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Genetic Analysis of Powdery Mildew Resistance in an Introgression Line of Wheat

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any degree or examination elsewhere.
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Jahukastekindla introgressiivse suvenisuliini geneetiline analüüs

IRENA JAKOBSON

TTÜ KIRJASTUS

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LIST OF PUBLICATIONS

The present dissertation is based on the following publications, which will be referred to in the text by their Roman numerals:

I **Jakobson I**, Peusha H, Timofejeva L, Järve K. (2006). Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* x *Triticum militinae* hybrid line. *Theor Appl Genet* 112: 760–769. https://doi.org/10.1007/s00122-005-0181-2

II **Jakobson** I, Reis D, Tiidema A, Peusha H, Timofejeva L, Valarik M, Kladivova M, Simkova H, Dolezel J, Järve K. (2012). Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat–*Triticum militinae* introgression line. *Theor Appl Genet* 125: 609–623. https://doi.org/10.1007/s00122-012-1856-0

III Ivaničová Z, **Jakobson I**, Reis D, Šafář J, Milec Z, Abrouk M, Doležel J, Järve K, Valárik M. (2016). Characterization of new allele influencing flowering time in bread wheat introgressed from *Triticum militinae*. *New Biotechnology* 33(B): 718–727. https://doi.org/10.1016/j.nbt.2016.01.008

IV Janáková E, **Jakobson I**, Peusha H, Abrouk M, Škopová M, Šimková H, Šafář J, Vrána J, Doležel J, Järve K, Valarik M. (2019). Divergence between bread wheat and *Triticum militinae* in the powdery mildew resistance *QPm.tut-4A* locus and its implications for cloning of the resistance gene. *Theor Appl Genet* 132: 1061–1072. https://doi.org/10.1007/s00122-018-3259-3

Some supporting data not included in the papers is additionally presented.

AUTHOR'S CONTRIBUTION TO THE PUBLICATIONS

I The author designed all and performed the majority of the experiments for studies, analysed the data and wrote the manuscript.

II The author designed and performed the majority of the experiments for studies, analysed the data and participated in writing the manuscript.

III The author performed genotyping, development of mapping populations, QTL and statistical analysis, participated in study design and drafted the manuscript.

IV The author performed genotyping, development of mapping populations, and statistical analysis.

OTHER RELATED PUBLICATIONS:

Abrouk M, Balcárková B, Šimková H, Komínkova E, Martis M M, **Jakobson I**, Timofejeva L, Rey E, Vrána J, Kilian A, Järve K, Doležel J, Valárik M. (2017). The in silico identification and characterization of a bread wheat/*Triticum militinae* introgression line. *Plant Biotechnol J* 15(2): 249–256. doi:10.1111/pbi.12610.

Tsõmbalova J, Karafiátová M, Vrána J, Kubaláková M, Peuša H, **Jakobson I**, Järve M, Valárik M, Doležel J, Järve K. (2017). A haplotype specific to North European wheat (*Triticum aestivum* L.). *Genet Resour Crop Evol* 64(4): 653–664. http://dx.doi10.1007/s10722-016-0389-9.

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

INTRODUCTION

Wheat was one of the first domesticated food crops, having been cultivated since the start of settled agriculture about 10,000 years ago and now it is one of the world's most important food crops. Wheat provides about 20% of the calories consumed by humans and covers 15% of the cultivated area of the world (FAOSTAT 2015, http://faostat.fao.org). Over the last decades the area of wheat cultivation in Estonia has grown considerably. In 2010-2016, share of cereals in exports of agricultural products and food preparations from Estonia grew twice, in total up to 10%. More than half of the growth was due to wheat export (Puura and Silla, 2017).

Fungal pathogens represent the most relevant biotic stresses for wheat. Caused by *Blumeria graminis DC. f.* sp. *tritici* (*Bgt*), powdery mildew is one of the most devastating diseases of wheat in areas with maritime or semi-continental climate. Fungal infection can cause yield losses and, through effects on flour proteins, can also affect the quality of grain (Bennett 1984; Leath and Bowen, 1989; Griffey et al., 1993; Everts et al., 2001; Conner et al., 2003; Samobor et al., 2006). In the course of time, severity of powdery mildew has substantially increased both on the global level and, more specifically, in Europe (Morgunov et al., 2012). Control of fungal diseases through the application of fungicide is expensive and has a negative impact on natural eco-systems. Moreover, the pathogens can develop resistance to chemical fungicides. As an alternative, growing of cultivars carrying genetically based effective and durable resistance offers efficient, ecologically sound and economic base for the control of fungal diseases (Bhullar et al., 2010; Krattinger and Keller, 2015). Breeding for adequate levels of resistance to pathogens in high yielding wheat genotypes is one of the principal challenges and the most important goal in modern wheat breeding 2014).

Identification of resistance genes in cultivars currently grown in different countries has shown that, in most cases, powdery mildew is controlled by the deployment of genes conferring race-specific hypersensitive response to *Bgt* (Hsam et al., 2015). Genetically uniform agricultural ecosystems and widespread deployment of a small number of race-specific resistance genes in modern wheat cultivars has led to the rapid evolution of the *Bgt* and resulted in an increase in virulent strains within *Bgt* populations (Švec and Miklovičova, 1998; Hsam and Zeller, 2002; Parks et al., 2008; Cowger et al., 2009) or, through host jump, has even led to the emergence of new pathogen species (Wicker et al., 2013). Some genes start to lose effectiveness within 1–3 years of cultivation (Skinnes, 2002) or even before commercial deployment (Niewoehner and Leath, 1998; Parks et al., 2008). In this way, race-specific resistance provides only a temporary solution (McDonald and Linde, 2002; Huang and Röder, 2004; Parks et al., 2008). However, some wheat genotypes do not carry any known race-specific genes, yet they show a stable intermediate resistance to powdery mildew (Keller et al., 1999). The non-race-specific resistance – which retards infection, growth and reproduction of the pathogen at the adult plant stage – has been called "slow mildewing" (Roberts and Caldwell, 1970), "adult plant resistance" (Gustafson and Shaner, 1982) or "partial resistance" (Hautea et al., 1987). Due to the polygenic character in its base (Das and Griffey, 1994; Keller et al., 1999) adult plant resistance (APR) is considered more durable compared to race-specific resistance. However, if elevated resistance is an additive result of numerous small effects, the trait can hardly be included in breeding programmes.

Developments in DNA marker technologies have provided effective systems for mapping and subsequent incorporation of different resistant genes into wheat genome. DNA markers have provided a phenotype-neutral selection technology based on marker-trait associations in the absence of pathogen. The use of markers greatly facilitates selection of complex traits, such as durable disease resistance, and also can help to reduce the amount of non-target genetic background and diminish possible adverse linkage drag when a new gene has been transferred, for example, from wild relatives of wheat (Gill et al., 2011).

In the course of agricultural development and, specifically, since the start of intensive breeding at the end of the 19th century, early domesticates were gradually replaced, first by landraces and traditional varieties, and later on by modern cultivars. This has resulted in genetic bottlenecks and a loss of genetic diversity in the wheat gene pool (Christiansen et al., 2002; Raman et al., 2010; Nevo, 2011). Modern wheat varieties were bred from a limited number of landraces and their genomes are highly similar (Ravel et al., 2006; Chao et al., 2009). Introgressions from wild related species can be an attractive source of new resistance genes/alleles, including genes for resistance to powdery mildew (Warburton et al., 2006; Feuille et al., 2008; Gill et al., 2011).

The timopheevi group of tetraploid wheats (2n=28, A^tA^tGG) is a valuable resource for powdery mildew resistance (Dorofeev et al., 1976). Four powdery mildew resistance genes have been transferred from wheats of the timopheevii group, including *Pm6* (Jorgensen and Jensen, 1973), *Pm27* (Järve et al., 2000), *Pm37* (Perugini et al., 2008), and *MlAG12* (Maxwell et al., 2009). To our knowledge, no powdery mildew resistance gene originating from *T. militinae* has been transferred into the wheat genome up to now (McIntosh et al., 2017; http://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf).

In the laboratory of Dr. T. Enno (Institute of Experimental Biology, Estonia), several hybrid lines with improved resistance to powdery mildew were selected in the progeny of crosses between the common wheat cultivars (2n=42, AABBDD) and *Triticum militina*e (Peusha et al., 1995; 1996; Järve et al., 2002). These lines carry *T. militinae*-derived powdery mildew resistance genes which could be used in common wheat breeding.

The main objective of the present study was to localise the genetic factors affecting resistance to powdery mildew in the progeny of *Triticum aestivum / Triticum militinae* crosses.

ABBREVIATIONS

add	additive regression coefficient for association
APR	adult plant resistance
Avr	avirulence factors
BAC	bacterial artificial chromosome
BC_1	backcross to the parent of the first generation
Bgt	*Blumeria graminis* f. sp. *tritici*
BLAST	basic local alignment search tool
CAS	CRISPR associated protein
CC	coiled-coil
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (Spanish - International Maize and Wheat Improvement Center)
cM	centimorgan
CRISPR	clustered regularly interspaced short palindromic repeats
DAPI	4',6-diamidino-2-phenylindole
DH	double haploid
DI	disease index
DR	defense-responsive
DT	ditelosomic
ET	ethylene
ETI	effector-triggered immunity
F_1	first generation of a cross
F_2	second generation of a cross
FITC	fluorescein-5-isothiocyanate
FT	flowering time
GISH	genomic in situ hybridization
GLP	germin-like protein
HMA	heavy metal-associated domain
HR	hypersensitive response
ID	integrated domain
IWGSC	International Wheat Genome Sequencing Consortium
JA	jasmonic acid
LOD	logarithm of the odds (= LRS divided by 4.61)
LRR	leucine-rich repeat
LRS	likelihood ratio statistic
MAPK	mitogen-activated protein kinase
MARKKK	mitogen-activated protein kinase kinase kinase
MAS	marker-assisted selection
Mlo	mildew resistance locus
NB-LRR	nucleotide binding leucine-rich repeat
NBS	nucleotide-binding site
NIL	near isogenic line
NLR-ID	nucleotide binding leucine-rich repeat with integrated domain
P	level of significancy
PAMP	pathogen-associated molecular pattern
PR	pathogenesis-related

PRR	pattern recognition receptor
PTI	pattern-triggered immunity
QR	quantitative resistance
QTL	quantitative trait locus or loci
RFLP	restriction fragment length polymorphism
RGA	resistance gene analog
R-gene	resistance gene
RIL	recombinant inbred line
RLK	receptor-like kinase
RLP	receptor-like protein
SA	salicylic acid
SAR	systemic acquired resistance
SSR	simple sequence repeat
STS	sequence-tagged sites
TILLING	targeting induced local lesions in genome
TIR	Toll/interleukin-1 receptor/resistance protein
WAK	wall-associated receptor-like kinase

1 REVIEW OF THE LITERATURE

1.1 Two-layer defense system in plants

To stand up to the constant attacks of various potentially pathogenic microbes, plants have developed detection and response systems that identify pathogen signals and initiate the appropriate defense. A model of plant-pathogen interactions, called the 'zigzag' model, which encompasses a two-layer defense of the plant's immune system, was proposed by Jones and Dangl (2006). The first layer is a basal immune response providing broad-spectrum resistance to pathogens, which is mediated by pattern recognition receptors (PRRs) – located on the plant's cell surface, which detect pathogen-associated molecular patterns (PAMPs), such as bacterial flagellin or chitin, a component of fungal cell walls (Jones and Dangl, 2006; Boller and Felix, 2009; Dodds and Rathjen, 2010). Sensing of PAMPs initiates cascades of signalling and transcription events, known as pattern-triggered immunity (PTI), or non-host resistance. Inside the host cells host-adapted pathogens secrete effector molecules capable of suppressing the PTI.

The second layer of the defense system recognizes the pathogen-encoded effector proteins (virulence factors or *Avr* gene products), largely by intracellular receptors encoded by resistance genes(*R*-genes) of NB-LRR family containing nucleotide-binding domain and leucine-rich repeats (Jones and Dangl, 2006; Dodds and Rathjen, 2010; Thomma et al., 2011; Cook et al., 2015). Interaction between the product of the host's *R*-gene and the pathogen's *Avr*-gene determines whether there will be a compatible (susceptible) or incompatible (resistant) reaction in the host plant (Heitefuss, 2001). The incompatible reaction initiates effector-triggered immunity (ETI). Typically, ETI yields complete disease resistance phenotypes at the site of infection against pathogens containing the recognized effector, a process designated as race-specific resistance or host resistance. ETI often leads to the genetically programmed, rapid and localised death of the host cells, known as the hypersensitive response (HR). This process prevents further expansion of biotrophic pathogens in the host tissue (Heitefuss, 2001).

The *R*-gene products can recognize the products of the *Avr* genes directly via physical binding to the effector or indirectly, via a detection of modifications that effectors cause on a plant's target proteins (guard model) or via mimics of those targets (decoys) (Dodds and Rathjen, 2010). In the classical decoy model, the decoy and the corresponding NB-LRR protein are encoded by two separate genes. Recent reports have shown that decoy domains can be directly integrated into the corresponding NB-LRR, resulting in NLR-IDs (Sarris et al., 2016).

Recognition of a pathogen-derived signal triggers a defense reaction as a diverse set of cellular responses including changes in cellular redox status, generation of signaling molecules (e.g. salicylic acid (SA), jasmonic acid (JA), ethylene (ET), nitric oxide and reactive oxygen species), activation of the transcription in defense related genes and reinforcement of cell walls at the attempted infection sites (Lamb and Dixon, 1997; Chisholm et al., 2006; Underwood and Somerville, 2008). The SA-mediated signaling pathway has been associated with resistance to biotrophic pathogens which feed on living host tissue during their infection cycle. ET and JA-mediated signaling pathways, on the other hand, often mediate plant defense against necrotrophic pathogens that promote host cell death in the early stages of infection (Glazebrook, 2005).

Both defense layers share overlapping signaling pathways and induce similar defense responses, forming a complex network leading to defense responses (Panstruga et al., 2009). PTI and ETI responses are linked to the activation of the same set of the cellullar responses and the same defense-related genes, however, the processes are quantitatively and kinetically different (Asai et al., 2002, Tao et al., 2003). Recently, an alternative view of plant's innate immunity as a system that continually evolves to detect microbial invasion, was proposed (Cook et al., 2015). In an invasion model, the PTI and ETI are less viewed as strictly contrasting responses and rather as a continuous immune output resulting from variations between different invasion patterns that are derived from PAMPS, effectors or endogenous elicitors resulting from the infection (Cook et al., 2015).

1.2 Molecular basis of pathogen recognition (R-genes, RGA, RLK, RLP)

At least two classes of genes are involved in plant disease resistance: genes involved in the recognition process or receptor genes (*R*- and PRR-type genes) and pathogen-induced defense-responsive or defense related (DR) genes, functioning downstream of PTI- and ETI-initiated defense signaling pathways (Hu et al., 2008a; Kou and Wang, 2010).

Most molecularly studied plant resistance genes encode receptor proteins involved in host-pathogen recognition. Over 100 resistance genes have been cloned and characterised from various plant species, and the NB-LRR (Nucleotide binding Leucine-rich Repeat) proteins, containing a central domain with a nucleotide binding site (NBS) and a carboxyterminal domain containg multiple degenerate leucine-rich repeat residues (LRR), are prevailing (Ayliffe and Lagudah, 2004; McHale et al., 2006; Liu et al., 2007; Li et al., 2015; Sekhwal et al., 2015). In plant genomes, NB-LRRs form diverse gene families and can be subdivided into coiled-coil NBS-LRRs, which contain an N-terminal coiled-coil (CC) domain, and TIR-NBS-LRRs, which contain a Toll/interleukin-1 (TIR)-like domain and are absent in monocot genomes (Takken and Goverse, 2012). Similar receptor proteins, named Toll-like receptors, are involved in innate immunity in humans (Nürnberger et al., 2004). It was reported that usually the LRR domain recognizes the effectors of pathogens (Jones and Dangl, 2006), inducing structure changes of the NB-ARC domain and leading to activation of the signal transduction (Inoue et al., 2013). However, in some cases, the NB-ARC domain could also recognize the pathogen effector, such as N gene of tobacco could interact with pathogen effector molecule p50 (Burch-Smith et al., 2007).

The number of NB-LRRs in flowering plants varies across species. Using bioinformatics approaches, potential *R*-genes – as a large class of resistance gene analogs (RGA) – have been identified in sequenced plant genomes (Sekhwal et al., 2015). For example, in the *Arabidopsis* Col-0 genome, 149 proteins were predicted to be encoded by genes carrying NBS-LRR domains (Meyers et al., 2003). Genomes of model grass species rice and *Brachypodium distachyon* carry 458 and 212 NB-LRR-genes, respectively (Li et al., 2010). About 2.5% of the total 94,000 annotated genes in *T. aestivum* are NBS coding genes (Bouktila et al., 2014).

About 10% of receptors identified by extensive cross-plant-species database searches have been shown to include novel, highly variable integrated domains capable of detecting pathogen effectors by direct interaction (Sarris et al., 2016). For example,

two rice NB-LRR proteins, RGA5 and Pik-1 contain an additional heavy metal-associated domain (HMA) that is not found in other NB-LRRs. The HMA domains of the two immune receptors directly recognize several rice blast effectors, triggering a defense response (Cesari et al., 2014; Zhai et al., 2014; Maqbool et al., 2015). Sarris and co-authors (2016) suggest that NLR-IDs work in pairs of closely linked NB-LRRs, at least in the cases studied in detail to date.

A novel class of NB-LRR genes has been identified where products of closely linked, but divergently transcribed genes, are functioning in pairs and where resistance requires functioning of both genes (Cesari et al., 2014b; Williams et al., 2014). The protein products interact to form dimers or complexes of higher order. One member of the pair often carries one of several unique non-NB-LRR pathogen effector-sensing domains embedded at the C-terminus. The second member of the NB-LRR pair acts as a signal transducer, signaling the presence of the pathogen and activating the downstream host's defense machinery. In rice, these gene duos are important for blast resistance (Zhai et al., 2014). One pair has been described at the leaf rust resistance locus *Lr10* (Loutre et al., 2009) in wheat, and another in the *Rpg5* stem rust resistance locus in barley (Wang et al., 2013).

Following gene duplication and amplification events, numerous in the plant genome NB-LRR-genes and RGA often occur in clusters of linked paralogs (Hulbert et al., 2001; Jacob et al., 2013; Sekhwal et al., 2015).

Genome-wide expression profiling and transcriptome sequencing experiments have shown that the transcript levels of many NB-LRR-genes are significantly changed after the attack of a given pathogen (Arya et al., 2014; Rodamilans et al., 2014).

In addition to NB-LRRs, there is a second class of of immune receptors in plants, comprising receptor-like kinases (RLKs) and receptor-like proteins (RLPs) as cell-surface-localised receptors with an extracellular ligand-binding domain (Jones and Dangl, 2006; Dodds and Rathjen, 2010; Thomma et al., 2011; Cook et al., 2015). Besides the extracellular LRRs, other, non-LRR domains which recognize different elicitor classes have been found in RLKs and RLPs (Kaku et al., 2006). A wall-associated receptor-like kinase (WAK) has been found to confer resistance against fungal diseases in maize and rice (Zuo et al., 2015; Hurni et al., 2015). ZmWAK has been identified as the causal resistance protein in the *qHSR1* locus in maize, conferring resistance to head smut (*Sporisorium reilianum*) (Zuo et al., 2015). Hurni et al., (2015) identified the WAK-encoding gene *Htn1* in maize. Htn1 and qHSR1 share some structural homology, however, their extracellular domains have no sequence identity detectable by BLAST (Zuo et al., 2015). Some RLKs and RLPs recognize highly conserved ligands and can confer resistance against a broad range of pathogens. One of the first plant RLKs identified was rice *XA21* which confers resistance to most strains of the agronomically important bacterial blight pathogen *Xanthomonas oryzae* pv. o*ryzae* (Song et al., 1995).

Wall-associated kinases have been found to play a role not only in disease resistance (Diener et al., 2005; Li et al., 2009) but also in developmental processes such as root growth (Kaur et al., 2013) and gametophyte development (Wang et al., 2012). The number of RLK and RLP genes has incresed throughout the course of plant evolution (Krattinger and Keller, 2016). Interestingly, only 26 WAK-like genes are found in the model plant *Arabidopsis*, but in the rice genome the number of WAK and WAK-like genes is expanded compared to Arabidopsis and the rice genome contains more than 120 WAK like genes (Shiu et al., 2004; Zhang et al., 2005).

R-genes may require additional (supportive) non-NB-LRR-genes for their functioning. *Non-race-specific disease resistance 1* (*ndr1*), encoding a plasma membrane-localised protein, is required for *R*-genes to recognize bacteria and oomycetes in rice (Century et al., 1995). *Rar1* and *Rar2* (syn. *Nar-l* and *Nar-2*) are required for the *Mla12*-triggered resistance to barley powdery mildew (Freialdenhoven et al., 1994; Torp and Jørgensen, 1986). *Rar1* is required for several, but not all, *Mla* resistance genes, whereas *Rar2* has later turned out to be a mutated resistance gene *Mla12* (Jørgensen, 1996; Shen et al., 2003).

1.3 Defense-responsive or defense-related genes

In the downstream defense events, expression of defense-responsive or defense-related (DR) genes may be induced or suppressed upon pathogen infection or insect attack (van Loon et al., 2006; Hu et al., 2008a). Several types of DR proteins have been described in plant species and have been classified into 17 families of pathogenesis-related proteins (van Loon et al., 2006). DR genes were characterised as responding to a pathogen attack via changes in their expression level or via detection of posttranslational modifications in proteins encoded (Eulgem, 2005; Benschop et al., 2007). For example, in rice, a large number of defense-responsive genes has been identified by differential expression analysis (Vergne et al., 2010). Using transient gene silencing, a recent study identified 96 genes involved in resistance to the powdery mildew fungus in barley (Douchkov et al., 2014). In *Arabidopsis*, approximately 14% of all annotated genes may be related to pathogen defense (Bevan et al., 1998). Genes with known functions among the numerous isolated *DR*-genes encode a diverse array of enzymes. Map locations indicate that DR gene loci are not randomly distributed throughout the wheat genome but are rather located in clusters and/or in distal gene-rich regions of chromosomes (Li et al., 1999a).

However, causal effects of the DR genes are difficult to demonstrate due to the relatively small effects of individual genes and the presence of multiple gene family members that may play different roles in defense (van Loon et al., 2006).

Several lines of evidence suggest that germin-like proteins (GLPs) are involved in general plant defense responses (Lane, 2002; Breen and Bellgard, 2010), including the observation that the expression of certain GLPs is enhanced after infection with pathogens, the feeding of insects, or the application of chemicals, such as salicylic acid, hydrogen peroxide (H2O2), or ethylene (Zhang et al., 1995; Wei et al., 1998; Zhou et al., 1998; Federico et al., 2006; Lou and Baldwin, 2006; Zimmermann et al., 2006; Godfrey et al., 2007). Transient overexpression of certain barley GLP subfamilies resulted in enhanced resistance to the powdery mildew fungus, and for some subfamilies, silencing resulted in enhanced susceptibility to the pathogen (Zimmermann et al., 2006).

1.4 Other resistance-related genes

1.4.1 Resistance mediated by the loss-of-function genes

The *MILDEW-RESISTANCE* locus (*Mlo*) is a particular type of resistance gene first described in barley (*Hordeum vulgare* L) (Jorgensen JH, 1992).

Monogenic resistance mediated by recessive (*mlo*) alleles of the *Mlo* locus in barley differs from the above-described receptor gene / *DR*-gene system (Büschges et al., 1997). The wild-type *Mlo* gene is a negative regulator of resistance to powdery mildew in barley. So the presence of the wild-type *Mlo* gene allele modulates succeptible responses. Homozygous *mlo* mutant plants exhibit full broad-spectrum resistance to almost all known isolates of barley powdery mildew, whereas *Mlo* overexpression results in supersusceptibility (Wolter et al., 1993; Kim et al., 2002). The deduced amino acid sequence of MLO protein reveals no homologies to any other described plant resistance gene. The barley MLO protein resides in the plasma membrane, with the N terminus positioned extracellularly and the C terminus intracellularly, and consists of seven membrane-spanning domains (Büschges et al., 1997; Devoto et al., 1999). Two genes, *Ror1* and *Ror2*, which are required for full *mlo*-mediated resistance, have been previously described (Freialdenhoven et al., 1996; Peterhänsel et al., 1997).

Mlo-like genes have been described in the following plant species: tomato, pea, strawberry, pepper, bread wheat, cucumber, rose, tobacco, melon, grapevine, and apple (reviewed by Kusch and Panstruga, 2017). Wheat *Mlo*-like genes were first reported in 2002 and demonstrated to be associated with resistance to *Bgt* (Elliott et al., 2002).

A loss of function mutation in the *A. thaliana enhanced disease resistance 1* (*edr1*) gene resulted in inherited recessive resistance to powdery mildew caused by the fungus *G. olovinomyces* (Frye and Innes, 1998; Tang and Innes, 2002). The *edr1* gene in *A. thaliana* was isolated by positional cloning and found to encode a putative MAP kinase kinase kinase (MAPKKK), a negative regulator of ethylene responses in *A. thaliana* (Frye et al., 2001).

Recently it has been shown that the *mlo* and *edr1* mutants can be generated by genome editing (CRISPR/CAS) or TILLING strategies leading to enhanced powdery mildew resistance in wheat (Wang et al., 2014a; Acevedo-Garcia et al., 2017; Zhang et al., 2017).

1.4.2 Transmembrane proteins

At least two genes for broad spectrum partial resistance in wheat – *Lr34* and *Lr67* – encode transmembrane proteins (Krattinger et al., 2009; Moore et al., 2015).

Cloning of the wheat broad spectrum partial resistance gene *Lr34* (*Lr34/Yr18/Sr56Pm38*) has revealed that the gene encodes a protein with homology to ATP-binding cassette (ABC) transporters, a transkingdom superfamily of transmembrane proteins involved in the transport of a wide variety of substrates across cellular membranes (Krattinger et al., 2009). Sequencing of the genomic region in resistant and susceptible wheat cultivars identified differences which may lead to changes in the conformation of the protein and alter substrate specificity of the transporter (Krattinger et al., 2009).

It was shown that the broad spectrum partial resistance gene *Lr67/Yr46/Sr55/Pm46/Ltn3* encodes a predicted hexose transporter (Moore et al.,

2015) which facilitates the transport of hexoses across the plasma membrane (Büttner, 2010). The mechanism of disease resistance conferred by this transporter remains unknown, but alterations in hexose transport in infected leaves may explain for the influences on the growth of multiple biotrophic pathogen species. Thus, $LR67_{res}$ may cause reduced hexose transport through a dominant-negative interference mechanism by forming inactive heteromultimeric protein complexes (Moore et al., 2015).

1.5 Qualitative and quantitative resistance

Based on the phenotype and considering the mode of inheritance, plant resistance to pathogens was divided into two categories: complete resistance mediated by major *R*-gene(s) and incomplete (partial) resistance based on the additive effects of several genes, each contributing quantitatively to the level of plant defense. The former is qualitative resistance and the latter quantitative resistance (Poland et al., 2009; Clair, 2010). Thus, both phenotypic phenomenon and mode of inheritance are encompassed in the definition.

The 'qualitative' refers to an inheritance that is based on one or two major genes of large effect. The major race-specific genes confer very clear phenotypic effect, producing discrete classes of resistant and susceptible individuals that segregate in population, according to Mendelian principles (Niks et al., 2015). Thus, race-specific genes were the first class of resistance genes to be genetically defined and the molecular basis of the qualitative resistance has been studied widely (Ayliffe and Lagudah, 2004; McHale et al., 2006; Liu et al., 2007). Most molecularly studied plant race specific resistance genes encode receptor proteins involved in host-pathogen recognition.

