mv Pálma and Mv Magvas) were crossed with near-isogenic lines of Thatcher each carrying a different *Lr* gene [*Lr9*: Transfer/Thatcher*6 (R.L.6010); *Lr24*: Thatcher*6/Agent; *Lr25*: Thatcher*6/Transec; *Lr29*: Thatcher*6/Cs7D-Ag#11; *Lr35*: Thatcher*6/R.L.5711] and with Renan (*Lr37*). The F_1 plants were backcrossed to the recurrent parents. BC_1 plants were selected by means of MAS from different backcross generations and these were again backcrossed with the recurrent parent.

The choice of *Lr* genes for incorporation was based not only on their effectiveness, but also on whether reliable, closely linked PCR markers were available. These were used for MAS in backcross (BC) generations segregating for the *Lr* genes. The CTAB (cetyl-trimethyl-ammonium bromide) method (ROGERS and BENDICH 1985) and the DNeasy® Plant Mini Kit (Qiagen®) were used to isolate DNA. In each combination 10-15 plants were tested for leaf rust resistance in the greenhouse and field. In the seedling stage, the leaf rust resistance of the young plants was tested in the greenhouse parallel with the isolation of DNA, in order to monitor the efficiency.

The plants were inoculated in the 2-leaf stage with a mixture of leaf rust uredospores collected from varieties with various genetic backgrounds and multiplied in the greenhouse. PCR-based primers were used for the detection of the *Lr* genes (*Table 2*).

Table 2: **DNA markers used for marker assisted selection**

Lr gene	Marker	Marker type	Size of amplified product (bp)	References
Lr9	J13/1, J13/2	STS	1100	SCHACHERMAYR et al. 1994
Lr24	SC-H5/1, SC-H5/2	SCAR	700	DEDRYVER et al. 1996
Lr25	LR25F20, Lr25R19	SCAR	1800	PROCUNIER 2009
Lr29	UBC219	RAPD	1000	PROCUNIER et al. 1995
Lr35	BCD260F1/35R2	STS	900	SEYFARTH et al. 1999
Lr37	SC-Y15 F/R	SCAR	580	ROBERT ET AL. 1999

The PCR reactions were carried out as proposed by the authors cited in *Table 2*, after which the products were amplified using PTC-100 (MJ Research) and GeneAmp PCR System 9700 (Applied Biosystems) equipment. The amplified products were visible under UV light after electrophoresis on 1.2% agarose gels containing ethidium bromide. The presence of five leaf rust resistance genes (*Lr1*, *Lr10*, *Lr26*, *Lr34* and *Lr37*) was analysed in the Martonvásár wheat pool. Molecular markers *WR003* for *Lr1* (QIU et al. 2007), *ThLr10* for *Lr10* (FEUILLET et al. 2003), *IAG95* for *Lr26* (MOHLER et al. 2001), *csLV34* for *Lr34* (LAGUDAH et al. 2006) and *SC-Y15* for *Lr37* (ROBERT et al. 1999) were applied using the published PCR protocols.

Field tests

The field leaf rust resistance of the plants (36 Thatcher-based near-isogenic lines, 4 recurrent parents, donor parents, BC plants, control: Thatcher) was evaluated in an artificially inoculated nursery. Rows of a spreader variety, planted around the tested genotypes, were inoculated in development stage 37-39 on the Zadoks scale (ZADOKS et al. 1974) using the uredospore mixture also used in the greenhouse experiments. The spore suspension was injected into the spreader plants using a hyperdermic syringe. The pathogen then spread naturally from these primary sources of infection. The extent of infection at development stage 77-83 was evaluated in terms of severity (according to the modified Cobb scale; STUBBS et al. 1986) and host response (resistant, moderately resistant, intermediate, moderately susceptible and susceptible). The average coefficient of infection (ACI) was calculated from these two data by multiplying severity by an assigned constant value for the host response, for use in the statistical evaluation (STUBBS et al. 1986).

