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IDENTIFYING LEAF RUST RESISTANCE GENES AND MAPPING GENE *Lr37* ON THE MICROSATELLITE MAP OF WHEAT

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Abstract: Based on seedling resistance tests, five resistance genes (*Lr10*, *Lr3*, *Lr13*, *Lr14a* and *Lr37*) against leaf rust (*Puccinia triticina*) were identified in 16 cultivars of European winter wheat. STS and SCAR markers were used to verify the presence of the resistance genes *Lr37* and *Lr10* against leaf rust in cultivars, near-isogenic lines and segregating populations. The *Lr37* gene is present in a small translocation from *Triticum ventricosum* Ces. (*Aegilops ventricosa* Tausch) and is tightly linked with resistance genes *Yr17* and *Sr38*. The *Lr37* gene was identified in the cultivars Kris, Clever, Slade, Apache, Caphorn, Lorraine, Balthasar, Renan and confirmed by two PCR markers. The F_3 progenies of the crosses Kris (*Lr37*) × Nutka (*Lr37* not present) were used for map construction. Two STS/SCAR markers specific for *Lr37* were mapped in relation to nine polymorphic microsatellites on chromosome 2AS. The microsatellite marker *Xgwm1176* mapped relatively close to the STS and SCAR markers for *Lr37* with a linkage distance of 4.1 cM.

Key Words: STS, SSR, SCAR Markers, Wheat, Leaf Rust, Resistance Genes

INTRODUCTION

Leaf rust, caused by *Puccinia triticina*, is considered to be one of the most significant fungal diseases of bread wheat (*Triticum aestivum* L.) in Europe and worldwide. While not causing severe epidemics in the continent every year, the disease reaches epidemic level on several areas, including Switzerland, Hungary,

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Abbreviations used: STS - sequence tagged site; SSR – simple sequenced repeats; SCAR - sequence-characterized amplified regions; PCR - polymerase chain reaction.

Germany, Romania and regions of France, Italy and Poland [1-3]. Breeding wheat cultivars with resistance to leaf rust is the most effective, economical and environmentally friendly method of disease control. Greater knowledge on the identity of rust resistance genes present in lines and cultivars used as donors of resistance in wheat-breeding programs could greatly improve the efficiency of developing resistant cultivars.

Over 50 genes conferring resistance against leaf rust have thus far been recognized and identified using multipathotype testing [2, 4]. Park et al. [1] examined 91 cultivars produced in western Europe in 1995, and, in 78 cultivars, identified the following resistance genes against *Puccinia triticina*: *Lr1, Lr3a, Lr10, Lr13, Lr14a, Lr17b, Lr20, Lr26* and *Lr37. Lr37* was present in the cultivars Brigadier and Hussar. About 40% of the area sown in 1995 in Great Britain was occupied by cultivars with *Lr26*; all of the cultivars had other genes in addition to *Lr26*, with the gene combination *Lr26* + *Lr37* occupying over half of this area.

Rye and several *Aegilops,* and other wild relatives were used to transfer resistance genes into wheat cultivars for many years, with over 50 translocations of those genes recorded in the literature [5, 6]. Chromosome 1R of rye, in particular its short arm, has been extensively used in wheat breeding. Translocations 1RS.1AL and 1RS.1BL were and still are common in commercial cultivars all over the world, and four resistance genes - *Lr26, Yr9, Sr31* and *Pm8/Pm17* - are present in this segment of the rye chromosome [4].

A group of genes *Lr37, Yr17* and *Sr38*, conferring resistance against diseases such as leaf rust, yellow rust, stem rust, are located within a segment of *Triticum ventricosum* (*Ae. ventricosa*) chromosome 2NS translocated to the short arm of bread wheat chromosome 2AS. These genes are present in numerous cultivars, released in the last decade. The 7D chromosome-located resistance to eyespot, which is caused by *Tapesia* spp, has been the focus of interest in the French winter wheat cultivar VPM1 and in the cultivars derived from it, Rendezvous, Hyak and Madsen. In the above- mentioned cultivars, the rust resistance genes *Lr37, Yr17* and *Sr38* were also present and were found to be highly effective both in Australia and Europe. Both VPM1 and Rendezvous are present in the pedigree of many cultivars [1, 2, 7]. A cluster of the mentioned genes is present in Australian cultivars Trident and Sunbri as well as in derived lines and near-isogenic lines [4]

There are PCR assays available to identify *Lr37, Yr17* and *Sr38* resistance genes in lines and cultivars. Last year the website (www.maswheat.ucdavis.edu) provided useful information on resistance gene markers for wheat, including two new STS markers for *Lr37*.

