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ORIGINAL PAPER

Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat–*Triticum militinae* introgression line

Irena Jakobson · Diana Reis · Anu Tiidema · Hilma Peusha · Ljudmilla Timofejeva · Miroslav Valárik · Monika Kladivová · Hana Šimková · Jaroslav Doležel · Kadri Järve

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Abstract Introgression of several genomic loci from tetraploid *Triticum militinae* into bread wheat cv. Tähti has increased resistance of introgression line 8.1 to powdery mildew in seedlings and adult plants. In our previous work, only a major quantitative trait locus (QTL) on chromosome 4AL of the line 8.1 contributed significantly to resistance, whereas QTL on chromosomes 1A, 1B, 2A, 5A and 5B were detected merely on a suggestive level. To verify and characterize all QTLs in the line 8.1, a mapping population of double haploid lines was established. Testing for seedling resistance to 16 different races/mixtures of *Blumeria graminis* f. sp. *tritici* revealed four highly significant non-race-specific resistance QTL including the main QTL on chromosome 4AL, and a race-specific QTL on chromosome 5B. The major QTL on chromosome 4AL (*QPm.tut-4A*) as well as QTL on chromosome 5AL and a newly detected QTL on 7AL were highly effective at the adult stage. The *QPm.tut-4A* QTL accounts on average for 33–49 % of the variation in resistance in the double haploid population. Interactions between the main QTL *QPm.tut-4A* and the minor QTL were evaluated and discussed. A population of 98 F_2 plants from a cross of susceptible cv. Chinese Spring and the line 8.1 was created that allowed mapping the *QPm.tut-4A* locus to the proximal 2.5-cM region of the introgressed segment on chromosome 4AL. The results obtained in this work make it feasible to use *QPm.tut-4A* in resistance breeding and provide a solid basis for positional cloning of the major QTL.

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I. Jakobson (✉) · D. Reis · A. Tiidema · H. Peusha · L. Timofejeva · K. Järve
Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia
e-mail: irena.jakobson@ttu.ee

M. Valárik · M. Kladivová · H. Šimková · J. Doležel
Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Sokolovska 6, 77200 Olomouc, Czech Republic

Introduction

The obligate fungus *Blumeria graminis* DC. f. sp. *tritici* (Bgt), causal agent of powdery mildew disease, can infect bread wheat (*Triticum aestivum* L.) at any time from the first leaf stage until senescence. Two types of resistance to Bgt have been described. In a majority of wheat genotypes for which the genetic basis of the resistance has been studied, resistance is based on one or several Bgt-race-specific qualitative resistance gene(s) (*Pm1–Pm36, Pm40–Pm43*), which match fungal factor(s) involved in the mediation of the host–pathogen interaction (avirulence gene) and induce incompatibility in the interaction between the host and the pathogen (reviewed by Bent and Mackey 2007; Huang and Röder 2004). Resistance to different sets of fungal isolates conferred by qualitative resistance genes is typically tested on leaf samples (on the primary leaf) at the seedling stage of the host plant, but the resistance may be expressed throughout the life cycle of the host (Hsam and Zeller 2002). This type of race-specific resistance is overcome when the pathogen population changes, and thus may not be durable.

A non-race-specific partial resistance to Bgt, which has been less frequently analyzed in wheat, retards the infection, growth and reproduction of the fungus. Partial

resistance or slow mildewing is usually observed in adult plants (also known as adult plant resistance—APR, Gustafson and Shaner 1982), and wheats with APR generally exhibit low to intermediate resistance at the seedling stage (Bougot et al. 2006; Lan et al. 2010). However, to identify true partial resistance, the presence of race-specific resistance genes should be tested for, as some of the APR genotypes may carry (defeated) race-specific *Pm* gene(s) (Robe et al. 1996; Mingeot et al. 2002; Tucker et al. 2007; Lillemo et al. 2010).

Partial resistance is presumed to be a quantitative trait, which can be resolved into discrete genomic loci (quantitative trait loci (QTL), Keller et al. 1999; Chantret et al. 2001; Muranty et al. 2009; Tucker et al. 2006; Lan et al. 2009). If the trait is a result of numerous small additive effects, the resistance can hardly be included in breeding programs. However, partial resistance has been found to cosegregate even with a single locus, as shown for the *Lr34* (*Yr18/Pm38*) locus carrying resistance to several fungal pathogens (Spielmeyer et al. 2005). The *Lr34* gene has recently been predicted to encode an ABC transporter-like protein responsible for the pleiotropic disease resistance (PDR, Krattinger et al. 2009).

At the phenotypic level, differences between the qualitative race-specific and quantitative non-race-specific resistance may not be clear, one possible reason being that the results of tests strongly depend on the plant growth stage. For example, one of the resistance genes (*Pm37*) transferred into bread wheat from timopheevii wheats was first shown to exhibit a qualitative non-race-specific resistance to powdery mildew, while conferring full resistance to over 60 Bgt isolates in plants inoculated at Feeke's growth stage 2–3 (Large 1954; Srnić et al. 2005). Later it was shown to carry resistance to at least 14 Bgt isolates after testing leaf segments inoculated at the primary leaf stage (Perugini et al. 2008). Considering race specificity as the main characteristic of *Pm*-(seedling) resistance, this result is clearly uncommon. The *Pm37* line was fully resistant in the field when evaluated at Feeke's growth stages 8 and 10.1 (Srnić et al. 2005); however, no data are available on the level of resistance at later stages of plant growth. Thus, the question is whether the *Pm37* line carries atypically non-race-specific qualitative seedling resistance effective also at later stages of plant development, or the resistance is generated at an atypically early stage of plant development by a single gene responsible for APR—if a low level of infection (slow mildewing) is detected at the following growth stages.

Wheats of the timopheevii group are considered to be a useful source of disease resistance genes, from which, in addition to *Pm37*, several powdery mildew resistance genes (*Pm6*, *Pm27*, *MlAG12*) have been introduced into bread wheat (Jorgensen and Jensen 1973; Järve et al. 2000; Maxwell et al. 2009). Immunity to powdery mildew, leaf and yellow rusts, high resistance to stem rust, loose and dwarf smuts have also been reported as useful traits of *T. militinae*, a tetraploid wheat (A^tA^tGG) of the timopheevii group (Dorofeyev et al. 1976).

In our previous study, we described APR to powdery mildew transferred into bread wheat from *T. militinae* (Jakobson et al. 2006). The resistant introgression line 8.1 derived from a cross between *T. aestivum* and *T. militinae* exhibited effective APR in field tests and showed full resistance to a mix of *B. graminis* test isolates (races) at the seedling stage. The main *T. militinae*-origin QTL effective for APR and seedling resistance were both mapped to the distal part of wheat chromosome arm 4AL, between loci *Xgwm160* and *Xwmc232,* in a region estimated to be 20 cM in a mapping population derived from a cross between the introgression line 8.1 and cv. Tähti. The whole QTL region was designated *QPm.tut-4A*; however, it remained unclear whether seedling and adult plant resistance were due to the same or different loci of the region. Unfortunately, the *QPm.tut-4A* region showed a highly reduced level of recombination in the original mapping population, which hampered precise mapping of the locus.

In addition to the *QPm.tut-4A* QTL, minor QTLs for seedling resistance and/or APR were located on chromosomes 1A, 2A, 5A, 1B and 5B on a suggestive level (LOD <1.0–1.5) (Jakobson et al. 2006). As qualitative resistance genes *Pm3, Pm4* and *Pm16* were mapped in the regions of suggestive minor QTL on chromosomes 1A, 2A and 5B, respectively (Huang and Röder 2004; Chen et al. 2005), the observed seedling resistance might have been a result of pyramidizing of a set of race-specific *Pm* genes in the line 8.1, with each of the QTL being responsible for resistance to a subset of Bgt isolates in the tested artificial mix.

In this work, we developed two new mapping populations and used them for detailed phenotypic characterization and verification of loci contributing to powdery mildew resistance of the wheat–*T.militinae* introgression line 8.1 and for fine mapping of the major QTL.