Dihaploid programme

The anther cultures were initiated from greenhouse-grown materials. Anthers in the mid-uninucleate stage were cultured on liquid MN6 induction medium (CHU et al. 1990). The cultures were kept in the dark at 29°C for 30 days, after which the embryogenic structures were transferred to 190-2 regeneration medium containing 0.09 M sucrose (ZHUANG and JIA 1983). Plant regeneration took place at 26°C with a 16-h light, 8-h dark photoperiod regime. Green plantlets were transferred to individual test tubes containing hormone-free 190-2 regeneration medium with 0.03 M sucrose and were vernalized for six weeks. Colchicine treatment took place after the vernalization treatment in the test tubes, after which the plants were planted into soil and grown till maturity.

Results

Effectiveness of leaf rust resistance genes in Martonvásár

The field resistance of wheat genotypes carrying designated *Lr* genes has been tested for several decades in order to determine the efficiency of major leaf rust resistance genes. Each year Thatcher-based near-isogenic lines (NILs), each carrying a different resistance gene or allele, are sown in the experiments. The mean ACI values calculated from leaf rust infection data recorded in the artificially inoculated nursery in Martonvásár over the last seven years are presented in *Figure 1*. The results indicate that seven of the NILs carrying a single *Lr* gene or allele are still not infected by the pathogen or only to a negligible extent. Wheat lines carrying *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, *Lr29* and *Lr35* had excellent

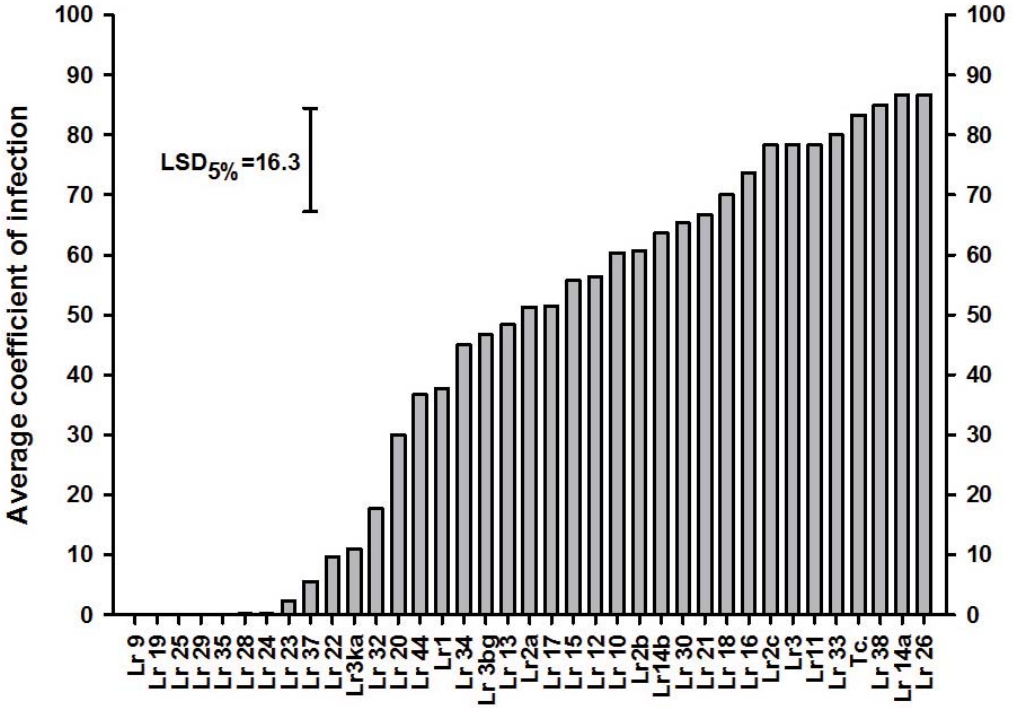

Figure 1: **Leaf rust infection of near-isogenic lines of Thatcher (Martonvásár, 2004-2009)**

resistance. In 2009 we detected moderately susceptible reaction (30-40MS) on wheat genotypes carrying *Lr37* in their genetic background, there was no sign of infection on them earlier. The line exhibiting the greatest degree of infection was the NIL carrying *Lr26*.

