Theor Appl Genet (2006) 112: 760–769
DOI 10.1007/s00122-005-0181-2

ORIGINAL PAPER

Irena Jakobson · Hilma Peusha
Ljudmilla Timofejeva · Kadri Järve

Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line

Received: 18 July 2005 / Accepted: 30 November 2005 / Published online: 17 December 2005

Abstract In the progeny of a cross between the common wheat cultivar Tähti and *Triticum militinae*, a member of the timopheevii group of tetraploid wheats, several hybrid lines were selected that are characterized by improved seedling and adult plant resistance (APR) to powdery mildew. An F_2 single-seed descendant mapping population segregating for seedling resistance and APR to powdery mildew was analysed for the identification of quantitative trait loci (QTL). The main QTL responsible for APR was detected on the long arm of chromosome 4A tightly linked to the *Xgwm160* locus on a *T. militinae* translocation explaining up to 54% of phenotypic variance. The same translocation influenced seedling resistance to powdery mildew upon inoculation of plants with a synthetic population of *Blumeria graminis* DC. f. sp. *tritici*, and explained 28–33% of the phenotypic variance.

Introduction

The obligate fungus *Blumeria graminis* DC. f. sp. *tritici* can infect plants from the first leaf stage until senescence. Resistance to the infection of powdery mildew fungus may be based on a race-specific gene-for-gene interaction of resistance gene(s) (*Pm*-genes) in wheat and avirulence gene(s) in the infecting fungus isolate. This type of plant–pathogen interaction is associated with the hypersensitive response and may not be durable. Most of the powdery mildew resistance genes identified in different wheat genotypes (*Pm1* – *Pm31*, McIntosh et al. 2003) confer complete resistance to different sets of fungus isolates in the seedling stage of the host plant (Hsam and Zeller 2002).

Some wheat genotypes exhibit a different type of resistance, which is non-isolate-specific and partial, retarding infection, growth and reproduction of the powdery mildew fungus. This resistance is generally observed in adult plants. Adult plant or durable resistance (APR) is a quantitative trait and can be resolved into discrete genetic loci (quantitative trait loci, QTL; Paterson et al. 1988).

Several sets of QTLs for adult plant powdery mildew resistance have been detected and mapped in different segregating wheat populations (Huang and Röder 2004). In the heritance of adult plant powdery mildew resistance, the additive effects of the detected QTLs prevail (Griffey and Das 1994; Keller et al. 1999; Chantret et al. 2001; Mingeot et al. 2002; Liu et al. 2001).

Pm-genes conferring powdery mildew resistance have been transferred to *Triticum aestivum* ($2n = 42$, AABBDD) from different species of the genus *Triticum*, including the timopheevii group of wheats (*Pm6, Pm27* from *Triticum timopheevii* (Zhuk.) Zhuk. ssp. *timopheevii*) (Shands 1941; Allard and Shands 1954; Järve et al. 2000). To our knowledge, APR to powdery mildew has not been transferred into *T. aestivum* from the timopheevii group of wheats.

The timopheevi group of tetraploid wheats ($2n = 28$) with the genome formula A^tA^tGG includes the wild form of timopheevi wheat known as *T. timopheevii* (Zhuk.) Zhuk. ssp. *armeniacum* (Jakubz.) van Slageren, the domesticated form *T. timopheevii* (Zhuk.) Zhuk. ssp. *timopheevii* (later abbreviated as *T. timopheevii*), and also a third, free-threshing form of wheat discovered by Zhukovsky in 1950, *Triticum militinae* Zhuk. et Migusch. (Zhukovsky and Migushova 1969). *T. militinae* is considered to be a spontaneous mutant of *T. timopheevii* (Dorofeev 1987); however, it has also been supposed to originate from an introgressive hybridization between *T. timopheevii* and *T. persicum* (Navruzbekov 1981; Järve et al. 2002).

Communicated by B. Keller

I. Jakobson (✉) · H. Peusha · L. Timofejeva · K. Järve
Department of Gene Technology,
Tallinn University of Technology,
Akadeemia tee 15, 19086 Tallinn, Estonia
E-mail: irena.jakobson@ttu.ee
Tel.: +372-6-204427

Using microsatellites derived from bread wheat, the genomic organization of *T. timopheevii* and *T. militinae* has been compared in a comparative mapping with that of hexaploid wheat (Salina et al. 2005). According to the results of this study, polymorphism between *T. militinae* and *T. timop*heevii (accession no. K-38555) is approximately at the same level as polymorphism between two accessions of *T. timopheevii* (*T. timopheevii* var. *timopheevii* and *T. timopheevii* var. *typica*).

Immunity to powdery mildew, leaf and yellow rusts, as well as high resistance to stem rust, loose and dwarf smuts have been reported as useful traits of *T. militinae* (Dorofeev et al. 1987). In three different climatic regions, *T. militinae* has been found to be unsusceptible to fungal diseases; mere traces of stem rust were detected (Migushova 1975). No resistance genes originating from *T. militinae* have been identified in common wheat.

The aims of this study were to evaluate powdery mildew resistance of hybrid wheat lines selected from the progeny of a cross between *T. aestivum* and *T. militinae*, and to estimate the number and genomic positions of the QTLs with significant effects on seedling resistance and APR.

Materials and methods

Plant material

As the female parent, the Finnish wheat cultivar Tähti was crossed with *T. militinae* Zhuk. et Migush. (accession no. K-46007 from the N.I.Vavilov Institute of Plant Industry, St.Petersburg, Russia). The self-sterile F_1 plants were backcrossed to Tähti once. BC_1F_2 plants were self-pollinated and hybrid population was advanced to BC_1F_4.

Random seeds from the hybrid population were planted. The somatic chromosome numbers of the selected plants were determined in root-tip cells using standard Feulgen staining procedures. One ear of each plant was pollinated with the pollen of the susceptible mother cultivar, the others were self-fertilized. Self-pollinated F_1 plants were advanced as hybrid lines.

From an F_1 plant of a backcross (hybrid plant 8/1 × Tähti), an F_2 population was derived and further used for microsatellite screening and QTL mapping. F_2 plants were selfed to produce F_2-derived F_3 families. The mapping population consisted of 134 F_2 plants and 130 F_2-derived F_3 families.