The quantitative resistance (QR) is defined as a trait of reduced, but not of absent disease based on multiple genes of small effect (Clair, 2010). Phenotypically, in a genetically variable population, quantitative plant-pathogen interactions produce a continuous distribution of phenotypes from susceptible to resistant, so individuals do not group into discrete classes which fit Mendelian segregation ratios (Clair, 2010; Roux et al., 2014; Niks et al., 2015). As a rule, statistical approaches and quantitative trait loci (QTL) analyses are used to dissect the genetic basis of resistance phenotype (Young, 1996; Clair, 2010). A QTL can be defined as a statistically significant association between allelic variation at a marker-genotype locus and a phenotypic trait that exhibits continuous variation in a genetically segregating or variable population (Doerge, 2002; Mackay et al., 2009; Clair, 2010). Separate QR-genes detected as QTL participating in the joint action can be mendelized, and their effects can be scored as effects of a single gene.

The QR is also known as a horizontal or field resistance (Nelson, 1978), adult plant resistance (APR, Gustafson and Shaner, 1982), slow mildewing (Roberts and Caldwell, 1970) or partial resistance (Hautea et al., 1987). Terms as an incomplete, minor-gene, broadspectrum or basal resistance are sometimes used as synonyms of QR (Niks et al., 2015). Different terms sometimes add an element of confusion as the phenotypic phenomenon often prevails without a known genetic basis of the phenomen (Niks et al., 2015). However, resistance that is quantitative, according to its phenotypic nature, may have a qualitative inheritance – the genes for race-specific resistance may produce partial disease reactions in the field (Cunfer, 2002), or varying levels of

resistance within a host population may result from the effects of a single gene with low heritability or low penetrance (Clair, 2010).

The genetic and molecular basis of the QR is less understood than that of the qualitative resistance, this is, in part, due to small effects of multiple QTL and, also, due to the difficulties arising from inconsistent phenotyping across different environments (Salvi and Tuberosa, 2005). To make genetic analysis of a QR phenotype even more complicated, genes underlying QTL may interact with each other (epistatic interaction) and/or with the environment. Sometimes the variation for a trait among plants of a population is more due to variation in non-genetic factors, such as a small variation in inoculum deposition, than to variation in genes (Clair, 2010; Niks et al., 2015).

It has been suggested that the same loci could be responsible for qualitative and quantitative traits (Robertson, 1989), but, also, completely different quantitative resistance mechanisms have been proposed (Poland et al., 2009; Roux et al., 2014). Several plausible hypotheses for mechanisms' underlying QR were proposed: i) QR is based on regulation of morphology and developmental traits; ii) QR is based on basal defense or defense signal transduction; iii) weak ETI is triggered by 'defeated' R proteins or the production of components of chemical warfare (review by Poland et al., 2009). Jones and Dangl (2006) suggested that, in absence of R-genes, PTI also confers a weak immune response that attenuates pathogen growth and contributes to basal defense. It has been proposed that poor suppression of PAMP-triggered defense by effectors, i.e. basal resistance, forms the base for QR for resistance to biotrophic pathogens (Niks et al., 2015).

Disease resistance QTL to several important rice diseases, such as bacterial blight, sheath blight, and rice blast were shown to co-localise with candidate *DR* genes (Ramalingam et al., 2003; Liu et al., 2004; Wu et al., 2004). The hypothesis that partial resistance is governed by defense genes is supported by recent experiments showing a successful use of defense gene-derived markers to improve selection for quantitative resistance to blast in rice (Liu et al., 2004; Wu et al., 2004). Moreover, in several instances, QTL for QR against pathogens has been shown to be associated with constitutively expressed pathogenesis-related genes or the products of so-called SAR genes associated with resistance reactions to pathogens (Pflieger et al., 2001; Liu et al., 2004; van Loon et al., 2006). Large-scale genetic mapping revealed that functional groups of barley genes involved in secretory processes and cell-wall reinforcement were significantly over-represented within QTL for resistance to powdery mildew (Schweizer and Stein, 2011).

R-gene loci frequently co-localise with resistance QTL. Kang and coworkers (2012) observed that about 63% of the disease-resistance QTL were located in the 2-Mb regions that flank the NBS-LRR genes. There are numerous markers that correspond to NBS-LRR genes positioned under individual QTL or meta QTL (MQTL) for powdery mildew resistance (Marone et al., 2013) and there are even examples of race-specific NB-LRR-genes that were identified in QTL studies (Paillard et al., 2012; Zhang et al., 2015). A few studies support the idea that QR may be contributed by defeated race-specific genes or by *R*-genes that have residual effects against virulent pathogens (Li et al., 1999b). Recently it was shown that *Lr22a*, which confers broad-spectrum APR to leaf rust, encodes an NB-LRR-like protein (Feuillet et al., 2003; Thind et al., 2017), as does the rice NB-LRR family *Pb1* gene (Hayashi et al., 2010). In some cases, these are altered or 'weak' forms of the genes (Li et al., 1999; Fukuoka et al., 2009). The rice – *Magnaporthe oryzae* pathosystem demonstrates that allelic differences at the same

locus can be responsible for two different types of resistance phenomena and shows the close linkage of gene-for-gene and quantitative disease resistance. The difference between race-specific qualitative resistance and QTL-mediated resistance was due to differences in two alleles at the same *Pi35* locus (Fukuoka et al., 2014).

A strategy of validation and functional analysis of the candidate genes for QTL has been established to characterize minor resistance QTL in rice (Hu et al., 2008a). The strategy is developed based on candidate gene hypothesis which has been used to predict the genes underlying QTL in crops (Faris et al., 1999; Liu et al., 2004; Ramalingam et al., 2003). Using this approach, ten genes (*Nrr, Wrky13, Wrky45, Gh3-1, Gh3-2, Gh3-8, pi21, Osdr8, Mpk6,* and *Pb1*) and one gene family cluster (*Glp*-s) contributing to resistance QTL have been reported for rice (Fu et al., 2011; Fukuoka et al., 2009; Hayashi et al., 2010; Hu et al., 2008a; Kou et al., 2010; Manosalva et al., 2009). Among the eleven characterised genes affecting quantitative resistance to different pathogens in rice, at least nine (*Wrky13, Wrky45, Gh3-1, Gh3-2, Gh3-8, Nrr, Osdr8, Mpk6*, and *Glp-s*) belong to the defense-responsive gene class. Functioning as a positive or negative regulator, each of these genes paticipates in defense response (reviewed by Kou and Wang, 2012).

Several genes that underlie disease QTL in different plant–pathogen systems have been identified in recent years, including *Lr34* (Krattinger et al., 2009), *Lr67* (Moore et al., 2015), *Fhb1* (Rawat et al., 2016), and *Yr36* (Fu et al., 2009) in wheat, *ZmWAK* in maize (Zuo et al., 2015), *Rhg1* in a soybean (Cook et al., 2012), *Rks1* (Huard-Chauveau et al., 2013). The cloned wheat APR genes include a cytoplasmic protein kinase gene – *Yr36* (Fu et al., 2009), an ABC transporter gene – *Lr34,* (Krattinger et al., 2009), a non-ABC transporter – *Lr67*,, (Moore et al., 2015). *Fhb1* is a new type of plant durable resistance genes conferring QR against *Fusarium* species in wheat. It has been shown that a pore-forming toxin-like (*PFT*) gene at *Fhb1* locus confers FHB resistance. *PFT* was predicted to encode a chimeric lectin with two agglutinin domains and an ETX/MTX2 toxin domain (Ravat et al., 2016). These genes, and the mechanisms by which they confer resistance, differ from previously cloned R-genes and from each other, suggesting that mechanisms involved in controlling QR are more diverse than those controlling qualitative resistance (Niks et al., 2015; French et al., 2016; Krattinger and Keller, 2016). An important feature of the cloned genes is that their phenotypic effects are sufficiently large for fine genetic mapping and detection of loss-of-function mutants.

A few findings support the emerging concept that a QTL may not necessarily resolve to a single locus but instead may be controlled by several contiguous loci. As was found by cloning a soybean gene *Rhg1* for resistance to a cyst nematode (*Heterodera glycines*), quantitative resistance may be based on the copy number of a set of three different genetically linked genes: the genes encode an amino acid transporter, an α-SNAP protein, and a protein with a WI12 (wound-inducible protein 12) region (Cook et al., 2012).

Resistance-related Kinase 1 (*Rks1*), a gene conferring broad-spectrum resistance to *Xanthomonas campestris* encodes an atypical kinase that mediates a quantitative resistance mechanism in plants by restricting bacterial spread from the infection site. Analysis of *Rks1* expression in multiple Arabidopsis accessions showed that resistance was associated with a higher level of *Rks1* expression, suggesting that regulation of expression is the major component of quantitative resistance to *Xanthomonas campestris* (Huard-Chauveau et al., 2013). In addition, expression levels of genes of the

Germin-Like Protein (Glp) family may underlie QR to both rice blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctonia solani*) in rice (Manosalva et al., 2009).

Thus not only defense-responsive genes, receptor genes, genes encoding transporters or altered NBS-LRRs, but also their copy number variations and different levels of expression appear to be contributors to quantitative resistance (Kou and Wang, 2012; Niks et al., 2015; French et al., 2016).

Qualitative resistance is often preferred in breeding programmes because of the large phenotypic effect, which aids accurate and fast selection of resistant progeny. The major limitation of race-specific resistance is its lack of durability: *R-gene*s are often rapidly overcome by new races carrying mutated *Avr* genes. The breakdown of the *R*-gene efficacy leads to the so-called 'boom and bust cycles' of plant–pathogen interactions under field conditions (McDonald and Linde, 2002).

Today breeding of wheat cultivars with partial resistance has been suggested as a promising and sustainable strategy to control the disease (Gupta et al., 2008; Krattinger and Keller, 2015), and some cases of MAS for quantitative resistance have been already reported in the literature (Anderson et al., 2007; Asea et al., 2012; Bai et al., 2012; Miedaner and Korzun, 2012). Due to a broader specificity and action of multiple loci, evolutionary pressure on pathogens is significantly decreased and QR tends to be more durable than race-specific resistance (Poland et al., 2009; Clair, 2010). Recent studies have shown that the cumulative effect of pyramiding many QTL loci can result in a high level of resistance (Fukuoka et al., 2015; Das and Rao, 2015; Yasuda et al., 2015; Ellur et al., 2016). Another way to achieve durable resistance is to pyramide in a cultivar different genes, including genes for race-specific resistance and genes with a minor or partial effect to prolong the period before a breakdown in the resistance is observed in the field (Liu et al., 2000; Singh et al., 2001; Zhao et al., 2003). Furthermore, in some cases genes for partial resistance may effectively enhance the resistance phenotypes of the race-specific genes, as was found for the partial resistance genes *Sr2* and *Lr34* (German and Kolmer, 1992; Vanegas et al.,2008).

1.6 Wheat – powdery mildew pathosystem

1.6.1 Powdery mildew of wheat

Cereal powdery mildew – *Blumeria graminis* – is an ascomycete fungal species evolved into at least eight *formae speciales* each specifically infecting one host species (Inuma et al., 2007). *Blumeria graminis* f.sp*. tritici* (hereafter called *Bgt*) is the causal agent of powdery mildew on common wheat (*Triticum aestivum* L.). However, the modern *Bgt* isolates still maintain their ability to infect wild tetraploid wheat, even though globally their main host is hexaploid wheat (Wicker et al., 2013). Powdery mildew occurs almost everywhere that wheat is grown. The fungus can infect plants at any time, from the first leaf stage until senescence. Spores of the fungus germinate on the leaf surface. Powdery mildew produces greyish, cottony colonies, mostly on the upper leaf surface, although some pustules may develop on the underside of the leaf or on a stem, heads and awns (Glawe, 2008; Mwale et al., 2014).

Heavily infected plant seedlings gradually become yellow and dry, losing the capability of photosynthesis. Infection of seedlings causes a decrease in the number and survival of tillers, while infection of the penultimate and flag leaves affects the number of kernels per head, and kernel weight and can cause a yield reduction (Leath and

Bowen, 1989; Everts and Leath, 1992; Conner et al., 2003). The loss can be as severe as 50% if the flag leaf becomes severely diseased during the stages of heading and filling (Griffey et al., 1993).

The *Bgt* is an obligate biotroph that feeds exclusively on living epidermal cells of wheat. Wheat-*Bgt* interaction is characterised by the establishment of structures inside epidermal plant cells called haustoria, which allow the pathogen to take up nutrients from the host (reviewed by Glawe, 2008). According to the genome analysis of barley powdery mildew, *Blumeria graminis* f. sp. *hordei*, genes coding for enzymes of primary and secondary metabolism, carbohydrate-active enzymes and transporters are absent from the fungal genome, indicating an exclusively biotrophic lifestyle (Spanu et al., 2010).

The *Bgt* reproduces asexually by wind-dispersed conidia (haploid spores) or sexually by ascospores. The asexual lifecycle takes 7-10 days under optimal conditions, and this rapid succession leads to a fast spread of the disease (Glawe, 2008). Cool, humid weather, with temperatures between 15 °C - 20 °C, form a favorable environment for fungal invasion, but infection is also possible between 5 °C and 30 °C. In the late summer, after the crop has reached its maturity, sexual reproduction of isolates of opposite mating types results in the formation of overwintering chasmothecia. Ascospores in chasmothecia serve as survival structures, but, in most environments, their role in initiating the disease is much less important than that of the conidia. Conidia produced on grasses and volunteer wheat also maintain inoculum until wheat is planted. In autumn-sown wheat, infections that do not result in visible symptoms can maintain the fungus on leaves through the winter (Frank and Ayers, 1986).

Bgt possesses an expanded genome of at least 174 Mb, much larger than is commonly observed for fungal genomes (Gregory et al., 2007). About 6,540 genes were annotated in the *Bgt* genome and over 85-90% of the genome was classified as transposable element sequences, making it the most repetitive fungal genome sequenced so far (Parlange et al., 2011; Wicker et al., 2013). *Bgt* populations evolve quickly, showing a high rate of variation. Multiple races of the fungus exist at the same time and new ones continue to be produced as a result of mutations as well as genetic recombination due to sexual reproduction. Transposable elements can generate high levels of genetic variation, independent of sexual recombination, and contribute to genome flexibility responsible for rapid adaptation of fungal populations to environmental conditions (Parlange et al., 2011).

The ability for fast adaptation of *Bgt* is demonstrated by its recent host range expansion to the hybrid cereal triticale. In several European countries, a 'new' powdery mildew on triticale has emerged through a host range expansion of wheat powdery mildew during the last ten years (Troch et al., 2013).

1.6.2 Race-specific resistance to powdery mildew in wheat

In terms of disease phenotype, resistance to the infection of powdery mildew in wheat may be race-specific or non-race-specific (Hsam and Zeller, 2002; Huang and Röder, 2004; Cowger et al., 2012). Race-specific resistance is a complete resistance that is effective only to a specific set of powdery mildew isolates. Usually, this type of resistance is associated with a rapid hypersensitive response. According to a simple gene-for-gene hypothesis (Flor, 1971), this type of plant-pathogen interaction is based on the race-specific interaction of the resistance gene (R-gene) product in wheat and the avirulence (*Avr*) gene product in the infecting *Bgt* isolate. However, new data

suggest that the control of avirulence in *Bgt* is more complex than a canonical gene-for-gene interaction model suggests. Genetic analysis and functional studies have identified several *Bgt* genes involved in the determination of pathogen avirulence against the *Pm3* allelic series in wheat (Bourras et al., 2015; Bourras et al., 2016). A new model proposed for race-specificity of the *Pm3* gene involves at least three components: a resistance gene (*R*-gene) allele in wheat, an allele-specific avirulence effector (*Avr*) in patogen and a pathogen-encoded suppressor of avirulence (*Svr)* (Bourras et al., 2015; 2016). The final outcome will depend on the combination of avirulence genes and avirulence gene suppressors in the pathogen. Resistance is mediated only by an *Avr/R/svr* combination where the resistance gene recognizes its cognate avirulence effector in absence of the suppressor. In absence of the cognate *Avr* or in presence of the *Svr* suppressor, the interaction results in susceptibility.

Race-specific resistance is typically tested at the seedling stage of the host plant, but it is expected that resistance is expressed throughout the lifecycle of the host (Hsam and Zeller, 2002; Huang and Röder, 2004). Estimations of the effectiveness of major powdery mildew resistance genes at the adult plant stage are usually obtained from field observations of donor lines, and genetic analysis as the basis for assessing their effects under field conditions is not the rule (Mohler et al., 2011). The true portion of seedling resistance genes contribution to field resistance is yet to be determined. However, the resistant *Pm3a* allele in line 2174 plays a critical role in the resistance expressed not only in seedlings but also in adult plants (Chen et al., 2009). Wheat resistance genes *Pm50*, *Pm53, Pm54* and *Pm58* were at first detected as major QTL in the field trials and later were designated as *Pm* genes (Mohler et al., 2013; Hao et al., 2015; Petersen et al., 2015; Wiersma et al., 2017).

1.6.3 Partial or quantitative resistance to powdery mildew in wheat

Some wheat cultivars/landraces are resistant to a broad spectrum of powdery mildew isolates at the adult stage and have, for years, maintained their resistance to infection while in commercial cultivation. Providing effective resistance against powdery mildew in the south-eastern United States for half a century, winter wheat cultivar Knox, and its derivative cv. Massey are the best-known examples (Griffey and Das, 1994; Liu et al., 2001). Chinese wheat landrace Pingyuan 50 has shown adult-plant resistance to powdery mildew in the field for over 60 years (Asad et al., 2014). Resistance to powdery mildew in line 2174 has remained effective since its release in 1997 (Chen et al., 2009). Wheats with adult plant resistance (APR) to powdery mildew generally exhibit low to intermediate resistance at the seedling stage (Gustafson and Shaner, 1982). The APR resistance tends to be non-race-specific and is usually not associated with a rapid hypersensitive response. Usually APR resistance is not complete, i.e. to a small extent, all stages of the infection process including sporulation, may be found in plants. Early in the season, pustules may develop on the lower leaves of resistant cultivars, but not on upper leaves later in the season. The frequency of infection, the number of spores that successfully infect the plant, the size of pustules and the production of spores are reduced (Cunfer, 2002).

It has been possible to dissect the genetic basis of the partial resistance or APR to *Bgt* in a number of wheat genotypes (Keller et al., 1999; Chantret et al., 2000; Chantret et al., 2001; Tucker et al., 2007; Lillemo et al., 2008; Muranty et al., 2008; Lan et al., 2009; 2010; Asad et al., 2012; 2013; 2014). The number of QTL for *Bgt* resistance detected in mapping populations at the adult stage of plant growth is extremely

variable, from one in cv. Atlantis / cv. Cortez mapping population (Mohler et al., 2011) to 18 in cv. Forno / cv. Oberkülmer mapping population (Keller et al., 1999). Mapping of quantitative resistance loci typically yields multiple QTL of small to moderate effect and the additive effects of the detected QTL prevail in the inheritance of adult plant powdery mildew resistance phenotype.

As wheat race-specific genes in the background can mask the effect of non-race-specific resistance, the presence of R-genes should be tested at first, if identification of genes responsible for true partial resistance is planned (Keller et al., 1999; Wang et al., 2005; Lillemo et al., 2010).

1.7 Powdery mildew resistance genes and QTL detected in wheat

All detected powdery mildew resistance genes in wheat were formally named '*Pm*-genes' (Powdery mildew) and, to date, more than 62 designated *Pm* genes (*Pm1–Pm62*, *Pm18 = Pm1c, Pm22 = Pm1e, Pm23 = Pm4c, Pm31 = Pm21*) and about 60 temporarily named loci/alleles have been detected in wheat and assigned to specific chromosomes (McIntosh et al, 2011, 2012, 2013, 2014, 2017; Li et al., 2017; Liu et al., 2017; Wiersma et al., 2017; Tan et al., 2018; Sun et al., 2018; Zhang et al., 2018; Zou et al., 2018).

Some wheat *Pm* genes occur as multiple allele series in a chromosome locus, with each member conferring specific resistance against a subset of pathogen races. Multiallelic genes include *Pm1* (*Pm1a–Pm1e*) on chromosome 7A (Hsam et al., 1998; Singrün et al., 2003), *Pm2* (*Pm2a–Pm2c*) on chromosome 5D (Briggle 1969; McIntosh and Baker, 1970; Ma et al., 2015; Xu et al., 2015), *Pm3* (*Pm3a–Pm3t*) on chromosome 1A (Srichumpa et al., 2005; Yahiaoui et al., 2006; Bhullar et al., 2010), *Pm4* (*Pm4a–Pm4e*) on chromosome 2A (Singrün et al., 2003; Hao et al., 2008; Schmolke et al., 2012; Li et al., 2017), *Pm5* (*Pm5a–Pm5e*) on chromosome 7B (Hsam et al., 2001; Huang et al., 2000a, 2003) and *Pm24* (*Pm24a–Pm24b*) on chromosome 1D (Huang et al., 2000b; Xue et al., 2012).

Most of the known powdery mildew resistance genes are dominant race-specific genes, although some recessive race-specific genes in wheat have been identified. With the exception of *PmTm4* (Hu et al., 2008a), all powdery mildew resistance genes which were detected in the distal bin on the long arm of the chromosome 7B are recessive. The cluster of the recessive genes includes *Pm5* (5 alleles detected, Hsam et al., 2001; Huang et al., 2003) and four temporarily named genes *PmH* (Zhou et al., 2005), *mljy, mlsy* (Huang et al., 2002), *mlxbd* (Huang et al., 2000a; Xue et al., 2009), which are closely linked to *Pm5*. Two recessive genes – *Pm9* and *mlRD30* – were detected on the long arm of the chromosome 7AL close to the dominant *Pm1* locus (Schneider et al., 1991; Singrün et al., 2004). Other examples of recessive genes include recessive *PmLK906* and *pmX*, both located on chromosome 2AL close to the dominant multiallelic *Pm4* (Niu et al., 2010; Fu et al., 2013), *Pm26* and *Pm42* on chromosome 2BS (Rong et al., 2000; Hua et al., 2009), *MlHubel* and *PmSE5785* on chromosome 2D (Peng et al., 2014; Wang et al., 2016), *pm2026* on chromosome $5A^{m}L$ (Xu et al., 2008), *PmY212* on chromosome 5DL (Sun et al., 2006), *pmCH89* on chromosome arm 4BL (Hou, et al., 2015), *Pm47* (*PmHYLZ)* on the short art of chromosome 7B (Xiao et al., 2013) and *Pm61* on chromosome arm 4AL (Sun et al., 2018).

Three wheat *Pm* genes, namely *Pm38* – on chromosome 7DS (Lillemo et al., 2008), *Pm39* – on chromosome 1BL (Lillemo et al., 2008) and *Pm 46* – on chromosome 4DL (Herrera-Fossel et al., 2014) confer partial resistance to powdery mildew in the adult plant stage. These broad-spectrum genes not only provide resistance to *Bgt*, but resistance to all races of leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis* f. sp. *tritici*), and stem rust (*Puccinia graminis* f. sp. *tritici*) (Lillemo et al., 2008 Herrera-Fossel et al., 2014).

Over the past two decades, successful applications of quantitative-genetic methodology have facilitated identification of numerous QTL conferring quantitative resistance to powdery mildew. To date, more than 100 quantitative trait loci (QTL) have been reported in different wheat cultivars and lines, including the Swiss winter wheat cv. Forno (Keller et al., 2009), French winter wheat lines RE714, Festin, Courtot, and RE9001 (Chantret et al., 2000, 2001; Mingeot et al., 2002; Bougot et al., 2006), North American winter wheats Massey and USG3209 (Liu et al., 2001; Tucker et al., 2007), Japanese wheat cultivar Fukuho-komugi and Israeli wheat cultivar Oligoculm (Liang et al., 2006), CIMMYT wheat lines Opata 85, W7984 (Börner et al., 2002) and Saar (Lillemo et al., 2008), Australian wheat cultivar Avocet (Lillemo et al., 2008), Chinese wheat cultivars Bainong 64 (Lan et al., 2009), Lumai 21 (Lan et al., 2010) and landrace Pingyuan 50 (Asad et al., 2014), German spring wheat cv. 'Naxos (Lu et al., 2012) and Italian cv. Strampelli (Asad et al., 2013). The QTL for powdery mildew resistance have been mapped on all wheat chromomes and some of the detected QTL for adult plant resistance have been mapped in the genome regions where major *Pm* gene/s was/were previously detected (e.g. *Pm3*, Liang et al., 2006; Xu et al., 2006; *Pm4b*, Mingeot et al., 2002 and *Pm5*, Keller et al., 1999; Marone et al., 2013). A meta-analysis of resistance to powdery mildew QTL yielded 24 regions on 18 wheat chromosomes which were found to be common among at least two independent studies (Marone et al., 2013; Lillemo and Lu, 2015).

1.8 Powdery mildew resistance genes and QTL cloned/sequenced in wheat

There is a significant gap between the number of genetically detected wheat resistance genes/QTL and the number of cloned or sequenced genes. Due to the large size of wheat genome (17 Gb), its allohexaploid nature (AABBDD), and high content of repetitive DNA (more than 85%; IWGSC, 2018), fine mapping and map-based cloning are laborious in common wheat (Gupta et al., 2008; Shewry, 2009; Uauy, 2017). Using different approaches, five dominant *Bgt* race-specific wheat resistance genes – *Pm2, Pm3, Pm8, Pm21* , *Pm60* - , two genes for partial resistance - *Pm38* and *Pm46* – and *TaMlo* have been cloned or sequenced (Yahiaoui et al., 2004; Krattinger et al., 2009; Hurni et al., 2013; Moore et al., 2015; Sanchez-Martin et al., 2016; Xing et al., 2017; He et al., 2018; Zou et al., 2018; Yu et al., 2005). All cloned race-specific *Pm* genes belong to the NBS-LRR resistance gene family and encode a typical intracellular coiled-coil nucleotide-binding site and leucine-rich repeat domain protein (Yahiaoui et al., 2004; Hurni et al., 2013; Sanchez-Martin et al., 2016; Xing et al., 2017; Zou et al., 2018).

Pm3 is the best characterised race-specific powdery mildew resistance gene. The *Pm3b* was identified by map-based cloning as a member of a cluster of genes encoding CC-NBS-LRR proteins (Yahiaoui et al., 2004). Seventeen functional alleles of the *Pm3*

sharing high sequence identity (> 97%) have been found (Srichumpa et al., 2005; Yahiaoui et al., 2006; Bhullar et al., 2009; Brunner et al., 2010). Resistance specificity of *Pm3* is based on change of one or a few amino acids. Change of a single amino acid converts the susceptible Pm3CS protein into the protein of the resistant phenotype. Thus, the recognition specificity of *Pm3* is controlled by a very limited amount of amino acids in the LRR domain (Yahiaoui et al., 2004; Yahiaoui et al., 2006).

Originating from rye (*Secale cereale* L.) ***Pm8*** gene is an ortholog of *Pm3* (Hurni et al., 2013). A BLAST search revealed that *Pm8* is similar to the *Pm3* alleles showing 87% identity at the DNA level and 80% at the protein level to the *Pm3* allele *Pm3CS*. The product of the *Pm3* was identified as a dominant suppressor of *Pm8* and the suppression most likely involves post-translational processes such as the formation of non-functional protein complexes (Hurni et al., 2014).

Sequence comparison of multiple flow-sorted independently derived mutant chromosomes (MutChromSeq) of six mutants has been used to analyse the ***Pm2*** gene (Sánchez-Martín et al., 2016). The approach unambiguously identified a NB-LRR type gene encoding domains with homology to a coiled coil, a nucleotide binding site and leucine-rich repeats on chromosome 5D as a single candidate gene that was verified by sequencing additional mutants.

Pm60, a powdery mildew resistance gene derived from *T. urartu*, was cloned by combining genetic mapping and RNA-sequencing. Two linked genes of 70% similarity encoding NB-containing proteins were mapped in the resistance locus. Virus-induced gene silencing, single-cell transient expression and stable transformation assays demonstrated that one of the two genes - designated *Pm60* - confers resistance to powdery mildew (Zou et al., 2018). However, yeast two-hybrid, bimolecular fluorescence complementation and luciferase complementation imaging assays showed that *Pm60* protein interacts with its neighboring NB-containing protein, suggesting that they might be functionally related (Zou et al., 2018).