Marker assisted selection and gene pyramiding

The agronomic traits of Thatcher NILs are not adapted to Hungarian conditions, but in most cases these are the only source of resistance genes. The phenotype of the French variety Renan, which carries gene *Lr37*, is more similar to that of Hungarian varieties. A marker assisted backcross programme was set up to incorporate leaf rust resistance genes into four Martonvásár varieties (Mv Emma, Mv Madrigál, Mv Magvas, Mv Pálma). Combinations between Martonvásár wheat varieties and resistance sources carrying single Lr genes were first created for genes *Lr9*, *Lr24*, *Lr25* and *Lr29*, after which the programme was expanded to include two genes conferring adult plant resistance (*Lr35* and *Lr37*). All selected *Lr* genes provide an excellent level of resistance to the Hungarian leaf rust population, while the recurrent parents chosen have good agronomic and quality traits. Mv Pálma, Mv Emma and Mv Madrigál are susceptible to the pathogen, while Mv Magvas is moderately resistant.

After the PCR reaction conditions were optimised, all primers worked. This way MAS could be made in the segregating generations. Until now lines in the BC_2-BC_6 generation have been developed for various crosses (*Table 3*). The agronomic traits of BC_5 and BC_6 lines are very similar to those of the Martonvásár parent. As the linkage of the markers to the resistance genes is not complete, MAS of *Lr* genes was complemented in each case by phenotypic analysis. Only plants that were resistant to leaf rust and were found to carry the relevant resistance gene were used to create the next backcross generation.

Since the use of *Lr* genes singly increases the danger of genetic vulnerability, combinations of lines carrying different genes were developed in order to pyramid the genes. The aim was to create genotypes carrying several resistance genes simultaneously, in the hope that these would have more durable resistance to leaf rust than those carrying a single gene. To date, different gene combinations have been developed for the four Martonvásár varieties. A doubled haploid (DH) programme has been set up based on anther culture in order to stabilise the gene combinations. The raising of DH plants is now underway for most of the combinations. So far, plants carrying two *Lr* genes in a stable condition have been identified for three combinations (Mv Emma *Lr9*+*Lr24*, Mv Pálma *Lr9*+*Lr24* and Mv Pálma *Lr9*+*Lr29*).

Identification of designated leaf rust resistance genes using molecular markers

The second field in which molecular markers are used in wheat resistance breeding is the determination of designated resistance genes in genotypes where the genetic background has not yet been clarified. The presence of a number of genes is currently being analysed in wheat varieties and breeding lines bred in Martonvásár or used as parents in the breeding programme. Tests have been carried out for the presence of a total of 10 *Lr* genes (*Lr1*, *Lr9*, *Lr10*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr34*, *Lr35* and *Lr37*) in the breeding material, but so far only five genes have been found to be present.

Lr1

In hexaploid wheat *Lr1* is located at the distal end of the long arm of chromosome 5D. This gene was the first designated *Lr* gene and can still be found in many wheat varieties all over the world (MCINTOSH et al. 2008). *Lr1* is one of the sequenced resistance genes, this way a functional marker is available for detection (QIU et al. 2007). Among 74 Martonvásár wheat varieties released since the beginning of the wheat breeding programme 11 carry the *Lr1* resistance gene (Martonvásári 17, Mv Irma, Mv Madrigál, Mv Matador, Mv Summa, Mv Magvas, Mv Mezőföld, Mv Tamara, Mv Mazurka, Mv Hombár and Mv Laura).