Materials and methods

Plant material

The line 8.1 resistant to powdery mildew was obtained from a cross of spring wheat cv. Tähti with tetraploid *Triticum militinae* and was found to carry nine introgressions from *T. militinae* in seven chromosomes altogether (Jakobson et al. 2006). Via microspore culture (Touraev et al. 1996; A. Touraev, personal communication), 1–20 DH plants were generated from each F_3 or F_4 plant heterozygous in at least three regions of the *T. militinae* introgressions. Altogether, 350 double haploid (DH) plants

were generated from 79 F_3 and 17 F_4 plants from the progeny of the original cross 8.1 × Tähti (Jakobson et al. 2006). The DH plants were genotyped, and plants carrying different combinations of the introgressions from *T. militinae* and/or fragments of the original introgressions were identified. All *T. militinae* introgressions detected earlier in line 8.1 were represented in the set of derived DH plants, including the genotype carrying all the introgressions. The criteria employed to select the DH lines used in the resistance analysis of this study were to balance the distribution of parental alleles in all loci analyzed (1:1).

A new F_2 mapping population (98 plants) was obtained from a cross between susceptible cv. Chinese Spring (CS) and the resistant introgression line 8.1. F_3 and F_4 families of the CS × 8.1 cross were analyzed to verify the phenotypic effect in lines recombinant in the *T. militinae* introgression on chromosome arm 4AL.

To verify the physical location of markers for the *QPm.tut-4A* region, the region was transferred to chromosome arm 4AL of the susceptible CS 4AL ditelosomic line ($2n = 40 + tt$) by crossing the ditelosomic line with the resistant line 8.1 and generating the introgression ditelosomic line CS 4AL + *QPm.tut-4A*. The transfer of the *QPm.tut-4A* locus was verified by the ditelosomic line gaining resistance to Bgt, and by molecular markers.

In addition, wheat nullisomic–tetrasomic lines N4AT4B and N4AT4D were used to verify the physical location of markers. Seeds of the ditelo-4AL line as well as N4AT4B and N4AT4D lines were kindly provided by Dr. S. M Reader (John Innes Centre, Norwich, UK) and Prof. T. R. Endo (Kyoto University, Japan), respectively.

4AL chromosome-specific DNA

Chromosome-specific DNA from CS 4AL and CS 4AL + *QPm.tut-4A* introgression line were prepared by amplifying DNA of flow-sorted chromosome arms 4AL. Briefly, chromosomes 4AL, representing 50,000 CS 4AL and 50,000 CS 4AL + *QPm.tut-4A* telosomes, were sorted using a FACSVantage SE flow cytometer (Becton–Dickinson, San José, USA). Liquid suspensions of mitotic chromosomes were prepared according to Vrána et al. (2000). The identity of sorted chromosomes and presence of contaminating chromosomes in sorted fractions were determined by fluorescence in situ hybridization (FISH) with probes for telomeric repeat, *Afa* and GAA microsatellite according to Kubaláková et al. (2003). Purity of sorted fractions reached 89 and 87.5 %, respectively, as estimated by FISH. Contamination was represented by a mixture of chromosomes, with chromosome 1D being the major component (50 % of the contaminated chromosomes).

DNA of flow-sorted chromosomes was amplified by multiple displacement amplification (MDA) using illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare Bio-Sciences Corp., Piscataway, USA) as described in Šimková et al. (2008). The 50,000 CS 4AL telosomes yielded 29.5 ng purified DNA, which was split into three independent MDA reactions providing 5.7, 5.1 and 5.2 μg amplified DNA, respectively. Similarly, the 50,000 4AL + *QPm.tut-4A* telosomes provided 44.6 ng DNA, which was used as a template for three MDA reactions yielding 5.8, 4.9 and 6 μg amplified DNA, respectively. The individual samples were combined to reduce a possible bias introduced by the MDA.

Fungal material

A total of 13 characterized Bgt isolates, 4 natural Bgt mixtures and 1 artificial Bgt mixture were used to analyze seedling resistance. Nine of the characterized Bgt isolates (no. 2.1, 2.7, 9.8, 9.21, 10, 12, 15, 16, and 17) were derived via single-conidium propagation from the Bgt test isolates, kindly provided by Dr. F. Felsenstein and Dr. S. L. K. Hsam (Technical University of Munich, Germany). This set included two pairs of single-conidium-derived subisolates (2.1 and 2.7; 9.8 and 9.21) which were derived from test isolates nos. 2 and 9. The remaining four characterized Bgt isolates (v5, v11, v13, v17) were purified from a local Estonian Bgt mixture Vääna 2007. The Bgt isolates were maintained on common wheat cv. Kanzler and were characterized by repeated tests on differentiating wheat lines possessing known resistance genes (Lutz et al. 1992; Huang and Röder 2004). With the exception of the two pairs of subisolates (2.1 and 2.7; 9.8 and 9.21), each of the Bgt isolates had a specific pattern of virulence response (Online Resource 1B). The four natural Bgt mixtures included powdery mildew populations Dacke and Revelj kindly provided by Dr. Tuvesson (Lantmännen SW Seed, Sweden) representing local natural powdery mildew population in Svalöf, Sweden. The remaining two natural mixtures Jõgeva and Vääna represented local Bgt populations from southern and northern Estonia in 2007, respectively (Online Resource 1C). Artificial mixture of the analyzed test isolates (TTU) was used to test both seedling resistance on detached leaf segments, and adult plant resistance on whole plants in the growth chamber.

Marker analysis

Total genomic DNA was extracted from young leaf tissues (approximately 300 mg) according to the method described by Huang et al. (2000), with minor modifications.

Radioactive PCR amplifications of microsatellite (SSR) fragments were performed as described by Röder et al. (1998) and Bryan et al. (1997). Amplified DNA fragments were separated on 5 or 6 % polyacrylamide denaturing gel and autoradiographed. SSR markers were selected from

the GrainGenes 2.0 database (http://wheat.pw.usda.gov). Microsatellite markers gwm0832, gwm0855 and gwm0982 were kindly provided by Dr. M. Röder (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany). In addition, markers designed for the coding sequences of *Pm3* and *waxy* genes (Yahiaoui et al. 2004; Tommasini et al. 2006; http://maswheat.ucdavis.edu/protocols/Waxy/), PCR markers derived for the RFLP marker psr119 (http://wheat.pw.usda.gov/GG2/) and the RGA fragment rga3.1 amplified from *T. militinae* cDNA were mapped in the F_2 8.1 × Tähti population. Primers for rga3.1 were: forward: GCAAGTATGAGAAAGTTAATG GCT, reverse: ATCAGGGCATGATTTATTGTCCAT. Primers for the marker psr119 were: forward: TGGGAA GATGAAGGCGAAG; reverse: GCTCGGACTTCTTC AGTTGC.

A codominant CAP marker was derived for the region homologous to the rice *PDR23*-like gene (NM_001073407) identified in the survey sequence of chromosome arm CS 4AL (Mayer et al., unpublished). Primers for the promoter region (forward: ATTGTTGGCATCGTCTCCAC) and the first exon of the gene (reverse: CACAGCTGTTTTCTCAG GTACAA) with estimated product size of 984 bp were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). The primers were used to amplify the *OsPDR23*-like fragment from chromosome-specific DNAs of CS 4AL and CS 4AL + *QPm.tut-4A*. Amplification of chromosome-specific DNAs allowed a direct PCR product sequencing without interference of homeologous genomes, and a detected SNP affecting the *Fsp*BI restriction site was used to map the fragment in the CS × 8.1 population. STS marker csLV34 developed for the *Lr34/Yr18/Pm38* locus on chromosome 7DS (Lagudah et al. 2006) was used to map *Lr34* in the CS × 8.1 population.

A total of 92 microsatellite markers polymorphic between cv. Tähti and *T. militinae* were tested in the F_2 8.1 × Tähti population; 30 of them were segregating in the population and were mapped to 34 loci in addition to the preexisting F_2 8.1 × Tähti map of 42 loci (Jakobson et al. 2006). A total of 350 DH plants were tested for the alleles of all markers mapped in the F_2 8.1 × Tähti population. Ninety eight plants from the CS × 8.1 cross were tested for alleles of 25 markers mapped on the chromosome arm 4AL as well as for alleles of a total of 11 markers in the regions of detected minor QTL, and for alleles of 5 markers mapped on chromosome 7DS in the region where the CS resistance gene *Lr34* is located (http://maswheat.ucdavis.edu/protocols/Lr34/index.htm).