The ***Pm21***, transferred from the wild *Haynaldia villosa*, confers durable broad spectrum resistance to *Bgt* throughout all stages of plant growth (Chen et al., 1995). Resently, two groups have reported cloning *Pm21* as a gene encoding a typical CC-NBS-LRR protein of 909 amino acids named *DvRga2* (Xing et al., 2017 and *NLR1-V (*He et al., 2018). The genes encode identical proteins with minor indel polymorphisms in the second intron. Thus He et al., (2018) speculate that *DvRga2* and *NLR1-V* may be allelic.

Distinct of the above mentioned proteins structures were revealed when two wheat genes - ***Pm38*** and ***Pm46*** - for partial resistance to *Bgt* were cloned, suggesting that the formally designed *Pm* genes, likely comprise a heterogeneous group of functionally diverse genes.

Both *Pm38* (synonym *Lr34/Yr18/Sr57/Pm38*) and *Pm46* (synonym *Lr67/Yr46/Sr55/Pm46 Ltn3*) are genes for resistance with pleiotropic effect. *Pm38* and *Pm46* have been cloned as leaf rust genes *Lr34* and *Lr67,* respectively (Krattinger et al., 2009; Moore et al., 2015) and both encode transmembrane proteins.

The ***Pm38*** encodes a protein with homology to ATP-binding cassette (ABC) transporters, a transkingdom superfamily of transmembrane proteins involved in the transport across cellular membranes of a wide variety of substrates (Krattinger et al., 2009). The ***Pm46*** gene encodes a predicted hexose transporter (Moore et al, 2015) which facilitates the transport of hexoses across the plasma membrane (Büttner 2010). It has been suggested that small sequence differences between the resistant and susceptible alleles may lead to changes in the conformation of the protein and alter

substrate specificity of the transporter. In both cases – *Pm38 and Pm46* – the mechanism of disease resistance conferred by these transporters remains unknown (Krattinger et al., 2009; Moore et al., 2015).

Wheat *Mlo*-like genes were first reported in 2002 and demonstrated to be associated with resistance to *Bgt* (Elliott et al., 2002). TaMLO protein is a membrane-bound protein and its molecular weight is about 60 kD (Büschges et al., 1997; Yu et al., 2005). The full-length cDNA of wheat ***TaMlo*** gene is 89% identical to barley *Mlo*, and the main domains of barley and wheat *Mlo* are highly conserved (Yu et al., 2005). *TaMlo* was localised on 2AL, 2BL and 2DL wheat chromosomes (Chen et al., 1995).

A full-length cDNA sequence of *Edr1*-like gene in wheat, ***TaEdr1,*** has been cloned (Niu et al., 2005). High similarity in structure of *TaEdr1*, *Edr1* and *HvEdr1* allowed to suggest that an *Edr1* pathway is functioning in wheat, and the pathway might play a role in regulation of defense responses (Tang et al., 2005; Xiao et al., 2005). The expression of *TaEdr1* was enhanced by *Bgt* infection in leaves, stems, spikes, and in roots. (Niu et al., 2005).

It has been demonstrated that *TaLrk* (*T. aestivum* leaf rust kinase)*, Ta-Ja2* (*T. aestivum jasmonate*-regulated protein gene)*, TaPr-1* (*T. aestivum pathogenesis related* protein)*, TaPr-2* and *TaPr-5* proteins play a positive role in powdery mildew resistance in wheat. However, neither map positions nor systemic signal pathways of powdery mildew resistance have been reported in wheat genome as yet (Niu et al., 2009).

1.9 Wide crosses for the improvement of wheat powdery mildew resistance

For more than 60 years, the search for new resistance genes in progenies of distant hybridization events has been an effective way to increase diversity of resistance to pathogens, *inter alia*, to the causal agent of powdery mildew in wheat (Pridham, 1939; Shands, 1941; Allard, 1949; Mujeeb-Kazi and Kimber, 1985; Warburton et al., 2006; Haijar and Hodgkin, 2007; Gill et al., 2008; Feuillet et al., 2008; Kaur et al., 2018). More than half of the registered major powdery mildew resistance genes (*Pm* genes) listed in the wheat gene catalogue (McIntosh et al., 2008-2017), are derived by wide hybridization from related species belonging to the primary (such as *T. monococcum, T. turgidum, Aegilops tauschii*), secondary (*T. timopheevii, T. araraticum*, *T. carthlicum*, *Aegilops speltoides, Aegilops longissima*) or tertiary gene pools (*Ae. ovata, Ae. umbellulata, Secale cereale, Haynaldia villosa* (syn*. Dasypyrum villosum), Elytrigia intermedium, Thinopyrum ponticum*) (McIntosh et al., 2008-2017; Alam et al., 2011; Shah et al., 2018).

The Triticeae tribe contains more than 500 species (Feuillet et al., 2008; http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi,), and on the basis of genomic constitution, wild relatives of common wheat can be classified into primary, secondary and tertiary gene pools (Feuillet et al., 2008). Hexaploid landraces (AABBDD), cultivated and wild tetraploids with the AABB genome (*T. turgidum, T. dicoccum* and *T. dicoccoides*) and diploid species with the AA *(T. monococcum*, including var. *boeticum* and var. *urartu*) and DD (*Aegilops tauschii*) genomes belong to the primary gene pool species. In general, genetic transfers from the abovementioned genomes can be generated by direct hybridization and homologous recombination.The polyploid *Triticum* and *Aegilops* species – which share at least one homologous genome with

common wheat – such as *T. timopheevii* (A^tA^tGG) and also the diploid S-genome of *Aegilops* species that are closely related to the B genome of wheat (Kilian et al., 2007), form the secondary gene pool. Here, gene transfer also takes place in the process of direct crosses, however, reduced chromosome pairing and suppressed recombination between the non-homologous genomes are common problems for these crosses. The tertiary gene pool contains diploid and polyploid species with genomes not homologous to cultivated wheat genomes. Gene transfer from these species can only be achieved by special techniques that assist homoeologous exchange, like using the *ph1* allele of common wheat, the facilitation of chromosome breaking and exchange by irradiation, or callus culture mediated induction of translocations (Mujeeb-Kazi and Rajaram, 2002; Qi et al., 2007; Feuillet et al., 2008). Different sophisticated strategies, generally referred to as chromosome engineering (Qi et al., 2007), are used to facilitate the transfer from diploid and tetraploid donors. Intermediary hybrids (synthetic wheats or wheat-alien amphiploids) are first developed, followed by the cytological detection of wheat-alien chromosomal additions, substitutions and translocations (Zeller and Hsam, 1983).

Reduced or supressed recombination may be considered a general drawback in the reduction of the size of introgressions from species within the secondary or tertiary gene pools (Qi et al., 2007; Li et al., 2008). If the introgression originates from species of the secondary or tertiary gene pool, the rate of decline of linkage disequilibrium (LD) between closely linked genes is slow (Summers and Brown, 2013; Feuillet et al., 2008). This means that additional crosses and special methods are needed to select the new resistance gene within a genetic background that meets requirements for both agronomic traits and for resistance to diseases. The delay in the introduction of a useful gene can be described as 'competition drag', characterizing the additional time needed to raise both the yield and quality to the standard of the current market-leading cultivars (Summers and Brown, 2013).

Only a handful of introduced powdery mildew resistance genes, such as *Pm2a*, *Pm4a, Pm4b, Pm6, Pm8* and *Pm21*, are actually used in commercial wheat breeding (Friebe et al., 1996; Olson et al., 2010). Yield reduction – often associated with disease resistance genes reported in wheat and other cereals – is the most common cause of the limited use of transferred resistance genes (Worland et al., 1989; Singh and Huerta-Espino, 1997; Ortelli et al., 1996; Brown, 2002). The reason for yield reduction may lie in the large chromosomal fragments that are transferred, carrying additional genes that confer undesirable traits, such as poor agricultural, yield or quality performances (a phenomenon known as 'linkage drag' (Young and Tanksley, 1989). For example, a large yield penalty is associated with *Pm16*, a gene for resistance to powdery mildew introgressed from wild emmer wheat (*Triticum dicoccoides*) and located on the translocated 5B chromosome (Chen et al., 2005). Advanced breeding lines carrying *Pm16* had on average about 15% lower yield than those without the gene (Summers and Brown, 2013). Up to now it is not clear whether this yield loss is a direct effect of the *Pm16* gene itself or an indirect cost of genes linked to *Pm16*.

However, transfer of a large chromosomal fragment may, in some cases, have a positive effect on the yield. The positive influence of *T. timopheevii* 2G chromosome fragments on number of grains in an ear has been shown (Timonova et al., 2012).

In some cases, chromosomal fragments with desirable genes may be too small to be detected by standard cytological methods (Luo et al., 2009; He et al., 2009; Lu et al., 2016), as for example, in the case of alien introgressions carrying *Pm40* and *Pm43*

powdery mildew resistance genes, both originating from *Thinopyrum intermedium* (Luo et al., 2009; He et al., 2009), and both located by the laborious whole-genome mapping process. Another example is line 'Pubing 74', obtained from distant hybridization between the common wheat cv. Fukuho and *A. cristatum,* displaying a high resistance to powdery mildew, both at the seedling and adult stages. The new powdery mildew resistance gene *PmPb74* was mapped on chromosome 5D, while no cytologically detectable signals were observed by genomic *in situ* hybridization techniques (Lu et al., 2016).

Another factor complicating the use of wild relatives for resistance breeding in bread wheat is the possible suppression of resistance. Lutz et al., (1994) showed that synthetic amphiploids generally express lower levels of resistance to powdery mildew than the respective diploid parental lines. It was proposed that, due to the action of suppressor genes localised in the A and/or B genome(s) of the *T. durum* parents, the *T. tauschii*-derived resistance genes might not be expressed in the genetic background of syntetic hexaploid genotypes (Assefa and Fehrman, 2000). The suppression of resistance to leaf rust, stripe rust and tan spot in the synthetic hexaploids has also been observed (Chevre et al., 1989; Innes and Kerber, 1994; Siedler et al., 1994; Ma et al., 1995). The studied suppressor genes were found to be specific to particular resistance genes and their genetic locations were found differing (Kema et al., 1995; Ma et al., 1995; Assefa and Fehrmann, 2004). For example, suppression of *Pm8*-mediated resistance was detected in some wheat lines with the *Secale cereale*-derived 1BL.1RS translocation (Hanusova et al., 1996; McIntosh et al., 2011) and correlated with the presence of the *Pm3* locus on the wheat chromosome 1AS (McIntosh et al., 2011). Later, it was shown that the absence of the *Pm8* function in certain 1BL.1RS-containing wheat lines is not caused by gene loss or mutation but is based on suppression by the wheat gene *Pm3*, an ortholog of rye *Pm8* (Hurni et al., 2013). This result was confirmed in a transient single-cell expression assay and in transgenic lines with combined *Pm8* and *Pm3* transgenes. An expression analysis revealed that suppression is not a result of gene silencing, and it has been suggested that a post-translational mechanism is involved in the suppression of *Pm8*. The formation of a heteromeric protein complex might result in inefficient or absent signal transmission for the defense reaction (Hurni et al., 2014).

1.10 Wheats of the timopheevii group in common wheat improvement

1.10.1 Wheats of the timopheevii group – a description

With reference to a set of morphological traits, genetic compatibility during hybridization, and natural habitat, timopheevii wheats form a separate group within the tetraploid wheats (2n = 4x = 28). The group includes wild form of the timopheevii wheat known as *Triticum timopheevii* (Zhuk.) Zhuk. *var. araraticum* (Jakubz.) (syn. *T. timopheevii* subsp. *armeniacum)* (Jakubz.) MacKey) (further abbreviated as *T. araraticum*), the domesticated form *Triticum timopheevii* (Zhuk.) Zhuk. *var. timopheevii* (further abbreviated as *T. timopheevii*), and a third form of wheat, discovered by P.M.Zhukovsky in 1950, *Triticum militinae Zhuk. et Migusch.* (further abbreviated as *T. militinae*) (Zhukovsky and Migushova, 1969; Goncharov, 2002).

The wild form of timopheevii wheat, *T. araraticum,* is found throughout the Middle East and surrounding areas (southeastern Turkey, northern Iraq, western Iran, Armenia,

and Azerbaijan) (Dorofeev et al., 1976). The *T. araraticum* is morphologically very similar to Emmer wheat and their areas of distribution overlap in Turkey, Iraq, and Iran (Zohary and Hopf, 2000). However, the two species are genetically isolated (Wagenaar, 1961; Feldman, 1966) and are cytogenetically clearly distinct (Badaeva et al., 1994a, b; Jiang and Gill, 1994; Kilian et al., 2009; Mori et al., 2009; Badaeva et al., 2016).

T. timopheevii is a domesticated form which was cultivated as a population mixed with *T. monococcum* (Chelta-Zanduri population) in western Georgia, where it was discovered by P.M. Zhukovsky in 1922. According to Dekaprelevich (1954), the total area of *T. timopheevii* cultivation did not exceed 300–400 ha in the 1930s. At present, the species is not cultivated anymore, and is maintained *ex situ* in seed banks and special collections (Dorofeev et al., 1979; Knüpffer, 2009).The domesticated form is described by Zhukovsky (1928) as follows: characteristic to the species, the plants have thick pubescence consisting of long white tough hairs on the leaf sheats, a thinner pubescence is found on leaf-blades. Ears are compact (40-45 spikelets /10 cm of spike rachis, broad, pyramidal, flattened, the width considerably exceeds the thickness. Only forms with pubescent ears are known. Ears brittle at maturity; segments of the rachis are pubescent with comparatively thin short hairs. Awns are soft, thin, 4-7 cm in length. Glumes are thin and membraneous. Empty glumes are much shorter than the lower flowering ones (Zhukovsky, 1928).

Both *T. araraticum* and *T. timopheeviii* have non-free-threshing hulled ears. Ecologically, *T. timopheevii* is a species of a cool moist alpine climate, being undemanding for warmth and resistant to excess of humidity. As well as *T. araraticum,* the *T. timopheevii* grain has high bread-making quality (Zhukovsky, 1971; Dorofeev et al., 1976). Even in the northern region of Russia (district Leningrad) the protein content has been found to be 19.4% in *T. timopheevii* and up to 30% in *T. araraticum.*

Although the wild timopheevii wheat, *T. araraticum*, is considered to be the progenitor of the domesticated *T. timopheevii* (Feldman, 1977), the *T. araraticum* has not been found in western Georgia, where the domesticated timopheevii wheat has been grown. Armenia and Azerbaijan are the only parts of Transcaucasia where *T. araraticum* has been found. To clarify history of the domestication of timopheevii wheats, Mori et al. (2009) analysed the molecular variation at 23 microsatellite loci in the chloroplast genome of 57 accessions representing wild timopheevii wheat *(T. araraticum Jakubz.*) and six accessions representing domesticated timopheevii wheat *(T. timopheevii (Zhuk.) Zhuk.)*. None of the *T. araraticum* plastotypes collected in Transcaucasia were closely related to the *T. timopheevii* plastotype. On the other hand, the plastotypes found in northern Syria and southern Turkey showed closer relationships with *T. timopheevii*. These results indicate that the domestication of timopheevii wheat might have occurred not in Transcaucasia, but in a region of southern Turkey and northern Syria, and the already domesticated timopheevii wheat might have been introduced into Georgia (Mori et al., 2009).

Wide intraspecific diversity accompanied by chromosomal aberrations – mainly translocations – has been found in *T. araraticum* (Kawahara and Tanaka, 1981, Badaeva et al., 1994a; 1995a). On the contrary, *T. timopheeviii* is characterised by high karyotype stability with respect to chromosomal aberrations, having significantly lower variability than its wild relative, *T. araraticum*. Nine accessions of *T. timopheevii* have been found to be karyotypically identical according to C-banding (Badaeva et al., 1994b). This high karyotypic stability has been considered to be caused by the relatively young age of the domesticated timopheevii wheat. Supporting the hypothesis of a monophyletic origin

of domesticated timopheevii wheat, Moori and co-authors (2009) have demonstrated a limited cpDNA variation in domesticated timopheevii wheat accessions. However, hybridization patterns of tested in *T. araraticum* DNA probes differs from those found in *T. timopheevii,* as shown for Spelt-1 and Spelt-52 (Salina et al., 2006b; Zoshchuk et al., 2007; Badaeva et al., 2016).

The third member of the timopheevii group of wheats, *T. militinae,* was found among the plants of a *T. timopheevii* collection plot by Zhukovsky in 1950 (Jakubziner, 1969, Zhukovsky and Migushova, 1969). *T. militinae* differs from *T. timopheevii* in a number of morphological characters. The plants are 100-120 cm in height, a little lower than *T. timopheevii* plants. Morphologically, vegetative organs of *T. militinae* resemble those of *T. timopheevii* with the exception of the awn-pointed slightly carinate empty glume which has been considered to be similar to that of *T. persicum* var. *fuliginosum* (Zhukovsky and Migushova, 1969). Ears are dark, short (3.5-4.5 cm), wide and even more compact than than those of *T.timopheevii* (50-70 spikelets /10 cm of spike rachis, Dorofeev, 1976), differing from the latter by naked grains and black awns. *T. militinae* is the only free-threshing tetraploid species in the *Boeoticum* subgenus. The leaves of both *T. militinae* and *T. araraticum* are covered with a sparse, rigid pubescence, different from the specific long ciliate pubescence (rare, but very long trichomes) on *T. timopheevii* leaves.

T. militinae is considered to be a spontaneous mutant of *T. timopheevii* (Dorofeev et al., 1976), but it has also been supposed to originate from an introgressive hybridization between *T. timopheevii* and *T. persicum* (Navruzbekov, 1981) or to be a result of an unknown hybridization of *T. timopheevii* (Gocharov, 2002).

The analysis of meiosis in *T. timopheevii/T. aestivum* and *T.militinae/T. aestivum* hybrids indicates differences between *T. timopheevii* and *T.militinae* genomes resulting in pairing incompatibility of one of the *T. militinae* chromosomes, compared to the affinity of *T. timopheevii /T. aestivum* chromosome sets (Shnaider, 1986; 1988; Järve et al., 2002).

Cytologically, the 1A chromosome of *T. militinae* is slightly longer compared to the same chromosome of *T. timopheevii*, and has therefore a more submetacentrical character (Badaeva et al., 1988). In C-banding pattern, *T. militinae* has been found to be similar to *T. timopheevii*, with the differences in *T. militinae* karyotype not exceeding the intraspecific variability of *T. timopheevii* (Badaeva et al., 1994b). The genomic organization of *T. timopheevii* and *T. militinae* has been compared (Salina et al., 2006a). According to this study, polymorphism between *T. militinae* (accession No K-46007; N.I. Vavilov Institute of Plant Industry) and *T. timopheevii* (K-38555) was approximately at the same level as polymorphism between the two accessions of *T. timopheevii* (*T. timopheevii* var. *timopheevii* and *T. timopheevii* var. *typica*).

1.10.2 Crossability of timopheevii wheats and other wheat species

The timopheevii group species are geneticaly isolated from other wheat species. All three timopheevii wheats cause cytoplasmic male sterility when crossed with *T. aestivum* (Wilson and Ross, 1962; Kobylyanskyi and Fadeeva, 1986; Brown-Guedira et al., 1997). Thus the recipient wheat cultivar (*T. aestivum*) is generally used as the female parent in wide hybridization programmes. F_1 hybrids between common wheat and *T. timopheevii* or *T. militinae* are self-sterile and can be maintained by backcrossing them as females with *T. aestivum*. Self-fertility is usually fully restored after 2-3 backcrosses. A series of subsequent self-pollinations are carried out to stabilize hybrid

forms and recover the original wheat genome. However, persistence of cytological instability and aberrance of morphological traits from these in the parental common wheat cultivar are possible even at BC1F18–BC1F20 generations (Badaeva et al., 1991; Leonova et al., 2002; Gordeeva et al., 2009; Timonova et al., 2013).

Due to the genetic incompatibility and differences in genomic structures, yield of hybrid grains in crosses of *T. aestivum* with *T. timopheevii* and *T. militinae* is extremely low (1-5 % of pollinated florets) (Shands, 1941; Allard and Shands, 1954; Peusha and Shnaider, 1983;-Kobylyanskyi and Fadeeva, 1986).

To facilitate the introgression of desirable genes from related diploid and tetraploid species, the synthetic hexaploid wheat (artificial amphiploids) may be used and forms developed with patricipation of timopheevii wheats have been generated (Friebe et al., 1996; Goncharov et al., 2007; Miko et al.; 2015). The production of wheat amphiploids is often a laborious task, in some cases, even 20 generations were insufficient to obtain cytogenetically stable plant material (reviewed by Goncharov et al., 2007). However, *T. kiharae* accessions have been produced from crosses of *T. timopheevii* (A^tA^tGG) and *T. tauschii* (DD), and these forms have been used for the development of immune wheat lines, characterised by their complex resistance to fungal pathogens (Maystrenko et al., 1996; Motsny et al., 2000 Laikova et al., 2004; Laikova et al., 2013).

1.10.3 Genome analyses and chromosome substitutions in crosses of timopheevii wheats and *T. aestivum*

Tetraploid wheats, emmer (genomic formula AABB) and timopheevii (genomic formula A^tA^tGG) originate from common diploid progenitors - *Aegilops speltoides* and *Triticum urartu*; however, they evolved from two independent hybridization events and represent two evolutionarily divergent lineages of polyploid wheats (Dvořák et al., 1993; Mori et al., 1995; Brown-Guedira et al., 1996; Rodriguez et al., 2000a; Kilian et al., 2007).

From their diploid ancestor *T. urartu,* both lineages inherited a 4AL.5AL reciprocal translocation on chromosomes 4A and $4A^t$ (Naranjo et al., 1987; Jiang and Gill, 1994; 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). There is no evidence suggesting the presence or absence of inversions on chromosome $4A^t$ of the timopheevii wheat. The genome of timopheevi wheats is characterised by four species-specific translocations ($6A^tS$/1GS, 1GS/4GS, 4GS/$4A^tL$, $4A^tL$/$3A^tL$) and paracentric inversion in the long arm of chromosome 6A (Jiang and Gill, 1994; Rodriguezet al., 2000; Salina et al., 2006).

Wheats of the timopheevi group were not involved in the formation of the genome of common wheat (*T. aestivum,* genomic formula AABBDD) and their genomes are only partially homologous to the common wheat genome (Dvořák et al., 1993; Mori et al., 1995; Rodriguez et al., 2000b; Kilian et al., 2007; Dvořák et al., 2017).

Despite absence of full homology, chromosomes of the A^t and G genomes pair with A- and B-genome chromosomes, respectively, in the presence of the *Ph* gene which suppresses homoeologous pairing (Feldman, 1966; Dvořák, 1983; Gill and Chen, 1987). In F1 hybrids from the cross between *T. timopheevii* and *T. aestivum,* chromosomes of B and G genomes conjugated in 30% of the cases, and chromosomes of A and A^t genomes, in 70% of the cases (Feldman, 1966). Thus exchanges occurred predominantly between the homoeologous chromosomes of related genomes (A and

A^t, B and G), however, in some crosses the G/D genome chromosome substitutions were also detected (Badaeva et al., 1991, 2000, Gordeeva et al., 2009; Timonova et al., 2013). Spontaneous substitutions involving all A^t- and G- genome chromosomes have been identified (Brown-Guedira et al., 1996b). with high frequency of introgressions in chromosomes 1A, 2A, 2B, 5A, 5B, and 6B (Badaeva et al., 1991, 2000; Gordeeva et al., 2009). Introgressions in 7A were characterised by medium frequencies, while introgressions in 4A, 3A and 3B were rarely found (Badaeva et al., 1991, 2000; Gordeeva et al., 2009; Timonova et al., 2013). Low substitution frequencies are usually characteristic of the chromosomes modified by the species-specific translocations that arose during the formation and evolution of *T. aestivum* and *T. timopheevii,* probably decreasing their compensation ability (Rodriguez et al., 2000; Badaeva et al., 2000). Considering the differences between chromosomes 4A and $4A^t$ (Jiang and Gill, 1994; Rodriguez et al., 2000), 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).

On the other hand, the pattern and frequency of chromosomal substitutions depends on the genotype of the parental common wheat cultivar (Badaeva et al., 1991; 2000; Brown-Guedira et al., 1996b; Gorgeeva et al., 2009). The effect of the parental wheat genotype on the formation of individual chromosome substitutions may be illustrated by the surprisingly great differences detected in the occurrence and frequency of G/D genome chromosome substitutions in different crosses (Badaeva et al., 1991, 2000).

The data on the karyotypical analysis of chromosome substitutions in *T. aestivum* x *T. militinae* hybrids is limited (Badaeva et al., 2000). The pattern of chromosome substitutions appears similar to that of *T. timopheevii* crosses.

1.10.4 Genetic maps of timopheevii wheats

To explore genetic relationships in the Triticeae and to facilitate transfer of agronomically important genes, genetic maps have been developed for di-, tetra-, and hexaploid wheat species (GrainGenes 2.0; https://wheat.pw.usda.gov/GG3/maps). In contrast to other wheat species, mapping of timopheevii wheats has received relatively little attention. The first full genome linkage maps of timopheevii wheats, based on two mapping populations (*T. timopheevii* var. *timopheevii* × *T. timopheevii* var. *typica* and *T. timopheevii* K-38555 × *T. militinae*) – containing 121 and 103 hexaploid wheat SSR markers, respectively – were reported by Salina and co-authors (2006a). Later, additional markers have been integrated into the maps. Dobrovolskaja and co-authors (2009. 2011) tested 767 genomic SSRs and EST-derived SSR markers of the bread wheat genome and/or S genome of the *Ae. speltoides.* 59.5% of tested markers were amplified in the genomes of four accessions of the timopheevii wheat and 23% of tested markers detected polymorphism (Dobrovolskaa et al., 2009).

Transferability of common wheat markers to *T. timopheevii* was generally better for the A genome-specific markers than for B genome-specific markers. Markers specific for the D genome of *T. aestivum* have been mapped both to A^t and G genomes of *T. timopheevii* (Salina et al., 2006a; Dobrovolskaja et al., 2009). Comparative mapping has shown that most of the S genome SSR markers of the *Ae. speltoides* have been located on the G genome chromosomes of *T. timopheevii* (Dobrovolskaya et al., 2011). The order of common wheat markers involved has generally shown high co-linearity between the chromosomes of *T. timopheevii* and *T. aestivum*. However, discrepancies

between *T. timopheevii* and *T. aestivum* genetic maps were noticed (Salina et al., 2006a; Dobrovolskaya et al., 2009). The *T. timopheevii* maps constructed revealed at least two so far unknown rearrangements on chromosomes $4A^t$ and $6A^t$: an introgression from chromosome 4A into the short arm of $6A^t$, and an inversion of the major part of the long arm of chromosome $6A^t$ (Salina et al., 2006a; Dobrovolskaya et al., 2009).

In attempts to map more precisely *T. timopheevii*-derived resistance genes and to find closely linked markers for MAS, partial genetic linkage maps for regions introgressed from chromosome 2G (Brown-Guedira et al., 2003; Tsilo et al., 2008; Wu et al., 2009), 4G (Zhang et al., 2012), $2A^t$ and 5G (Leonova et al., 2008), $5A^t$ (Malihipour et al., 2017), 6G (Järve et al., 2000), $6A^t$ (Leonova, 2015) and $7A^t$ (Perugini et al., 2008; Maxwell et al., 2009) have been constructed. Partial genetic linkage maps for regions of a chromosome arm $3A^tS$ were created in an attempt to locate a wedge-type (W-type) spikelet disarticulation gene in *T. timopheevii* (Li and Gill, 2006).

Suppression of recombination may be the determinant obstacle in mapping translocated *T. timopheevii* chromosome segments in crosses with hexaploid wheat (Devos et al., 1993; Järve et al., 2000; Kammholz et al., 2001; Tsilo et al., 2008). For example, depending on the mapping population, genetic distance between two stem rust resistance genes (*Sr36* and *Sr40)* on chromosome 2G in *T. timopheevii* accessions has been estimated as 21.9 cM (Dyck, 1992) and 3 cM, respectively (Tsilo et al., 2008).