Lr10

The *Lr10* gene originates from bread wheat and is located on chromosome arm 1AS. *Lr10* is the first cloned resistance gene in wheat, according to literature it has a CC-NBS-LRR structure. Functional markers were designed to identify this resistance gene in the wheat genome (FEUILLET et al. 2003). *Lr10* can be found in many wheat varieties with different geographical origin. In released Martonvásár varieties we could find it in 15 of them (Martonvásári 13, Mv Matador, Mv Martina, Mv Kucsma, Mv Emese, Mv Palotás, Mv Prizma, Mv Matild, Mv Mambo, Mv Béres, Mv Garmada, Mv Hombár, Mv Gorsium, Mv Kemence and Mv Laura).

Lr26

Earlier results (KŐSZEGI et al. 2000) showed that *Lr26*, located on the 1BL.1RS translocation, was frequently found in Martonvásár varieties. This was confirmed by recent results for 59 Martonvásár varieties, 36 of which contain the 1BL.1RS translocation (61%). However, *Lr26* was detected at different frequencies in varieties registered before and after 2000. The 1BL.1RS translocation was present in 77.1% of the 35 older varieties, while this figure dropped to 37.5% in the 24 most recently registered genotypes. The

Table 3: **Progenies developed in the backcross programme (Martonvásár, 2009)**

Variety	*Lr9*	*Lr2 4*	*Lr25*	*Lr29*	*Lr35*	*Lr37*
Mv Emma	BC_6	BC_6	BC_6	BC_6	BC_4	BC_4
Mv Madrigál	BC_6	BC_6	BC_6	BC_4	BC_5	BC_5
Mv Magvas	BC_6	BC_4	BC_5	BC_6	BC_5	BC_4
Mv Pálma	BC_6	BC_5	BC_5	BC_6	BC_5	BC_2

1BL.1RS translocation is also found in a large number of the varieties of non-Martonvásár origin used in the breeding programme, being detected in 53.4% of the wheat varieties and breeding lines examined.

Lr34

Pedigree analysis of Martonvásár varieties demonstrated that Bezostaya 1, or its ancestor Bezostaya 4, was present in the pedigree of almost all varieties. In addition to registered Martonvásár varieties, a number of Martonvásár breeding lines were tested, along with crossing partners of other origin. *Lr34* was found in 64 of 226 wheat varieties and lines examined (28.3%). The gene was detected in 34 of 128 varieties and lines of Martonvásár origin (26.6%), but the molecular marker identified the gene in only twelve of 73 registered varieties tested (Martonvásári 3, Martonvásári 13, Martonvásári 17, Mv Emese, Mv Garmada, Mv Gorsium, Mv Laura, Mv Mambó, Mv Pálma, Mv Palotás, Mv Táltos and Mv Vilma).

Lr37

Varieties of Western European origin are also used as parents in the Martonvásár crossing programme. Over the last few decades many varieties carrying *Lr37* have been bred in Western Europe, so it was expected that the genome of the foreign crossing partners and of some of the Martonvásár wheat varieties and lines might contain this gene. The results of the PCR amplifications indicated its presence in Western European, North American and Eastern European cultivars and lines. Most of the lines carrying *Lr37* originated from Switzerland, but it was also identified in French varieties, in three lines bred in the USA, in one breeding line from Serbia and in one Austrian variety. The analysis also showed the presence of the gene in the Martonvásár breeding material. Among the registered varieties Mv Vekni carries this leaf rust resistance gene and it has also been detected in several breeding lines.

Discussion

Although major resistance genes have many disadvantages (AYLIFFE et al. 2008), they are still widely used in wheat resistance breeding. In recent years developments in molecular marker techniques and marker identification have facilitated the spread of MAS. This is particularly true in the field of breeding wheat for leaf rust resistance, where PCR-based markers are already available for almost half of the 60 or more designated resistance genes and alleles. Furthermore, all the effective resistance genes designated so far can be traced in segregating progeny populations by means of MAS.