Recently, the *Lr37* resistance gene was identified in the following cultivars: Beaufort and Brigadier (UK), Terza and Titlis (CH), and Rapor and Renan (F) by Winzeler et al. [2] and in Admiral, Andante, Prophet, Torfrida, Brigadier, Hussar and Zodiac by Singh et al. [7]. Ambrozkova et al. [8] examined a translocation from *T. ventricosum* and three resistance genes (*Lr37, Yr17* and *Sr38*) in 12 cultivars registered in the Czech Republic, and in 7 French and UK

cultivars, and verified the results of the reaction type test using the SCAR marker SC-Y15 [8]. The resistance gene *Lr37* was identified in the following cultivars based on a marker test: Corsaire, Apache, Bill, Brigadier, Eureka, Hussar, Torfrida, Renan, Rendezvous and Rapier. In our previous preliminary experiments, the marker for *Lr37* was examined in 37 European cultivars, and was identified using the SCAR marker SC-Y15 in the following accessions: in cultivars Titlis, Terza, Rapor, Kris, Clever, Slade and Brigadier and Thatcher *Lr37* near-isogenic lines [10]. However, the location of the *Lr37* resistance gene on the microsatellite map of wheat is still unknown.

The aim of this study was to identify the *Lr37* resistance gene in European wheat cultivars, in particular those registered in France and Poland, using the reaction type resistance test, to verify two PCR markers and to flank the introgressed segment (25 - 38 cM) from *T. ventricosum* carrying a cluster of three genes *Lr37, Yr17* and *Sr38,* using the available SSR markers on chromosome 2A of wheat.

MATERIAL AND METHODS

Plant material

Seeds of the wheat cultivars Apache, Balthasar, Bill, Caphorn, Lorraine, Bonpain, Charger, Eveil, Isengrain, Soisson and Trémie were supplied by Florimond-Desprez, France, and seeds of Kris, Clever, Nutka, Zentos, Renan and Opata 85 came from the collection of the Institute of Plant Genetics (IPG), Poznań, Poland. Seeds of the Thatcher near-isogenic lines (NILs) were supplied by Prof. R.F. Park, University of Sydney, Camden, Australia and by Dr. M. Csosz, Cereal Research Institute, Szeged, Hungary. Details on all the near-isogenic lines used were given in our previous paper [9]. Plants of the F_3 generation, derived from F2 by single seed descend, of the segregating population progeny of the crosses Kris × Nutka, and Kris × Zentos were supplied by the Szelejewo Breeding Co.

Seedling resistance test

Multipathotype testing of the 13 cultivars and 16 near-isogenic lines listed in Tab. 3 was conducted in a greenhouse. Prior to inoculation, all healthy material was grown in air-filtered cabinets in a glasshouse at a temperature between 15 and 25°C with a 14-hour photoperiod (daylight supplemented by 400 W Na-lamps). After inoculation with a spore suspension in mineral oil (Soltrol 170), the sets were placed in a dew chamber at 15°C for 24 hours, and then for 9 days in a climatic chamber maintained at 22°C with a 16-hour photoperiod given by metal halide lamps (350 μE). Infection types on the differentials were read 10 days after inoculation.

A set of 12 isolates of *P. triticina* was used, and a set of differential cultivars was included in each testing to check the identity and purity of the isolate, as well as the infection type. Infection types were scored according to Stakman et al. (1962) [11].

DNA extraction

DNA was isolated from 7-day-old seedling leaves via a modified CTAB method described by Stępień et al. [10]. The extracts were stored at -20ºC until used.

Tab. 1. Thermocycle temperature profiles for all the primer sets used to identify STS and SCAR markers for the *Lr37* and *Lr10* resistance genes.

Gene	Marker	The size of amplified marker fragments	Cycle condition	Reference
Lr37	SC-Y15 F/R	580bp	94ºC–4 min, 35 cycles (94ºC–1 min, 65ºC–1 min, 72ºC-1 min), 72ºC-5 min	*
Lr37	CslVrgal3'F/R	383bp	94ºC–10 min, 40 cycles (94ºC–1 min, 55ºC–1 min, 72ºC–1 min),72ºC–10 min	**
Lr10	F1.2245 Lr10-6/r2	310bp	94ºC–3 min, 35 cycles (94ºC–45 s, 57ºC–45 s, 72ºC–30 s), 72ºC–3 min	***

Data kindly provided by *Robert, O., **by Lagudah, E.S., ***by Feuillet, C.