Parental plants of the studied hybrid lines were resistant to the synthetic population of *B. graminis* at the seedling stage (score 0–2).

Disease resistance

During the first 15 days, the plants were grown in the greenhouse. On the tenth day of growth, the reaction to 11 test-isolates of *B. graminis* (DC. et Marat*) speer* f. sp. *tritici* (kindly provided by Dr. F. Felsenstein, Freising-Weihenstephan, Germany) and to a synthetic population of mixed test-isolates was estimated on detached leaves and scored (0, no visible symptoms; 9, heavy sporulation, Lutz et al. 1992). Five days later, the plants were planted in the field in a completely randomized design, ten plants in a 1 m row. Strips of plants of the susceptible cultivar Saratovskaya-29 surrounded every fifth row.

For the estimation of APR under natural infection with the native population of the pathogen, the mildew was assessed on the upper two leaves as a visually estimated percentage of leaves covered with mildew. If the leaves became senescent, the previous score for that leaf was used in calculating the cumulative mildew cover for the upper two leaves. Disease severity was expressed as a disease index (DI) on a 0–9 scale (Yu et al. 2001). The plants were scored twice, at first in the ear emergence stage (DI 1) and the second time 14 days later (DI 2) in the milky ripe stage.

In the mapping population, APR was tested in 2 years (in 2002 and 2004). In 2004, F_2-derived F_3 families were grown in two replications (five plants in a block) using a completely randomized block design. In all the 130 F_3 families, adult resistance was scored for four individual F_3 plants per replication (1,040 F_3 plants were tested altogether).

The parental lines were included as controls in all experiments.

Microsatellite marker analysis

A total of 129 simple sequence repeats (SSR) were used to screen the parental lines: *gwm* markers (Röder et al. 1998), *psp* markers, kindly provided by Dr. P. Stephenson (Norwich, UK), *wmc* markers (Gupta et al. 2002), and *BARC* marker, developed by P. Cregan, Q. Song and associates (http://www.wheat.pw.usda.gov/) (Table 1).

Total genomic DNA was extracted from young leaf tissue (approximately 300 mg) frozen in liquid nitrogen, according to the method described by Huang et al. (2000) with minor modifications. Radioactive PCR amplifications of microsatellite fragments were performed as described by Röder et al. (1998) or Bryan et al. (1997), the annealing temperature depending on the type of the primer. Amplified DNA fragments were separated on a 5 or 6% polyacrylamide denaturing gel and autoradiographed.

QTL mapping

The linkage groups were established with the Map Manager QTX Version b16 software for genetic mapping of Mendelian markers and QTLs (Meer et al. 2002; Chmielewicz and Manly 2002) with a minimal LOD score of 3 and a maximum genetic distance of 30 cM.

Table 1 List of microsatellite markers used in the screening of hybrid lines

Chromosome	Markers
1A	Xpsp2999, Xpsp3027, Xpsp3151, Xgwm33, Xgwm99, **Xgwm136**
2A	Xpsp3029, Xpsp3039, Xpsp3088, **Xpsp3142**, Xpsp3153, Xgwm47, Xgwm71, Xgwm294, Xgwm296, Xgwm311, Xgwm356, Xgwm359, Xgwm372, Xgwm382, Xgwm512
3A	Xpsp3047, Xgwm2, Xgwm155, Xgwm162, Xgwm369, Xgwm480, Xgwm666
4A	Xpsp3028, Xpsp3058, Xpsp3119, **Xpsp3142**, Xgwm160, Xgwm610, Xgwm637, Xbars153, Xbarc52, Xbarc184, **Xwmc219,** Xwmc232, Xwmc283, **Xwmc313, Xwmc497**
5A	Xgwm126, **Xgwm156**, Xgwm186, Xgwm205, Xgwm293, **Xgwm304**, Xgwm410, Xgwm415, Xgwm595, Xgwm617, Xgwm639, Xgwm666, Xwmc415, Xwmc492
6A	Xpsp3029, Xpsp3071, Xpsp3152, Xgwm427, Xgwm459, Xgwm570, Xgwm617
7A	Xpsp3050, Xpsp3094, Xpsp3114, Xgwm60, Xgwm130, Xgwm260, Xgwm276, Xgwm350, Xgwm573, Xgwm635, Xgwm666
1B	Xpsp3000, Xpsp3100**, Xgwm33**, Xgwm153, Xgwm264, Xgwm274, Xgwm550
2B	Xpsp3030, Xgwm16, Xgwm47, Xgwm71, Xgwm210, Xgwm382, Xgwm410, Xgwm501, Xgwm526
3B	Xpsp3003, Xpsp3030, Xpsp3035, Xpsp3078, Xpsp3081, Xpsp3112, Xpsp3144, Xgwm112, Xgwm247, Xgwm264, Xgwm285, Xgwm493
4B	Xpsp3030, Xpsp3078, Xgwm66, Xgwm368
5B	Xpsp3037, Xpsp3065, Xgwm66, Xgwm68, Xgwm604, Xgwm639, Xgwm213, Xgwm159
6B	Xpsp3009, Xpsp3079, Xpsp3112, Xpsp3131, Xpsp3139, Xgwm132, Xgwm133, Xgwm508, Xgwm518, Xgwm613, Xgwm626, Xgwm921
7B	Xpsp3033, Xpsp3081, Xgwm16, Xgwm46, Xgwm68, Xgwm112, Xgwm274, Xgwm302, Xgwm333, Xgwm573, Xgwm611
1D	Xpsp3037, Xpsp3137, **Xgwm33**, Xgwm147, Xgwm232, Xgwm337, Xgwm458
2D	Xpsp3058, Xgwm71, Xgwm311, Xgwm210, Xgwm296, Xgwm382
3D	Xpsp3019, Xgwm2, Xgwm71, Xgwm383
4D	Xpsp3007, Xpsp3079, Xpsp3103, Xpsp3112, Xgwm624
5D	Xgwm16, Xgwm174, Xgwm192, Xgwm205, Xgwm358, Xgwm565, Xgwm639
6D	Xpsp3058, Xpsp3200
7D	Xpsp3035, Xpsp3079, Xpsp3094, Xpsp3113, Xpsp3123, Xgwm350, Xgwm635

The primers printed in bold were not polymorphic between Tähti and *Triticum militinae*

Genetic distances between markers were estimated using the mapping function of Kosambi (1944). The chromosomal alignment of linkage groups was deduced from the published wheat maps (Röder et al. 1998; Gale et al. 1995; Somers et al. 2004) and from the *GrainGenes* database (http://www.wheat.pw.usda.gov).