Seedling resistance

For the seedling resistance tests, plants were grown in a growth chamber under controlled environmental conditions (16/8 h day/night, temperature 22–23 °C, humidity 50–70 %, light irradiance 150 μEm^{-2} s^{-1}). A leaf of a 10-day-seedling (Feeke's stage 1; Large 1954) was cut into four segments; the segments were cultured separately on benzimidazole agar in plastic boxes (Hsam and Zeller 1997) and inoculated with four different Bgt isolates/subisolates or Bgt mixtures. Seedling resistance was scored 10 days after inoculation according to Lutz et al. (1992) (0, no visible symptoms; 1–9, increasing amount, size and density of mycelium and conidia, Online Resource 1A). In each experiment, the response of a DH line to a Bgt isolate/subisolate/mixture was tested using leaf segments of four different plants of the same genotype. In 90–97 % of assessments, the scores for four plants of a DH line were identical and/or differed by not more than for one point of the score. The average of scores for four plants was used in the QTL analysis.

Seedling resistance to Bgt isolates and/or Bgt mixtures was analyzed in the DH population of 120 lines (480 plants altogether), while the response to the two pairs of subisolates (9.8/9.21 and 2.1/2.7) was analyzed in a population expanded to 222 DH lines (888 plants altogether). The whole population was tested simultaneously.

Furthermore, F_2 plants and F_3 and F_4 families from the (CS × 8.1) cross were tested with subisolates 9.21, 9.8, 2.7 and 2.1.

Adult plant resistance

Adult plant resistance of the DH population was tested in the field in Jõgeva in 2006 (71 DH lines) and 2007 (123 DH lines including the lines tested in 2006). Plants were sown in a completely randomized design, ten plants of one genotype in a 1-m row. To provoke natural infection, rows of plants of the susceptible cultivars Luja and Tjalve surrounded each block of five rows. In 2009, 139 DH lines (including the lines tested in 2006 and 2007) and 98 F_2 plants from the CS × 8.1 cross were tested for APR in a growth chamber (16/8 h day/night, temperature 22–23 °C, humidity 50–70 %, light irradiance 150 μEm^{-2} s^{-1}), where in all stages of growth the tested plants were surrounded by susceptible plants (cv. Opata and cv. Kanzler) heavily infected with the artificial Bgt mixture (TTU). The parental lines were included as controls in all experiments. Disease severity in adult plants was visually estimated as percentage of leaf surface covered with mildew, expressed as a disease index (DI) on a 0–9 scale according to Yu et al. (2001) (0: 0 % of leaf coverage; 1: <5 %; 3: <15 %; 5: <25 %; 7: <50 % and 9: 50 % of leaf coverage or more). The average value for APR was based on scores of ten, five and four plants per tested DH line in seasons 2006, 2007 and 2009, respectively. Plants were scored twice, in the ear emergence stage (Feeke's stage 10.5) and, 2 weeks later,

in the milky ripe stage (Feeke' stage 11.1). In 2006 and 2007, mildew was separately assessed on the upper two leaves, the flag leaf and the penultimate leaf (F-1). In 2009, only the flag leaf was assessed.

QTL mapping and statistical analysis

Map Manager QTX Version QTXb16 (Meer et al. 2002; Chmielewicz and Manly 2002) was used (minimal LOD score of 3.5 and maximum genetic distance of 30 cM) to add the new markers to the preexisting F_2 map and to establish linkage groups in the DH population. Genetic distances between markers were estimated using the mapping function of Kosambi. The association between phenotype and marker genotype was investigated using single marker regression. The positions of the QTL were determined by simple interval mapping (SIM) and multiple trait interval mapping using the same software. 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. QTL effects were estimated as the percentage of phenotypic variation explained by the QTL. QTL detected only on a suggestive level in all tests were not included in the further analysis.

Statistical analysis was performed with the SAS Version 9.3 software (SAS Institute Inc., North Carolina, USA, 2011). Single-factor and multi-factor analyses of variance (ANOVA) were performed using the general linear model (PROC GLM) to assess the effect of markers located closest to the corresponding QTL as factors. Type III sum of squares and adjusted least square means were used. Log10-transformed data were used in all statistical and QTL analyses.

Results

Phenotypic analysis of powdery mildew resistance

At the seedling stage (Feeke's stage 1), the introgression line 8.1 was resistant to all tested Bgt isolates, subisolates and mixtures (score 0–1.1, Table 1). The scores of seedling resistance for cv. Tähti varied between 3.6 and 5.2 in tests with Bgt isolates/subisolates/mixtures, with the exception of the reaction to the Bgt mixture Vääna, to which the response in cv. Tähti was scored as high as 8.2 ± 0.18 (Table 1). For all Bgt races tested, seedling resistance showed a continuous one-peak distribution both in the DH population and in the F_2 CS × 8.1 population. However, the distributions were skewed and deviated slightly from a normal distribution (Online Resource 2). The deviating distribution of resistance scores may be explained by the fact that the major QTL counted for approximately 50 % of the total effect on resistance (Jakobson et al. 2006). In most experiments, a transgressive segregation toward the susceptible parent was detected, illustrating the fact that the susceptible parent carries an unidentified locus (loci) responsible for a low level of resistance. The population mean did not always correspond to the parental mean (Table 1). Powdery mildew resistance scores were highly significantly correlated with each other upon infection of the DH population with different powdery mildew isolates/subisolates/mixtures at the seedling stage ($r = 0.57$–0.90; $P < 0.001$, Online Resource 3). Correlation between the scores of seedling resistance to two subisolates derived from the same test isolate (Bgt subisolate pairs 2.1/2.7 and 9.8/9.21) was not higher than the correlation between the scores after inoculation with different Bgt test isolates in the DH population. In the F_2 progeny of the cross CS × 8.1, the correlation between the seedling resistance scores was lower than in the DH population ($r = 0.33$–0.64; $P < 0.01$–0.001).

The scores for the seedling stage resistance correlated significantly with the results of APR tests for the DH population ($r = 0.35$–0.84; $P < 0.01$, Online Resource 3). In the APR tests, the infection level in the field tests (2006 and 2007) was much lower than that in the growth chamber in 2009 where DH plants were grown on a high background of the Bgt mixture TTU (Table 1). According to the field assessments of powdery mildew on the Estonian Variety List cultivars, carried out at the Jõgeva Plant Breeding Institute in 1998–2010, the spectrum of virulence of the local natural powdery mildew population changed in 2006–2007 when several wheat cultivars lost their high level of resistance (Peusha et al. 2008, A. Ingver, personal communication). The line 8.1 still remained highly resistant in 2007; however, the mean value for APR rose from almost full immunity in 2006 to DI = 1.5 on the penultimate leaf in 2007, while still no mildew was detected on the flag leaf (Table 1).

In the DH population, APR estimations at the ear emergence stage and at the milky ripe stage were highly correlated in the field experiments [correlation rate 0.93, ($P < 0.001$) in 2006 and 0.96 ($P < 0.001$) in 2007] and had a lower, but still highly significant correlation rate ($r = 0.77$; $P < 0.001$) in the growth chamber experiment. Therefore, only the value of the milky ripe stage score is presented in this study.