1.10.5 Genes for resistance to fungal diseases transferred from wheats of the timopheevii group

Since the middle of the last century, genes conferring resistance to different pathogenes have been successfully transferred from timopheevii wheats to bread wheat via sexual hybridization (Pridham, 1939; Shands, 1941; Allard, 1949; Allard and Shands, 1954; Nyquist, 1957, 1963; Atkins, 1967; Maan and McCracken, 1969; McIntosh and Gyarfas, 1971). The domesticated *T. timopheevii* is considered to have a complex resistance to powdery mildew, stem, leaf and yellow rusts, *Fusarium* head blight, dwarf and loose smut (Dorofeev et al., 1976). Genes conferring resistance to stem rust (*Sr36* and *Sr37*), leaf rust (*Lr18*, *LrTt1* and *LrTt2*) and resistance to powdery mildew (*Pm6* and *Pm27*) were transferred from domesticated *T. timopheevii* (McIntosh et al., 2008; 2011; http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp).

Incorporation of of two *T. timopheevii*-derived resistance genes, ***Sr36*** and ***Pm6,*** into common wheat was a result of recombination between related 2B and 2G chromosomes (Allard and Shands, 1954; Nyquist, 1957; 1963; Gyarfas, 1978; Jorgensen and Jensen, 1973). The stem rust resistance gene *Sr*36 was transferred to the short arm of the wheat chromosome 2B (Allard and Shands, 1954; Nyquist, 1957; Gyarfas, 1978), with powdery mildew resistance gene *Pm6* linked to the stem rust resistance gene. However, genetic analysis has shown that *Pm6* is located on the long arm of the common wheat chromosome 2B (Nyquist, 1957; McIntosh and Gyarfas, 1971; Jorgensen and Jensen, 1973). It has been reported that the *T. timopheevii* translocation carrying *Sr36* in cv. Cook is large and spans across both arms of 2B, covering – according to GISH analysis - about 80% of chromosome 2B (Chemayek et al., 2017). Bariana et al. (2001) and Tsilo et al. (2008) have reported microsatellite markers closely linked to the *Sr36* locus. Markers suitable for MAS have also been developed for *Pm6* (Liu et al., 1998; Tao et al., 2000; Wang et al., 2000; Ji et al., 2007).

The *T. timopheevii*–derived 2G/2B introgression carrying *Sr36/Pm6* genes has been widely used in germplasms in North America, Europe, Australia, South Africa and in Kenya and Ethiopia (McIntosh et al., 1995, 2011). *Sr36* has been characterised as a gene of durable resistance, which remains effective in cultivars that are grown for a long period of time in large-area fields, and expresses a near-immune level of resistance (although some *Sr36*-virulent fungal races are now known) (Knott, 1989). *Pm6* is still one of the most effective resistance genes in China, where it is especially effective as a gene for adult resistance for powdery mildew in the field, though lines carrying *Pm6* may be susceptible at the seedling stage (Ji et al., 2008). In some commercial wheat varieties, *Sr36* and *Pm6* have been pyramided with other *Sr/Pm* genes (Jorgensen and Jensen, 1973; Huang and Roder, 2004; Olson et al., 2010). In Estonia, the combination of *Pm6* and *Pm2* is not effective (our unpublished data).

Pm27*,* another powdery mildew resistance gene derived from domesticated *T. timopheevii*, was identified in the hybrid line 146-155-T. According to the results of monosomic analysis, powdery mildew resistance in the line 146-155-T is conferred by a single dominant gene located on chromosome 6B (Enno et al., 1998; Järve et al., 2000). Using RFLP and microsatellite markers, a *T. timopheevii* translocation was confirmed on chromosome 6B of the hybrid line 146-155-T. Translocaton breakpoints were located, showing that the introgression region originates from the chromosome 6G of *T. timopheevii* and was transferred into the 146-155-T by homeologous recombination (Järve et al., 2000). By now, *Pm27* has lost its effectiveness in Estonia.

A *T. timopheevii*-derived QTL for resistance to powdery mildew (***QPm.icg-6D***) was detected in an introgressive hexaploid wheat line. It has been concluded that *QPm.icg-6D* is located in the region of a 6DS.6DL-6A^{t}L translocation (Leonova, 2015).

The stem rust resistance gene ***Sr37*** was transferred to the short arm of wheat chromosome 4B from the short arm of chromosome 4G (McIntosh and Gyarfas, 1971). So far, *Sr*37 has not contributed to wheat improvement (McIntosh et al., 1995, 2011), as the transferred 4G chromosomal segment is associated with undesirable agronomic traits (linkage drag). However, the *Sr37* has proven effective against the newly emerged Ug99 races of *Puccinia graminis* f. sp. *tritici* and successful attempts to reduce the size of the 4G chromosome segment carrying *Sr37* have been made (Zhang et al., 2012).

In close proximity to each other, three *T. timopheevii*-derived resistance genes/QTL have been located on chromosome 5BL: ***Lr18, LrTt2*** and ***QLr.icg-5B****.* The gene for resistance to leaf rust ***Lr18*** has been transferred from *T. timopheevii* to chromosome 5B of common wheat as a specific terminal segment derived from chromosome 5G (McIntosh et al., 1983; Yamamori, 1994; Friebe et al., 1996). The *Lr18* was characterised as an effective in all growth stages gene for resistance to leaf rust and has been used in several cultivars. The recessive mode of the gene action adds to the complexity of selection for *Lr18*, but markers which can be effectively utilized for MAS have recently been reported (Sadeghabad et al., 2017).

The dominant leaf rust resistance gene ***LrTt2*** was transferred to the long arm of chromosome 5B into the background of cv. Saratovskaya 29 from the 5G chromosome of the *T. timopheevii* ssp. *viticulosum* (Leonova et al., 2004). Authors considered it unlikely that *LrTt2* is an allele of the *Lr18* locus (Leonova et al., 2010; 2011). A major *T. timopheevii* derived QTL, ***QLr.icg-5B,*** was detected in same region of introgression on chromosome 5BL (Leonova et al., 2011; Timonova et al., 2013).

A recessive gene for resistance to leaf rust at the seedling stage, temporarily designated as ***LrTt1***, was mapped in a *T. timopheevii*-original introgression region on chromosome 2A in a line 842 (Leonova et al., 2004).

Two *T. timopheevii*-originated minor QTL for partial resistance to leaf rust, ***QLr.icg-2A*** and ***QLr.icg-1A***, were detected on chromosomes 2A and 1A, respectively (Leonova et al., 2010; 2011; Timonova et al., 2013).

A wheat line derived from *Triticum timopheevii* Zhuk, 'TC 67' – with a high level of resistance to Fusarium head blight – has been described (Cao et al., 2009). Two *T. timopheevii*-derived QTL for FHB resistance were detected at intervals *cfd6.1-barc48* and *cfd39-cfa2185* on chromosome 5AL, explaining up to 10% and up to 21% of phenotypic variation for disease (type 1 resistance) and Fusarium-damaged kernel (FDK) (type IV resistance) under field conditions, respectively (Malihipour et al., 2017).

A leaf rust resistance gene (Bai et al., 1998) and a gene for seedling resistance to *Septoria nodorum* blotch, *SnbTM* (Ma and Hughes, 1995; Cao et al., 2001) were transfed from domesticated *T. timopheevii* to durum wheat chromosomes 1A and 3A, respectively.

According to Dorofeev (1976), poor yields and hulled brittle ears make it difficult to use the wild form of timopheevii wheat, *T. araraticum*, in wheat breeding. However, *T. araraticum* plants are characterised by a high content of grain protein (up to 30%) and accessions resistant to powdery mildew and loose smut have been found (Dorofeev et al., 1976). With a wide range of reaction types to powdery mildew and rust pathogens, resistance to foliar diseases (leaf, stem and stripe rust, powdery mildew) was noticed in a collection of 301 *T. araraticum* accessions from Armenia, Azerbaijan, Iraq and Turkey (Brown-Guedira et al., 1996a). No powdery mildew resistance was detected in the accessions from Iran. All tested *T. araraticum* accessions were resistant to Septoria blotch (caused by *Septoria tritici* Roberge in Desmasz.) and a high percentage of them were resistant to tan spot (caused by *Pyrenophora tritici-repentis* (Died.) Drechs.) (Brown-Guedira et al., 1996a). Up to now, genes for resistance to leaf rust – *Lr50,* stem rust – *Sr40*, powdery mildew – *Pm37* and *MlAG12,* have been transferred from the wild form of the *T. araraticum* (McIntosh et al., 2008; 2011; http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp).

The gene ***Lr50*** – conferring resistance to leaf rust – is located on the long arm of the wheat chromosome 2B (Brown-Guedira et al., 2003). *Lr50* has not been deployed in wheat improvement, as North American races of *P. triticina* are virulent to this resistance gene.

The stem rust resistance gene – ***Sr40*** – was transferred to the short arm the 2B chromosome of hexaploid wheat from the Turkish accession of *Triticum timopheevii* ssp. *armeniacum* (PGR6195) (Dyck, 1992; Friebe et al., 1996). The location of *Sr40* on chromosome 2BS was validated through molecular mapping (Wu et al., 2009). So far, *Sr40* has not been deployed in commercial cultivars (McIntosh et al., 1995), however, it was shown that the *Sr40* provides effective level of seedling and adult plant resistance against the new virulent race of stem rust, Ug99 (Wu et al., 2009).

The dominant powdery mildew resistance genes ***Pm37*** and ***MlAG12*** from two different accessions of wild *T. timopheevii* subsp. *armeniacum* (*araraticum*), both from Iraq, were transferred into hexaploid wheat onto the long arm of chromosome 7A (Srnic´et al., 2005; Perugini et al., 2008; Maxwell et al., 2009).

Pm37 has shown itself to be highly effective in the field in southeastern U.S. and confers resistance to more than 60 different isolates of *Bgt*. So far, no virulence to

Pm37 has been detected. The gene was mapped on the long arm of chromosome 7A. According to the authors, *Pm37* is linked to the *Pm1* locus, but the gene is not an allele of *Pm1*. *Pm37* was located about 16.2 cM proximal to *Pm1* (Perugini et al., 2008).

The temporary designed gene ***MlAG12*** (Maxwell et al., 2009) was introgressed into the background of soft red winter wheat cv. Saluda. The gene provided consistent resistance over six seasons of field evaluations in North Carolina and secured resistance at the 2-3 leaf stage in greenhouse. The introgression carrying the resistance gene was mapped to the chomosome arm 7AL. Linkage analysis showed that *MlAG12* was flanked by markers *Xwmc346* and *Xwmc273* at distances of 6.6 cM and 8.3 cM, respectively. According to the map, *MlAG12* is allelic, or in close linkage with the *Pm1* locus. Nevertheless, a detached leaf test with ten differential powdery mildew isolates indicated that the resistance pattern of *MlAG12* was different from all designated alleles at the *Pm1* locus. The authors also presume that *MlAG12* is not an allele of the *Pm37* locus as, according to marker analysis, the introgression is distal to the introgession for *Pm37* (Maxwell et al., 2009).

Immunity to powdery mildew, leaf and yellow rusts, a high resistance to stem rust, loose and dwarf smuts have been reported as the useful traits of *T.militinae* (Dorofeev et al., 1979). In three different climatic regions, *T. militinae* was found to be unsusceptible to fungal diseases; only traces of stem rust were detected (Dekaprelevich, 1954; Jakubziner, 1969; Migushova, I975). *T. militinae* has rarely been used to improve disease resistance in common wheat (Peusha and Shnaider, 1983; Järve et al., 2002). No resistance genes originating from *T. militinae* have been identified in common wheat (McIntosh et al., 2017).

2 AIMS OF THE STUDY

The main objectives of the present study were to map the genetic factors behind the resistance to powdery mildew (*Bgt*) in a common wheat introgression line and, exploring potential negative associations with other agronomically important traits, to evaluate usefulness of detected factors in breeding.

To this end, the following objectives were set:

- to estimate the number and chromosome locations of *Triticum militinae*-derived introgressions in the genomes of resistant to *Bgt* introgression lines selected from the progeny of crosses between hexaploid wheat *Triticum aestivum* and tetraploid wheat *Triticum militinae*;

- to select a resistant to powdery mildew introgression line for further genetic analysis and to develop a map of the *T. militinae* introgressions in the genome of the selected resistant hybrid line (line 8.1);

- to estimate the number and genomic positions of the QTL for powdery mildew resistance at the seedling and adult stages of plant growth in F2 and F3 mapping populations from the cross of line 8.1 to cv. Tähti;

- to confirm the involvement of QTL for resistance in a doubled haploid (DH) mapping population derived from the cross of line 8.1 to cv. Tähti;

- to precisely locate the *QPm.tut-4A* locus, the main QTL for *T. militinae*-derived powdery mildew resistance in the common wheat introgression line 8.1;

- to confirm the efficacy of the detected *T. militinae*-derived *QPm.tut-4A* for resistance to *Bgt* in different genetic backgrounds;

- to evaluate the possible effect of the *T. militinae* derived introgressions in line 8.1 on flowering time and other yield-related morphological traits.

3 MATERIALS AND METHODS

3.1 Plant material used in the study:

- *Triticum militinae* Zhuk. et Migush. (accession No K-46007; N. I. Vavilov Institute of Plant Industry, St.Petersbourg, Russia),
- *T. aestivum*: spring wheat cvs. Tähti, Mooni, Opata and landrace Chinise Spring (CS),
- BC_1F_4 population (cv. Tähti x *T. militinae*) x cv. Tähti,
- CS N4AT4B and CS N4AT4D nullisomic–tetrasomic lines (provided by Dr. S. M. Reader (John Innes Centre, Norwich, UK.),
- CS 4AL ditelosomic line (2n = 40 + tt), (provided by Dr. S. M. Reader),
- CS N5BT5D and CS N5BT5A nullisomic lines, (provided by Dr. S. M. Reader),
- CS *ph1b* mutant line (provided by Dr. S. M. Reader).

3.2 Lines and mapping populations developed in this work:

- Single seed descendant introgression lines 8.1, 8.5, 8.7 and 8.9 resistant to *Bgt* (**Publ. I**).
- F_2 and $F_{2\text{-}3}$ mapping population (134 F_2 plants and 122 F_3 families) obtained from the cross (line 8.1 x cv. Tähti) (**Publ. I**).
- Double haploid (DH) mapping population of 350 DH lines developed from the cross (line 8.1 x cv. Tähti) (**Publ. II**).
- F_2 mapping population (98 lines) obtained from a cross between susceptible cv. CS and resistant introgression line 8.1 (**Publ.11**). The F_3-F_6 progeny (8425 hybrid plants) was analysed to verify the phenotypic effect of the *QPm.tut-4A* locus and to search plants recombinant in chromosome arm 4AL (**Publ. IV**).
- F_2 and F_3 mapping population (94 plants/families) created from cross (cv. Mooni x DH81) (**Publ. III**).
- F_2 mapping population (140 plants) created from the cross (cv. Opata x DH81).
- Ditelosomic introgression line CS 4AL+*QPm.tut-4A* (2n=40+tt) for constructing a chromosome-specific BAC library (**Publ. II**).
- *ph1* mapping population of 1225 BC_1F_2 plants homozygous for the *ph1* locus and heterozygous for the *QPm.tut-4A* locus (**Publ. IV**).
- a derivative of cv. Tähti carrying singly the 4AL translocation with *QPm.tut-4A* from *T. militinae* – near-isogenic line T312.30.38.16 (F_3BC_5 (DH312 x Tähti) x Tähti),
- derivatives of cv. Mooni carrying singly the 4AL translocation with *QPm.tut-4A* locus from *T. militinae* - near isogenic lines MooniR ($F_3BC_{7\text{-}9}$ (DH303 x cv. Mooni) x cv. Mooni).

3.3 Experimental schema (Fig. 1)

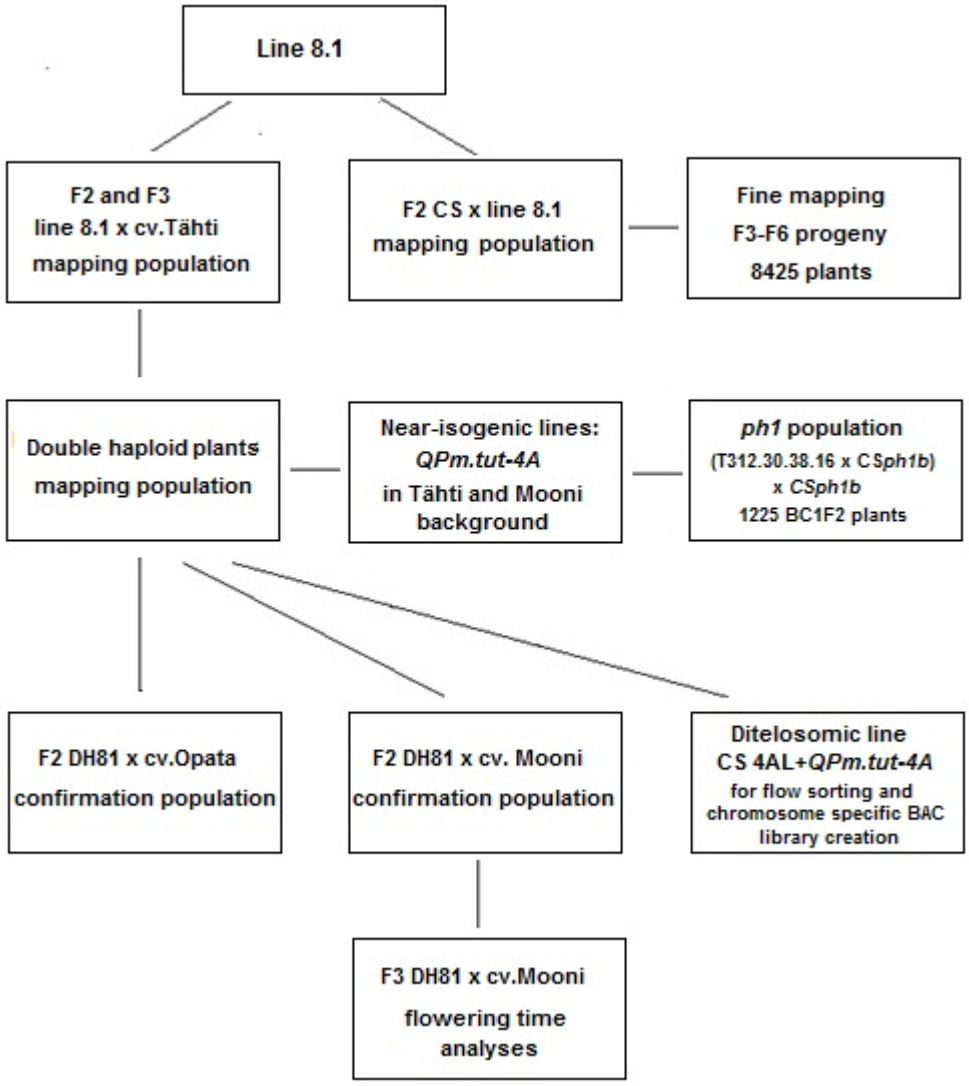


Figure 1. *Experimental schema*.

3.4 Fungal material and resistance tests

A detailed description of fungal material and the tests for seedling resistance as well as field experiments for the evaluation of adult plant resistance are provided in the publications **Publ. I** and **Publ. II.** Powdery mildew disease on detached seedling leaves was scored on a 0 – 9 scale (0 - no visible symptoms, 9 – heavy sporulation, Lutz et al., 1992). Disease severity on adult plants was expressed as disease index (DI) on a 0 – 9 scale (Yu et al., 2001).

3.5 Evaluation of flowering time (FT) and morphological traits

The method and populations for evaluation of FT is described in **Publ. III**.
113 DH lines (6 plants per line), grown in a controlled environment, were evaluated for plant height, spike length, spikelet number and spike density (spikelet number per 10 cm of spike), and 1000 kernel weight (**unpublished data**).

3.6 Marker analysis

The methods for extraction of genomic DNA, amplification of molecular markers (SSR and STS markers, markers derived from BAC contigs), conditions of PCR amplification and analysis of the DNA fragments are described in **Publ. I, II, III** and **IV.**

3.7 QTL and statistical analysis

Linkage group creation, QTL mapping and statistical analysis are described in **Publ. I, Publ. II** and **Publ. III**.
QTL is designated according to the guidelines for nomenclature of QTL in wheat (McIntosh et al., 2008).

4 RESULTS AND DISCUSSION

4.1 Marker analysis of *T. militinae*-derived introgression lines (Publ. I)

The tetraploid wheat species *T. militinae* (2n=28) was used as a source of resistance to powdery mildew for common wheat (*T. aestivum*, 2n=42). Four single seed descendant introgression lines were advanced from a heterogeneous BC_2F_4 population, produced from direct hybridization between a hexaploid wheat genotype, Finnish cultivar Tähti, and the tetraploid species of the timopheevii group, *T. militinae*, followed by one generation of backcrossing.

129 wheat SSR markers from the published maps of *T. aestivum* (**Publ. I**) were used to detect *T. militinae*-derived introgressions in genomes of four hybrid lines (8.1, 8.5, 8.7 and 8.9). 93% of the used microsatellite markers showed polymorphism between *T. militinae* and cv. Tähti genomes, while 40% of markers were scored as codominant and 45% as null alleles in *T. militinae*. In the four introgression lines analysed, *T. militinae / T. aestivum* replacements were detected in 73 loci which according to the published wheat maps (Gale et al., 1995; Röder et al., 1998; Sommers et al., 2004; Sourdille et al., 2004) could be aligned to chromosomes. The number of chromosomes with detected introgressions varied from 6 to 8 per line. The replacements in chromosomes 4A, 5A and 7A were detected in all four lines. Altogether, six chromosomes of the A genome and four chromosomes of the B genome were involved in *T. militinae* translocations, while no introgressions were found in the D genome.

Although timopheevii wheats in general are hardly crossable with hexaploid wheat (Dorofeev et al., 1976), a few interspecific hybrids have been described (Skurygina, 1984; Brown-Guerdila et al., 2003; Badaeva et al., 1995b; 2000; Budashkina and Kalinina, 2001). As might be expected, the G/D chromosome substitutions in *T. aestivum* x *T. timopheevii* hybrids are rare (Badaeva et al., 1991, 2000; Timonova et al., 2013), but even less frequently, introgressions in chromosome 4A have been detected (Badaeva et al., 1991, 2000; Gordeeva et al., 2009; Badaeva et al., 2010). It has been concluded that the spectra of substitutions and rearrangements depend on the genotype of parental forms (Badaeva et al., 2010).

One possible reason for the lack of introgressions on chromosome 4A could be incompatibility between the parental chromosomes 4A and $4A^t$ explained by the fact that timopheevii wheats do not carry the $4A^tL.7GS$ translocation characteristic of the chromosome 4A in hexaploid wheat, but carry a distal $4A^tL.3A^tL$ translocation. This modification results in the absence of collinearity between the distal regions of chromosomes 4AL and $4A^tL$ (Jiang and Gill, 1994; Maestra and Naranjo, 1999; Rodriguez et al., 2000; Salina et al., 2006; Dobrovolskaya et al., 2009).

In addition, it was recently demonstrated that some northern European cultivars of common wheat, including cv. Tähti, have a specific haplotype'A' of the distal region of chromosome 4A (Tsõmbalova et al., 2017). The haplotype is characterised by a deletion which may affect the A^t / A pairing in meiosis. Two tetraploid timopheevii wheats, *T. timopheevii* (hulled) and *T. militinae* (free-threshing), both A^tA^tGG, carry in the distal region of chromosome arm 4AL a haplotype similar to specific haplotype 'A'. It is possible that success in obtaining a 4AL introgression in our case was due to the fact that both parents of the hybrid lines (cv. Tähti and *T. militinae*) carry the haplotype, increasing the compatibility between the distal regions of 4AL in cv.Tähti and the corresponding region of *T. militinae* on chromosome 7G.

4.2 Resistant to powdery mildew hybrid line 8.1 (Publ. I)

The introgression lines 8.1, 8.5, 8.7 and 8.9 were evaluated for their resistance to *Bgt* at the seedling stage by the detached-leaf assay and were tested over 3 years in field trials at the adult plant stage. Line 8.1, which showed a high and stable level of APR in all field trials, was chosen for further genetic analysis. The line exhibited not only effective APR in field tests but was fully resistant to a mix of *Bgt* isolates (races) at the seedling stage. Resistance at the seedling stage correlated with the adult resistance, which allowed us to suggest that the same genetic system is involved in the formation of powdery mildew resistance in the line in both stages of the growth.

Plants of the resistant introgression line 8.1 are higher than those of the original spring wheat cv Tähti, the spike is longer and of low compactness (speltoid type). Compared to cv. Tähti, spring line 8.1 is characterised by a slower rate of development.

4.3 The genetic map of the *T. militinae*-derived introgression regions in line 8.1 (Publ. I, II, III)

According to primary analysis, the resistant to powdery mildew line 8.1 carried *T. militinae*-derived introgressions in seven chromosomes (1A, 1B, 2A, 4A, 5B, 5A and 7A) (**Publ. I**). However, on the basis of the markers' segregation in the F_2 mapping population (line 8.1 x cv.Tähti), nine linkage groups (LOD >3) were created (**Publ. I**). By comparing with the published hexaploid wheat SSR maps (Röder et al., 1998; Somers et al., 2004; Sourdille et al., 2004; GrainGenes 2.0), the introgressions were assigned to seven chromosomes, and two linkage groups were assigned to chromosome 2A and 5A. At this stage of work, the genetic map of the introgressive part of the genome of line 8.1 comprised 42 loci spanning 251 cM (**Publ. I**).

Considering the density of molecular markers and the number of available recombination events as key factors affecting the mapping of QTL (Yang et al., 2012), about a hundred additional markers were tested for polymorphism and 40 new loci were added to the genetic map of line 8.1 (**Publ. II; Publ. III**). No additional chromosomes carrying introgressions were detected by the additional screening.

The size of introgressions in the genome of line 8.1 ranged from a few centimorgans on the chromosome arm 2AL to the almost complete substitution of chromosome 5A. Two linked markers, originating from the genome of *T. militinae* remained unlinked to any linkage group in line 8.1.

The order of SSR markers in linkage groups of introgressions on chromosomes 1A, 2A, 5A, 7A and 5B was in good agreement with the published linkage maps of common wheat. However, by comparison with the published maps (Gale et al., 1995; Röder et al., 1998; Somers et al., 2004; Sourdille et al., 2004), the genetic distance between the markers in the detected translocations was significantly reduced on some chromosomes. Markers assigned to the chromosome 5B showed a distorted segregation, favoring the cv. Tähti allele. Segregation distortion favoring the *T. militinae* alleles was observed for some markers on the chromosome arm 5AL and for all markers on chromosome 4A.

Recombination was fully supressed in the *T. militinae*-origin introgression segment on the chromosome arm 4AL. In the F_2 (8.1 x Tähti) mapping population, no recombination in the introgressed segment was detected between the 14 markers

mapped to the region. Hovewer, according to the published genetic maps, the region of introgression on chromosome arm 4AL covered a distance of 10–26 cM.

In F_3-F_4-F_5 progenies of the cross (8.1 x Tähti) and in the DH population additional markers allowed us to detect a few auxiliary recombinants (between the two groups of loci *Xwmc232–Xgwm160* and *Xrga3.1–Xpsr119* (Fig. 1; **Publ. II**) in the region of the *T. militinae* introgression on chromosome 4AL.

By analysis of DAPI vs GAA (FITC) bivariate flow karyotype, the chromosomal origin of the *T. militinae* introgression on 4AL was identified as a result of homeologous recombination with *T. militinae* chromosome 7G (Abrouk et al., 2017). The 4AL introgression overlaps almost the entire segment of the 4A.7B translocation characteristic of the chromosome 4A in hexaploid wheat (Jiang and Gill 1994; Devos et al., 1995; Maestra and Naranjo, 1999; Rodriguez et al., 2000b). Recently, high degree of divergence with respect to both their gene content and nucleotide sequence has been revealed by a comparison of chromosome 4A segment's sequence in the native (CS) and introgressive forms (line 8.1) (Janakova et al., 2018). Apparently the lack of chromosome pairing prevented recombination between the chromosomal segment of *T. militinae* and the corresponding chromosomal region in cv. Tähti or CS.