The association between phenotype and marker genotype was investigated using single marker regression. The positions of the detected QTLs were determined using simple interval mapping (SIM) and composite interval mapping (CIM). The free-regression model was applied. The likelihood ratio statistic (LRS) threshold for declaring the statistical significance of association was calculated empirically for each experiment using the permutation test, at 1,000 iterations. Confidence interval was estimated by bootstrap analysis using the same software.

QTL effects were estimated as the percentage of phenotypic variation explained by QTL.

Statistical analysis

Log_{10}-transformed data was used in all statistical and QTL analyses. Chi-square analyses were performed to test the significance of deviations of observed segregation ratio from theoretical expectations. The ANOVA (analysis of variance) was performed to determine the significance of differences between the genotypes. Components of variance were computed considering the effects of the environment (year) and the genotype as random. Estimates of variance components δ^2_G (genetic variance), δ^2_E (environmental variance), δ^2_{GxE} (genotype × environment interaction variance) and δ^2_{Err} (error variance) were calculated.

As the ANOVA showed no significant effect of replications (blocks) for the disease resistance in F_3, we have further used the mean of the data from two blocks (eight plants) for each family to search for the QTLs. The phenotypic correlation coefficient of adult plant powdery mildew resistance between F_2 and F_3 progenies was calculated.

Results

Hybrid lines

In 1995, the Finnish cultivar of spring wheat Tähti was crossed as a female parent with *T. militinae;* approximately 5% of pollinated florets gave a seed. The F_1 hybrids between the common wheat and *T. militinae* are self-sterile and, usually, two to three backcrosses are needed to fully restore the self-fertility. In this study, the F_1 hybrids were maintained by backcrossing them once as females with *T. aestivum*. The fertile F_2 hybrids were

grown as a population without isolating the ears. The derived F_4 hybrid population consisted of phenotypically heterogeneous but cytogenetically stable plant material and showed improved resistance to powdery mildew in field tests (data not shown). The four hybrid lines (8/1, 8/4, 8/7 and 8/9) derived in this study were single-seed descendant lines advanced from plants randomly selected from the hybrid population.

Disease resistance in hybrid lines

The self-pollinated F_2 families were divided into three groups and tested for APR in the field conditions during 3 years (2002–2004). In general, hybrid lines expressed higher levels of APR than the parent cultivar Tähti (Fig. 1), the average DI of lines derived from the hybrid population 4.8 ± 1.8 being significantly lower as compared to the DI of Tähti 11.9 ± 0.1.

Significant differences both between the lines and between the years ($P < 0.0001$), as well as a highly significant line-by-year (genotype × environment) interaction were detected ($P < 0.0001$) by ANOVA. Genotypic differences between the lines explained 32% of the phenotypic variation for APR in the hybrid lines and the line-by-year interaction explained 29% of the variance.

APR in the mapping population

Hybrid line 8/1 showed a high and stable level of APR and for further marker analysis and QTL detection, a single-plant descendant F_2 mapping population was derived from a cross of this line with Tähti.

Two adult plant disease assessments corresponding to the beginning of ear emergence (DI 1) and milky ripe (DI 2) development stages were carried out in the mapping population, both in 2002 and in 2004. The distribution of the DI assessments is presented in Fig. 2. For all four assessments, DIs showed a continuous variation with one peak and with a distribution slightly deviated from normal. Transgressive segregation towards the susceptible parent was observed. The average of DI of F_2 plants and F_3 families from the cross (8/1 × Tähti) was approximately the same as the mean of parental DIs, indicating an additive inheritance for APR in the line 8/1.

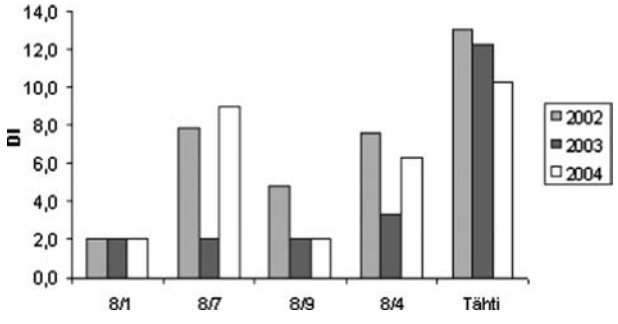

Fig. 1 Phenotypic distribution of four hybrid lines from the cross Tähti × *T. militinae* for APR across 3 years

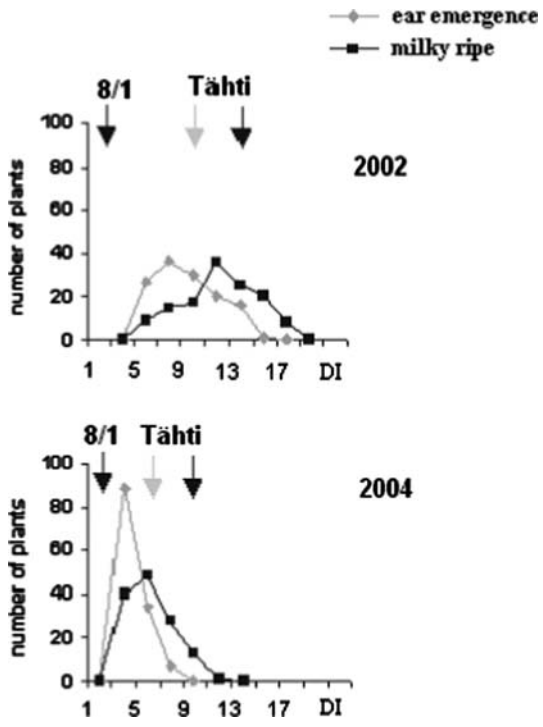

Fig. 2 Distribution of the adult plant powdery mildew DI of F_2 plants (2002) and $F_{2:3}$ families (2004) derived from the cross 8/1 × Tähti in the first and second assessment. The adult plant powdery mildew DI of two parents (8/1 and Tähti) is indicated

The contribution of the two variance components (genotype of the F_2 plant or F_3 family and time of the assessment) to the DI variability was calculated from ANOVA, separately for either of the years. Both variance components were found to be significant ($P < 0.0001$). The genotype effect explained about 43 and 47% of the total trait variance in 2002 and 2004, respectively. As the effect of the time of assessment explained about 30% of the phenotypic variance, both DI 1 and DI 2 scorings were further separately used for QTL mapping.