Experiments carried out in an artificially inoculated field nursery indicated that several *Lr* genes still provide complete or excellent protection against this pathogen in Hungary. The incorporation of six of these genes into Martonvásár wheat varieties is now in progress. The aim is to develop sources adapted to Hungarian conditions, with far better agronomic traits than the original donor varieties. NILs developed from the same recurrent variety and each carrying a different *Lr* gene can be crossed with each other to pyramid resistance genes at the genotype level (NELSON 1978) which could result in better resistance if 'undefeated' resistance genes are introgressed into a single plant genotype (PINK 2002). Alternatively, multiline varieties can be produced from a mixture of lines (BROWNING and FREY 1969). The multiline concept can be further refined using the 'mix and match' approach (PINK and PUDDEPHAT 1999), in which the line population forming the multiline variety is compiled on the basis of matching virulence. The aim of the programme cannot be to use lines containing a single resistance gene as varieties. Matching virulence has now been identified for almost all *Lr* genes in all wheat-growing areas of the world (MCINTOSH et al. 1995), so if any line carrying a resistance gene that is still effective today were to be grown on a larger area, virulent pathotypes would soon multiply in the pathogen population.

The presence of *Lr1* and *Lr10* genes were assumed in the Martonvásár gene pool, but this was the first time we could prove it using molecular markers. During investigations to detect designated resistance genes, a reduction in the proportion of varieties carrying the *Lr26* resistance gene was noted among wheat varieties registered in recent years. This process has accelerated, primarily due to the greater value attached to technological quality traits. Varieties carrying the 1B/1R translocation have poorer bread-making quality due to the presence of storage proteins of the secalin type (DHALIWAL et al. 1987). As expected *Lr34* was found in many Martonvásár varieties. Although this gene alone is capable of reducing the level of infection to almost half, as reported by SINGH and RAJARAM (2002) and confirmed in the present work, resistance that is both excellent and durable can only be achieved if *Lr34* is combined with 2 or 3 other genes (SINGH and RAJARAM 1992). *Lr37* can be detected at high frequency in Western European wheat varieties. This is not the result of targeted resistance breeding against leaf rust, as this pathogen rarely causes serious economic losses in countries with a cool maritime climate. Another rust species, stripe rust, however, often causes damage to wheat fields. *Lr37* originated from A*egilops ventricosa* and the chromosome segment that became translocated into the wheat genome also carries the *Yr17* resistance gene for yellow rust. This gene was successfully used by Western European breeders to fight the pathogen for a number of years, but it has now lost its effectiveness (BAYLES et al. 2000). Unfortunately, pathotypes virulent to *Lr37* have also appeared (ROBERT et al. 2000) and the virulence was also observed in Martonvásár during the 2008/2009 wheat season.

Experience gained so far suggests that markers flanking *Lr* genes can be used simply and effectively in marker assisted backcross programmes. Nevertheless, as the linkage between markers and resistance genes is not complete, regular phenotypic monitoring will be required if satisfactory parental genotypes are to be selected. According to our earlier results (GÁL et al. 2007) the ratio of false positive plants for the genes *Lr9*, *Lr24*, *Lr25* and *Lr29* was 1.3, 4.0, 9.5 and 7.6%, respectively. However, molecular markers can prove the presence of the requested resistance gene in the genetic background and in the case of plants carrying adult plant resistance genes - like *Lr35* and *Lr37* - this is the only way to choose appropriate parents for crossing programme. The

use of MAS, whereby breeders select for molecular markers linked to *Lr* genes, enables the pyramiding of more than one effective resistance gene. With the help of molecular markers, resistance genes are easy to detect in wheat varieties of unknown parentage. This information can then be used to design crossing programmes.

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References

AYLIFFE M, SINGH R, LAGUDAH E, 2008: Durable resistance to wheat stem rust needed. Curr Opin Plant Biol 11, 187-192.

BANSAL UK, HAYDEN MJ, VENKATA BP, KHANNA R, SAINI RG, BARIANA HS, 2008: Genetic mapping of adult plant leaf rust resistance genes *Lr48* and *Lr49* in common wheat. Theor Appl Genet 117, 307-312.