QTL mapping

Five chromosome regions on chromosomes 1A, 4A, 5A, 5B and 7A were found to carry significant and/or highly significant *T. militinae*-origin QTL for seedling resistance in the DH population, and three of them (on chromosomes

Table 1 Mean of scores for seedling resistance to powdery mildew of the cv. Kanzler, CS, parental lines cv. Tähti and line 8.1, DH and F_2 CS × 8.1 plants derived from their cross, plus standard errors, minimum (Min.) and maximum (Max.) scores for resistance to Bgt subisolates, isolates and mixtures and the same values for the APR scores

	cv. Kanzler	CS	cv. Tähti	Line 8.1	DH lines (8.1 × Tähti)						F_2 CS × 8.1					
					Parental mean	Number of plants	Mean	Stand. err.	Min.	Max.	Parental mean	Number of plants	Mean	Stand. err.	Min.	Max.
Seedling resistance																
Bgt subisolates																
2.1	6.1	4.9	4.0	0.3	2.2	215	2.7	0.08	0.5	5.3	2.6	98	1.1	0.13	0.0	5.0
2.7	6.5	5.3	4.5	0.5	2.5	222	2.8	0.09	0.0	5.8	2.9	98	1.7	0.15	0.0	6.0
9.8	5.3	4.4	4.2	0.1	2.2	222	1.9	0.11	0.0	6.0	2.3	98	1.2	0.14	0.0	6.0
9.21	6.4	4.8	5.0	0.8	2.9	222	3.4	0.10	0.5	7.0	2.8	98	1.5	0.15	0.0	6.0
Bgt isolates																
10	5.9	4.2	4.1	0.4	2.3	120	3.4	0.09	1.0	6.0						
12	5.9	5.2	4.4	0.3	2.4	120	1.8	0.12	0.0	5.0						
15	6.0	3.0	3.9	0.1	2.0	116	3.0	0.14	0.8	6.3						
16	6.1	5.0	5.0	0.2	2.6	120	2.3	0.15	0.0	5.8						
17	6.7	6.5	5.2	0.8	3.0	120	2.5	0.15	0.0	6.0						
V5	5.3		4.8	0.0	2.4	114	2.2	0.15	0.0	6.0						
V11	6.8		5.1	0.6	2.9	120	2.3	0.15	0.0	5.8						
V13	5.4		4.8	0.0	2.4	114	2.0	0.15	0.0	6.0						
V17	5.3		4.3	0.1	2.2	114	1.6	0.13	0.0	5.3						
Bgt mixtures																
Dacke	6.3		4.3	1.1	2.7	117	2.3	0.14	0.0	6.0						
Revelj	5.5		3.6	0.4	2.0	117	1.2	0.11	0.0	4.8						
Jõgeva	5.3	4.9	4.5	0.1	2.3	116	1.5	0.16	0.0	6.0						
Vääna	8.3	8.1	8.2	1.1	4.6	116	4.5	0.18	1.0	8.8						
TTU	6.4		4.9	0.1	2.5	116	1.8	0.17	0.0	6.8						
Adult plant resistance																
2006 (flag)	–		3.4	0.0	1.7	71	1.2	0. 15	0.0	4.0						
(F-1)	–		6.4	0.0	3.7	71	2.0	0.21	0.0	7.0						
2007 (flag)	–		4.4	0.0	2.7	123	1.3	0.12	0.0	5.0						
(F-1)	–		6.0	1.5	4.0	123	3.3	0.15	0.0	7.2						
2009 (flag)	–	9.0	9.0	3.0	6.0	139	5.2	0.2	3.0	9.0	6.0	98	5.3	0.16	3.0	9.0

4A, 7A and 5A) were also detected in APR assessments (Table 2, Online Resource 4). The *T. militinae*-origin main QTLs on chromosome 4AL were also detected in the QTL analysis of the F_2 CS × 8.1 population.

The main QTL

The *T. militinae* introgressed segment on chromosome 4AL includes 14 loci mapped on the chromosome arm (Fig. 1) and, according to different published maps, covers a distance of 10–26 cM (GrainGenes 2.0).

On the published maps (GrainGenes 2.0), the locus *Xbarc153* has been mapped to the distal part of chromosome arm 4AL, and based on the fact that no segregation of a Tähti allele of barc153 was detected in the F_2 8.1 × Tähti population (Jakobson et al. 2006), we suggested that the *T. militinae* introgression on chromosome 4A is an internal introgression resulting from two independent recombination events in the original *T. aestivum* × *T. militinae* cross. However, analyzing the distal part of the chromosome using the chromosome arm 4AL-specific DNA (both from CS and CS 4AL + *QPm.tut-4A*), DNA from N4AT4B and N4AT4D, and the segregation data for the F_2 CS × 8.1 population, we now conclude that cv. Tähti and line 8.1 carry a null allele not only for the locus *Xbarc153-4AL*, but also for loci *Xbarc52-4AL, Xwmc497-4AL, Xgwm350-4AL* and *Xwmc219-4AL* (Fig. 1), all located on the distal end of the chromosome arm 4AL (GrainGenes 2.0). The findings indicate that the distal end of 4AL has undergone additional rearrangements in cv. Tähti or its progenitor.

Markers barc153, barc52, wmc497 and wmc219 were not amplified from the *T. militinae* genome, while the *T. militinae* allele for the marker gwm350 was not amplified in line 8.1.

In the F_2 8.1 × Tähti mapping population, the main QTLs for seedling resistance and APR were located on the

Table 2 Powdery mildew resistance QTL detected in the DH population on inoculation with different Bgt races at the seedling stage and QTL for adult plant resistance to powdery mildew detected in the same population

	2.1	2.7	9.8	9.21	10	12	15	16	17	V5
Chromosome 4A (*Xwmc232–Xrga3.1*)										
R^2	40	36	66	48	39	57	64	51	52	54
P	***	***	***	***	***	***	***	***	***	***
(LRS)	(114.5)	(99.2)	(235.4)	(146.1)	(73.7)	(102.2)	(121)	(85.0)	(88.1)	(88.9)
Add	−0.23	−0.22	−0.48	−0.28	−0.19	−0.39	−0.32	−0.46	−0.40	−0.43
Chromosome 5A (*Xgwm666–Xcfd30-Xbarc319*)										
R^2	11	3	23	9	18	19	33	15	21	14
P	***	*	***	***	***	***	***	***	***	***
(LRS)	(25.1)	(8)	(58.1)	(20.8)	(28.8)	(25.5)	(47.7)	(18.9)	(28.5)	(17.1)
Add	−0.10	−0.06	−0.23	−0.10	−0.11	−0.19	−0.21	−0.20	−0.21	−0.23
Chromosome 7A (*Xgwm635–Xbarc70-waxy*)										
R^2	8	5	11	3	20	8	18	8	6	9
P	***	**	***	*	***	*	***	*	*	**
(LRS)	(17.3)	(10.3)	(21.6)	(6.1)	(32.4)	(9.5)	(23.0)	(9.6)	(8.2)	(10.3)
Add	−0.09	−0.07	−0.19	−0.07	−0.13	−0.12	−0.16	−0.13	−0.12	−0.15
Chromosome 5B (*Xgwm205 –Xgwm213*)										
R^2	12	6	9	11	17		5			
P	***	**	***	***	***		*			
(LRS)	(26.6)	(12.9)	(20.9)	(25.4)	(26.7)		(5.9)			
Add	−0.11	−0.07	−0.17	−0.12	−0.10		−0.08			
Chromosome 1A (*Xpsp2999–Xwmc24*)										
R^2	11	8	11	15	11	13	11	8	13	11
P	***	***	***	***	***	**	**	**	***	**
(LRS)	(24.7)	(17.7))	(26.2)	(34.9)	(16.6)	(15.9)	(14.1)	(10.3)	(16.7)	(13.4)
Add	−0.12	−0.10	−0.20	−0.14	−0.08	−0.15	−0.13	−0.16	−0.19	−0.16

	V11	V13	V17	Dacke	Revelj	Jõgeva	Vääna	TTU	APR[1]2006	APR[1]2007	APR[2]2009
Chromosome 4A (*Xwmc232–Xrga3.1*)											
R^2	40	46	45	42	41	70	33	60	46	24	30
P	***	***	***	***	***	***	***	***	***	***	***
(LRS)	(62.1)	(71.0)	(68.6)	(64.3)	(62.0)	(141.5)	(46.4)	(106)	(43.1)	(34)	(48.4)
Add	−0.40	−0.43	−0.41	−0.35	−0.39	−0.62	−0.26	−0.57	−0.31	−0.17	−0.19
Chromosome 5A (*Xgwm666–Xcfd30-Xbarc319*)											
R^2	18	16	13	11	11	21	15	24	16	14	22
P	***	***	**	**	**	***	***	***	**	***	***
(LRS)	(23.7)	(19.8)	(15.5)	(13.7)	(13.7)	(27.4)	(19.5)	(32)	(12.1)	(19)	(35.1)
Add	−0.24	−0.27	−0.25	−0.15	−0.15	−0.28	−0.15	−0.29	−0.17	−0.11	−0.14
Chromosome 7A (*Xgwm635–Xbarc70-waxy*)											
R^2	7	7	5	16	24	13	7	9	28	9	5
P	*	*	*	***	***	**	*	**	***	**	*
(LRS)	(8.7)	(8.8)	(6.3)	(19.8)	(31..5)	(16..5)	(7.7)	(11)	(23.1)	(11)	(6.2)