Both the genotype and the year had a significant effect on the total DI variance over 2 years ($P < 0.0001$), the effect of genotype × environment (year) interactions was not significant ($P = 0.138$). The significant correlation of the corresponding DIs estimated in different years ($r = 0.46$ and 0.49 for DI 1 and DI 2, respectively; $P < 0.001$) indicated that the same genetic factor was acting in different environments.

Microsatellite marker analysis in hybrid lines

Totally, 93% of the 129 microsatellite markers analysed showed a polymorphism between the *T. militinae* and Tähti genotypes. The 120 polymorphic microsatellites revealed 174 loci in the genome of hybrid wheat, on average 1, 45 loci per marker.

Table 2 Translocations in the hybrid lines

Chromosome	8/1	8/7	8/9	8/4
1A	+	+	–	–
2A	+	–	–	–
3A	–	+/–	+	+
4A	+	+/–	+/–	+/–
5A	+	+	+/–	+/–
7A	+/–	+/–	+/–	+–
1B	+/–	+/–	–	–
3B	–	+	+	+
5B	+	–	–	–
6B	–	+/–	+/–	+/–

+ *T. militinae* translocation; – no translocation; +/– heterozygote

In the four hybrid lines, *T. militinae*/*T. aestivum* replacements were detected in 73 loci (42%). The number of translocations per hybrid line varied from 6 to 8 (Table 2). According to the C-banding data, in *T. timopheevii*- and *T. militinae*-derived introgressive lines, substitutions of the whole chromosome were far more frequent than translocations (Badaeva et al. 1991, 2000). On the contrary, our molecular study identified intercalary translocations while no whole chromosome substitutions were detected. The translocations ranged from a few centimorgans to the almost complete chromosome arm substitutions. In our hybrid lines, altogether six chromosomes of the A genome and four chromosomes of the B genome were involved in translocations (Table 2), while no translocations were found in the D genome. However, according to Badaeva et al. (1991, 2000), the G/D chromosome substitutions have been detected in *T. timopheevii*- and *T. militinae*-derived introgressive lines.

Microsatellite marker analysis in the mapping population

A total of 37 markers showing polymorphism between 8/1 and Tähti produced 42 segregating fragments in the mapping population derived from the cross 8/1 × Tähti. 40% of markers were scored as codominant, 45% as null alleles in *T. militinae* and 15% amplified independently segregating fragments from Tähti and *T. militinae* genomes.

The mapping of 42 loci resulted in 9 linkage groups (LOD >3). Colinearity of markers with the published maps was observed and seven linkage groups could be assigned to a chromosome of hexaploid wheat (Fig. 3).