Examples of translocations introgressed from *T. timopheevii* showing no or an extremely low level of recombination in crosses with hexaploid wheat have been referred to earlier (Devos et al., 1993; Järve et al., 2000; Kammholz et al., 2001; Bariana et al., 2001; Jia et al., 1996; Lukaszewski, 2015). Depending on the size and structure of the introgression or substitution, the recombination frequency can be reduced to 0% (Canady et al., 2006). Factors suggested to be responsible for recombination suppression include genetic background, a high level of sequence diversity, chromosomal rearrangements, hemizygous state (Lukaszewski et al 2015).

4.4 Mapping and verification of the QTL for resistance to powdery mildew in line 8.1

4.4.1 An analysis of the F_2 and F_3 mapping population from the cross of line 8.1 and cv. Tähti (Publ. I)

Preliminary data indicated that APR to *Bgt* in line 8.1 may be determined by a polygenic system. QTL analysis in F_2 and F_3 (line 8.1 x cv. Tähti) mapping populations confirmed this assumption. At the adult plant stage, two QTL for APR, located on chromosomes 4A and 5A, were considered significant in F_2 and F_3 mapping populations. On a suggestive level, minor QTL on chromosomes 2A, 5B, 1B were detected. If the suggestive QTL were taken into account, up to 69% of the total trait variance was explained.

The QTL on chromosome 4A was located between loci *Xgwm160* and *Xwmc232* and explained up to 35% of the APR variance in 2002, and up to 54% in 2004, in F_2 and F_3 mapping populations, respectively. Despite the differences in environmental conditions, the results of the QTL mapping were remarkably consistent for F_2 plants and F_3 families in field trials of 2002 and 2004 (**Publ. I**). A major QTL for powdery mildew resistance at the seedling stage was mapped in the same introgression region on the distal part of chromosome 4A, between loci *Xgwm160* and *Xwmc232,* as the QTL for APR. The whole QTL region was designated as *QPm.tut-4A.* It remained unclear

whether the seedling resistance and the adult plant resistance were evoked by the same or different loci in the QTL region.

Minor QTL for APR and seedling resistance were detected on a suggestive level (LOD <2.0) also on chromosomes 1A, 2A, 1B, and 5B.

4.4.2 Validation of the QTL for non-race-specific resistance to powdery mildew using a DH mapping population (Publ. II)

Analysis of a low share of a minor QTL in the summary trait variance is complicated in several aspects, such as number of replications used to generate phenotypic data, population size and marker density (Young, 1996; Asins, 2002; Collard et al., 2005). For a correct evaluation of its effect, low-share QTL requires repeated tests in controlled conditions to minimize random effects. Accurate QTL analysis also relies on precise phenotyping. 350 double haploid (DH) lines generated in this study allowed repetitive characterisation and validatation of QTL in tests with different pathogen isolates. In order to evaluate random variation in test results, 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 – No. 2 and No. 9 – and tested in the DH population. The results of these tests allowed us to conclude that detected small differences between two isolates in seedling resistance were on the same level as differences in results of testing twice the same fungus isolate.

In the DH population generated from the cross (line 8.1 x cv.Tähti), five chromosomal 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, and three of the QTL (on chromosomes 4A, 7A and 5A) were also detected in APR assessments (**Publ. II**). With the exception of the newly detected in the DH population minor QTL on chromosome 7A, the remaining QTL were earier found as suggestive in the F_2 (8.1 x Tähti) mapping population (**Publ. I**). The newly detected QTL on chromosome 7AS was identified in the region of microcollinearity with the QTL region on chromosome 4AL (Devos et al., 1995; Khlestkina et al., 2010).

The main QTL on 4A (*QPm.tut-4A*) was found to account, on average, for 35-49% of seedling resistance variance and for 33-52% of APR variance. Each of the detected minor QTL was responsible for 10-17% of the resistance variance.

4.4.3 Analysis of the effect of *the QPm.tut-4A* (Publ. II)

For the analysis, the DH population was divided into two subpopulations consisting of genotypes carrying different alleles of *QPm.tut-4A*: the subpopulation 4A-A carrying the cv. Tähti allele and the subpopulation 4A-B carrying the *T. militinae* allele. Resistance scores for the seedlings inoculated with a set of different Bgt isolates/mixtures in the two subpopulations were compared, and, in average, the difference between the two subpopulations was 2.8 ± 0.15, varying in different tests from 1.7 to 3.8 (Fig. 2). 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' being the most aggressive (Fig. 2). However, for the genotype carrying all five QTL, the mean scores of seedling resistance still remained higher than the scores for the original introgression line 8.1.

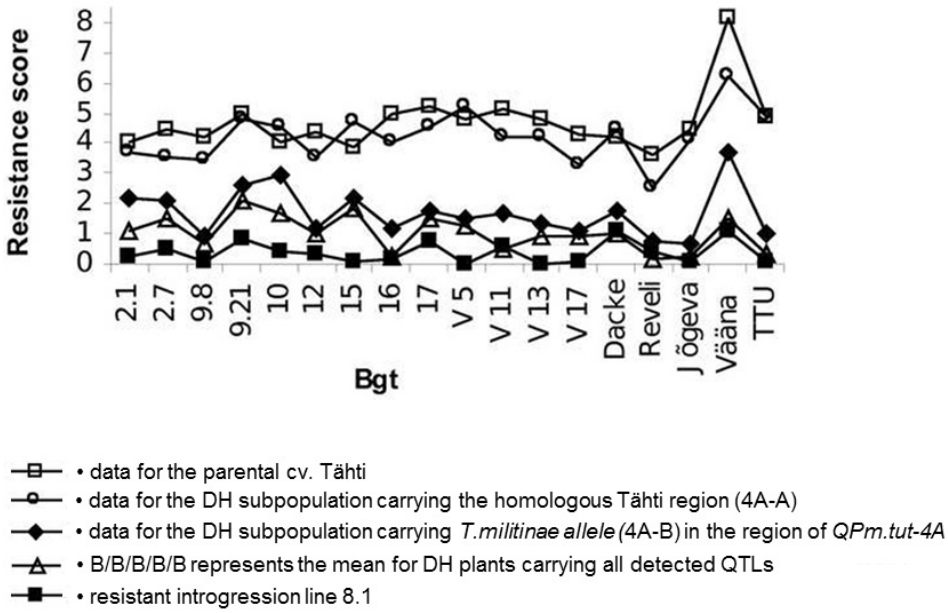


***Figure 2**. Mean of seedling resistance scores upon inoculation of DH lines with and without QPm.tut-4A, DH plants carrying all detected QTL for Bgt resistance, cv Tähti and line 8.1 with different subisolates, isolates and mixtures of Bgt*

In the three APR experiments performed, the protective effect of *QPm.tut-4A* varied with an average of 2.3 ± 0.2 for the DI difference between the 4A-A and 4A-B genotypes. Under conditions of an extremely high level of powdery mildew density (growth chamber experiment in 2009), when the infection level in cv. Tähti and the 4A-A subpopulation was high (DI = 9.0 and 7.0, respectively), the average DI in the 4A-B subpopulation remained at 4.6 ± 0.8, (two time lower) indicating that *QPm.tut-4A* has a limited 'capacity' and accounts for partial resistance, a feature usual for APR.

Although minor QTL effectively improve the resistance, if present along with *QPm.tut-4A,* they do not significantly improve resistance if they are, either alone or all together, present in the absence of *QPm.tut-4A*. DH134, for example, carries all four minor QTL (on chromosomes 1A, 1B, 5B, 7A), but not the *QPm.tut-4A,* and its resistance score in different tests at the seedling stage varies widely from intermediate / moderately susceptible to highly susceptible (data not shown).

4.4.4 Efficiency of the *QPm.tut-4A* in different genetic backgrounds (Publ. II; unpublished data)

It has been observed that the quantitative trait loci responsible for phenotypic trait variance in a primary mapping population may not operate in the same manner in a different genetic background (Collard et al., 2005). Due to interactions with alleles in other loci or epistasis, the effect of a QTL may differ or the QTL may not be effective in different genetic backgrounds (Holland, 2001; Liao et al., 2001).

To test whether *QPm.tut-4A* itself leads to enhanced powdery mildew resistance, we introduced the translocation on chromosome 4A into a susceptible wheat background. Effectiveness of the major *QPm.tut-4A* was verified in genetic backgrounds of susceptible to powdery mildew landraces/culltivars CS, Opata and Mooni. Ninety eight F_2 plants from the cross (CS x line 8.1) were tested for alleles of 25 markers mapped on the 4AL chromosome arm, as well as for alleles of 11 markers mapped in the regions of minor QTL detected in this study, and for the alleles of 5 markers mapped on chromosome 7DS including makers for *Lr34* (Lagudah et al., 2009).

The *T. militinae*-origin main QTL on chromosome 4AL were confirmed in a QTL analysis of the F_2 (CS x 8.1) population. The *QPm.tut-4A* generated 35–38% of the seedling resistance variance, and 52% of the APR variance (**Publ. II**). However, previously detected minor QTL were not confirmed in this mapping population. One possible reason for that might be a relatively small mapping population. Simulation and experimental studies have shown that the power of minor QTL detection is low when populations of less than 200 plants are analysed (Beavis, 1998).

Analysis of the *QPm.tut-4A* locus in the progeny of crosses (cv. Opata x DH81) and (cv. Mooni x DH81) confirmed transferability of the *QPm.tut-4A* to a new genetic background (unpublish data). Detached-leaf *Bgt* assay was used to screen for seedling resistance to four *Bgt* isolates in one hundred and forty F_2 plants from a cross between cv. Opata and DH81 and ninety four F_2 plants from a cross between cv. Mooni and DH81. Plants were genotyped with two markers flanking the *QPm.tut-4A* (*Xwmc232* and *Xgwm160*). Fig. 3 represents the average scores for seedling resistance to four different *Bgt* isolates in F_2 populations, divided into groups according to the presence/absence of *T. militinae* alleles in the region of the *QPm.tut-4A*. As well as in the genetic background of cv. Tähti, where it was originally introgressed, the *QPm.tut-4A* remained effective in three different *T. aestivum* backgrounds (cvs. CS, Opata, Mooni). In all cases (3 populations and 4 isolates), the average resistance score for F_2 plants homozygous for *T. militinae* allele in *QP.tut-4A* locus was higher than resistance score for plants of line 8.1 (Fig. 3).

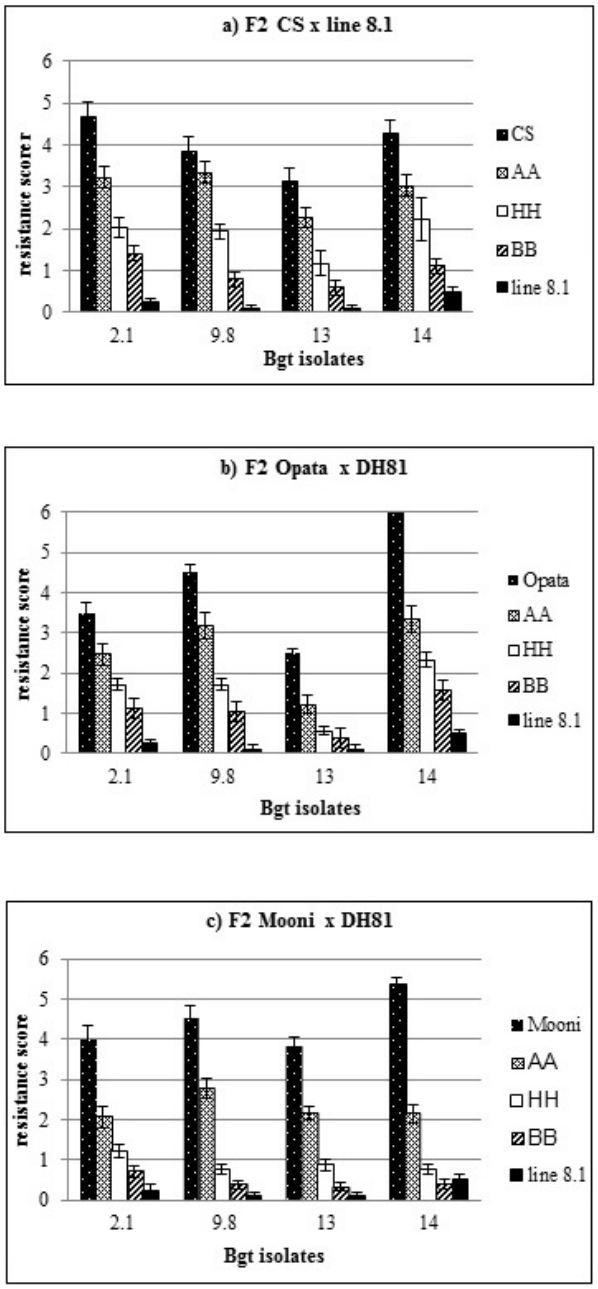


Figure 3. *The effect of alternative alleles in the QPm.tut-4A region of the 4AL chromosome arm on seedling resistance to four Bgt isolates in different F2 populations. AA – homozygous CS, Opata or Mooni allele, AB – heterozygous, BB – homozygous T. militinae allele.*

Creating near-isogenic lines (NILs) by marker-assisted backcrossing (MAB) is another way to confirm the efficacy of the new QTL in different genetic backgrounds (Collard and Mackill, 2008). MAS for quantitative resistance has been reported in the literature (Salameh et al., 2010; Asea et al., 2012; Bai et al., 2012). Here, by means of

marker-assisted backcrosses and selection, near isogenic (NI) introgression lines carrying *QPm.tut-4A* as the single introgression, were generated from crosses (DH312 x Tähti) and (DH303 x Mooni). Two microsatellite markers located on the *T. militinae*-origin introgression on chromosome 4A, *Xgwm160* and *Xgwm832*, were used to control the transfer of the introgression. Markers linked to introgressions on chromosomes 5A, 2A and 5B were used to select plants which had lost these introgressions in the course of backcrossing. Detached-leaf *Bgt* assay was used to control resistance to powdery mildew throughout different steps of backcrossing. Field trials (2017-2018) were used to control APR for powdery mildew in BC_7F_2-BC_9F_2 inbred lines. The BC_9F_2 plants that carried the *T. militinae* origin introgression and, accordingly, the *QPm.tut-4A,* showed a substantial improvement in resistance to powdery mildew, while morphological traits, in large, remained unchanged (data not shown). Results confirm the effectiveness of *QPm.tut-4A* in controlling resistance to powdery mildew. Thus, *QPm.tut-4A* is a novel source of powdery mildew resistance and has a good prospect to be used in breeding. Markers *Xgwm832* and *Xgwm160* located in the region of the introgression on 4A can be used in MAS.

A comparative analysis of DH lines showed that *QPm.tut-4A* does not require any other QTL for its activity. Resistance to powdery mildew in near isogenic lines of Tähti and Moon background confirmed this conclusion.

4.4.5 Line 8.1 carries a non-race-specific resistance

At the seedling stage (Feeke's stage 1), introgression line 8.1 was resistant to all tested *Bgt* isolates, subisolates and mixtures thereof (**Publ. I** and **Publ. II**). With the exception of the minor QTL on chromosome 5B, *QPm.tut-4A* and all minor QTL were detected in all tests of inoculation with 16 different *Bgt* isolates and mixtures.

Observed in line 8.1 non-race-specific resistance might be evoked by a set of pyramided race-specific *Pm* genes, or by a single *Pm* gene with appropriate specificity or by gene(s) of unknown type. For example, the region of the minor QTL on chromosome 1A for seedling resistance to powdery mildew involves the *Pm3* gene locus. However, functional markers for this gene (Yahiaoui et al., 2004; Tommasini et al., 2006) were used here to analyse cv. Tähti and line 8.1, and no functional allele of *Pm3* was detected (**Publ. II**).

The minor QTL on chromosome 5B (*Xgwm205–Xgwm213*) was revealed at the seedling stage only in a subset of *Bgt* tests (**Publ. II**, Table 2), indicating that the QTL may act in a race-specific manner. Advanced studies are needed to clarify the identity of the detected minor QTL and *Pm* loci.

4.5 Towards the precise mapping of the *QPm.tut-4A* region

4.5.1 Ditelosomic line 'CS DT4AL+*QPm.tut-4A*' (Publ. II and IV)

With the objective to create chromosome arm 4AL specific BAC library, the *QPm.tut-4A* region was transferred to the chromosome arm 4AL of the susceptible CS 4AL ditelosomic line (2n = 40 + tt). For this purpose, the ditelosomic line was crossed with the resistant line 8.1, followed by flow-sorting of the chromosome arms 4AL carrying the introgression from *T. militinae*. A single recombinant ditelosomic plant was identified and selfed, generating the ditelosomic introgressin line CS DT4AL+*QPm.tut-4A*. Transfer of the

QPm.tut-4A locus was verified by gaining resistance to *Bgt*, by cytological analysis and by testing with molecular markers (**Publ. II**).

The flow karyotype derived from the DAPI-stained chromosomes of the DT4AL+*QPm.tut-4A* line included a distinct peak corresponding to the 4AL telosome (4AL+*QPm.tut-4A*) (Abrouk et al., 2017), which enabled it to be sorted. The flow-sorted chromosome arms 4AL with the *QPm.tut-4A* region were used for:

i) preparation of chromosome-specific DNA CS 4AL+*QPm.tut-4A* , which was used to verify the physical location of new markers generated for the introgression region (Janakova et al., 2018);

ii) construction of a 4AL-7G-specific BAC library in order to acquire sequences of the *QPm. tut-4A* region of line 8.1. The 43,008 clones generated provided a 6.8-fold coverage of the 4AL-7G chromosome arm carrying the *QPm.tut-4A* locus (www.olomouc.ueb.cas.cz/dnalib/taapmt4alha).

However, it should be noted that now the status of chromosome flow sorting is such that almost any wheat chromosome and also chromosomes in many crops can be isolated to a reasonable purity (Dolezel et al., 2014; Tsõmbalova et al., 2016; Abrouk et al., 2017). Sequencing libraries of DNA amplified from the *T. militinae* chromosome 7G, which was the original location of the *QPm.tut-4A* locus in *T. militinae* genome, have been constructed (Abrouk et al., 2017).

4.5.2 Fine mapping of the *QPm.tut-4A* locus (Publ. IV)

Unfortunately, the *T. militinae*-derived introgression on chromosome 4A has shown a highly reduced level of recombination, hampering the precise mapping of the *QPm.tut-4A* locus (**Publ. II**). It is still possible that the *QPm.tut-4A* locus includes not a single gene for resistance, but a cluster of *T. militinae*-derived genes responsible for non-race-specific powdery mildew resistance at the seedling and adult plant stages of plant growth. Further attempts to generate new plants recombinant in the region of the *QPm.tut-4A* locus were taken.

In wheat, the frequency of recombination can be increased through selection of appropriate parents or by manipulating *Ph1*, the major locus controlling chromosome pairing in the polyploid species (Griffiths et al., 2006; Qi et al., 2007). Both possibilities were used in this work.

In the population obtained after crossing the susceptible cv. Chinese Spring (CS) with the resistant line 8.1, the first observed recombination event was found in the set of 98 F2 plants and the *QPm.4A-tut* was linked to the upper (proximal) part of the translocation. The *QPm.tut-4A* locus was delimited to a narrow region (*Xwmc232 / Xgpw3079 / Xbarc70 / Xgwm832 / Xgpw356 / Xgpw7051*; with an estimated size of 2.5 cM (**Publ. II**). Genotyping of the extended mapping population of 8425 F3-F6 individuals with respect to markers *owm82* and *Xgwm160* revealed 30 new recombination events. Testing these recombinant individuals for their reaction to the four powdery mildew isolates showed that the resistance phenotype was fully correlated with the presence of the *T. militinae* segment between markers *owm202* and o*wm223* (Fig. 4(A)).The net effect of the mapping reduced genetic length of the segment harboring *QPm.tut-4A* to 0.012 cM. Segregation of 102 new developed markers observed in the extended CS × 8.1 mapping population was used to improve the genetic map of the *QPm.tut-4A* region (**Publ. IV**).

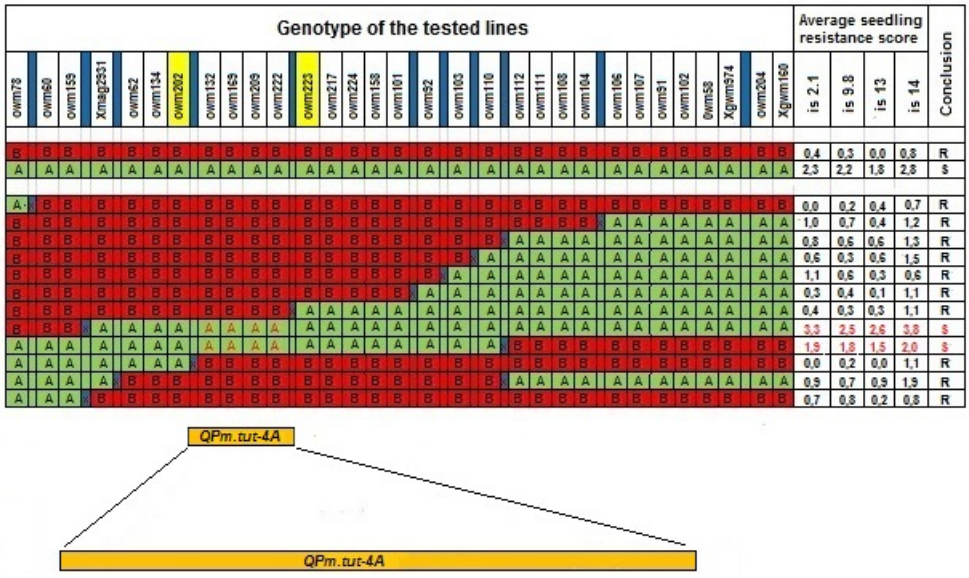
In hexaploid wheat, the pairing of homoeologs is strongly restricted by the action of the *Ph1* locus (Riley and Chapman, 1958; Sears, 1977; Gill et al., 1993). A "*ph1*

population" was created to induce a higher rate of recombination between the introgression containing *QPm.tut-4A* and its presumed homoeologous segment on CS 4AL. The "*ph1* population" was derived by self-pollination of 22 (out of 107 screened) BC_1F_1 ([line 8.1 × ph1b] × ph1b) plants simultaneously homozygous for the *ph1b* allele and heterozygous for the *QPm.tut-4A* segment. Genotyping of the resulting 1255 BC_1F_2 progeny revealed 155 recombination events between loci *owm82* and *Xgwm160*, equivalent to a 33-fold increase in recombination, as compared to the rate observed in the presence of *Ph1*. The 0.012 cM *QPm.tut-4A* region was split into eight subregions

Testing these recombinant individuals for their reaction to the four powdery mildew isolates allowed to reduce the length of the region containing the *QPm.tut-4A* and restricted it by markers *owm169* and *owm228* (Fig. 4(B)).The newly available genomic sequence of CS (IWGSC, 2018) made it possible to determine the length of the 4AL segment flanked by these markers in CS genome as 640 kbp. The original CS segment replaced by *QPm.tut-4A* locus in line 8.1 harbors 16 predicted genes (Janakova et al., 2018).

The physical length of the corresponding sequence in *T. militinae* genome was evaluated by a chromosome walking approach which was initiated by Dr M. Valarik (Olomouc, Czech Republic) (Abrouk et al., 2017; Janakova et al., 2018). In total, 26 BAC clones were sequenced to provide a complete sequence of 480.2 kbp segment of the *QPm.tut-4A* region in line 8.1 bounded by markers *owm169* and *owm228*. Sequence comparison revealed that the corresponding segment (*owm169–owm228*) in *T. militinae* genome harbored 12 predicted genes, eight of which lacked a homolog in the CS segment. Thus the introgression *T. militinae* region was 25% shorter than the corresponding region in CS. A comparison of the segments sequences revealed a high degree of divergence with respect to both their gene content and nucleotide sequence. Apparently, the absence of homology between the original wheat and the introgressive locus *QPm.tut-4A* was the main reason for the suppression of recombination.

A)



B)

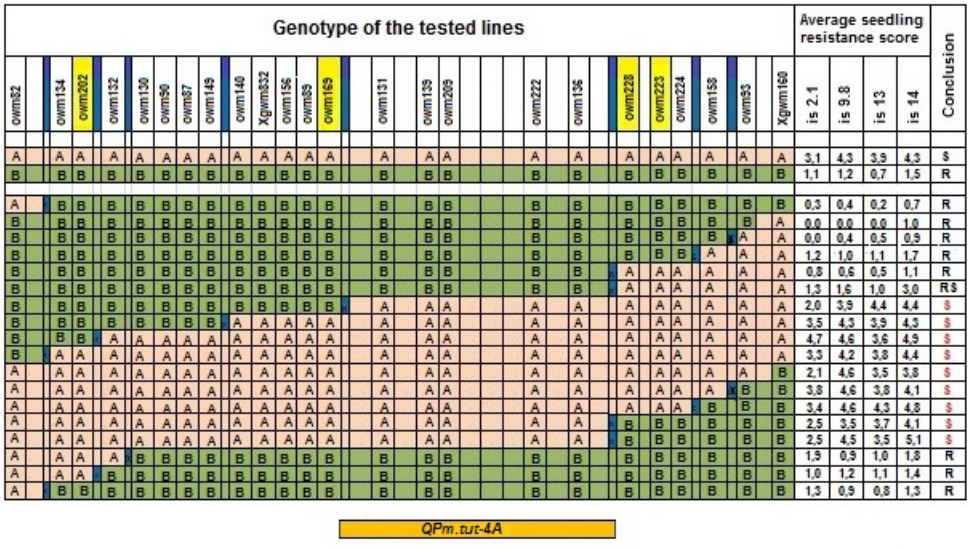


Figure 4. *Fine mapping of the QPm.tut-4A region in the recombinant progeny of the cross (line 8.1 x CS) (A) and progeny of the cross (line 8.1 x ph1 CS) (B).*
A - CS allele; B – T. militinae allele

4.1 *QPm.tut-4A* is a new a source of resistance to powdery mildew in wheat

A comparison with the published QTL for powdery mildew, resistance located on chromosome 4A confirmed the uniqueness of *QPm.tut-4A.* So far, several QTL for adult plant resistance to powdery mildew have been reported on chromosome 4A, however, most of them are of minor effect and can be detected only in specific environments. Two QTL mapped on chromosome 4AS in the regions *Xgwm111 – Xpsp934* and *Xglk128 – Xcdo475* in a segregating wheat/spelt population, explained for 7-14% of the phenotypic variance (Keller et al., 1999). One QTL for adult plant powdery mildew resistance in the winter wheat line RE174 was detected on chromosome 4A (*XgbxG036- XgbxG542*) in the study carried out by Chantret and co-authors (2001). Over two years, the QTL explained for 5-6% of the genetic variability. A minor QTL has been detected in the French wheat cultivar Courtot, between markers *Xgdm61* and *Xgwm397* (Bougot et al., 2006). In southeastern USA, in the soft red winter wheat germplasm AGS 2000, a major *QPm.uga-4A* was mapped in the interval of markers *tPt-4753–wPt-3515,* near the centromere region of chromosome 4A (Hao et al., 2015). The position of the *T. militinae* derived *QPm.tut-4A* does not co-locate with the abovementioned QTL. Only a minor QTL – *QPm.osu-4A*, which was identified in the US hard winter wheat line 2174, was linked to the *Xgwm160* marker, but this QTL was assigned to the most distal part of 4AL (Chen et al., 2009). All QTL detected on chromosome 4A affect adult plant resistance to powdery mildew (Keller et al., 1999; Chantret et al., 2001; Mingeot et al., 2002; Bougot et al., 2006; Chen et al., 2009).