BARABÁS Z, MATUZ J, 1983: A levélrozsda és a lisztharmat epidémia, illetve különféle rezisztencia típusok befolyása őszi búza genotípusok termésére (Yield of winter wheat genotypes as affected by leaf rust and powdery mildew epidemics as well as by the type of resistance). Növénytermelés 32, 193-207.

BAYLES RA, FLATH K, HOVMØLLER MS, DE VALLAVIEILLE-POPE C, 2000: Breakdown of the *Yr17* resistance to yellow rust of wheat in northern Europe. Agronomie 20, 805-811.

BROWN-GUERDIRA GL, SINGH S, FRITZ AK, 2003: Performance and mapping of leaf rust resistance to wheat from *Triticum timopheevii* subsp. *armeniacum*. Phytopathology 93, 784-789.

BROWNING JA, FREY KJ, 1969: Multiline cultivars as a means of disease control. Ann Rev Phytopathol 7, 355-382.

CHERUKURI DP, GUPTA PK, CHARPE A, KOUL S, PRABHU KV, SINGH RB, HAQ QMR, 2005: Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. Euphytica 143, 19-26.

CHU CC, HILL RD, BRULE-BABEL AL, 1990: High frequency of pollen embryoid formation and plant regeneration in *Triticum aestivum* L. on monosaccharide containing media. Plant Sci 66, 255-262.

DANNA CH, SACCO F, INGALA LR, SAIONE HA, UGALDE RA, 2002: Cloning and mapping of genes involved in wheat-leaf rust interaction through gene-expression analysis using chromosome-deleted near-isogenic wheat lines. Theor Appl Genet 105, 972-979.

DEDRYVER F, JUBIER MF, THOUVENIN J, GOYEAU H, 1996: Molecular markers linked to the leaf rust resistance gene *Lr24* in different wheat cultivars. Genome 39, 830-835.

DHALIWAL AS, MARES DJ, MARSHALL DR, 1987: Effect of 1B/1R chromosome translocation on milling and quality characteristics of bread wheats. Cereal Chem 64, 72-76.

FEUILLET C, TRAVELLA S, STEIN N, ALBAR L, NUBLAT A, KELLER B, 2003: Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100, 15253-15258.

GÁL M, VIDA G, UHRIN A, BEDŐ Z, VEISZ O, 2007: Incorporation of leaf rust resistance genes into winter wheat genotypes using marker-assisted selection. Acta Agron Hung 55, 149-156.

GOLD J, HARDER D, TOWNLEY-SMITH F, AUNG T, PROCUNIER JD, 2002: Development of molecular marker for rust resistance genes *Sr39* and *Lr35* in wheat breeding lines. Electronic J Biotechnol 2, 35-40.

GUPTA SK, CHARPE A, KOUL S, PRABHU KV, HAQ QM, 2005: Development and validation of molecular markers linked to an *Aegilops umbellulata*-derived leaf-rust-resistance gene, *Lr9*, for marker-assisted selection in bread wheat. Genome 48, 823-830.

HELGUERA M, KHAN IA, DUBCOVSKY J, 2000: Development of PCR markers for the wheat leaf rust resistance gene *Lr47*. Theor Appl Genet 100, 1137-1143.

HELGUERA M, KHAN IA, KOLMER J, LIJAVETZKY D, ZHONG-QI L, DUBCOVSKY J, 2003: PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci 43, 1839-1847.

HELGUERA M, VANZETTI L, SORIA M, KHAN IA, KOLMER J, DUBCOVSKY J, 2005: PCR markers for *Triticum speltoides* leaf rust resistance gene *Lr51* and their use to develop isogenic hard red spring wheat lines. Crop Sci 45, 728-734.

HERRERA-FOESSEL SA, SINGH RP, HUERTA-ESPINO J, WILLIAM HM, GARCIA V, DJURLE A, YUEN J, 2007: Identification and molecular characterization of leaf rust resistance gene *Lr14a* in durum wheat. Plant Dis 92, 469-473.