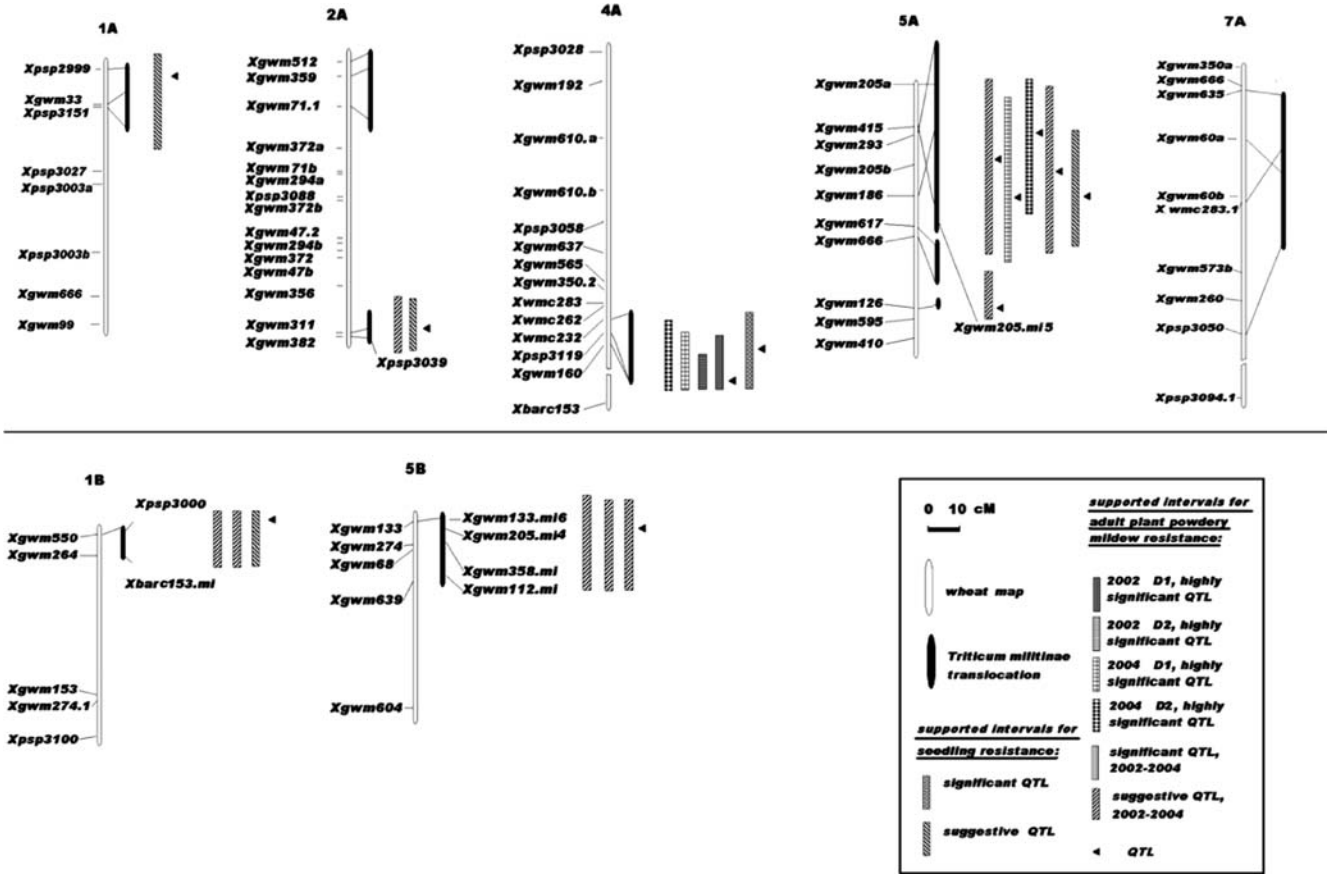

Fig. 3 The map of *T. militinae*-derived translocations and powdery mildew resistance QTLs in the mapping population derived from a cross between the hybrid line 8/1 and cultivar Tähti. Only chromosomes with *T. militinae* translocations are represented. Multiple loci detected by single markers have a suffix (1–6) added following the marker name. Suffix mi designates a *T. militinae* allele

Table 3 Powdery mildew resistance QTLs detected at the different plant stage for F2 plants or for F3 families issued from cross between 8/1 and Tähti

Chromosome	Marker interval	Adult plant resistance												Seedling plant resistance		
		2002						2004								
		DI 1			DI 2			DI 1			DI 2					
		R^2	LRS	Add Dom	R^2	LRS	Add Dom	R^2	LRS	Add Dom	R^2	LRS	Add Dom	R^2	LRS	Add Dom
SIM																
4A	Xgwm232–Xgwm160	27	36.6***	−0.330.01	35	51.4***	−0.290.03	54	90.4***	−0.32−0.11	41	61.0***	−0.35−0.04	28	35.7***	−0.290.18
5A	Xgwm186–Xgwm415	5	6.3 *	−0.14−0.08				7	8.7*	−0.100.10						
	Xgwm666–Xgwm126	5	9.6*	−0.32−0.01												
2A	Xgwm311–Xgwm382										5	6.2*	−0.13−0.01	6	6.5*	−0.12−0.20
1A	Xpsp2999–Xpsp3151													6	6.7*	0.010.08
CIM																
4A	Xgwm232–Xgwm160	25	36.2***	−0.320.01	34	53.0***	−0.28−0.02	49	84.8***	−0.32−0.11	40	55.5***	−0.33−0.03	33	42.9***	−0.290.22
5A	Xgwm186–Xgwm415	6	8.4*	−0.48−0.56	4	13.9**	−0.05−0.13	6	14.3**	−0.100.08	6	8.2*	0.210.68	5	7.6*	0.47−0.64
	Xgwm666–Xgwm126	7	12.1*	3.487.58												
2A	Xgwm311–Xgwm382										5	6.4*	−0.13−0.02	6	6.9*	−0.12−0.09
1A	Xpsp2999–Xpsp3151													5	7.4*	0.06−0.16
1B	Xgwm3000	5	7.7*	−0.130.04	4	5.6*	−0.070.08							4	6.4*	0.00−0.20
5B	Xgwm133.mi6–Xgwm205.mi1				4	5.6*	0.74−1.8	5	9.8*	−0.931.56	6	6.4*	0.771.68			

R^2 percentage of variance explained by individual QTL; *LRS* likelihood ratio statistic; *add.* additive effect due to a substitution of a Tähti allele by a corresponding *T. militinae* allele in the line 8/1; *dom.* dominance effect due to a substitution of a Tähti allele by a corresponding *T. militinae* allele in the line 8/1

P level of significancy: $*P < 0.05$; $**P < 0.01$; $***P < 0.0001$

The genetic map covered the introgressive part of the genome of line 8/1, comprising 38 loci and spanning 251 cM. Four amplification products remained unlinked, three of them originated from the genome of *T. militinae*. Four markers showed a distorted segregation favouring the Tähti allele, three of them were clustered in a tightly linked group (LOD >15) assigned to chromosome 5B; one marker with distorted segregation remained unlinked to any other marker.

Compared to the published genetic maps of wheat (Röder et al 1998; Gale et al. 1995), the genetic distance of the detected translocations was significantly reduced on some chromosomes (chromosome 7A). This may indicate regions with reduced recombination in some of the translocated segments. *T. timopheevii* translocations showing no or extremely low levels of recombination in crosses with hexaploid wheat have been referred to earlier (Devos et al. 1993; Järve et al. 2000; Kammholz et al. 2001).

QTL analysis

The microsatellite map and DI assessments were used to identify the genetic associations. The data of two assessments in different years were used separately for QTL mapping.

A QTL with a LRS score greater than the threshold required for declaring a highly significant QTL linkage to a locus was detected by SIM and CIM analyses (Table 3). The highly significant QTL for powdery mildew resistance on chromosome 4A had the highest LRS score ($P < 0.0001$) both in 2002 and in 2004. The QTL peaked at the microsatellite marker *Xgwm160* in an *Xwmc232 – Xpsp3119* introgressive translocation interval (Fig. 5).

Four LRS curves (DI1 and DI2 in 2002 and in 2004) for chromosome 4A showed identical peaks despite the marked difference in the distribution of the two DI scores in a year and the differences between the results obtained for F_2 and F_3 progenies, indicating that the genetic factors contributing to APR are not environmentally sensitive. The major QTL explained up to 35% of the variance in 2002, and up to 54% in 2004. The allele for improved resistance originated from *T. militinae* and acted in a nearly additive fashion (Table 3). The additivity of the main QTL is consistent with the results from the field studies based on classical quantitative genetic analysis.

An additional QTL with significant LRS score was detected by CIM analysis (considering *Xgwm160* as a cofactor; Table 3). The QTL was located on chromosome 5A; however, its precise location depended on the year of the experiment. The minor QTL explained 4–6% of trait phenotypic variance.

On chromosomes 1B, 2A, 5A and 5B, suggestive minor QTLs for APR were detected, either in 2002 or in 2004 (Table 3). The non-reproducible QTL effect on these chromosomes may be a false positive effect rather than that of an environment-specific gene. However, reporting suggestive linkages may be useful, taking into account that non-significance in statistical terms might not denote insignificance in biological terms (Freymark et al. 1993).