Table 2 continued

	V11	V13	V17	Dacke	Revelj	Jõgeva	Vääna	TTU	APR[1]2006	APR[1]2007	APR[2]2009
Add	−0.15	−0.14	−0.12	−0.18	−0.24	−0.23	−0.09	−0.18	−0.22	−0.07	−0.10
Chromosome 5B (*Xgwm205–Xgwm213*)											
R^2		5			5	8	11	7			6
P		*			*	*	**	*			*
(LRS)		(5.5)			(5.3)	(9.6)	(13.2)	(7.8)			(7.1)
Add		−0.11			−0.09	−0.12	−0.14	−0.15			−0.07
Chromosome 1A (*Xpsp2999–Xwmc24*)											
R^2	7	12	10	7	8	13	15	12	7		5
P	*	**	**	*	*	**	***	**	*		*
(LRS)	(8.5)	(15.1)	(11.6)	(8.2)	(9.9)	(16.2)	(19.2)	(15)	(4.9)		(7.1)
Add	−0.14	−0.19	−0.16	−0.11	−0.13	−0.23	−0.16	−0.23	−0.11		−0.05

R^2 percentage of variance explained by individual QTL, *LRS* likelihood ratio statistics, *Add* additive regression coefficient for association, APR^1 summary score for APR on the flag and penultimate leaves, APR^2 score for APR on the flag leaf

P level of significance * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

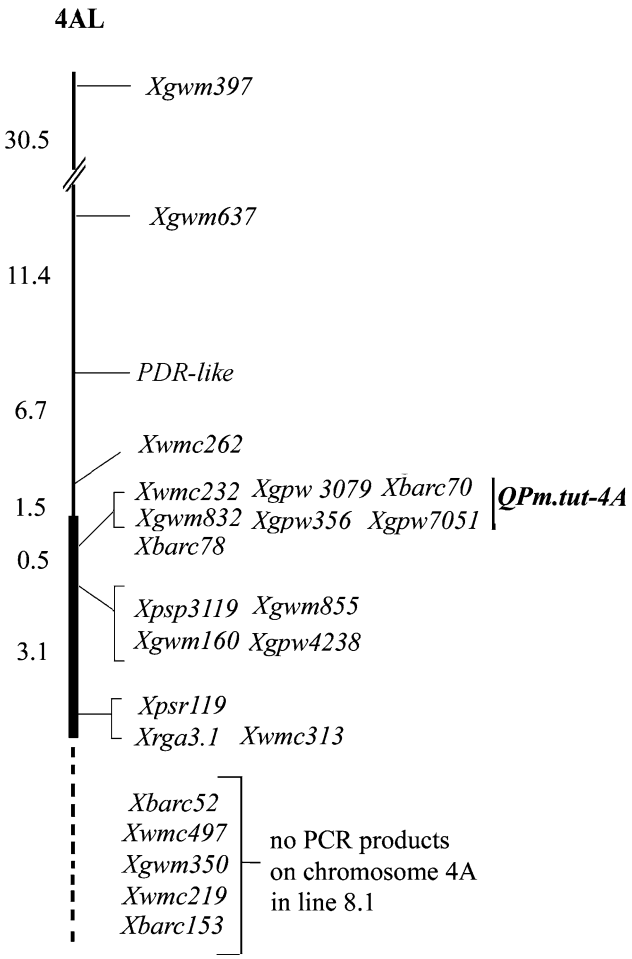

Fig. 1 Genetic map of the region introgressed from *T. militinae* (*bold*) on chromosome 4A of the line 8.1. *QPm.tut-4A* region is marked by a *vertical bar*. Distances of the map are calculated on the basis of segregation in F_2 CS × 8.1 population with the exception of the 3.1 cM distance between the loci *Xrga3.1/Xpsr119/Xwmc313* and *Xgwm160/Xgpw4238/Xpsp3119/Xgwm855* which is calculated according to the segregation in 8.1 × Tähti population

T. militinae introgression on 4AL; however, no recombination in the introgressed segment was detected (Jakobson et al. 2006). In F3-F4-F5 progenies of the cross 8.1 × Tähti and in the DH populations, the addition of new markers allowed us to detect a few recombinants (between the two groups of loci *Xwmc232–Xgwm160* and *Xrga3.1–Xpsr119;* Fig. 1) in the region of the *T. militinae* introgression (2,508 haplotypes were analyzed in total). Major QTLs both for seedling resistance and for APR still covered almost the whole *T. militinae* introgression on chromosome arm 4AL with the exception of the region of *Xrga3.1*and *Xpsr119* loci (Online Resource 4). Subsequently, a recombinant genotype (plant no. 88) carrying the proximal part of the original *T. militinae* introgression was detected in the F_2 progeny from the CS × 8.1 cross. Analysis of seedling resistance and APR in the F_2, F_3 and F_4 progenies of the recombinant plant diminished the QTL responsible for

Table 3 Effect of alternative alleles in loci of the *T. militinae* 4A introgression on seedling and adult plant resistance to powdery mildew in the progenies of the CS × 8.1 cross

CS × 8.1	*T.militinae* introgression on chromosome 4A										Number of plants	Seedling resistance to powdery mildew subisolates[a]				APR*
	Xwmc232	Xgpw3079	Xgwm832	Xbarc70	Xgpw356	Xgpw7051	Xgpw4238	Xgwm855	Xpsp3119	Xgwm160		2.1	2.7	9.8	9.21	
F_2	A	A	A	A	A	A	A	A	A	A	19	1.5 ± 0.35	3.6 ± 0.42	2.9 ± 0.35	2.2 ± 0.50	6.8 ± 0.21
	H	H	H	H	H	H	H	H	H	H	55	1.2 ± 0.18	1.4 ± 0.14	0.9 ± 0.13	1.6 ± 0.16	5.1 ± 0.20
	B	B	B	B	B	B	B	B	B	H	23	0.5 ± 0.12	0.7 ± 0.11	0.6 ± 0.15	0.6 ± 0.23	4.3 ± 0.27
F_2 plant no. 88	H	H	H	H	H	H	A	A	A	A	1	3	3	1	2	5
F_3 derived from the plant no. 88	A	A	A	A	A	A	A	A	A	A	7	2.5 ± 0.63	3.4 ± 0.60	3.2 ± 0.58	4.4 ± 0.42	5.5 ± 0.96
	H	H	H	H	H	H	A	A	A	A	16	1.9 ± 0.27	1.6 ± 0.21	0.9 ± 0.24	1.8 ± 0.23	3.8 ± 0.33
	B	B	B	B	B	B	A	A	A	A	7	1.4 ± 0.30	1.1 ± 0.26	0.3 ± 0.18	1.6 ± 0.30	3.8 ± 0.49
F_4 derived from the plant no. 88	A	A	A	A	A	A	A	A	A	A	30	4.2 ± 0.13	–	4.7 ± 0.36	–	3.7 ± 0.47
	H	H	H	H	H	H	A	A	A	A	21	2.9 ± 0.41	–	3.1 ± 0.26	–	3.5 ± 0.29
	B	B	B	B	B	B	A	A	A	A	40	2.1 ± 0.45	–	1.1 ± 0.26	–	2.0 ± 0.34

A homozygous CS allele, *H* heterozygous locus, *B* homozygous *T. militinae*-derived allele

* Tested in growth chamber after inoculation with the Bgt mixture TTU

[a] Standard errors are attached

powdery mildew resistance both at the seedling and adult plant stage to the loci *Xwmc232/Xgpw3079/Xbarc70/Xgwm832/Xgpw356/Xgpw7051* (Table 3; Fig. 1). Considering the fact that no segregation between seedling resistance and adult plant resistance was detected, we assume that the locus designated *QPm.tut-4A* is involved in powdery mildew resistance at both stages of plant growth. At the seedling stage, *QPm.tut-4A* was effective against all tested Bgt isolates and mixtures in the DH population and was responsible for 33–70 % of the mean trait variance (Table 2). The same QTL was found to be responsible for 24–46 % of the mean trait variance in adult plants in the DH population. In the F_2 CS × 8.1 population, *QPm.tut-4A* generated 35–38 % of the seedling resistance variance, and 52 % of the APR variance.