STS and SCAR markers analysis

A polymerase chain reaction (PCR) was performed in a 25-µl reaction volume containing: 2 µl 50 ng/µl of genomic DNA, 2.5 µl 10 x PCR buffer (50 mM KCL, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.8, 0.1% Triton X-100), 1µl 2.5 mM dNTPs (Amersham Pharmacia Biotech Inc), 12.5 pmol of each primer (GENSET SA), 16 µl MQ H_2O, and 0.5 µl (2 U/µ) DyNAzyme^{TM} II DNA Polymerase (Finnzymes). Amplification was performed in a PTC-200 thermocycler (MJ-Research, USA); the cycle conditions for two/the two/two of the primer sets are listed in Tab. 1. The amplification products were separated on 1.5% agarose gel in 1 x TBE buffer (0.178 M Tris-borate, 0.178 M boric acid, 0.004 M EDTA) and stained with ethidium bromide. The 20 µl PCR products were combined with 3 µl of the loading buffer (0.25% bromophenol blue, 30% glycerol), which was added to prepare the samples for agarose-gel electrophoresis. The PCR products were electrophoresed at 3 V/cm for about 3 hours, visualized under UV light and photographed (Syngen UV visualiser).

Microsatellite analysis

Thirty-three microsatellite markers which were mapped on chromosome 2AS in the ITMI population were selected for thise analysis, and the polymorphic SSR markers are presented in Tab. 2 [12].

PCR reaction and fragment detection were performed as described by Röder et al. [12]. Fragment analysis was carried out using ALFexpress an automated laser fluorescence sequencer (Amersham Biosciences). Fragment sizes were calculated using Fragment Analyser V 1.02 (Amersham Biosciences) by comparison with internal size standards.

Tab. 2. Annealing temperature (T_m) and fragment size of polymorphic SSR markers in the wheat cultivars: Kris, Nutka, Zentos.

Locus	Location	T_m (ºC)	Fragment size (bp) in		
			Kris	Nutka	Zentos
Xgwm0636	2AS	50	102	108	107
Xgwm0296b	2AS	55	null	135	136
Xgwm0359	2AS	55	213	-*	215
Xgwm0497	2AS	55	142	-	131
Xgwm0558	2AS	55	115	120	117
Xgwm0895	2AS	55	149	-	145
Xgwm0071a, b	2AS	60	128/119	-	125 /115
Xgwm0095	2AS	60	118	120	-
Xgwm0512	2AS	60	null	183	183
Xgwm0830	2AS	60	119	126	130
Xgwm1053	2AS	60	130	132	-
Xgwm1115	2AS	60	123	121	120
Xgwm0372	2AS	60	309	322	313
Xgwm1176	2AS	60	263	259	-
Xgwm1198	2AS	60	150	-	152

*- not examined

Linkage analysis

The linkage relationship between STS and SCAR markers for the *Lr37* gene and the SSR markers was established with MAPMAKER/Exp version 3.0b [13]. Markers were placed with a LOD threshold of 3.0. The Kosambi function was applied to convert the recombination fractions into map distances [14].

RESULTS AND DISCUSSION

The presence of the resistance genes *Lr3, Lr10, Lr13, Lr14a* and *Lr37* was postulated on the base of a multipathotype tests (Tab. 4). The results of a seedling inoculation procedure performed in a greenhouse using a set of 12 different isolates of this pathogen are presented in Tab. 3.

The results of the resistance tests indicated the presence of resistance gene *Lr37* in the cultivars Kris, Clever, Apache, Caphorn, Lorraine, Balthasar and Renan. Its identification in Apache, Renan and Bill concurs with the results of Ambrozková et al. [8], while this is the first report of its presence in the cultivars Balthasar, Caphorn, Complet, Kris and Clever. The resistance gene *Lr37* was identified in combination with *Lr10* and *Lr13* in three cultivars Caphorn, Kris and Clever and in combination with *Lr13* and *Lr14a* in cv. Balthasar. Resistance gene *Lr14a* is postulated in cvs. Isegrain and Soisson as a single gene, and in Renan in combination with *Lr37*. Cultivar Nutka, registered in Poland, has *Lr13* only, and Zentos has no resistance gene and was susceptible to all the isolates of *Puccinia triticina* used in the experiments (Tab. 3). The single resistance gene *Lr10* is postulated in Bonpain and Eveil; however, this gene is not effective in Europe, except in combination with other resistance genes [2].

Tab. 3. Seedling infection types* displayed by Thatcher NILs with *Lr* genes and cultivars, when tested with different isolates of *Puccinia triticina* at 22°C.