Thus, two QTLs located on two different chromosomes (4A and 5A) control the quantitative resistance to powdery mildew in the mapping population at the adult plant stage and explain up to 38 and 55% of total trait variance in F_2 and F_3, respectively. If the suggestive QTLs on 5B, 1B, 2A and 5A are included, up to 69% of total trait variance can be explained.

To demonstrate the selective power of *Xgwm160* in the selection for APR to powdery mildew, F_2 plants and F_3 families were grouped according to the genotypes in this locus (Fig. 4). At the adult plant stage, the average disease severity in plants with the Tähti alleles was twice as high as in plants with the *T. militinae* alleles. However, the DI score for plants with homozygous *T. militinae* alleles in *Xgwm160* locus remained higher than the score for line 8/1, indicating, that not only the main QTL on chromosome 4A is responsible for the high and stable level of APR in this hybrid line.

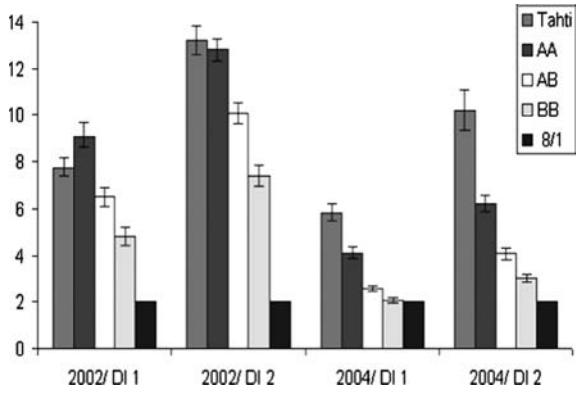

Fig. 4 The effect of alternative alleles in the QTL region of chromosome 4A (*Xgwm160*) on APR in the mapping population for the two assessments, in 2002 and in 2004. *AA* homozygous Tähti allele, *AB* heterozygous, *BB* homozygous *T. militinae*-derived allele

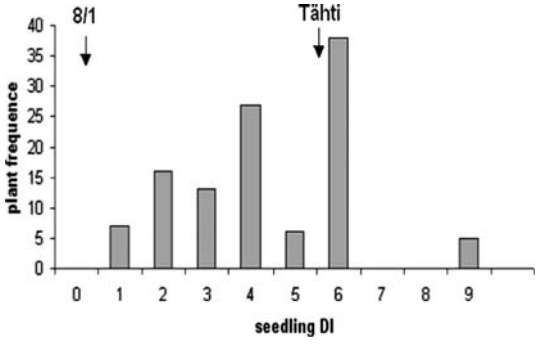

Fig. 5 Distribution of the seedling plant powdery mildew DI for F_2 plants in the mapping population (*0* no visible symptoms; *9* heavy sporulation). The scores for the parents are indicated by *arrows*

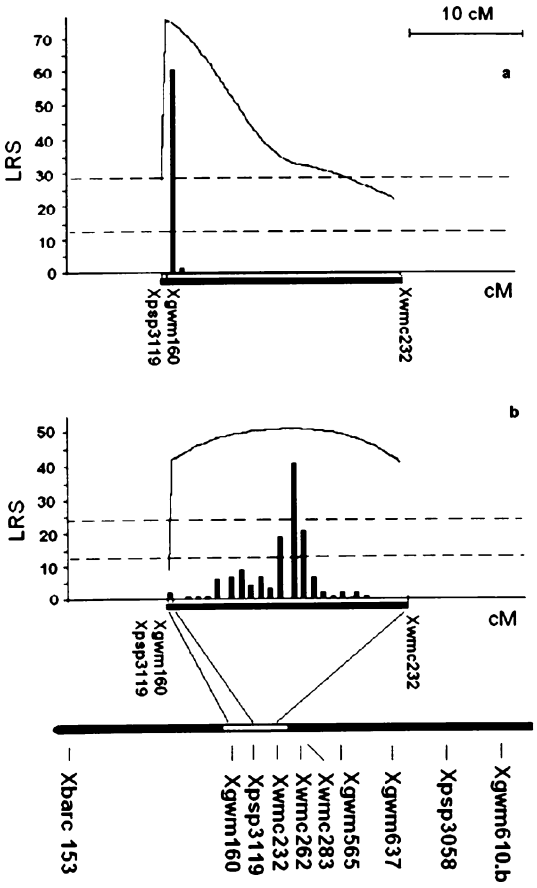

Fig. 6 The likelihood plots of QTLs associated with the adult plant (**a** 2002, DI 1) and seedling resistance (**b** 2002) to powdery mildew on chromosome 4A in the 8/1 × Tähti mapping population. The estimations of confidence intervals by bootstrap resampling are plotted as histograms. The *horizontal dashed lines* represent the significant and the highly significant LRSs

Seedling resistance

Hybrid line 8/1 was resistant to the synthetic population of *B. graminis* DC. f. sp. *tritici* at the seedling stage (score 0–2).

The seedling resistance score in the mapping population showed a continuous distribution (Fig. 5). To avoid the necessity for a qualitative distinction between the resistant/susceptible plants, a quantitative interpretation of the seedling tests of F_2 plants was conducted (Chantret et al. 2000).

A QTL with a significant effect on the seedling resistance to powdery mildew was detected on chromosome 4A by both SIM and CIM analyses. LRS values above the highly significant threshold (Table 3) were registered over the entire translocation on this chromosome. The peak for the LRS score was 9–12 cM away from the *Xgwm232* locus, between loci *Xgwm232* and *Xgwm160* (Fig. 6). The allele conferring resistance originated from *T. militinae*.

Suggestive QTLs for seedling resistance were detected on chromosomes 1A, 1B, 5A (all of them in Tähti alleles) and 2A (*T. militinae* allele) (Table 3). A suggestive QTL for seedling resistance originating from the Tähti genome may explain the transgressive segregation of seedling resistance in the mapping population (Fig. 5).

The main QTL for seedling resistance explained about 33% of phenotypic variance for the trait, each of the suggestive QTLs added about 4–6% (Table 3). Thus, up to 53% of total trait variance could be explained by the QTLs.