Minor QTLs

In the DH population, four *T. militinae*-derived significant QTLs with minor effects were identified on chromosomes 1A, 5A, 5B and 7A (Table 2). With the exception of the minor QTL on chromosome 7A, the presence of the remaining QTLs was suggested earlier in the F_2 8.1 × Tähti mapping population (Jakobson et al. 2006).

The new QTL was identified on chromosome 7AS in the region between loci *Xgwm635* and *waxy* with the peak on the *Xbarc70* locus (Table 2; Fig. 2; Online Resource 4). SIM analysis revealed the QTL in all seedling resistance tests, and, on average, it was found to be responsible for 10 % of the trait variance (Table 2). In the field tests for APR, the QTL was statistically (highly) significant and was responsible for 9–28 % of the APR variance. However, in the growth chamber test for APR, the QTL on chromosome 7A was detected only on a suggestive level. In multiple trait analysis, where a higher threshold was selected for highly significant LRS, the QTL was highly significant only in seedling tests with Bgt isolate no. 10 and Bgt mixture Revelj. The QTL on chromosome 7AS is located in a region which shows microcollinearity to the QTL region on chromosome 4AL (Khlestkina et al. 2010; Figs. 1, 2).

A 185 cM map (27 loci) covering almost the entire length of the *T. militinae*-substituted chromosome 5A was created for the line 8.1, and a highly significant powdery mildew QTL was mapped between loci *Xgwm666* and *Xbarc319* with the peak on the *Xcfd30* locus (Table 2; Fig. 2; Online Resource 4). The QTL was detected in all seedling and APR assessments and was on average responsible for 16 % of seedling resistance variance.

The 95 % confidence interval of the QTL on chromosome 1A covered the whole 18-cM *T. militinae* introgression (*Xgwm2999–Xwmc24*) in the SIM analysis of the DH population of 120 lines, with peaks of the LRS curves

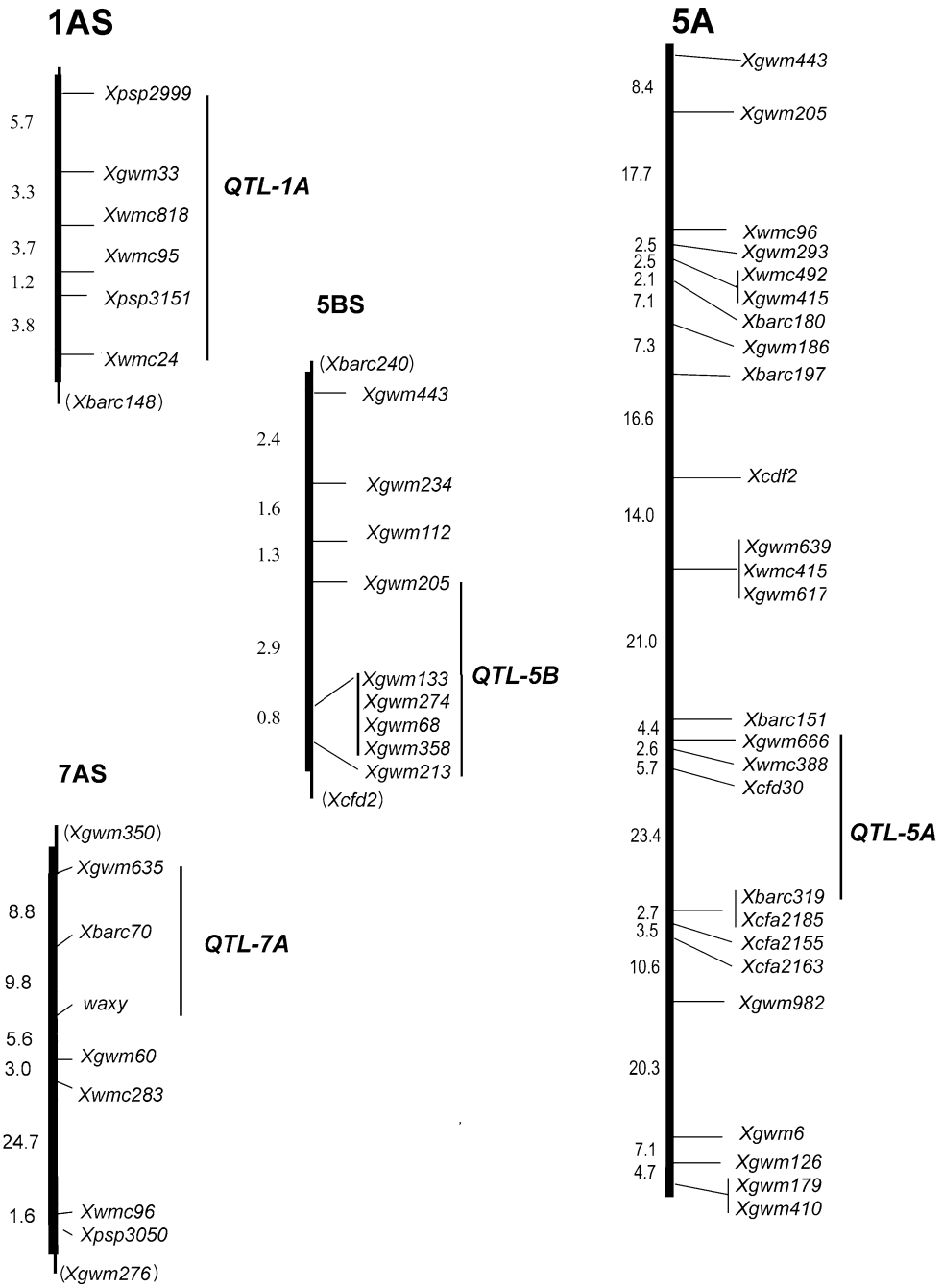

Fig. 2 Genetic maps of the regions introgressed from *T. militinae* (*bold*) on chromosomes 1A, 5A, 7A and 5B. QTL regions are marked by *vertical bars*. Markers are ordered according to the data of the DH population, and distances of the map are calculated on the basis of segregation in the F_2 8.1 × Tähti population and in the DH population. Markers flanking the introgression and loci not segregating in the F_2 8.1 × Tähti population are given in *parentheses*. In comparison to this study, the suggestive QTLs (Jakobson et al. 2006) were earlier located in the regions *Xpsp2999–Xpsp3151* (on chromosome 1A), *Xgwm133–Xgwm205* (on chromosome 5B), *Xgwm186–Xgwm415* and *Xgwm666–Xgwm126* (both on chromosome 5A)

varying in their positions in tests with different Bgt isolates (Table 2; Fig. 2; Online Resource 4). However, in experiments with subisolates 9.8 and 9.21, an expanded population of 222 DH lines was tested, and the QTL was localized in the *Xgwm33–Xwmc818* region. As the region of the *T. militinae* introgression on chromosome 1A involves the *Pm3* gene locus, functional markers developed for this gene (Yahiaoui et al. 2004; Tommasini et al. 2006) were used to analyze cv. Tähti and the line 8.1, and no functional allele of *Pm3* was detected. The QTL on chromosome 1A explained about 10 % of seedling resistance variance.

While the minor QTL on chromosome 1A was detected on at least a suggestive level in all seedling resistance tests, the minor QTL on chromosome 5B (*Xgwm205–Xgwm213*) was revealed only in a subset of tests (Table 2; Online Resource 4). No LSR peaks indicating a QTL on chromosome 5B were detected after inoculation with Bgt isolates/mixtures 12, 16, 17, V5, V17 and Dacke, indicating that the QTL acts in a race-specific manner. Race-specific resistance gene *Pm16* derived from *T. dicoccoides* has been located in the same region on chromosome 5B (Chen et al. 2005); however,

Pm16 confers resistance to Bgt test isolates 12, 16 and 17 (Huang and Röder 2004).

Two minor QTLs, located on chromosomes 2A and 1B, were detected only on a suggestive level (LRS <9.8) in seedling resistance analysis and, for both of them, the favorable allele improving resistance originated from the common wheat parent Tähti. The QTLs on 2A and 1B were not included in further analyses.