Close to the *Xbarc78* locus, association mapping of historical wheat germplasm in different environments has detected a linkage disequilibrium cluster bearing powdery mildew resistance genes (Crossa et al., 2007). *Xbarc78* is located in the *T. militinae* introgression region of *QPm.tut-4A* on chromosome 4AL.

Two race-specific genes have been mapped in the terminal chromosomal bin 4AL-0.8-1.00 (Geng et al., 2016; Sun et al., 2018). *Pm61*, a recessive gene for resistance to powdery mildew, was detected in the Chinese wheat landrace – Xuxusanyuehuang and was mapped within a 0.46 cM genetic interval between markers *Xgwm160* and *Xicsx79* (Sun et al., 2018). The second *Pm* gene was dominant, and was temporarily designated *MLIW30.* Its location was flanked by SSR markers *XB1g2000.2* and *XB1g2020.2* on a 0.1 cM interval (Geng et al., 2016). *MLIW30* was effective both at seedling and adult plant stages and was derived from a *T. turgidum* ssp. *dicoccoides* accession IW30 (Isreal).

As well as *QPm.tut-4A, MLIW30* confers incomplete resistance rather than immunity to powdery mildew at the seedling and adult plant stages. Both genes originate from the tetraploid wheat species, *T. militinae* (A^tA^tGG) and *T. diccoides* (AABB), respectively. However, wild emmer wheat is the tetraploid ancestor of common wheat (Nevo et al., 2013), while timopheevii wheats originate from an evolutionarily divergent lineage (Dvořák and Zhang, 1990; Dvořák et al., 1993).

The *Pm61, MLIW30, QPm.osu-4A* and *QPm.tut-4A* have been mapped to the same region of chromosome arm 4AL, with *Xgwm160* being a shared common marker. However the *QPm.tut-4A* has been located to a loci proximal to *Xgwm160*, whereas *MLIW30, Pm61* and *QPm.osu-4A* are located distal to *Xgwm160*.

Thus, *QPm.tut-4A* is a new source of resistance to powdery mildew which shows stable effects across all tested environments, its utilization in resistance breeding should be highly valuable.

4.2 The effect of *T. militinae* introgressions in line 8.1 on flowering time and yield-related traits (Publ. III, unpublished data)

Transfer of resistance genes from relative wheat species into cultivated wheat generally involves unpredictable effects on agronomic and quality performance (Buerstmayer et al., 2011; Summers annd Brown, 2013). The introgressed alien fragments often come as large linkage blocks that may carry genes with a potentially negative impact on the traits selected for in the elite varieties.

Plants of the introgression line 8.1 are higher than those of the original spring wheat cv. Tähti. The spike of the 8.1 plants is longer and of lower compactness (speltoid type). Development of 8.1 plants is slower compared to cv. Tähti plants (**Publ. III**).

One of the objectives of the present study was to detect potential associations between the resistance to powdery mildew and agronomically important traits, such as flowering time (FT) and yield-related traits.

4.2.1 Flowering time

FT is an important trait that plays a major role in regional and seasonal adaptation of wheat. FT affects the reproductive success of wheat, and in elite breeding material, it strongly affects yield components (Zhang et al., 2008). Late maturity plants generally have a greater grain yield than early maturity plants, however the late-maturing lines can be adversely affected by biotic and abiotic stresses. Allelic variation of vernalisation (*Vrn*) and photoperiod (*Ppd*) genes is considered to be the main source of flowering time plasticity which enables wheat to grow and successfully flower in different climate zones (Distelfeld et al., 2009).

Using gene-specific primers and genotyping-by-sequencing, *Vrn-A1, Vrn-A3* and *Ppd-A1* genes were mapped in line 8.1 within the regions of *T. militinae* introgressions on chromosomes 5A, 7A and 2A, respectively (**Publ. III**). In addition, the introgression region on chromosome 5A includes the region in which the *Vrn2* gene has been located (Dubcovsky et al., 1998; Yan et al., 2004).

In at least one experiment, QTL analysis detected in the regions of *T. militinae* introgressions significant QTL for FT, indicating that the *T. militinae* alleles of *Vrn-A1, Vrn3, Ppd-A1* and possibly also of *Vrn-A2* may affect flowering time in *T. aestivum* (**Publ. III)**.

In the DH mapping population, the major QTL for FT was located on chromosome 5A in the region delimited by markers *Xgwm666-Xgwm982*, with a peak in the position of the *Vrn-A1* gene. *Vrn-A1*, a gene determining the spring/winter growth habit or vernalization response, has been earlier shown to have a primary effect in controlling ear-emergence time (Kato et al., 1999). The major QTL was responsible for 38-70% of FT variation in different trials. The effect of the major QTL in the region of *Vrn-A1* locus on chromosome 5A was confirmed in two additional mapping populations (**Publ. III**). The *T. militinae* allele of *Vrn-A1* was designated *Vrn-A1f*-like, as its promoter region resembles the *Vrn-A1f* allele from *T. timopheevii* (Golovnina et al., 2010). In field

conditions the replacement of the *Vrn-A1* allele with the *VRN-A1f-like* allele causes a FT delay of 3 to18 days, depending on the genetic background.

Besides the main QTL on chromosome 5A, a significant QTL flanking the major QTL of *Vn-A1* on chromosome 5A and minor significant QTL for FT were identified on four chromosomes: 1A, 2A, 4A and 7A (**Publ. III**). Despite the fact that the introgression line 8.1 flowered later than cvs. Tähti and Mooni in all experiments, not all *T. militinae* alleles in the detected QTL increased the flowering time. While the *Vrn-A1f-like* allele, as well as (to a lesser extent) the *T. militinae* allele of *Vrn3* delayed flowering in the common wheat background, the *T. militinae* alleles of *Ppd-A1*, as well as the significant QTL flanking the major QTL of *Vn-A1* on chromosome 5A, showed an opposite effect, accelerating flowering by 1-2 days each (**Publ. III**). However, the minor QTL accelerating the flowering were only detected in the DH population. Further investigations involving larger populations and/or additional genetic backgrounds are needed to prove these QTL, which, if verified, could find an application in wheat breeding.

4.2.2 Yield-related traits

Yield-related traits such as plant height and spike morphology are important traits not only of the plant architecture but also of the wheat crop performance (Flintham et al., 1997; Wang and Li, 2008; Youssefian et al., 1992; Liu et al., 2018). Spike morphology has direct impacts on grain number and grain size, two main components of grain yield (Jantasuriyarat et al., 2004). There is a negative correlation between plant height and yield (Law et al., 1973). Shorter plants exhibit improved assimilate partitioning to developing spikes, resulting in a higher rate of floret survival, increased grain number per spike, and improved harvest index (Flintham et al., 1997; Youssefian et al., 1992). In common wheat, plant height is associated with lodging, tall plants being much more susceptible to lodging, particularly when plants are grown under high fertilizer regimes (Hedden, 2003).

In order to evaluate the possible impact of *T. militinae* introgressions on agronomically impotant traits, a preliminary QTL analysis of a selection of morphological traits was carried out in the mapping population of 113 DH lines tested for FT in Estonia in 2009 (**Publ. III**). It was shown that all *T. militinae*-origin introgressions in line 8.1 are involved in modification of some morphological trait (Posti, 2008; **unpublished**), altogether 18 significant and highly significant QTL for morphological traits were detected (Tabl. 1). Plant morphology was most affected by the large *T. militinae*-origin introgression on chromosome 5A, with both negative and positive effects on wheat crop performance. The introgression region where the main QTL for powdery mildew resistance on chromosome 4A (*QPm.tut-4A*) was detected had a relatively low influence on spike length. The detected QTL explained about 6% of trait variation, however, the recombination in the region was suppressed, and additional efforts are needed to evaluate the possible link between the resistance locus *QPm.tut-4A* and the crop performance.

Table 1. *QTL for yield-related traits detected in the DH mapping population*

Trait	Chr	Closest marker/or marker interval	R^2 (%)	*P*	Additive effect	Allele increasing trait score
Plant height	5A	*Xgwm415-Xgwm639*	9	***	5,5	mil
	5A	*Xcfa2185-Xgwm0982/Xcfa2155*	23	***	7.52	mil
	2AS	*PpdA*	4	*	2,5	mil
	7A	*Xpsp3050-Xwmc96*	10	***	- 4,83	Tä
Spike length	5A	*Xgwm415-Xgwm639*	10	***	0,72	mil
	5A	*VrnA1*	12	***	- 0,7 ä	Tä
	5A	*Xgwm0982/Xcfa2155*	24	***	0,91	mil
	5A	*Xgwm6-Xgwm410*	24	***	1,02	mil
	4A	*Xgwm160*	**6**	**	- 0,61	Tä
	7A	*Xpsp3050-Xwmc96*	7	**	- 0,57	Tä
	5B	*Xgwm213*	5	*	- 0,33	Tä
	unknown	*Xgwm610*	8	**	- 0,56	Tä
Spikelet No/ear	5A	*Xgwm319-Xgwm0982/Xcfa2155*	11	***	0,7	mil
	1B	*Xpsp3000*	5	*	- 0,42	Tä
	1A	*Xgwm33*	4	*	- 0,47	Tä
Spike density	5AL	*Xgwm6-Xgwm126*	15	***	- 1,57	Tä
	5B	*Xgwm358-Xgwm213*	7	**	0,95	mil
	unknown	*Xgwm610*	10	**	1,15	mil
1000 kernel weight	5A	*Xgwm415-Xbarc180*	12	***	2,44	mil
	5A	*Xgwm0982/Xcfa2155-Xgwm179*	17	***	- 3,07	Tä
	1A	*Xpsp2999*	11	**	- 2,2	Tä
	2AL	*Xgwm382*	6	*	1,56	mil
	5B	*Xgwm213*	8	**	- 1,9	Tä

R2 - percentage of variance explained by individual QTL;
add. - additive effect due to a substitution of a Tähti (Tä) allele by the corresponding *T. militinae* (mil) allele in the line 8/1;
P - level of significancy: * P<0.05; ** P<0.01; *** P<0.0001

CONCLUSIONS

The study revealed the polygenic nature of *Bgt* (*Blumeria graminis tritici*) resistance in the common wheat line 8.1 derived from a cross between tetraploid species *Triticum militinae* and common wheat cultivar Tähti. Underlying the resistance phenotype in line 8.1 in the seedling stage of plant growth, quantitative trait loci (QTL) for resistance were identified as introgessions from *T. militinae* on chromosomes 1A, 4A, 5A, 5B, and 7A. Three of the introgressions (on chromosomes 4A, 5A and 7A) also carry a QTL for adult plant resistance to powdery mildew.

Both in seedlings and in adult plants, the *QPm.tut-4A,* located in the distal part of chromosome arm 4AL, was identified as the locus most effectively improving resistance to powdery mildew. *QPm.tut-4A* explained 35-49% and 33-52% of resistance variance in seedlings and adult plants, respectively.

QPm.tut-4A is effective upon inoculation with all tested *Bgt* isolates and mixtures and very likely confers race-non-specific resistance.

The *T. militinae*-origin *QPm.tut-4A* provides partial resistance to *Bgt*. The detected minor QTL additionally improve the resistance, but their presence is not required for the activity of *QPm.tut-4A*. Transferability of the *QPm.tut-4A* to different genetic backgrounds was confirmed in the study.

In introgressive wheat line 8.1, the *QPm.tut-4A* locus was mapped to a 480 kbp region of the segment between markers *owm169* and *owm228* introgressed into chromosome 4A from *T. militinae*. The SSR markers *Xgwm160* and *Xgwm832* flank the region and should provide a useful tool for marker-assisted selection (MAS).

QTL analysis of flowering time (FT) in a DH mapping population generated from the cross (line 8.1 x cv. Tähti) led to the identification of five loci originating from *T. militinae* and affecting the plants' flowering time. The QTL of the most significant effect was located on chromosome 5A in the region of *Vrn-A1* gene where the *T. militinae*-origin *Vrn-A1f*-like allele significantly delayed flowering.

QTL for morphological traits (plant height, spike length and density, and 1000-kernel-weight) were detected in regions of the *T. militinae* introgressions in line 8.1. A QTL for a spike length was detected in the region of *QP.tut-4A* on chromosome 4A. *T. militinae* introgressions that can positively affect wheat yield were detected in line 8.1. Additional analysis is needed to better evaluate the effect of the introgressed regions on wheat performance.

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 QTL.

QPm.tut-4A is now included in resistance breeding of spring wheat in Estonia.

REFERENCES

Abrouk M, Balcárková B, Šimková H, Komínkova E, Martis M M, Jakobson I, Timofejeva L, Rey E, Vrána J, Kilian A, Järve K, Doležel J, Valárik M. (2017). The in silico identification and characterization of a bread wheat/*Triticum militinae* introgression line. *Plant Biotechnol J* 15: 249–256.

Acevedo-Garcia J, Spencer D, Thieron H, Reinstädler A, Hammond-Kosack K, Phillips AL, Panstruga R. (2017). mlo-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach. *Plant Biotechnol J* 15(3): 367–378.

Alam MA, Xue F, Wang C, Ji W. (2011). Powdery mildew resistance genes in wheat: identification and genetic analysis. *J Mol Bio Res* 1(1): 20-39.

Allard RW and 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.

Allard RW. (1949). A cytogenetic study dealing with the transfer of genes from *Triticum timopheevii* to common wheat by backcrossing. *Acad J Agric Res*78: 33-64.

Anderson JA, Chao S, Liu S. (2007). Molecular breeding using a major QTL for Fusarium head blight resistance in wheat. *Crop Sci* 47: 112–119.

Arya P, Kumar G, Acharya V, Singh AK. (2014). Genome-wide identification and expression analysis of NBS-encoding genes in *Malus* x *domestica* and expansion of NBS genes family in Rosaceae. *PLoS One* 9: e107987.

Asad MA, Bai B, Lan C, Yan J, Xia X, Zhang Y, He Z. (2014). Identification of QTL for adult-plant resistance to powdery mildew in Chinese wheat landrace Pingyuan 50. *Crop J*: 308–314.

Asad MA, Bai B, Lan CX, Yan J, Xia XC, Zhang Y, He ZH. (2012). Molecular mapping of quantitative trait loci for adult-plant resistance to powdery mildew in Italian wheat cultivar Libellula. *Crop Pasture Sci* 63: 539–546.

Asad MA, Bai B, Lan CX, Yan J, Xia XC, Zhang Y, He ZH. (2013). QTL mapping for adult plant resistance to powdery mildew in Italian wheat cv. Strampelli. *J Integr Agric* 5: 756–764.

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu W-L, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J. (2002). MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature* 415, 977–983.

Asea G, Vivek BS, Lipps PE, Pratt RC. (2012). Genetic gain and cost efficiency of marker-assisted selection of maize for improved resistance to multiple foliar pathogens. *Mol Breeding* 29: 515–527.

Asíns MJ. (2002). Present and future of quantitative trait locus analysis in plant breeding. *Plant Breeding* 121: 281–291.

Assefa S and Fehrman H. (2000). Resistance to wheat leaf rust in *Aegilops tauschii* Coss. and inheritance of resistance in hexaploid wheat. *Genet Resour Crop Evol* 47: 135–140.

Assefa S and Fehrman H. (2004). Evaluation of *Aegilops tauschii* Coss. for resistance to wheat stem rust and inheritance of resistance genes in hexaploid wheat. *Genet Resour Crop Evol* 51: 663–669.

Atkins RE. (1967). Inheritance of reaction to race 15B of wheat stem rust in derivatives of Frontana and *Triticum timopheevii* Zhuk. *Iowa State J Sci* 41: 305–311.

Ayliffe MA and Lagudah ES. (2004). Molecular genetics of disease resistance in cereals. *Annals of Botany* 94: 765–773.

Badaeva ED and Gill BS. (1995). Spontaneous chromosome substitutions in hybrids of *Triticum aestivum* with *T. araraticum* detected by C-banding technique. *Wheat Inf Serv* 80(1): 26–31.

Badaeva ED, Badaev NS, Enno TM, Zeller FJ, Peusha HO. (1995b). Chromosome substitution in progeny of hybrids *Triticum aestivum* x *Triticum timopheevii*, resistant to brown rust and powdery mildew. *Russ J Genet* 31: 75–77.

Badaeva ED, Badaev NS, Gill BS, Filatenko AA. (1994a). Intraspecific karyotype divergence in *Triticum araraticum (Poaceae)*. *Plant Syst Evol* 192: 117–145.

Badaeva ED, Boguslavskyi RL, Badaev NS. (1988). Cytogenetical investigation of cereals. Tetraploid wheats of Zanduri group. *Genetika* 24(8): 1411–1418 (in Russian).

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

Badaeva ED, Budashkina EB, Bilinskaya EN, Pukhalskiy VA. (2010). Intergenomic chromosome substitutions in wheat interspecific hybrids and their use in the development of genetic nomenclature of *Triticum timopheevii* chromosomes. *Russ J Genet* 46(7): 769–785.

Badaeva ED, Filatenko AA, Badaev NS. (1994b). Cytogenetic investigation of *Triticum timopheevi* (Zhuk.) Zhuk. and related species using the C-banding technique. *Theor Appl Genet* 8: 622–628.

Badaeva ED, Jiang J, Gill BS. (1995a). Detection of intergenomic translocations with centromeric and noncentromeric breakpoints in *Triticum araraticum*: mechanism of origin and adaptive significance. Genome 38: 976–981.

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). *Genetika* 36: 1663–1673 (in Russian).

Badaeva ED, Ruban AS, Zoshchuk SA,Surzhikov SA, Knüpffer H, Kilian B. (2016). Molecular cytogenetic characterization of *Triticum timopheevii* chromosomes provides new insight on genome evolution of *T. zhukovskyi*. *Plant Syst Evol* 302(8): 943–956.

Bai B, He ZH, Asad MA, Lan CX, Zhang Y, Xia XC, Yan J, Chen XM, Wang CS. (2012). Pyramiding adult-plant powdery mildew resistance QTLs in bread wheat. *Crop Pasture Sci* 63(7): 606–611.

Bai D, Knott DR, Zale J. (1998). The transfer of leaf resistance from *Triticum timopheevii* to durum and bread wheat and the location of one gene on chromosome 1A. *Can J Plant Sci* 78: 683–687.

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA. (2001). Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. *Aust J Agri. Re*. 52: 1247–1255.

Beavis WB. (1998). QTL analyses: power, precision, and accuracy. In: Patterson AH (ed). Molecular dissection of complex traits. CRC Press, Boca Raton.

Bennett FGA (1984). Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. *Plant Pathol* 33(3): 279–300.

Benschop JJ, Mohammed S, O'Flaherty M, Heck AJR, Slijper M, Menke FKH. (2007). Quantitaitve phosphoproteomics of early elicitor signaling in Arabidopsis. *Mol Cell Proteomics* 6: 1198–1214.

Bevan M. Bent E, Love K, Goodman H, Dean C, Bergkamp R, Dirkse W, et al. (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. *Nature* 391: 485–488.

Bhullar NK , Zhang Z, Wicker T, Keller B. (2010). Wheat gene bank accessions as a source of new alleles of the powdery mildew resistance gene *Pm3*: a large scale allele mining project. *BMC Plant Biol* 10: 88–100.

Bhullar NK, Street K, Mackay M, Yahiaoui N, Keller B. (2009).Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus**.** *Proc Natl Acad Sci* 106: 9519–9524.

Boller T and Felix G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu Rev Plant Biol* 60: 379–406.

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder S, Weber WE. (2002). Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theor Appl Genet* 105: 921–936.

Bougot Y, Lemoine J, Pavoine MT, Guyomar'ch H , Gautier V, Muranty H, Barloy D. (2006). A major QTL effect controlling resistance to powdery mildew in winter wheat at the adult plant stage. *Plant Breeding* 125: 550–556.

Bouktila D, Khalfallah Y, Habachi-Houimli Y, Mezghani-Khemakhem M, Makni M, Makni H. (2014). Large-scale analysis of NBS domain-encoding resistance gene analogs in Triticeae. *Genet Mol Biol* 37: 598–610.

Bourras S, McNally KE, BenDavid R, Parlange F, Roffler S, Praz CR, Oberhaensli S, Menardo F, Stirnweis D, Frenkel Z, Schaefer LK, Flückiger S, Treier G, Herren G, Korol AB, Wicker T, Keller B. (2015). Multiple avirulence loci and allele-specific effector recognition control the *Pm3* race-specific resistance of wheat to powdery mildew. *Plant Cell* 27: 2991–3012.

Bourras S, McNally KE, Müller MC, Wicker T, Keller B. (2016). Avirulence genes in cereal powdery mildews: The gene-for-gene hypothesis 2.0. *Front Plant Sci* 7: 241–247.

Breen J and Bellgard M. (2010). Germin-like proteins (GLPs) in cereal genomes: gene clustering and dynamic roles in plant defence. *Funct Integr Genomics* 10: 463–476.

Briggle LW. (1966). Three loci in wheat involving resistance to *Erysiphe graminis* f. sp*. tritici*. *Crop Sci*: 6: 461–465.

Brown-Guedira GL, Gill BS, Bockus WW, Cox TS, Hatchett JH, Leath S, Peterson CJ, Thomas JB, Zwer PK. (1996a). Evaluation of a collection of wild timopheevi wheat for resistance to disease and arthropod pests. *Plant Dis* 80: 928–933.

Brown-Guedira GL, Gill BS, Cox TS, Leath S. (1997). Transfer of disease resistance genes from *Triticum araraticum* to common wheat. *Plant Breeding* 116: 105–112.

Brown-Guedira GL, Singh S, Fritz AK. (2003). Performance and mapping of leaf rust resistance transferred to wheat from *Triticum timopheevii* subsp. a*rmeniacum. Phytopathology* 93(7): 784–789.

Brown-Guerdira GL, Badaeva ED, Gill BS. (1996b). Chromosome substitutions of *Triticum timopheevii* in common wheat and some observations on the evolution of polypioid wheat species. *Theor Appl Genet* 93: 1291–1298.

Budashkina EB and Kalinina NP. (2001). Development and genetic analysis of common wheat introgressive lines resistant to leaf rust. *Acta Phytopathol. Entomol. Hungarica* 36: 61–65.

Buerstmayr M, Lemmens M, Steiner B, Buerstmayr H. (2011). Advanced backcross QTL mapping of resistance to Fusarium head blight and plant morphological traits in a *Triticum macha* × *T. aestivum* population. *Theor Appl Genet* 123: 293–306.

Burch-Smith TM, Schiff M, Caplan JL, Tsao J, Czymmek K, et al. (2016). A novel role for the TIR domain in association with pathogen-derived elicitors. *PLOS Biology* 14(1): e1002374.

Burdon JJ, Barrett LG, Rebetzke G, Thrall PH. (2014). Guiding deployment of resistance in cereals using evolutionary principles. Evolutionary Applications published by John Wiley & Sons Ltd 7 609–624.

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Töpsch S, Vos P, Salamini F, Schulze-Lefert P. (1997). The barley *Mlo* gene: A novel control element of plant pathogen resistance. *Cell* 88: 695–705.

Büttner M. (2010). The *Arabidopsis* sugar transporter (AtSTP) family: an update. *Plant Biol* 12, 35–41.

Canady MA, Ji Y, Chetelat RT. (2006). Homeologous recombination in *Solanum lycopersicoides* introgression lines of cultivated tomato. *Genetics* 174(4): 1775–1788.

Cao W, Fedak G, Armstrong K, Xue A, Savard ME. (2009). Registration of spring wheat germplasm TC 67 resistant to Fusarium head Blight. *Journal of Plant Registrations* 3: 104–106.

Cao W, Hughes GR, Ma H, Dong Z. (2001). Identification of molecular markers for resistance to *Septoria nodorum* blotch in durum wheat. *Theor Appl Genet* 102: 551–554.

Casselman AL, Vrebalov J, Conner JA, Singhal A, Giovannoni J, Nasrallah ME, Nasrallaha JB. (2000). Determining the physical limits of the *Brassica* S locus by recombinational analysis. *Plant Cell* 12: 23–33.

Century KS, Holub EB, Staskawicz BJ. (1995). *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. *PNAS* 92(14): 6597–6601.

Cesari S, Kanzaki H, Fujiwara T, Bernoux M, Chalvon V, Kawano Y, Shimamoto K, Dodds P, Terauchi R, Kroj T. (2014). The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. *The EMBO J* 33: 1941–1959.

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

Chao S, Zhang W, Akhunov E, Sherman J, Ma Y, Luo M-C, Dubcovsky J. (2009). Analysis of gene-derived SNP marker polymorphism in US wheat (*Triticum aestivum* L.) cultivars. *Mol Breeding*23: 23–33.

Chemayek B, Bansal U, Qureshi N, Zhang P, Wagoire WW, Bariana HS. (2017). Tight repulsion linkage between *Sr36* and *Sr39* was revealed by genetic, cytogenetic and molecular analyses. *Theor Appl Genet* 130: 587–595.

Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ. (1995). Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. *Theor Appl Genet* 91: 1125–1128.

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 Breeding* 124: 225–228.

Chen Y, Hunger RM, Carver BF, Zhang H, Yan L. (2009). Genetic characterization of powdery mildew resistance in US hard winter wheat. *Mol Breeding* 24: 141–152.

Chevre AM, Jahier J, Trotter M. (1989). Expression of disease resistance genes in amphiploid wheats-*Triticum tauschii* Coss. *Cereal Resour Comm* 17: 23–29.

Chisholm ST, Coaker G, Day B, Staskawicz BJ. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124: 803–814.

Christiansen MJ, Andersen SB, Ortiz R (2002). Diversity changes in an intensively bred wheat germplasm during the 20th century. *Mol Breeding* 9: 1–11.

Clair DASt. (2010). Quantitative disease resistance and quantitative resistance loci in breeding. *Ann Rev Phytopathol* 48: 247–268.

Collard BCY and Mackill DJ. (2008).Marker assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos Trans R Soc B* 363: 557–572.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* 142: 169–196.

Conner RL, Kuzyk AD, Su H. (2003). Impact of powdery mildew on the yield of soft white spring wheat cultivars. *Can J Plant Sci* 83: 725–728.

Cook DE, Lee TG, Guo X, Melito S, Wang K, Bayless AM, Wang J, Hughes TJ, Willis DK, Clemente TE, Brian W. Diers BW, Jiming Jiang J, Hudson ME, Bent AF. (2012). Copy number variation of multiple genes at *Rhg1* mediates nematode resistance in soybean. *Science* 338: 1206–1209.

Cook DE, Mesarich CH, Thomma BP. (2015). Understanding plant immunity as asurveillance system to detect invasion. *Ann Rev Phytopathol* 53: 541–563.

Cowger CH, Miranda L, Griffey C, Hall M, Murphy JP. (2012). Wheat powdery mildew. In: Disease Resistance in Wheat. (ed) Sharma I. Wallingford, Oxfordshire; Cambridge, MA: CABI. pp 84–119.

Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R. (2007). Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics* 177: 1889–1913.

Cunfer BM. (2002). Powdery mildew. In: Bread wheat. Improvement and production. (ed): Curtis BC, Rajaram S, Macpherson GH, Food and agriculture organization of the united nations, Rome. www.fao.org/docrep/006/y4011e/y4011e0l.htm

Dangl JL and Jones JDG. (2001). Plant pathogens and integrated defence responses to infection. *Nature* 411: 826–833.

Das G and Rao GJ. (2015). Molecular marker assisted gene stacking for biotic and abioticstress resistance genes in an elite rice cultivar. *Front Plant Sci* 6: 698.

Das MK and Griffey CA. (1994). Heritability and number of genes governing adult-plant resistance to powdery mildew in Houser and Redcoat winter wheats. *Phytopathology* 84 : 406–409.

Dekaprelevich LL. (1954). Species, varieties and cultivars of Georgian wheats. *Proc Georgictrt Acad Sci* 8: 361 (in Russian).

Devos KM, Dubcovsky J, Dvořák J, Chinoy CN, Gale MD. (1995). Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. *Theor Appl Genet* 91: 282–288.

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.

Devoto A, Piffanelli P, Nilsson I, Wallin E, Panstruga R, von Heijne G, Schulze-Lefert P. (1999). Topology, subcellular localization, and sequence diversity of the Mlo family in plants. *J Biol Chem* 274: 34993–35004.