Discussion

The objective of this study was to elucidate the genetic basis of adult plant powdery mildew resistance transferred from *T. militinae* into the Finnish spring wheat cultivar Tähti. Simultaneously, hybrid lines were advanced from seeds randomly selected from a heterogeneous hybrid population, powdery mildew resistance levels were assessed and chromosomal segments introgressed from *T. militinae* were mapped in the selected hybrid lines.

In the F_2 backcross-mapping population derived from the hybrid plant 8/1, a *T. militinae*-origin genomic region on chromosome 4A was responsible for up to 54% of the APR variance. Despite the differences in environmental conditions, the results of the QTL mapping were remarkably consistent for F_2 plants and F_3 families in 2002 and 2004, respectively.

Thus far, three QTLs for adult plant resistance (APR) to powdery mildew have been mapped on chromosome 4A (Huang XQ, Röder MS 2004). Two QTLs mapped on chromosome 4AS in the regions *Xgwm111–Xpsp934* and *Xglk128–Xcdo475* in a segregating wheat/spelt population explained 7–14% of the phenotypic variance (Keller et al. 1999). One QTL for adult powdery mildew resistance in the winter wheat line RE174 was detected on chromosome 4A (*XgbxG036–XgbxG542*) in the study by Chantret et al. (2001). This QTL explained 5–6% of the genetic variability over 2 years. However, the position of the QTL for APR in the line 8/1 does not coincide with the abovementioned QTLs.

The QTL for powdery mildew resistance at the seedling stage shows a highly significant LRS score at the same introgressive translocational region on the chromosome 4A (*Xwmc232–Xgwm160*, Table 3). This chromosomal region is probably responsible for the correlation found between the DIs for resistance at the seedling and adult plant stages (0.29 and 0.38, for adult plant data set in 2002 and 2004, $P < 0.001$). The exact positions of the highest LRS values for the adult and seedling stage resistance differ somewhat, although the confidence intervals for both peaks overlap (Fig. 6), permitting either a single gene or a cluster of genes to be involved in the resistance at different stages of plant growth. A cluster of genes related to resistance seems to be the case, taking into account that the resistance allele for APR on chromosome 4A acted in a near additive

fashion and the QTL for seedling resistance was inherited as a recessive locus (Table 3).

It has been repeatedly observed that disease resistance genes may be located in a complex region containing different race specifics (Hammond-Kosack and Jones 1997; Hsam and Zeller 2002). A number of loci for resistance to different pathogens (*H25, Lr28* and *Lr30, Sr7*, *YrMin* and *YrND, Stb7)* have been located on chromosome 4A (McIntosh et al. 2003). The gene for race-specific resistance to *Mycosphaerella graminicola* in the spring wheat, *Stb7*, has been mapped precisely at the distal end of chromosome 4AL in a region closely linked to *Xgwm160* (McCartney et al. 2003). Since homologous relationships have been found between disease resistance loci (Hammond-Kosack and Jones 1997), it is possible that the genome of the line 8/1 includes a cluster of *T. militinae*-derived genes on chromosome 4A, responsible for the non-race-specific powdery mildew resistance in the seedling and adult plant stages of plant growth. Further analysis of recombinant plants will allow us to map this region precisely.

It has been suggested that the same loci could be responsible for qualitative and quantitative traits (Robertson 1985). A race-specific gene for resistance to powdery mildew, *Pm 16*, derived from wild emmer (*Triticum dicoccoides*) has been mapped on chromosome 4AL (Reader and Miller 1991; McIntosh et al. 2003). However, Chen et al. (2005) showed that a SSR marker located on the short arm of chromosome 5B, *Xgwm159*, is closely linked to *Pm16* (genetic distance 5.3 cM), and suggested that *Pm16* might be located on a translocated 4A.5BS chromosome. In our mapping population, the amplification product of *Xgwm159* primers was not segregating, however, the hybrid line 8/1 carries a *T. militinae* translocation in the region of *Xgwm213–Xgwm68–Xgwm66* close to the marker *Xgwm159* on chromosome 5B. Markers *Xgwm66, Xgwm68* and *Xgwm213* have been mapped on chromosome 5B in the ITMI mapping population (Röder et al. 1998), and, in our mapping population, they form a linkage group with no linkage to the *Xgwm160–Xwmc232* translocation on chromosome 4A. Therefore we suggest that rather than *Pm16*, some unknown gene(s) located on the *Xgwm160-Xwmc232* translocation on chromosome 4A is involved in the detected QTL for APR in the line 8/1.

In 8/1, the suggestive QTLs ($P < 0.05$) were detected for APR and for seedling resistance, all of them explaining 4–6% of the respective phenotypic variance. It has been indicated that the minor QTLs for non-race-specific powdery mildew resistance could be less effective or "defeated" alleles of *Pm* genes (Nass et al. 1981; Chantret et al. 1999). For some of the minor QTLs detected in the line 8/1, this may be the case. A residual effect on APR has been demonstrated for the resistance gene allele *Pm4b* (Chantret et al. 1999). *Pm4b* originates from *T. carthlicum* (synonym *Triticum persicum)* (McIntosh et al. 2003). Since it has been assumed that *T. militinae* has arisen as a result of an introgressive hybridization between *T. timopheevii* and *T. persicum* (Navruzbekov 1981), the introgressed into 8/1 chromosomal segment on chromosome 2A with minor QTLs for APR and seedling resistance may include a homologous to resistance gene *Pm4* region. Further analysis is required to prove this suggestion.

In the hybrid line 8/1, 54% of APR and 33% of variance in seedling resistance can be explained by the major QTLs detected on chromosome 4A. Suggestive QTLs on different chromosomes add up to 69 and 53%, respectively, of the phenotypic variance, which is a slightly higher percentage than Kearsey and Farquhar (1998) have found to be an average (46%). Even if the detected effect of the *T. militinae* translocation on chromosome 4A is one of several clustered genes, it may be considered as a new genetic factor for marker-assisted selection for *T. militinae*-derived resistance to powdery mildew (Fig. 4).

Acknowledgements The authors gratefully acknowledge Prof. A. Veske and Prof. E. Truve for their constructive comments to improve this paper. This work was supported by Estonian Science Foundation (grant No 4720) and Estonian Ministry of Agriculture.