	Isolate												
Thatcher NIL/ Cultivar	b9201-2c3	b9506-2b	b950506-a	b9407-1ca3	b950019-a	b950365-d	b347	b01M196-d	FSA9-A	B93841C1	B9387-1A1	B9834-E
Lr 1	;=	;=	3+	0;	;=	;=	3+	;-	3+	;	;=	;1
Lr 2a	;12	;12	;	;12	;12c	;1	3+	X=	X-	;12-	;12-	X++3
Lr 3	;	;-	;	X-	;1	X++3+	3+	;1	X	3+	3+	;1
Lr 3bg	;	;	;=	3+	;1	X++	3+	;1	3+	3+	3+	;1
Lr 3ka	;	;-	;1	;	;1	X+	3+	;	;	3+	3+	;1
Lr 10	;12	;12	33+	;12	3+	X-	33+	3+	;12	;12-	33+	3+
Lr 13	3+	3+	3+	XX-	12c	;12	X+	3+	X++	3+	X++	3+
Lr 14a	3+	3+	X++3	3+	X++	3+	X++	X++3	3+	3+	3+	3+
Lr 15	;12-	;12-	3+	;1	;1	;1	3+	3+	;1	3+	;1C	;12
Lr 16	2++3	2++3	22+	2-	2c	12	2	12	2	2++3	2++	3+
Lr 17	Y-	Y=	3+	;1-	;1	;1	3+	;12-	;1	3+	Y++	Y++3+
Lr 20	;1N	;N	3+	3+	3+	;1N	3+	;1N	;N	3+	;N	;N
Lr 23	;12-	;12-	;1	;12-	;12	;	;	;1+	X=	;12C	;1C	;12+
Lr 24	;1	;1	;1	;	;	;	;	;1	;1	;	;	;1C
Lr 26	3+	3+	0;	X=	0;	0;	0;	0;	X+	3+	;12	0;
Lr 37	Y++3	X++	X++	X++3+	X++3	Y++	3+	3+	3+	3+	X++3	3+
Nutka	3+	3+	3+	X++3	X=	X++	X++3	3+	X	-**	-	-
Kris	;1-	;1	X-	;	X-	;12	;12	3+	;1	-	-	-
Clever	X=	X	X=	;12	X=	X=	X++3	X++3+	X-	-	-	-
Zentos	3+	3+	3+	3+	3+	3+	3+	3+	-	-	-	-
Renan	Y++	X-	X	X+	X++	X-	3+	3+	X++3	-	-	3+
Apache	XX-	X-	X+	X+	;12	XX+	X++3	-	-	X++3	X-	3+
Caphorn	;1-	;1-	;1+	;1	;12C	;12-	Y-	-	-	-	;12	3+
Lorraine	;1	;	;1	;12	;	X	-	-	-	-	X++	;
Balthazar	Y	;12	XX-	;12-	;12C	X=	-	-	-	X++3	-	Y++3
Charger	;12	;12	X++3	;12	X=	;12	-	-	-	-	XX-	3+
Soissons	X++	3+	X++	3+	X++	3+	X++	-	-	3+	3+	-
Trémie	;1	;12	33+	;1	- *	-	-	-	-	;1+	2+3+	-
Isengrain	X++3	3+	X++3	3+	-	-	-	-	-	3+	3+	-

*infection types follow Stakman et al. [11], **cultivar not tested with the corresponding isolate

The identification of the postulated resistance genes *Lr37* and *Lr10* was confirmed by the PCR marker assay in all the cultivars and lines examined in

this paper (Tab. 4). Reliable molecular markers are not available for the other resistance genes identified in this paper [10].

It may be concluded that both of the PCR markers, of 580bp and 383bp in size and used to identify resistance gene *Lr37*, gave results that concurred with the multipathotype results in various genetic backgrounds of bread wheat. Both SCAR and STS markers were used to identify the 2S segment from ***T. ventricosum***, which possesses the genes ***Lr37, Yr17*** and ***Sr38*** in the segregating progeny F_3, derived from two crosses: Kris × Nutka, and Kris × Zentos. The STS and SCAR markers cosegregated in 99 F_3 plants out of 100 in the segregating population of Kris × Nutka. In the plants of the Kris × Zentos progeny, full agreement was found between the two markers in 94 plants of F_3.

Tab. 4. Postulated resistance genes against *Puccinia triticina* in selected European wheat cultivars and identification of PCR markers for the genes *Lr10* and *Lr37*.