Being clearly more effective at the seedling stage of plant growth, each of the *T. militinae*-derived minor QTL detected in the analysis of seedling resistance still affected APR on at least a suggestive level and/or in at least one of the years of testing. On a significant or highly significant level, only the minor QTL located on chromosome 5A was stably involved in APR variance (Table 2; Online Resource 4).

Markers linked to the minor QTL in the DH population were tested for linkage to powdery mildew resistance in the F_2 CS × 8.1 population, and no significant *T. militinae*-derived QTL was detected. However, in the CS × 8.1 population, an additional QTL originating from CS was detected using the STS marker csLV34 for the *Lr34/Yr18/Pm38* locus on chromosome 7DS. The QTL was responsible for 10 % of APR in the F_2 CS × 8.1 population and it was not detected in the seedling tests.

Considering the well-known homology of distal ends of chromosome arms 4AL, 7AS and 7DS, and the location of the pleiotropic disease resistance gene *Lr34/Yr18/Pm38* on 7DS, we generated primers for a 4AL-specific *PDR-like* fragment identified in the 454 survey sequence of the CS 4AL chromosome arm (Mayer et al. unpublished). The fragment was amplified in CS and cv. Tähti, but not in *T. militinae,* placing the gene out of the *T. militinae* introgression. The *PDR-like* gene was mapped in the F_2 CS × 8.1 population, approximately 7.5 cM proximal to the *QPm.tut-4A* region on chromosome 4AL (Fig. 1). The locus was not linked to powdery mildew resistance in the analyzed population.

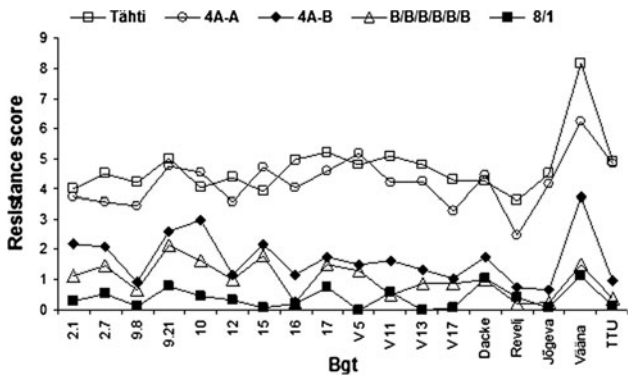

Fig. 3 Mean of seedling resistance scores upon inoculation with different subisolates, isolates and mixtures of *Blumeria graminis* DC. f. sp. *tritici* (Bgt). Data for the parental cv. Tähti, the resistant introgression line 8.1 together with data for the DH subpopulations carrying *T. militinae* allele (4A-B) in the region of *QPm.tut-4A* and carrying the homologous Tähti region (4A-A). B/B/B/B/B represents the mean for DH plants carrying all detected QTLs

Analysis of the effect of detected QTL

For the analysis, the DH population was divided into two subpopulations consisting of genotypes carrying different alleles of *QPm.tut-4A*: subpopulation 4A-A carrying the cv. Tähti allele and subpopulation 4A-B carrying the *T. militinae* allele. The alleles of the remaining QTL were distributed evenly between the two subpopulations. With an average value of 2.8 ± 0.15, the difference in seedling responses of the two subpopulations 4A-A and 4A-B after inoculation with different Bgt isolates/mixtures varied from 1.7 to 3.8 (Fig. 3). In both subpopulations, the pattern of average response at the seedling stage to different Bgt isolates/mixtures was similar to that of the cv. Tähti, with the Bgt mixture Vääna again being the most aggressive (Fig. 3). However, for all Bgt isolates/mixtures, the mean scores of seedling resistance and APR for the genotype carrying all five QTL still remained higher than the scores for the original introgression line 8.1 ($P < 0.01$). In the three APR experiments, the protective effect of *QPm.tut-4A* varied with an average of 2.3 ± 0.2 for the DI differences between 4A-A and 4A-B genotypes. Under conditions of extremely high level of powdery mildew background (growth chamber experiment in 2009), when the infection level in cv. Tähti and the 4A-A subpopulation was very high (DI = 9.0 and 7.0, respectively), DI in the 4A-B subpopulation remained at 4.6 ± 0.8, indicating that *QPm.tut-4A* has a limited 'capacity' and accounts for partial resistance, a feature usual for APR.

For each of the detected QTLs, separate single-way ANOVAs revealed highly significant ($P < 0.0001$) differences in seedling resistance (data not shown), and the combined effect of all detected QTLs on the phenotypic variation, computed by summarizing the R^2 values for the individual QTL, was 0,98. This result does not explain the difference in resistance scores between the original line 8.1 genotype and the genotype carrying five detected QTL, and indicates on an overestimation of the effect of individual QTL.

To compute the effect of the QTL taking into account the other four QTLs, multifactorial ANOVA was performed on a model of the five QTLs and their pairwise interactions. The proportion of resistance variation (R^2) for the model was 0.79, which is in agreement with the results graphically presented in Fig. 3. Multifactorial ANOVA revealed highly significant effects of the main QTL (*QPm.tut-4A*) and the QTL on chromosome 7A ($P < 0.0001$), while QTL on chromosomes 1A and 5B as factors showed significant differences in resistance

($P < 0.05$) (Online Resource 5). The effect of the QTL on chromosome 5A was detected only as a significant interaction ($P < 0.05$) between the QTL on chromosomes 4A and 5A. The second significant interaction was revealed between the main QTL and the QTL on chromosome 7A ($P < 0.05$), showing that the resistance to powdery mildew in DH plants carrying the main QTL responded differently to the presence of the QTL on chromosome 7A compared to the resistance of DH plants lacking *QPm.tut-4A*.

Discussion

Introgressions from wild related species are an attractive source of important genes (gene alleles) for the wheat gene pool narrowed down by domestication and breeding (Feuillet et al. 2008). Introgression of genome segments from tetraploid *T. militinae* into the bread wheat cv. Tähti resulted in a genotype (line 8.1) with a high level of resistance to powdery mildew. In our previous work, we have detected several QTLs for powdery mildew resistance in line 8.1; however, only one of them showed a reliable and reproducible effect (Jakobson et al. 2006). An introgression on chromosome arm 4AL involving the main *QPm.tut-4A* locus was found to be responsible for at least half of the resistance variance in the wheat line and improved the resistance of the "mildly susceptible" cv. Tähti to an agronomically sufficient level.

The introgressed segment on chromosome 4AL showed no recombination in the original F_2 mapping population 8.1 × Tähti of 134 plants, and only a few recombinants were detected in the enlarged F3–F5 population. Inhibition of recombination of introgressed segments in plants has been reported before and, depending on the size and structure of the introgression or substitution, the recombination frequency can be reduced to 0 % (Canady et al. 2006). In wheat, frequency of recombination can be increased by selection of appropriate parents or by manipulating *Ph1*, the major locus controlling chromosome pairing in polyploid wheat (Griffiths et al. 2006). To verify the previously suggested minor QTL, to characterize the QTLs and their interaction and to overcome mapping problems due to the reduced/altered recombination frequency, we created two new mapping populations (a DH population generated from the cross 8.1 × Tähti and a population generated from the cross CS × 8.1).

Analysis of a quantitative trait, i.e., a total effect of multiple genes, is complicated in several aspects (Asíns 2002). Above all, low share of a QTL in the summary trait variance requires repeated tests for correct evaluation of its effect. If, as in this study, an agronomically useful source of non-race-specific resistance is sought, race specificity has to be analyzed after inoculation of stable (homozygous) plant material with a representative set of characterized pathogen races. A total of 350 double haploid plants generated in this study allowed us to select representative populations of stable genotypes to characterize and validate the QTL independently and in combinations. As the DH plants of this study were generated from the heterozygous F3–F4 plants of the original 8.1 × Tähti cross, the DH populations to be analyzed for resistance could be selected from plants carrying new combinations of alleles in the chromosome regions of interest.