References

Allard RW, Shands RG (1954) Inheritance to resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevii*. Phytopathology 44:266–274

Badaeva ED, Budashkina EB, Badaev NS, Kalinina NP, Shkutina FM (1991) General features of chromosome substitutions in *Triticum aestivum* × *T. timopheevii* hybrids. Theor Appl Genet 82:227–232

Badaeva ED, Prokofieva ZD, Bilinskaya EN, Obolenkova LA, Solomatin DA, Zelenin AV, Pukhalskyi VA (2000) Cytogenetic analysis on hybrids resistant to yellow rust and powdery mildew obtained by crossing common wheat (*Triticum aestivum* L., AABBDD) with wheats of Timopheevi group (A^tA^tGG) (in Russian). Genetika 36:1663–1673

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Chantret N, Pavoine MT, Doussinault G. (1999) The race-specific resistance gene to powdery mildew, *MlRE*, has a residual effect on adult plant resistance of winter wheat line RE714. Phytopathology 89:533–539

Chantret N, Mingeot D, Sourdille P, Bernard M, Jacquemin JM, Doussinault G (2001) A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. Theor Appl Genet 103:962–971

Chantret N, Sourdille P, Röder M, Tavaud M, Bernard M, Doussinault G (2000) Location and mapping of the powdery mildew resistance gene MIRE and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor Appl Genet 100:1217–1224

Chen XM, Luo YH, Xia XC, Xia LQ, Chen X, Ren ZL, He ZH, Jia JZ (2005) Chromosomal location of powdery mildew gene *Pm16* in wheat using SSR marker analysis. Plant Breed 124:225–228

Chmielewicz KM, Manly KF (2002) User manual for QTX. Roswell Park Cancer Institute

Devos KM, Millan T, Gale MD (1993) Comparative RFLP maps of homoeologous group 2 chromosomes of wheat, rye, and barley. Theor Appl Genet 85:784–792

Dorofeev VF, Udaczin RA, Semenova LV, Novikova MV, Gradchaninova OD, Shitova IP, Merezhko AF, Filatenko AA (1987) World wheats. Agropromizdat, Leningrad, pp 75–87

Freymark PJ, Lee M, Woodman WL, Martinson CA (1993) Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). Theor Appl Genet 87:537–544

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Proceedings of the 8th international wheat genetics symposium, vol 1. China Agricultural Scientech Press, Beijing, pp 29–40

Griffey CA, Das MK (1994) Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Sci 34: 641–646

Gupta PK, Balyan H, Edwards K, Isaac P, Korzun V, Röder M, Gautier M-F, Joudrier P, Schlatter A, Dubcovsky J, De la Pena R, Khairallah M, Penner G, Hayden M, Sharp P, Keller B, Wang R, Hardouin J, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Hsam SLK and Zeller FJ (2002) Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In: Belanger RR et al (eds) The powdery mildews. A comprehensive treatise, pp 219–238

Huang X, Zeller FJ, Hsam SLK, Wenzel G, Mohler V (2000) Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome 43:298–305

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137:203–223

Järve K, Peusha HO, Tsymbalova J, Tamm S, Devos KM, Enno TM (2000) Chromosomal location of a *T. timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome 43:377–381

Järve K, Jakobson I, Enno T (2002) Tetraploid wheat species *Triticum timopheevii* and *Triticum militinae* in common wheat improvement. Acta Agron Hung 50(4):463–477

Kammholz SJ, Campbell AW, Sutherland MW, Hollamby GJ, Martin PJ, Eastwood RF, Barclay I, Wilson RE, Brennan PS, Sheppard JA (2001) Establishment and characterisation of wheat genetic mapping populations. Aust J Agric Res 52:1079–1088

Kearsey MJ, Farquhar GL (1998) QTL analysis in plants; where are we now? Heredity 80:137–142

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. Theor Appl Genet 98:903–912

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Liu SX, Griffey CA, Saghai-Maroof MA (2001) Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci 41:1268–1275

Lutz J, Limpert E, Bartoa P, Zeller FJ (1992) Identification of powddery mildew resistance genes in common wheat (*Triticum aestivum* L.). I. Czechoslovakian cultivars. Plant Breed 108:33–39

McCartney CA, Brüle-Babel AL, Lamari L, Somers DJ (2003) Chromosomal location of race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6. Theor Appl Genet 107:1181–1186

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat http://www.wheat.pw.usda.gov/ggpages/wgc/ 2003/)

Meer JM, Manly KF, Cudmore RH (2002) Software for genetic mapping of Mendelian markers and quantitative trait loci. Roswell Park Cancer Institute

Migushova EF (1975) About the origin of wheat genomes (in Russian). Bull Appl Bot Genet Breed 55(3):16

Mingeot D, Chantret N, Baret PV, Dekeyser A, Boukhatem N, Sourdille P, Doussinault G, Jacquemin JM (2002) Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. Plant Breed 121:133–140

Nass HA, Pedersen WL, MacKenzie DR, Nelson RR (1981) The residual effects of some ''defeated'' powdery mildew resistance genes in isolines of winter wheat. Phytopathology 71: 1315–1318

Navruzbekov NA (1981) Toward the origin of *T. militinae* Zhuk. Et Migush. In: Botanical and genetic resources of dagestan flora. Makhachkala (in Russian)

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Reader SM, Miller TE (1991) The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat. Euphytica 53:57–60

Robertson DS (1985) A possible technique for isolating genic DNA for quantitative trait in plants. J Theor Biol 117:1–10

Röder MS, Korzun V, Wendehake K, Plasche J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149: 2007–2023

Salina EA, Leonova IN, Efremova TT, Röder MS (2005) Wheat genome structure: translocations during the course of polyploidization. Funct Integr Genom 6:71–80

Shands RG (1941) Disease resistance of *Triticum timopheevii* transferred to common winter wheat. Amer Soc Agron J33:709–712

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Zhukovsky PM, Migushova EF (1969) A highly immune endemic gene pool for breeding of resistant cultivars of common wheat *via* distant hybridization. Proc Agric Sci 2:9–20

Yu DZ, Yang XJ, Yang LJ, Jeger MJ, Brown JKM (2001) Assessment of partial resistance to powdery mildew in Chinese wheat varieties. Plant Breed 12: 279–284