Cultivar	Postulated *Lr* genes	Gene *Lr10*	Gene *Lr37*	
		Marker	Marker SC-Y15	Marker cslVrgal3'
Apache	*Lr13, Lr37*	-	+	+
Balthazar	*Lr13, Lr14a, Lr37*	-	+	+
Bill	*Lr37***	-	+	+
Caphorn	*Lr10, Lr13, Lr37*	+	+	+
Lorraine	*Lr3, Lr37*	-	+	+
Bonpain***	*Lr10*	+	-	-
Charger	*Lr10, Lr13*	+	-	-
Eveil***	*Lr10*	+	-	-
Isengrain	*Lr14a*	-	-	-
Soisson	*Lr14a*	-	-	-
Tremie	*Lr10, Lr13*	+	-	-
Renan	*Lr14a, Lr37*	-	+	+
Kris	*Lr10, Lr13,Lr37*	+	+	+
Clever	*Lr10, Lr13, Lr37*	+	+	+
Nutka	*Lr13*	-	-	-
Zentos	No resistance genes	-	-	-
Opata* 85	*Lr10,Lr13,Lr14b, Lr30,Lr34*	+	-	-

*according to McIntosh *et al.* [4], **according to Ambrozková *et al.* [8], ***according to Winzeler *et al.* [2].

From the wheat microsatellite map of Röder et al. [12], thirty three primer pairs located on the short arm of chromosome 2A were used to identify polymorphism between Kris and Nutka, as well as between Kris and Zentos. Among the 33 markers, 19 produced polymorphism between Kris-Nutka and Kris-Zentos.

Nine out of 19 markers that showed polymorphism between Kris and Nutka (*Xgwm1176, Xgwm296b, Xgwm636, Xgwm830, Xgwm1053, Xgwm1115, Xgwm95, Xgwm372* and *Xgwm558*), as well as 10 out of 19 markers that showed polymorphism between Kris and Zentos (*Xgwm359, Xgwm296b, Xgwm636, Xgwm830, Xgwm497, Xgwm1115, Xgwm71a, Xgwm372 Xgwm1198* and

Xgwm512) were used to screen the F_3 populations. The fragment sizes of the polymorphic SSR markers were listed in Tab. 2. The 9 primers pairs that produced polymorphism between Kris and Nutka, and the STS/SCAR markers for the *Lr37* resistance gene were mapped in the distal region of chromosome 2AS (Fig. 1). The microsatellite marker *Xgwm1176* was relatively close to the *Lr37* STS markers, with a linkage distance of 4.1 cM. The other 9 microsatellite markers that showed polymorphism between the two parents, Kris and Zentos were not used for mapping. This is the first report on the microsatellite mapping of the resistance gene *Lr37* in wheat. Chromosome walking with number of STS markers will be necessary to clone groups of resistance genes, originating from *Triticum ventricosum.*

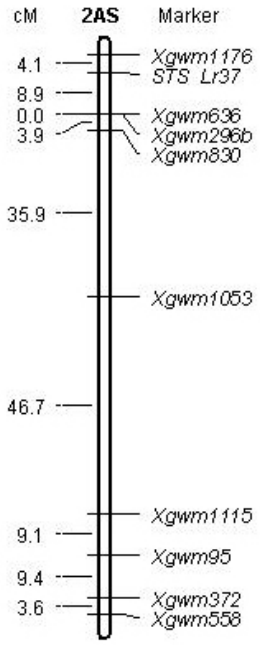

Fig. 1. A genetic map of the *Lr37* region on chromosome 2AS. Nine microsatellite loci (*Xgwm1176*, *Xgwm296b*, *Xgwm636*, *Xgwm830*, *Xgwm1053*, *Xgwm1115*, *Xgwm95*, *Xgwm372*, *Xgwm558*) and one STS locus are shown. The locus names are indicated on the right side of the map. Kosambi map distances (cM) are shown on the left side.

Resistance genes present in translocation from wild species, such as that from *Triticum ventricosum*, are particularly useful in increasing both the resistance of wheat cultivars to important pathogens, as well as their biodiversity.

The following criteria should be taken in account when DNA markers are considered for use in breeding programs [15-17]:

- the linkage between the marker and the gene of interest, in order to avoid false positives;

- the reliability and reproducibility of the marker;
- the cost and reliability of field screening.

The markers for resistance gene *Lr37* examined in this paper, as well as several markers used in our previous papers will be useful in the identification of clusters of resistance genes in cultivars and lines. [9, 10]. New resistance genes that can be as effective as *Lr29*, *Lr35*, *Lr47* and others are required for breeding programs in Europe, because the resistance of *Lr37* and *Lr17* has recently been overcome in America and is likely to be overcome in Europe [4,18,19].

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