Diener AC and Ausubel FM. (2005*). Resistance to Fusarium Oxysporum 1*, a dominant Arabidopsis disease-resistance gene, is not race specific. *Genetics* 171(1): 305–321.

Distelfeld A, Li C, Dubcovsky J. (2009). Regulation of flowering in temperate cereals. *Curr Opin Plant Biol* 12: 178–184.

Dobrovolskaya O, Boeuf C, Salse J, Pont C, Sourdille P, Bernard M, Salina E. (2011). Microsatellite mapping of *Ae. speltoides* and map-based comparative analysis of the S, G, and B genomes of Triticeae species. *Theor Appl Genet* 123: 1145–1157.

Dobrovolskaya OB, Sourdille P, Bernard M, Salina EA. (2009). Chromosome synteny of the A genome of two evolutionary wheat lines. *Russ J Genet* 45: 1368–1375.

Dodds PN and Rathjen JP. (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet* 11: 539–548.

Doerge RW. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nat. Rev. Genet*. 3: 43–52.

Dolezel J, Vrana J, Capal P, Kubalakova M, Buresova V, Simkova H. (2014). Advances in plant chromosome genomics. *Biotechnol. Adv* 32, 122–136.

Dorofeyev VF, Jakubtsiner MM, Rudenko MI, Migushova EF, Udachin RA, Merezhko AF, Semenova LV, Novikova MV, Gradchaninova OD, Shitova IP. (1976). The wheats of the world. (eds.) Brezhnev DD, Dorofeyev VF, Kolos Publ, Leningrad, 487 pp. (in Russian).

Douchkov D, Lück S, Johrde A, Nowara D, Himmelbach A, Rajaraman J, Stein N, Sharma R, Kilian B, Schweizer P. (2014). Discovery of genes affecting resistance of barley to adapted and non-adapted powdery mildew fungi. *Genome Biol* 15: 518.

Dubcovsky J, Lijavetzky D, Appendino L, Tranquilli G. (1998). Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. *Theor Appl Genet* 97: 968–975.

Duveiller E, Singh RP, Nicol JM. (2007). The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. *Euphytica* 157: 417–430.

Dvorák J and Zhang HB. (1990). Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proc Natl Acad Sci USA* 87: 9640–9644.

Dvořák J, di Terlizzi P, Zhang HB, Resta P. (1993). The evolution of polyploid wheats: identification of the A genome donor species. *Genome* 36: 21–31.

Dvořák J. (1983). The origin of wheat chromosomes 4A and 4B and their genome reallocation. *Can J Gen Cytol* 25(3): 210–214.

Dyck PL. (1992). Transfer of a gene for stem rust resistance from *Triticum araraticum* to hexaploid wheat. *Genome* 35, 788–792.

Elliott C, Zhou F, Spielmeyer W, Panstruga R, Schulze-Lefert P.(2002). Functional conservation of wheat and rice *mlo* orthologs in defense modulation to the powdery mildew fungus. *Mol Plant-Microbe Interac* 15: 1069–1077.

Ellur RK, Khanna A, Yadav A, Pathania S, Rajashekara H, Singh VK, Krishnan SG, Bhowmick PK, Nagarajan M, Vinod KK, Prakash G, Mondal KK, Singh NK, Prabhu KV, Singh AK (2016). Improvement of Basmati rice varieties for resistance to blast and bacterialblight diseases using marker assisted backcross breeding. *Plant Sci*. 24: 2330–2341.

Enno T, Peusha H, Timofeyeva L, Tohver M, Yakobson I, Priilinn O. (1998). Identification of chromosomal translocations in common wheat, derivative of *Triticum timopheevii*. *Acta Agronomica Hung* 46: 209–216.

Eulgem T. (2005). Regulation of the Arabidopsis defense transcriptiome. *Trends Plant Sci* 10: 71–78.

Everts KL and Leath S. (1992). Effect of early season powdery mildew on development, survival and yield contribution of tillers of winter wheat. *Phytopathology* 82: 1273–1278.

Everts KL, Leath S, Finney PL. (2001). Impact of powdery mildew and leaf rust in milling and bakong quality of soft red winter wheat. *Plant Dis* 85: 423–429.

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS. (1999). Candidate gene analysis of quantitative disease resistance in wheat. *Theor Appl Genet* 98: 219–225.

Federico ML, Iñiguez-Luy FL, Skadsen RW, Kaeppler HF. (2006). Spatial and temporal divergence of expression in duplicated barley germin-like protein-encoding genes. *Genetics* 174(1): 179–190.

Feldman M. (1977). Historical aspects and significance of the discovery of wild wheats. University of Missouri, Columbia, Stadler Symp 9: 121–145.

Feldtnan M. (1966). Identification of unpaired chrornosornes in F1 hybrids involving *T. aestiwrm* and *T. timopheevii*. *Can J Genet Cytol* 8: 144–151.

Feuillet C, Langridge P, Waugh R. (2008). Cereal breeding takes a walk on the wild side. *Trends Genet* 24: 24–32.

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B. (2003). Mapbased isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *P. Natl Acad. Sci. USA* 100: 15253–15258.

Flintham JE, Börner A, Worland AJ, Gale MD. (1997). Optimizing wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes. *J Agric Sci* 128: 11–25.

Flor HH. (1971). Current status of gene for gene concept. *Annu Rev Phytopathol* 9: 275–296.

Food Agric. U.N. 2015. FAOSTAT database. Rome: FAO. http://faostat.fao.org/site/291/default.aspx

Frank JA and Ayers JE. (1986). Effect of triadimenol seed treatment on powdery mildew epidemics on winter wheat. *Phytopathology* 76: 254–257.

Freialdenhoven A, Peterhansel C, Kurth J, Kreuzaler F, Schulze-Lefert P. (1996). Identification of genes required for the function of non-race-specific mlo resistance to powdery mildew in barley. *Plant Cell* 8: 5–14.

Freialdenhoven A, Scherag B, Hollricher K, Coilinge DB, Thordal-Christensen H, Schulze-Lefert P. (1994). *Nar-l* and *Nar-2*, two loci required for Mla_{12} specified race-specific resistance to powdery mildew in barley. *Plant Cell* 6: 983–994.

French E, Kim B-S, Iyer-Pascuzzi A S. (2016). Mechanisms of quantitative disease resistance in plants. *Sem Cell Dev Biol* 56: 201–208.

Friebe B, Jiang JM, Raupp WJ, McIntosh RA, Gill BS. (1996). Characterization of wheat alien translocations conferring resistance to diseases and pests: current status. *Eyphytica* 91: 59–87.

Frye CA and Innes RW. (1998). An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell* 10: 947–956.

Frye CA, Tang D, Innes RW. (2001). Negative regulation of defense responses in plants by a conserved MAPKK kinase. *P Natl Aca Sci USA* 98: 373–378.

Fu BS, Chen Y, Li N, Ma HQ, Kong ZX, Zhang LX, Jia HY, Ma ZQ (2013). *pmX*: a recessive powdery mildew resistance gene at the *Pm4* locus identified in wheat landrace Xiaohongpi. *Theor Appl Genet* 126: 913–921.

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J. (2009). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science* 323: 1357–1360.

Fu J, Liu H, Li Y, Yu H, Li X, Xiao J, Wang S. (2011). Manipulating broad-spectrum disease resistance by suppressing pathogen-induced auxin accumulation in rice. *Plant Physiol* 155: 589–602.

Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K. (2009). Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* 325: 998–1001.

Fukuoka S, Saka N, Mizukami Y, Koga H, Yamanouchi U, Yoshioka Y, Hayashi N, Ebana K, Mizobuchi R, Yano M. (2015). Gene pyramiding enhances durable blast disease resistance in rice. *Plant Physiol* 5: 7773–7779.

Fukuoka S, Yamamoto S-I, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N, Yasuda N, Fujita Y, Nguyen TTT, Koizumi S, Sugimoto K, Matsumoto T, Yano M. (2014). Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. *Sci Rep* 4: 4550–4556.

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM. (1995). Genetic maps of hexaploid wheat. *Proceedings of the 8th International Wheat Genetics Symposium*, China Agricultural Scientech Press, Beijing, China 1: 29–40.

Geng M, Zhang J, Peng F, Liu X, Lv X, Mi Y, Li Y, Li F, Xie C, Sun Q. (2016). Identification and mapping of *MLIW30*, a novel powdery mildew resistance gene derived from wild emmer wheat. *Mol Breeding* 36: 130.

German SE and Kolmer JA. (1992). Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. *Theor Appl Genet* 84(1–2): 97–105.

Gill BS, Friebe BR, White FF. (2011). Alien introgressions represent a rich source of genes for crop improvement. *PNAS* 108(19): 7657–7658.

Gill BS, Huang L, Kuraparthy V, Raupp WJ, Wilson DL, Friebe B. (2008). Alien genetic resources for wheat leaf rust resistance, cytogenetic transfer, and molecular analysis. *Aust J Agric Res* 59: 197–205.

Gill KS, Gill BS, Endo TR, Mukai Y. (1993). Fine mapping of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. *Genetics* 134: 1231–1236.

Gill, B. S, Chen, P. D. (1987). Role of cytoplasrn-specific introgression in the evolution of polyploid wheats. *Proc Natl Acud Sci USA* 84: 6800 6804.

Glawe DA (2008). The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annu Rev Phytopathol* 46: 27–51.

Glazebrook J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol* 43: 205–227.

Godfrey D, Able AJ, Dry IB. (2007). Induction of a grapevine germin-like protein (vvglp3).gene is closely linked to the site of erysiphe necator infection: a possible role in defens? *Mol Plant-Microb Interact* 20(9): 1112–1125.

Golovnina KA, Kondratenko EY, Blinov AG, Goncharov NP. (2010). Molecular characterization of vernalization loci *VRN1* in wild and cultivated wheats. *BMC Plant Biol* 10: 168.

Goncharov NP (2002). Comparative genetics of wheat and their related species. Shumny VK. (ed.) Siberian University Press, Novosibirsk, p 169–176.

Goncharov NP, Bannikova SV, Kawahara T. (2007). Wheat artificial amphiploids involving the *Triticum timopheevii Genome*: their studies, preservation and reproduction. *Genet Resour Crop Evol* 54: 1507–1516.

Gordeeva EI, Leonova IN, Kalinina NP, Salina EA, Budashkina EB (2009). Comparative cytological and molecular analysis of common wheat introgression lines containing genetic material of *T. timopheevii* Zhuk. *Russ J Genet* 45: 1428–1437.

GrainGenes2.0 https://wheat.pw.usda.gov/GG3/maps

Gregory TR, Nicol JA, Tamm H, Kullman B, Kullman K, Leitch IJ, Murray BG, Kapraun DF, Greilhuber J, Bennett MD. (2007). Eukaryotic genome size databases. *Nucleic Acids Res* 35: D332–D338.

Griffey CA, Das MK, Stromberg EL. (1993). Effectiveness of adult-plant resistance inreducing grain yield loss to powdery mildew in winter wheat. *Plant Dis* 77: 618–622.

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G. (2006). Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. *Nature* 439: 749–752.

Gupta PK, Mir RR, Mohan A, Kumar J. (2008). Wheat genomics: present status and future prospects. *Int J Plant Genomics* Article ID 896451, 36 p.

Gustafson GD and Shaner G. (1982). Influence of plant age on the expression of slow-mildewing resistance in wheat. *Phytopathology* 72: 746–749.

Gyarfas J. (1968). Transference of disease resistance from *Triticum timopheevii* to *Triticum aestiwtm*. M.Sc. Thesis. University of Sydney, Australia.

Hajjar R and Hodgkin T. (2007). The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica* 156: 1–13.

Hanusova R, Hsam SLK, Bartos P, Zeller FJ. (1996). Suppression of powdery mildew resistance gene *Pm8* in *Triticum aestivum* L (common wheat) cultivars carrying wheat-rye translocation T1BL.1RS. *Heredity* 77: 383–387.

Hao Y, Liu A, Wang Y, Feng D, Gao J, Li X, Liu S, Wang H. (2008). *Pm23*: a new allele of *Pm4* located on chromosome 2AL in wheat. *Theor Appl Genet* 117: 1205–1212.

Hao Y, Parks R, Cowger C, Chen Z, Wang Y, Bland D, Murphy JP, Guedira M, Brown-Guedira G, Johnson J. (2015). Molecular characterization of a new powdery mildew resistance gene *Pm54* in soft red winter wheat. *Theor Appl Genet* 128: 465–476.

Hautea RA, Coffman WR, Sorrells ME,Bergstrom GC. (1987). Inheritance of partial resistance to powdery mildew in spring wheat. *Theor Appl Genet* 73: 609–615.

Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, Kanamori H, Yamane H, Hayano-Saito Y, Matsumoto T, Yano M, Takatsuji H. (2010). Durable panicle blast-resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J* 64: 498–510.

He H, Zhu S, Zhao R, Jiang Z, Ji Y, Ji J, Qiu D, Li H, Bie T. (2018). *Pm21*, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. *Mol Plant* 11(6):879–882.

He R, Chang Z, Yang Z, Yuan Z, Zhan H, Zhang X, Liu J. (2009). Inheritance and mapping of powdery mildew resistance gene *Pm43* introgressed from *Thinopyrum intermedium* into wheat. *Theor Appl Genet* 118: 1173–1180.

Hedden P. (2003). The genes of the green revolution. *Trends Genet* 19: 5–9.

Heitefuss R. (2001). Defence reactions of plants to fungal pathogens: principles and perspectives, using powdery mildew on cereals as an example. *Naturwissenschaften* 88: 273–283.

Herrera-Foessel SA, Singh RP, Lillemo M, Huerta‐Espino J, Bhavani S, Singh S, Lan C,·Calvo‐Salazar V, Lagudah ES. (2014). *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. *Theor Appl Genet* 127: 781–789.

Holland JB. (2001). Epistasis and plant breding. *Plant Breed Rev* 23 (ed.) Jules Janick.

Hou L, Zhang X, Li X, Jia J, Yang H, Zhan H, Qiao Q, Guo H, Chang Z. (2015). Mapping of powdery mildew resistance gene *pmCH89* in a putative wheat-*Thinopyrum intermedium* introgression line. *Int J Mol Sci* 16: 17231–17244.

Hsam NBO, Kowalczyk K, Zeller FJ, Hsam SLK. (2015). Characterization of powdery mildew resistance and linkage studies involving the *Pm3* locus on chromosome 1A of common wheat (*Triticum aestivum* L.). *J Appl Genetics* 56: 37–44.

Hsam SLK and Zeller FJ. (2002). Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In: Bélanger RR, Bushnell WR, Dick AJ, Carver TLW (eds.).The powdery mildews: A comprehensive treatise. Am. Phytopath. Soc. Press, St. Paul, MN, pp 219–238.

Hsam SLK, Huang XQ, Zeller FJ. (2001). Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). 6. Alleles at the *Pm5* locus. *Theor Appl Genet* 102: 127–133.

Hu K-M, Qiu D-Y, Shen X-L, Li X-H, Wang S-P. (2008a). Isolation and manipulation of quantitative trait loci for disease resistance in rice using a candidate gene approach. *Molecular Plant* 1(5): 786–793.

Hu TZ, Li HJ, Xie CJ, You MS, Yang ZM, Sun QX, Liu ZY. (2008b). Molecular mapping and chromosomal location of the powdery mildew resistance gene in wheat cultivar Tangmai 4. *Acta Agron Sin* 34: 1193–1198 (English abstract).

Hua W, Liu Z, Zhu J, Xie C, Yang T, Zhou Y, Duan X, Sun Q, Liu Z. (2009). Identification and genetic mapping of *Pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). *Theor Appl Genet* 119: 223–230.

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

Huang XQ, Hsam SLK, Mohler V, Röder MS, Zeller FJ. (2004). Genetic mapping of three alleles at the *Pm3* locus conferring powdery mildew resistance in common wheat (*Triticum aestivum* L.). *Genome* 47: 1130–1136.

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V. (2000b). Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. *Theor Appl Genet* 101: 407–414.

Huang XQ, Hsam SLK, Zeller FJ. (2000a). Chromosomal location of powdery mildew resistance genes in Chinese wheat (*Triticum aestivum* L. em Thell.) landraces Xiaobaidong and Fuzhuang 30. *J Genet Breed* 54: 311–317.

Huang XQ, Hsam SLK, Zeller FJ. (2002). Chromosomal location of genes for resistance to powdery mildew in Chinese wheat lines Jieyan 94- 1-1 and Siyan 94-1-2. *Hereditas* 136: 212–218.

Huang XQ, Wang LX, Xu MX, Röder MS. (2003). Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). *Theor Appl Genet* 106: 858–865.

Huard-Chauveau C, Perchepied L, Debieu M, Rivas S, Kroj T, Kars I, Bergelson J, Roux F, Roby D. (2013). An atypical kinase under balancing selection confers broad-spectrum disease resistance in arabidopsis. *PLoS Genet* 9(9): e1003766.

Hulbert SH, Webb CA, Smith SM, Sun Q. (2001). Resistance gene complexes: evolution and utilization. *Annu Rev Phytopathol* 39: 285–312.

Hurni S, Brunner S, Buchmann G, Herren G, Jordan T, Krukowski P, Wicker T, Yahiaoui N, Mago R, Keller B. (2013). Rye *Pm8* and wheat *Pm3* are orthologous genes and show evolutionary conservation of resistance function against powdery mildew. *Plant J* 76: 957–969.

Hurni S, Brunner S, Stirnweis D, Herren G, Peditto D, McIntosh RA, Keller B. (2014). The powdery mildew resistance gene *Pm8* derived from rye is suppressed by its wheat ortholog *Pm3*. *Plant J* 79: 904–913.

Hurni S, Scheuermann D, Krattinger SG, Kessel B, Wicker T, Herren G, Fitze MN, Breen J, Presterl T, Ouzunova M, Keller B. (2015). The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *PNAS* 112(28): 8780-8785.

Innes RL. and Kerber ER. (1994). Resistance to wheat leaf rust and stem rust in *Triticum tauschii* and inheritance in hexaploid wheat of resistance transferred from *T. tauschii. Genome* 37(5): 813–822.

Inoue H, Hayashi N, Matsushita A, Xinqiong L, Nakayama A, Sugano S, Jiang C-J, Takatsuji H. (2013). Blast resistance of CC-NB-LRR protein Pb1 is mediated by WRKY45 through protein–protein interaction. *PNAS* 110(23): 9577–9582.

Inuma T, Khodaparast SA, Takamatsu S. (2007). Multilocus phylogenetic analyses within *Blumeria graminis*, a powdery mildew fungus of cereals. *Mol Phylogenet Evol* 44: 741–751.

IWGSC (International Wheat Genome Sequencing Consortium) (2018) Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. *Science* 361:eaar7191.

Jacob F, Vernaldi S, Maekawa, T. (2013). Evolution and conservation of plant NLR functions. Front Immunol 4: 297.

Jakobson I, Tiidema A, Peusha H, Posti D, Ingver A, Järve K.(2009). QTL analysis of yield-related morphological traits and powdery mildew resistance in an introgressive line of bread wheat. In: “19th International Triticeae Mapping Initiative - 3rd COST Tritigen.“ August 31-September 4, 2009. Clermont-Ferrand, France.

Jakobson I, Islamov B, Peusha H, Tsõmbalova J, Timofejeva L, Kladivová M, Valárik M, Järve K. (2013). Efficiency of the *Triticum militinae*-derived powdery mildew resistance gene *QPm.tut-4A* on different genetic backgrounds. In: The International Conference "Plant Diseases and Resistance Mechanisms", February 20-22, 2013, Vienna, Austria. P37.

Jakubziner MM. (1969). Immunity of different wheat species. *Agricultural Biology* 4: 837–847 (in Russian).

Janáková E, Jakobson I, Peusha H, Abrouk M, Škopová M, Šimková H, Šafář J, Vrána J, Doležel J, Järve K, Valarik M. (2018). Divergence between bread wheat and *Triticum militinae* in the powdery mildew resistance *QPm.tut-4A* locus and its implications for cloning of the resistance gene. *Theor Appl Genet* 132: 1061–1072.

Jantasuriyarat C, Vales MI, Watson CJW, Rieralizarazu O. (2004). Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). *Theor Appl Genet* 108: 261–273.

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

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.

Ji JH, Qin B, Wang HY, Cao AX, Wang SL, Chen PD, Zhuang L, Du Y, Liu DJ, Wang XE. (2008a). STS markers for powdery mildew resistance gene *Pm6* in wheat. *Euphytica* 163: 159–165.

Jia J, Devos KM, Chao S, Miller TE, Reader SM, Gale MD. (1996). RFLP-based maps of the homologous group-6 chromosomes of wheat and their application in the tagging of *Pm12*, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. *Theor Appl Genet* 92: 559–565.

Jiang J and Gill BS. (1994). Different species-specific chromosome translocation in *Triticum timopheevii* and *T. turgidum* support diphyletic origin of polyploid wheats. *Chromosome Res* 2: 59–64.

Jones JD and Dangl JL. (2006). The plant immune system. *Nature* 444: 323–29.

Jorgensen JH and Jensen CJ. (1973). Gene *Pm*6 for resistance to powdery mildew in wheat. *Euphytica* 22: 4–23.

Jorgensen JH. (1992). Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. *Euphytica* 63: 141–152.

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N. (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *PNAS* 103(29): 11086–11091.

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. *Australian Acad J Agric Res* 52: 1079–1088.

Kang YJ, Kim KH, Shim S, Yoon MY, Sun S, Kim MY, Van K, Lee SH. (2012). Genome-wide mapping of NBS-LRR genes and their association with disease resistance in soybean. *BMC Plant Biol* 12: 139.

Kato K, Miura H, Sawada S. (1999). QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. *Theor Appl Genet* 98: 472–477.

Kaur R, Singh K, Singh J. (2013). A root-specific wall-associated kinase gene, *HvWAK1*, regulates root growth and is highly divergent in barley and other cereals. *Funct Integr Genomics* 13(2):167–177.

Kaur S, Jindal S, Kaur M, Chhuneja P. (2018). Utilization of wild species for wheat improvement using genomic approaches. In: Gosal S, Wani S. (eds). Biotechnologies of crop improvement, v 3. Springer, Cham, pp 105–150.

Kawahara T and Tanaka M. (1981): Intraspecific differentiations in chromosome structures of the wild tetraploid wheats. *Wheat Inf Serv* 52: 33.

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 x spelt population. *Theor Appl Genet* 98: 903–912.

Kema GHJ, Lange W, Van Silfhout CH. (1995). Differential suppression of stripe rust resistance in synthetic wheat hexaploids derived from *Triticum turgidum* subsp. *dicoccoides* and *Aegilops squarrosa*. *Phytopathology* 85: 425–429.

Khlestkina EK, Kumar U, Röder MS. (2010). Ent-kaurenoic acid oxidase genes in wheat. *Mol Breeding* 25: 251–258.

Kilian B, Özkan H, Deusch O, Effgen S, Brandolini A, Kohl J, Martin W, Salamini F. (2007). Independent wheat B and G genome origins in outcrossing *Aegilops* progenitor haplotypes. *Molec Biol Evol* 24: 217–227.

Kilian B, Özkan H, Pozzi C, Salamini F. (2009). Domestication of the Triticeae in the Fertile Crescent. In: Muehlbauer GJ, Feuillet C. (eds) Genetics and genomics of the Triticeae. Springer, New York, pp 81–119.

Kim MC, Panstruga R, Elliott C, Müller J, Devoto A, Yoon HW, Park HC, Cho MJ, Schulze-Lefert P. (2002). Calmodulin interacts with MLO protein to regulate defence against mildew in barley. *Nature* 416: 447–450.

Knott DR. (1989). The wheat rusts: Breeding for resistance. Springer-Verlag, Berlin.

Knüpffer H. (2009). Triticeae genetic resources in *ex situ* genebank collections. In: Feuillet C, Muehlbauer GJ (eds) Genetics and genomics of the Triticeae. Springer, Dordrecht, pp 31–79.

Kobylyanskyi VD, Fadeeva TS. (Eds).(1986). *Genetics* of cultivated plants. Cereals. Agropromizdat, Leningrad, 264p. (in Russian).

Kou Y and Wang S. (2010). Broad-spectrum and durability: understanding of quantitative disease resistance. *Curr Opin Plant Biol* 13: 181–185.

Kou Y and Wang S. (2012). Toward an understanding of the molecular basis of quantitative disease resistance in rice. *J Biotechl* 159: 283– 290.

Krattinger SG and Keller B. (2016). Trapping the intruder - immune receptor domain fusions provide new molecular leads for improving disease resistance in plants. *Genome Biol* 17: 23–25.

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 232: 1360–1363.

Kusch S and Panstruga R. (2017). mlo-based resistance: An apparently universal "weapon" to defeat powdery mildew disease. *Mol Plant-Microb Interac* 30: 179–189.

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta- Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B. (2009). Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theor Appl Genet* 119: 889–898.

Laikova LI, Arbuzova VS, Efremova TT, Popova OM. (2004). Resistance to fungal diseases in hybrid progeny from crosses between common wheat variety Saratovskaya 29 and the amphidiploid *Triticum timopheevii/Triticum tauschii* (AAGGDD). *Russ J Genet* 40(9): 1046–1050.

Laikova LI, Belan IA, Badaeva ED, Rosseeva LP, Shepelev SS, Shumny VK, Pershina LA. (2013). Development and study of spring bread wheat variety pmyati maystrenko with introgression of genetic material from synthetic hexaploid *Triticum timopheevii* Zhuk. × *Aegilops tauschii* Coss. *Russ J Genet* 49(1): 89–97.

Lamb C and Dixon RA. (1997). The oxidative burst in plant disease resistance. *Annu Rev Plant Physiol Plant Mol Biol* 48: 251–275.

Lan CX, Liang SS, Wang ZL, Yan J, Zhang Y, Xia XC, He ZH. (2009). Quantitative trait loci mapping for adult-plant resistance to powdery mildew in Chinese wheat cultivar Bainong. *Phytopathology* 99: 1121–1126.

Lan CX, Ni X, Yan J, Zhang Y, Xia XC, Chen X, He ZH. (2010). Quantitative trait loci mapping of adult-plant resistance to powdery mildew in Chinese wheat cultivar Lumai 21. *Mol Breeding* 25: 615–622.

Lane BG. (2002). Oxalate, germins, and higher-plant pathogens. *IUBMB Life* 53(2): 67–75.

Law CN, Snape JW, Worland AJ. (1973). The genetical relationship between height and yield in wheat. *Heredity* 40: 133–151.

Leath S and Bowen KL. (1989). Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. *Phytopathology* 79: 152–155.

Leonova I, Börner A, Budashkina E, Kalinina N, Unger O, Röder M, Salina E. (2004). Identification of microsatellite markers for a leaf rust resistance gene introgressed into common wheat from *Triticum timopheevii*. *Plant Breeding* 123: 93–95.

Leonova IN (2015). Genetic control of the resistance to fungal pathogens in common wheat with introgressions from *Triticum timopheevii* Zhuk. Doctoral Thesis, Novosibirsk (in Russian).

Leonova IN, Budashkina EB, Flath K, Weidner A, Börner A, Röder MS.(2010). Microsatellite mapping of a leaf rust resistance gene transferred to common wheat from *Triticum timopheevii*. *Cereal Res Commun* 38: 211–219.

Leonova IN, Budashkina EB, Kalinina NP, Röder MS, Börner A, Salina EA. (2011). *Triticum aestivum − Triticum timopheevii* introgression lines as a source of pathogen resistance gene. *Czech J Genet Plant Breeding* 47: 49–55.

Leonova IN, Röder MS, Budashkina EB, Kalinina NP, Salina EA. (2002). Molecular analysis of leaf rust resistant introgression lines obtained by crossing of hexaploid wheat *Triticum aestivum* with tetraploid wheat *Triticum timopheevii*. *Russ J Genet* 38(12): 1397–1403.

Leonova IN, Röder MS, Kalinina NP, Budashkina EB. (2008). Genetic analysis and localization of loci controlling leaf rust resistance of *Triticum aestivum* x *Triticum timopheevii* introgression lines. *Russ J Genet* 44(12): 1431–1437.