To demonstrate the reliability of the QTL analysis of this study, two single-conidium-derived subisolates (pairs: 2.1 and 2.7 or 9.8 and 9.21) were selected from the progeny of single-conidium-derived powdery mildew isolates nos. 2 and 9 and tested in a DH population expanded from 120 lines (i.e., 4 × 120 = 480 plants) to 222 lines (888 plants). The results of these tests allowed us to conclude that the small differences in seedling resistance we detected while testing different powdery mildew genotypes (isolates) were on the same level as differences between the results of testing the effect of the same fungus genotype twice as a pair of Bgt subisolates. In addition, these tests showed that although the significance level of the detected QTL was in general higher in the enlarged population (Table 2), no additional QTLs were detected.

Besides verifying and validating the non-race-specific character of the *QPm.tut-4A* locus, we have identified and characterized four minor QTLs for powdery mildew resistance in the DH population, three of which were suggested earlier (Jakobson et al. 2006). With the exception of the minor QTL on chromosome 5B, all minor QTLs also acted in a non-race-specific manner and contributed to the total resistance score after inoculation with all tested Bgt isolates and mixtures. Our work showed that in the DH population (cv. Tähti background), *QPm.tut-4A* effectively improved resistance both in the presence and absence of minor QTLs. Also, the analysis of the *QPm.tut-4A* locus in a CS × 8.1 progeny confirmed the transferability of the *QPm.tut-4A* effect to a different genetic background. Over the past 10 years, the line 8.1 has exhibited improved resistance in field tests and thus *QPm.tut-4A* is now included in resistance breeding of spring wheat in Estonia.

However, the final effect of *QPm.tut-4A* is not a full immunity, but partial resistance to powdery mildew. Although *QPm.tut-4A* was found to be effective in resistance tests with 13 different single-conidium-derived Bgt isolates, one cannot exclude the possible existence of a Bgt isolate to which *QPm.tut-4A* is not effective.

The second mapping population of the present work was obtained after crossing the susceptible cv. Chinese Spring (CS) with the resistant line 8.1. In this population, recombination was at least partially restored in the introgressed region of *QPm.tut-4A* on chromosome 4AL. In F_2

(CS × 8.1), the *QPm.tut-4A* locus was delimited to a narrow region (*Xwmc232/Xgpw3079/Xbarc70/Xgwm832/Xgpw356/Xgpw7051*; Fig. 1) with an estimated size of 2.5 cM. In addition to QTL for powdery mildew resistance originating from *T. militinae,* an additional QTL originating from CS and cosegregating with the pleiotropic resistance gene *Lr34/Yr18/Pm38* (a PDR-like gene) was detected in the F_2 CS × 8.1 population on chromosome 7DS. Recently, Krattinger et al. (2011) have found an expressed and putatively functional *Lr34* homologs located on chromosome 4A, designated *Lr34-B*. In the Arina × -Forno population, *Lr34-B* cosegregated with the locus *Xcdo475*, which is located on the Synthetic × Opata-4A map at a distance of 12.1 cM from the *Xwmc232* locus. We have mapped a PDR-like gene approximately 7.5 cM proximal to the *QPm.tut-4A* region on chromosome 4AL (Fig. 1), and the locus was not linked to powdery mildew resistance in our F_2 CS × 8.1 population. We conclude that *QPm.tut-4A* is not a homolog of the *Lr34/Yr18/Pm38* gene. Krattinger et al. (2011) considered it likely that the QTLs reported earlier on chromosome 4A mapped to different positions from *Lr34-B*, and concluded that the Lr34-B transporter may have a function other than durable disease resistance.

Association mapping of historical wheat germplasm in multienvironment trials has detected a linkage disequilibrium cluster bearing powdery mildew resistance genes close to the *Xbarc78* locus on chromosome 4AL (Crossa et al. 2007). However, no major powdery mildew gene (*Pm* gene) has been mapped at or near the position of *QPm.tut-4A*. On the other hand, two non-race-specific powdery mildew resistance genes (*Pm37* and *MlAG12*) have been transferred into bread wheat from timopheevii wheats, both showing full resistance to powdery mildew at the seedling stage and in adult plants (Srnić et al. 2005; Perugini et al. 2008; Maxwell et al. 2009). The line carrying *Pm37* remained fully resistant when tested at the Feeke's growth stage 8–10. In our study, adult plant resistance was evaluated later, at the Feeke's growth stage 10.5–11 when low levels of Bgt fungus were detected on lower leaves and stem of 8.1 plants. Although the origin and non-race-specific character of *QPm.tut-4A* are similar to those of *Pm37* and *MlAG12*, the chromosomal location of the genes is different, as both *Pm37* and *MlAG12* are located on the long arm of chromosome 7A. Nevertheless, considering the fact that the three genes were introduced into hexaploid bread wheat (AABBDD) via wide hybridization with a tetraploid species (A^tA^tGG) where homologous pairing between chromosomes might be disturbed, different location in the genome does not exclude the possibility that *QPm.tut-4A* is closely related to *Pm37* and *MlAG12*.

Evolutionarily, timopheevii wheats and emmer wheat originate from common diploid progenitors—*Aegilops speltoides* and *T. urartu*; however, they evolved from two independent hybridization events (Jiang and Gill 1994a) and represent two evolutionarily divergent lineages of polyploid wheats (Dvořák and Zhang 1990; Dvořák et al. 1993). Both lineages inherited a 4AL.5AL reciprocal translocation on chromosomes 4A and $4A^t$, from their diploid ancestor (Naranjo et al. 1987; Jiang and Gill 1994b; Devos et al. 1995). Chromosome 4A of emmer wheats underwent three more rearrangements in its evolution: a distal 4AL.7BS translocation, a pericentric inversion and a paracentric inversion in the region of 4AL.5AL.7BS translocations (Naranjo et al. 1987; Devos et al. 1995). No $4A^tL$.7GS translocation has been found in timopheevii wheats. As an alternative, a distal $4A^tL.3A^tL$ translocation was identified, resulting in the absence of collinearity between the distal regions of chromosomes 4AL and $4A^tL$ (Jiang and Gill 1994b; Maestra and Naranjo 1999; Rodriguez et al. 2000; Salina et al. 2006; Dobrovolskaya et al. 2009). There is no evidence of the presence or absence of inversions on chromosome $4A^t$. Considering the differences between chromosomes 4A and $4A^t$, it is not surprising that $4A^t$/4A substitutions or *T. timopheevii* translocations involving chromosome 4A are rarely detected in *T. aestivum* x *T. timopheevii* hybrids (Gordeeva et al. 2009; Badaeva et al. 2010).

The main QTL for powdery mildew resistance (*QPm.tut-4A*) in the introgression line 8.1 analyzed in the present work is located in the distal region of chromosome arm 4AL. During the evolution of wheat, this region was translocated from chromosome arm 7BS and covered at least 34 % of the physical length of 4AL (Hossain et al. 2004; Ishikawa et al. 2009). In the original cross of cv. Tähti with *T. militinae,* a considerable part of wheat 4AL was not homologous and even not homeologous with the *T. militinae* counterpart. In the course of stabilization of the 8.1 genome, a distal part of wheat chromosome 4AL may have recombined with the homeologous distal region of chromosome 7GS. This hypothesis is supported by the localization of a homeologous minor QTL for powdery mildew in a collinear region of chromosome arm 7AS in the line 8.1. However, the published *T. militinae* (*T. timopheevii*) maps do not cover the distal part of chromosome arm 7GS (Salina et al. 2006; Dobrovolskaya et al. 2009) and no additional markers specific for the *QPm.tut-4A* region are available. Thus, the exact origin of the 4A introgression cannot be resolved at present.

In conclusion, the major QTL *QPm.tut-4A* improves resistance to powdery mildew at the seedling stage and in adult plants, but it does not provide full immunity. *QPm.tut-4A* is effective upon inoculation with all tested Bgt isolates and mixtures at both evaluated stages of plant growth. Partial response and the lack of race specificity are common characteristics of APR; however, the

characteristics are exceptional for seedling resistance. The detected minor QTLs additionally improve the resistance, but their presence is not required for the activity of *QPm.tut-4A*. Work is in progress to show how effective *QPm.tut-4A* is on different genetic backgrounds. In parallel, efforts to develop markers for marker-assisted selection and cloning of the QTL are ongoing. Induction of chromosome breaks using ionizing radiation and stimulation of homeologous pairing using *ph1* mutants will be used to improve mapping resolution in the region carrying the main QTL for powdery mildew resistance.

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