HIEBERT CW, THOMAS JB, MCCALLUM BD, SOMERS DJ, 2008: Genetic mapping of the wheat leaf rust resistance gene *Lr60* (*LrW2*). Crop Sci 48, 1020-1026.

HIEBERT CW, THOMAS JB, SOMERS DJ, MCCALLUM B, FOX S, 2007: Microsatellite mapping of adult-plant resistance gene *Lr22a* in wheat. Theor Appl Genet 115, 877-884.

HUANG L, GILL BS, 2001: An RGA-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. Theor Appl Genet 103, 1007-1013.

HUSZ B, 1941: A beteg növény és gyógyítása (The diseased plant and its healing). Királyi Magyar Természettudományi Társulat, Budapest.

KHAN RR, BARIANA HS, DHOLAKIA BB, NAIK SV, LAGU MD, RATHJEN AJ, BHAVANI S, GUPTA VS, 2005: Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet 111, 846-850.

KOLMER J, 2008: *Lr63*, *Lr64*. Cited in: McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (eds), Catalogue of gene symbols for wheat: 2009 supplement, p 271 (Reference 10550, p 273). Ann Wheat Newsl 55, 256-278.

KNOTT DR, 1989: The wheat rusts: breeding for resistance. Springer-Verlag, Berlin.

KŐSZEGI B, LINC G, JUHÁSZ L, LÁNG L, MOLNÁR-LÁNG M, 2000: Occurrence of the 1RS/1BL wheat-rye translocation in Hungarian wheat varieties. Acta Agr Hung 48, 27-236.

KURAPARTHY V, SOOD S, CHHUNEJA P, DHALIWAL HS, KAUR S, BOWDEN RL, GILL BS, 2007: A cryptic wheat-*Aegilops triuncialis* translocation with leaf rust resistance gene *Lr58*. Crop Sci 47, 1995-2003.

LAGUDAH ES, MCFADDEN H, SINGH RP, HUERTA-ESPINO J, BARIANA HS, SPIELMEYER W, 2006: Molecular genetic characterization of the *Lr34*/*Yr18* slow rusting resistance gene region in wheat. Theor Appl Genet 114, 21-30.

MAGO R, MIAH H, LAWRENCE GJ, WELLINGS CR, SPIELMEYER W, BARIANA HS, MCINTOSH RA, PRYOR AJ, ELLIS JG, 2005: High-resolution mapping and mutation analysis separate the rust resistance genes *Sr31*, *Lr26* and *Yr9* on the short arm of rye chromosome 1. Theor Appl Genet 112, 41-50.

MATEOS-HERNANDEZ M, SINGH R, HULBERT SH, BOWDEN RL, HUERTA-ESPINO J, GILL BS, BROWN-GUEDIRA G, 2006: Targeted mapping of ESTs linked to the adult plant resistance gene *Lr46* in wheat using synteny with rice. Funct Integr Genomics 6, 122-131.

MCCARTNEY CA, SOMERS DJ, MCCALLUM BD, THOMAS J, HUMPHREYS DG, MENZIES JG, BROWN PD, 2005: Microsatellite tagging of the leaf rust resistance gene *Lr16* on wheat chromosome 2BSc. Mol Breed 15, 329-337.

MCINTOSH RA, WELLINGS CR, PARK RF, 1995: Wheat rusts - an atlas of resistance genes. Kluwer Academic Publishers, Dordrecht.

MCINTOSH RA, YAMAZAKI Y, DUBCOVSKY J, 2008: Catalogue of gene symbols for wheat. In: Komugi - Integrated wheat science database [Available online: http://www.shigen.nig.ac.jp/wheat/ komugi/ genes/symbolClassList.jsp; accessed 22 Nov 2009].

MEBRATE SA, OERKE EC, DEHNE HW, PILLEN K, 2008: Mapping of the leaf rust resistance gene *Lr38* on wheat chromosome arm 6DL using SSR markers. Euphytica 162, 457-466.

MELCHINGER AE, 1990: Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104, 1-19.

MOHLER V, HSAM SLK, ZELLER FJ, WENZEL G, 2001: An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8*/*Pm17* locus of common wheat. Plant Breed 120, 448-450.

NELSON RR, 1978: Genetics of horizontal resistance to plant diseases. Ann Rev Phytopathol 16, 359-378.

PINK DAC, 2002: Strategies using genes for non-durable disease resistance. Euphytica 124, 227-236.

PINK D, PUDDEPHAT I, 1999: Deployment of disease resistance genes by plant transformation - a ‚mix and match' approach. Trends Plant Sci 4, 71-75.

PRABHU KV, GUPTA SK, CHARPE A, KOUL S, 2004: SCAR marker tagged to the alien leaf rust resistance gene *Lr19* uniquely marking the *Agropyron elongatum* gene *Lr24* in wheat: a revision. Plant Breed 123, 417-420.

PRINS R, GROENEWALD JZ, MARAIS GF, SNAPE JW, KOEBNER RMD, 2001: AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103, 618-624.

PROCUNIER JD, 2009: Disease resistance. Leaf Rust Resistance *Lr29-Lr25*. Wheat CAP [Available online: http://maswheat.ucdavis.edu/ protocols/*Lr29*/index.htm; accessed 22 Nov 2009].

PROCUNIER JD, TOWNELY-SMITH TF, FOX S, PRASHAR S, GRAY M, KIM WK, CZARNECKI E, DYCK PL, 1995: PCR-based RAPD/ DGGE markers linked to leaf rust resistance genes *Lr29* and *Lr25* in wheat (*Triticum aestivum* L.). J Genet Breed 49, 87-92.

QIU JW, SCHURCH AC, YAHIAOUI N, DONG LL, FAN HJ, ZHANG ZJ, KELLER B, LING HQ, 2007: Physical mapping and identification of a candidate for the leaf rust resistance gene *Lr1* of wheat. Theor Appl Genet 115, 159-168.

RAUPP WJ, SUKHWINDER-SINGH, BROWN-GUERDIRA GL, GILL BS, 2001: Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr39* in wheat. Theor Appl Genet 102, 347-352.

ROBERT O, ABELARD C, DEDRYVER F, 1999: Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. Mol Breed 5, 167-175.

ROBERT O, DEDRYVER F, ROLLAND B, ABELARD C, JAUDEAU B, 2000: Relationships between molecular identification of the gene *Yr17* and adult plant resistance against stripe and leaf rust in bread wheat varieties. Acta Phytopathol Entomol Hung 35, 59-63.

ROGERS OS, BENDICH JA, 1985: Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5, 69-76.

SCHACHERMAYR G, SIEDLER H, GALE DM, WINZELER H, WINZELER M, KELLER B, 1994: Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theor Appl Genet 88, 110-115.

SEYFARTH R, FEUILLET C, SCHACHERMAYR G, WINZELER M, KELLER B, 1999: Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. Theor Appl Genet 99, 554-560.

SINGH RP, RAJARAM S, 1992: Genetics of adult-plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. Genome 35, 24-31.

SINGH RP, RAJARAM S, 2002: Breeding for disease resistance in wheat. In: Curtis BC, Rajaram S, Gómez Macpherson H (eds), Bread wheat improvement and production, 252-270. FAO, Rome.

STUBBS RW, PRESCOTT EE, SAARI EE, DUBIN HJ, 1986: Cereal disease methodology manual. CIMMYT, Mexico.

TAR M, PURNHAUSER L, CSÕSZ M, 2008: Identification and localization of molecular markers linked to the *Lr52* leaf rust resistance gene of wheat. Cereal Res Commun 36, 409-415.

ZADOKS JC, CHANG TT, KONZAK CF, 1974: A decimal code for the growth stages of cereals. Weed Res 14, 415-421.

ZHUANG JJ, JIA X, 1983: Increasing differentiation frequencies in wheat pollen callus. In: Hu H, Vega MR (eds), Cell and tissue culture techniques for cereal crop improvement, pp 431-432. Science Press, Beijing.