Li H, Zhou SY, Zhao WS, Su SC, Peng YL. (2009). A novel wall-associated receptor-like protein kinase gene, *OsWAK1*, plays important roles in rice blast disease resistance. *Plant Mol Biol* 69(3): 337–346.

Li J, Ding J, Zhang W, Zhang Y, Tang P, Chen J-Q, Tian D,·Yang S. (2010). Unique evolutionary pattern of numbers of gramineous NBS-LRR genes. *Mol Genet Genomics* 283: 427–438.

Li N, Jia HY, Kong ZX, Tang WB, Ding YX, Liang JC, Ma H, Ma Z. (2017). Identification and marker-assisted transfer of a new powdery mildew resistance gene at the *Pm4* locus in common wheat. *Mol Breeding* 37: 79.

Li W and Gill BS. (2006). Multiple genetic pathways for seed shattering in the grasses. *Funct Integr Genomics* 6: 300–309.

Li WL, Faris JD, Chittor JM, Leach JE, Hulbert SH, Liu DJ, Chen PD, Gill BS. (1999a). Genomic mapping of defense response gene in wheat. *Theor Appl Genet* 98: 226–233.

Li X, Kapos P, ZhangY. (2015). NLRs in plants. *Current Opinion in Immunology* 32: 114–121.

Li Z, Li B, Tong Y. (2008). The contribution of distant hybridization with decaploid *Agropyron elongatum* to wheat improvement in China. *J Genet Genomics* 35: 451–456.

Li ZK, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS. (1999b). A “defeated” rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. *Mol Gen Genet* 261: 58–63.

Liang SS, Suenaga K, He ZH, Wang ZL, Liu HY, Wang DS, Singh RP, Sourdille P, Xia XC. (2006). Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. *Phytopathology* 96: 784–789.

Liao CY, Wu P, Hu B, Yi KK. (2001). Effects of genetic background and environment on QTLs and epistasis for rice (*Oryza sativa* L.)panicle number. *Theor Appl Genet* 103: 104–111.

Lillemo M and Lu Q (2015). A meta analysis of partial resistance loci to powdery mildew in wheat. In: Paper presented at 14th international cereal rusts and powdery mildews conference, Helsingųr, Denmark, 5–8 July 2015.

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å. (2008). The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. *Theor Appl Genet* 116: 1155–1166.

Lillemo M, Bjørnstad A, Skinnes H. (2012). Molecular mapping of partial resistance to powdery mildew in winter wheat cultivar Folke. *Euphytica* 185: 47–59.

Lillemo M, Skinnes H, Brown JKM. (2010). Race specific resistance to powdery mildew in Scandinavian wheat cultivars, breeding lines and introduced genotypes with partial resistance. *Plant Breeding* 129: 297–303.

Liu B, Zhang S, Zhu X, Yang Q, Wu S, et al. (2004). Candidate defense genes as predictors of quantitative blast resistance in rice. *Mol Plant-Microbe Interact* 17: 1146–1152.

Liu DJ, Liu JY, Tao WJ, Chen PD. (1998). Molecular markers and breeding wheat for powdery mildew resistance. *Proceedings 9th International Wheat Genetics Symposium*, A.E. Slinkard (Ed), University of Saskatchewan, 3: 128–131.

Liu J, Liu D, Tao W, Li W, Wang S, Chen P, Cheng S, Gao D. (2000). Molecular marker facilitated pyramiding of different genes for powdery mildew resistance in wheat. *Plant Breeding* 119: 21–24.

Liu J, Liu X, Dai L, Wang G. (2007). Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. *J Genet Genom* 34: 765–776.

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.

Liu WX, Koo DH, Xia Q, Li CX, Bai F, Song YL, Friebe B, Gill BS. (2017). Homoeologous recombination-based transfer and molecular cytogenetic mapping of powdery mildew resistant gene *Pm57* from *Aegilops searsii* into wheat. *Theor Appl Genet* 130: 841–848.

Lou Y and Baldwin IT. (2006). Silencing of a germin-like gene in nicotiana attenuata improves performance of native herbivores. *Plant Physiol* 140(3): 1126–1136.

Loutre C, Wicker T, Travella S, Galli P, Scofield S, Fahima T, Feuillet C, Keller B. (2009). Two different CC-NBS-LRR genes are required for *Lr10*-mediated leaf rust resistance in tetraploid and hexaploid wheat. *Plant J* 60: 1043–1054.

Lu Q, Bjørnstad A, Ren Y, Asad MA, Xia X, Chen X, Ji F, Shi J, Lillemo M. (2012). Partial resistance to powdery mildew in German spring wheat 'Naxos' is based on multiple genes with stable effects in diverse environments. *Theor Appl Genet* 125: 297–309.

Lu Y, Yao M, Zhang J, Song L, Liu W, Yang X, Li X, Li L. (2016). Genetic analysis of a novel broad-spectrum powdery mildew resistance gene from the wheat-*Agropyron cristatum* introgression line Pubing 74. *Planta* 244(3): 713–723.

Lukaszewski AJ. (2015). Introgressions between wheat and rye. In: Molnįr-Lįng M, Ceoloni C, Doležel J (eds). Alien introgression in wheat. Springer, New York, pp 163–190.

Luo PG, Luo HY, Chang ZJ, Zhang HY, Zhang M, Ren ZL. (2009). Characterization and chromosomal location of *Pm40* in common wheat: a new gene for resistance to powdery mildew derived from *Elytrigia intermedium*. *Theor Appl Genet* 118: 1059–1064.

Lutz J, Hsam LK, Limpert E, Zeller FJ.(1994). Powdery mildew resistance in *Aegilops tauschii* Coss. and synthetic hexaploid wheats. Genetic Resources and Crop Evolution. *Genet Resour Crop Evol* 41: 151.

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 Breeding* 108: 33–39.

Ma H and Hughes GR. (1995). Genetic control and chromosomal location of *Triticum timopheevii*-derived resistance to *Septoria nodorum* blotch in durum wheat. *Genome* 7:91–97.

Ma H, Singh RP, Mujeeb-Kazi A. (1995).Suppression/ expression of resistance to stripe rust in synthetic hexaploid wheat (*Triticum turgidum* x *Triticum tauschii*). *Euphytica* 80: 87–93.

Ma P, Xu H, Xu Y, Li L, Qie Y, Luo Q, Zhang X, Li X, Zhou Y, An D. (2015). Molecular mapping of a new powdery mildew resistance gene *Pm2b* in Chinese breeding line KM2939. *Theor Appl Genet* 128: 613–622.

Maan SS and McCracken EV. (1969). Cytological stability of common wheats with leaf rust resistance derived from *Triticum timopheevii* Zhuk. *Euphytica* 18: 211–216.

Mackay TFC, Stone EA, Ayroles JF. (2009). The genetics of quantitative traits: challenges and prospects. *Nat Rev Genet* 10: 565–577.

Maestra B and Naranjo T. (1999). Structural chromosome differentiation between *Triticum timopheevii* and *T. turgidum* and *T. aestivum*. *Theor Appl Genet* 98: 744–750.

Malihipour A, Gilbert J, Brule-Babel A, Cao W. (2017). Mapping the A *Genome* for QTL conditioning resistance to Fusarium Head Blight in a wheat population with *Triticum timopheevii* background. *Plant Dis* 101: 11–19.

Manosalva PM, Davidson RM, Liu B, Zhu X, Hulbert SH, Leung H, Leach JE. (2009). A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. *Plant Physiology* 149: 286–296.

Maqbool A, Saitoh H, Franceschetti M, Stevenson C, Uemura A, Kanzaki H, Kamoun S, Terauchi R, Banfield MJ. (2015). Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. *Elife* 4:e08709. DOI: 10.7554/eLife.08709

Marone D, Russo MA, Laidò G, De Vita P, Papa R, Blanco A, Gadaleta A, Rubiales D, Mastrangelo AM. (2013). Genetic basis of qualitative and quantitative resistance to powdery mildew in wheat: from consensus regions to candidate genes. *BMC Genomics* 14: 562.

Maxwell JJ, Lyerly JH, Cowger C, Marshall D, Brown-Guedira G, Murphy JP. (2009). *MlAG12*: a *Triticum timopheevii*-derived powdery mildew resistance gene in common wheat on chromosome 7AL. *Theor Appl Genet* 119: 1489–1495.

Maystrenko OI, Laikova LI, Arbuzova VS, Popova OM. (1996). Development of analogues of common spring wheat cv. Saratovskaya 29 with complex resistance to powdery mildew, leaf and stem rust. *5th Int. Wheat Conf,* Ankara, Turkey, abstract 144.

McDonald BA and Linde C (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annu Rev Phytopathol* 40: 349–379.

McHale L, Tan X, Koehl P, Michelmore R (2006). Plant NBS-LRR proteins: adaptable guards. *Genome Biol* 7: 212–222.

McIntosh RA and Baker EP. (1970). Cytogenetic studies in wheat. IV. Chromosome location and linkage studies involving the *Pm*2 locus for powdery mildew resistance. *Euphytica* 19: 71–77.

McIntosh RA and Gyarfas J. (1971). *Triticum timopheevii* as a source of resistance to wheat stem rust. *Zeitschrift für Pflanzenzüchtung* 66: 240–248.

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC. (2014). Catalogue of gene symbols for wheat: 2013–2014 supplement. Komugi–wheat genetic resources database http://www.shigen.nig.ac.jp/wheat/komugi/ (http://maswheat.ucdavis.edu/CGSW/2013-2014_Supplement. pdf).

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Xia XC. (2017). Catalogue of Gene Symbols for Wheat: (2017). Supplement. Available online at: http://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ. (1998). Catalogue of Gene Symbols for Wheat. *Proc. 9th Int. Wheat Genet. Symp*, Saskatoon, Saskatchewan, Canade, 5. http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2012.pdf

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R. (2003). Catalogue of Gene Symbols for Wheat. Rome: Istituto Sperimentale per la Cerealicoltura. Available online from: http://wheat.pw.usda.gov/ggpages/wgc/2003/) (accessed March 2015).

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers WJ, Morris CF, Somers D, Appels R, Devos KM. (2008). Catalogue of gene symbols for wheat. In: McIntosh RA (ed), Gene symbols. http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/GeneSymbol.pdf

McIntosh RA, Zhang P, Cowger C, Parks R, Lagudah ES, Hoxha S. (2011). Rye-derived powdery mildew resistance gene *Pm8* in wheat is suppressed by the *Pm3* locus. *Theor Appl Genet* 123: 359–367.

McIntosh RA. (1983). Genetic and cytogenetic studies involving *Lr*18 resistance to *Puccinia recondita*. S. Sakamoto (Ed) *Proc. 6th Int. Wheat Genet. Symp*, Kyoto, Japan: 777–783.

Meyers BC, Kozik A, Griego A, Kuang HH, Michelmore RW. (2003). Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. *Plant Cell* 15(7): 1683–1683.

Miedaner T and Korzun V. (2012). Marker-assisted selection for disease resistance in wheat and barley breeding. *Phytopathology* 102:560–566.

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

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

Mohler V, Bauer A, Bauer C, Flath K, Schweizer G, Hartl L. (2011). Genetic analysis of powdery mildew resistance in German winter wheat cultivar Cortez. *Plant Breeding* 130: 35–40.

Mohler V, Bauer C, Schweizer G, Kempf H, Hartl L. (2013). *Pm50*: a new powdery mildew resistance gene in common wheat derived from cultivated emmer. *J Appl Genetics* 54(3): 259–263.

Moore JW, Herrera-Foessel S, Lan C, Schnippenkoetter W, Ayliffe M, Huerta-Espino J, Lillemo M, Viccars L, Milne R, Periyannan S, Kong X, Spielmeyer W, Talbot M, Bariana H, Patrick JW, Dodds P, Singh R, Lagudah E. (2015). A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. *Nature Gen* 47(12): 1494–1500.

Morgounov A, Tufan HA, Sharma R, Akin B, Bagci A, Braun HJ, Kaya Y, Keser M, Payne TS, Sonder K, McIntosh R. (2012). Global incidence of wheat rusts and powdery mildew during 1969–2010 and durability of resistance of winter wheat variety Bezostaya 1. *Eur J Plant Pathol* 132: 323–340.

Mori N, Kondo Y, Ishii T, Kawahara T, Valkoun J, Nakamura C. (2009). Genetic diversity and origin of timopheevi wheat inferred by chloroplast DNA fingerprinting. *Breed Sci* 59: 571–578.

Mori N, Liu Y-G, Tsunewaki K. (1995). Wheat phylogeny determined by RFLP analysis of nuclear DNA. 2. Wild tetraploid wheats. *Theor Appl Genet* 90: 129–134.

Motsny II, Lyfenko SF, Koval TN. (2000). Inheritance of resistance to fungal diseases in remote wheats–amphidiploid hybrids. *Tsitol Genet* 34(2): 46–56 (in Russia).

Mujeeb-Kazi A, Rajaram S. (2002). Transferring alien genes from related species and genera for wheat improvement. In: Curtis BC, Rajaram S, Macpherson GH. (eds.), Bread wheat: improvement and production. FAO Plant production and protection series, pp. 199–215.

Muranty H, Pavoine M-T, Jaudeau B, Radek W, Doussinault G, Barloy D. (2008). A quantitative approach detects three QTLs involved in powdery mildew resistance at the seedling stage in the winter wheat line RE714. *Australian Acad J Agric Res* 59(8): 714–722.

Mwale VM, Chilembwe EHC, Uluko HC. (2014). Wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*): Damage effects and genetic resistance developed in wheat (*Triticum aestivum* L). *Int J Plant Sci* 5(1): 1–16.

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).

Nelson RR. (1978). Genetics of horizontal resistance to plant diseases. *Ann Rev Phytopathol* 16: 359–378.

Nevo E, Korol AB, Beiles A, Fahima T (2013). Evolution of wild emmer and wheat improvement: population genetics, genetic resources, and genome organization of wheat's progenitor, *Triticum dicoccoides*. Springer, Berlin, pp 241–271.

Nevo E. (2011). Triticum. In: Wild Crop Relatives: Genomic and Breeding Resources, Cereals. C. Kole (ed.). Springer-Verlag Berlin Heidelberg, pp. 407–456.

Niewoehner AS and Leath S. (1998). Virulence of *Blumeria graminis* f. sp. *tritici* on winter wheat in the Eastern United States. *Plant Dis* 82 (1): 64–68.

Niks RE, Qi X, Marcel TC. (2015).Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. *Annu Rev Phytopathol* 53: 445–470.

Niu J and He D. (2009).Molecular basis of powdery mildew resistance in wheat (*Triticum aestivum* L.). *Afr J Biotechnol* 8: 4708–4716.

Niu J, Jia H, Yin J, Wang B, Ma Z, Shen T. (2010). Development of an STS marker linked to powdery mildew resistance genes *PmLK906* and *Pm4a* by gene chip hybridization. *Agric Sci Cn* 9(3): 331–336.

Niu JS, Zhang LZ, Hong DF, Wang YH (2005). Cloning, characterization and expression of wheat *EDR1* (enhanced disease resistance) gene. *J Plant Physiol Mol Biol* 31: 477–484.

Nürnberger T,Brunner F, Kemmerling B, Piater L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences *Immunol Rev* 198: 249–266.

Nyquist NE. (1957). Monosomic analysis of stem rust resistance of a common strain derived from *Triticum timopheevii*. *Agron J* 49: 222–223.

Nyquist NE. (1963). Inheritance of powdery mildew resistance in hybrids involving a common wheat strain derived from *Triticum timopheevii*. *Crop Sci* 3: 40–43.

Olson EL, Brown-Guedira G, Marshall DS, Jin Y, Mergoum M, Lowe I, Dubcovsky J. (2010). Genotyping of U.S. wheat germplasm for presence of stem rust resistance genes *Sr24*, *Sr36* and *Sr1RSAmigo*. *Crop Sci* 50: 668–675.

Ortelli S, Winzeler H, Winzeler M, Fried PM, Nösberger J. (1996). Leaf rust resistance gene *Lr9* and winter wheat yield reduction: I. Yield and yield components. *Crop Sci* 36: 1590–1595.

Paillard S, Trotoux-Verplancke G, Perretant M-R, Mohamadi F, Leconte M, Coedel S, de Vallavieille-Pope C, Dedryver F. (2012). Durable resistance to stripe rust is due to three specific resistance genes in French bread wheat cultivar Apache. *Theor Appl Genet* 125: 955–965.

Panstruga R, Parker JE, Schulze-Lefert P. (2009). SnapShot: plant immune response pathways. *Cell* 136: 978.

Parks R, Carbone I, Murphy JP, Marshall D, Cowger C. (2008). Virulence structure of the eastern US wheat powdery mildew population. *Plant Dis* 92: 1074–1082.

Parlange F, Oberhaensli S, Breen J, Platzer M, Taudien S, Šimková H, Wicker T, Doležel J, Keller B. (2011). A major invasion of transposable elements accounts for the large size of the *Blumeria graminis* f.sp. *tritici* genome. *Funct Integr Genomics* 11: 671–677.

Peng F, Song N, Shen H, Wu H, Dong H, Zhang J, Li Y, Peng H, Ni Z, Liu Z,Yang T, Li B, Xie C, Sun Q. (2014). Molecular mapping of a recessive powdery mildew resistance gene in spelt wheat cultivar Hubel. *Mol Breeding* 34: 491–500.

Perugini LD, Murphy JP, Marshall D, Brown-Guedira G. (2008). *Pm37*, a new broadly effective powdery mildew resistance gene from *Triticum timopheevii*. *Theor Appl Genet* 116: 417–425.

Peterhänsel C, Freialdenhoven A, Kurth J, Kolsch R, Schulze-Lefert P. (1997). Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death. *Plant Cell* 9: 1397–1409.

Petersen S, Lyerly JH, Worthington ML, Parks WR, Cowger C, Marshall DS,·Brown‐Guedira G, Murphy JP. (2015). Mapping of powdery mildew resistance gene *Pm53* introgressed from *Aegilops speltoides* into soft red winter wheat. *Theor Appl Genet* 128: 303–312.

Peusha H, Enno TM, Priilinn O. (1996). Genetic analysis of disease resistance in wheat hybrids, derivatives of *Triticum timopheevii* and *T. militinae*. *Acta Agron Hung* 44: 237–244.

Peusha H, Shnaider (Enno)TM. (1983). Crossability of common wheat with related species. *Proc Estonian Acad Sci Biology* 32: 241–244 (in Russian).

Peusha H, Stephan U, Hsam SLK, Felsenstein FG, Enno TM, Zeller FJ. (1995). Identification of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). IV. Breeding lines derived from wide crosses of Russian cultivars with species *T. timopheevii* Zhuk, *T. militinae* Zhuk. et Migush, *T. dicoccum* (Schrank.). Schuebl, *Aegilops speltoides* Taush. *Russian J Genetics* 31: 1–7 (in Russian).

Pflieger S, Palloix A, Caranta C, Blattes A, Lefebvre V. (2001). Defense response genes colocalize with quantitative disease resistance loci in pepper. *Theor Appl Genet* 103: 920–929.

Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ. (2009). Shades of gray: The world of quantitative disease resistance. *Trends Plant Sci* 14: 21–29.

Posti D. (2008). Saagikusega seotud tunnuste kaardistamine nisu (*Triticum aestivum* L.) haiguskindla hübriidliinii MSc Thesis TTU Tallinn.

Pridham JT. (1939). A successful cross between *Triticum vulgare* and *Triticum timopheevii*. *J Aust Inst Sci* 5: 160–161.

Puura E and Silla M. (2017). Exports of agricultural products and food preparations from the Baltic states and Finland in 2010−2016. In: Economic and Labour Market Trends. 66–79 https://www.stat.ee/valjaanne-2017_majanduse-ja-tooturu-trendid

Qi L, Friebe B, Zhang P, Gill BS. (2007). Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res* 15: 3–19.

Ramalingam J, Cruz CMV, Kukreja K, Chittoor JM, Wu J-L, Lee SW, Baraoidan M, GeorgeML, Cohen MB, Hulbert SH, Leach JE, Leung H. (2003). Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. *Mol Plant Microbe Interact* 16(1): 14–24.

Raman H, Stodart BJ, Cavanagh C, Mackay M, Morell M, Milgate A, Martin P. (2010). Molecular diversity and genetic structure of modern and traditional landrace cultivars of wheat (*Triticum aestivum* L.). *Crop Pasture Sci* 61: 222–229.

Ravel C, Praud S, Murigneux A, Canaguier A, Sapet F, Samson D, Balfourier F, Dufour P, Chalhoub B, Brunel D, Beckert M, Charmet G. (2006). Single-nucleotide polymorphism frequency in a set of selected lines of bread wheat (*Triticum aestivum* L.). *Genome* 49: 1131–1139.

Rawat N, Pumphrey M, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW, Akhunov E, Anderson JA, Gill B. (2016). Wheat *Fhb1* encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature Gen* 48: 1576–1580.

Riley R and Chapman V. (1958). Genetic control of the cytologically diploid behavior of hexaploid wheat. *Nature* 182:713–715.

Roberts JJ and Caldwell RM. (1970). General resistance slow mildewing to *Erysiphe graminis* f. sp. t*ritici* in Knox wheat. *Phytopathology* 60: 1310.

Robertson DS. (1989). Understanding the relationship between qualitative and quantitative genetics. In: Helentjaris T and Burr B (eds) Development and Application of Molecular Markers to Problems in Plant Genetics, 81–88. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Rodamilans B, San León D, Mühlberger L, Candresse T, Neumüller M, Oliveros JC, GarcíaJA. (2014). Transcriptomic analysis of *Prunus domestica* undergoing hypersensitive response to plum pox virus infection. *PLoS One* 9: e100477.

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

Rodriguez S, Maestra B, Perera E, Diez M, Naranjo T. (2000b). Pairing affinities of the B- and G- genome chromosomes of polyploid wheats with those of *Aegilops speltoides*. *Genome* 43: 814–819.

Rodriguez S, Perera E, Maestra B, Díez M**,** Naranjo T. (2000a). Chromosome structure of *Triticum timopheevii* relative to *T. turgidum*. *Genome* 43: 923–930.

Rong JK, Millet E, Manisterski J, Feldman M. (2000). A new powdery mildew resistance gene: introgression from wild emmer into common wheat and RFLP based mapping. *Euphytica* 115: 121–126.

Roux F, Voisin D, Badet T, Balagué C, Barlet X, Huard-Chauveau C, Roby D, Raffaele S. (2014). Resistance to phytopathogens *e tutti quanti*: placing plant quantitative disease resistance on the map. *Molecular Plant Pathology* 15(5): 427–432.

Sadeghabad AA, Dadkhodaie A, Heidari B, Razi H, Mostowfizadeh-Ghalamfarsa R. (2017). Microsatellite markers for the *Triticum timopheevi*-derived leaf rust resistance gene *Lr18* on wheat 5BL chromosome. *Breed Sci* 67: 129–134.

Salameh A, Buerstmayr M, Steiner B, Neumayer A, Lemmens M, Buerstmayr H. (2010). Effects of introgression of two QTL for Fusarium head blight resistance from Asian spring wheat by marker assisted backcrossing into European winter wheat on Fusarium head blight resistance, yield and quality traits. *Mol Breeding* 8: 485–494.

Salina EA, Leonova IN, Efremova TT, Röder MS. (2006a). Wheat genome structure: translocations during the course of polyploidization. *Funct Integr Genomics* 6: 71–80.

Salina EA, Lim KY, Badaeva ED, Shcherban AB, Adonina IG, V Amosova AV, Samatadze TE, Vatolina TY, Zoshchuk SA, Leitch AR. (2006b). Phylogenetic reconstruction of *Aegilops* section *sitopsis* and the evolution of tandem repeats in the diploids and derived wheat polyploids. *Genome* 49(8): 1023–1035.

Salvi S and Tuberosa R. (2005). To clone or not to clone plant QTLs: present and future challenges. *Trends in Plant Sci* 10(6): 297–304.

Samobor VM, Vukobratović VM, Jošt M. (2006). Effect of powdery mildew attack on quality parameters and experimental bread baking of wheat. *Acta agric Slov* 87(2)381–391.

Sarris PF, Cevik V, Dagdas G, Jonathan DG, Jones JDG, Krasileva KV. (2016). Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. *BMC Biology* 14: 8–26.

Schmolke M, Mohler V, Hartl L, Zeller FJ, Hsam SLK. (2012). A novel powdery mildew resistance allele at the *Pm4* locus from einkorn wheat (*Triticum monococcum*). *Mol Breeding* 29(2): 449–456.

Schneider DM, Heun M, Fischbeck G. (1991). Inheritance of the powdery mildew resistance gene *Pm9* in relation to *Pm1* and *Pm2* of wheat. *Plant Breeding* 107: 161–164.

Schweizer P and Stein N. (2011). Large-scale data integration reveals colocalization of gene functional groups with meta-QTL for multiple disease resistance in barley. *Mol Plant Microbe Interact* 24 (12): 1492–1501.

Sears ER. (1977). Induced mutant with homoeologous pairing in common wheat. *Can J Genet Cytol* 19: 585–593.

Sekhwal MK, Li P, Lam I, Wang X, Cloutier S, You FM. (2015). Disease resistance gene analogs (RGAs) in plants. *Int J Mol Sci* 16: 19248–19290.

Shah L, Rehman S, Ali A, Yahya M, Riaz MW, Si H, Ma C, Lu J. (2018). Genes responsible for powdery mildew resistance and improvement in wheat using molecular marker-assisted selection. *J Plant Dis Prot* 125: 145–158.

Shands RG. (1941). Disease resistance of *Triticum timopheevii* transferred to common winter wheat. *Amer Soc Agron Journal* 33: 709–712.

Shen Q-H, Zhou F, Bieri S, Haizel T, Shirasu K, Schulze-Lefert P. (2003). Recognition specificity and RAR1/SGT1 dependence in barley *Mla* disease resistance genes to the powdery mildew fungus. *Plant Cell* 15: 732–744.

Shewry PR. (2009). Wheat. *J. Exp. Bot*. 60: 1537–1553.

Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH. (2004). Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. *Plant Cell* 16: 1220–1234.

Shnaider TM. (1986). Meiosis in pentaploid and tetraploid interspecies wheat hybrids F_1. *Cytology and Genetics* (Kiev) 20: 327–330 (in Russian).

Shnaider, T.M. (1988). Behaviour of meiosis in wheat wide hybrids, obtained using a *ph* mutant. *Cytology and Genetics* (Kiev) 22: 18–22 (in Russian).

Siedler H, Obst A, Hsam LK, Zeller FJ. (1994). Evaluation of resistance to *Pyrenophora tritici-repentis* in *Aegilops tauschii* Coss. and synthetic hexaploid wheat amphiploids. *Genet Resour Crop Evol* 41: 27–34.

Singh S, Sidhu J, Huang N, Vikal Y, Li Z, Brar D, Dhaliwal H, Khush G. (2001). Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theor Appl Genet* 102: 1011–1015.

Skinnes H. (2002). Breakdown of race specific resistance to powdery mildew in Norwegian wheat. *Cereal Rusts Powdery Mildew Bull* 30.

Skurygina NA. (1984). Highly effective genes for resistance to populations of brown rust and powdery mildew in lines of common wheat derived from *T. timopheevii* Zhuk, and their identification. *Bull on Appl Bot Genet Breed* 85: 5–13 (in Russian).

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.

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270: 1804–1806.

Sourdille P, Robe P, Tixier MH, G. Doussinault G, Pavoine MT, Bernard M. (1999). Location of *Pm3g*, a powdery mildew resistance allele in wheat, by using a monosomic analysis and by identifying associated molecular markers. *Euphytica* 110: 193–198.

Sourdille P, Sukhwinder S, Cadalen T, Brown-Guerida GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M. (2004). Microsatellite-based deletion bin system for establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Funct Integr Genomics* 4: 12–25.

Spanu PD, Abbott JC, Amselem J, Timothy A. Burgis TA, Soanes DM, Stüber K, Ver E, van Themaat L, Brown JKM, Butcher SA, Gurr SJ, Lebrun M-H, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M,1 Brewer MT, Cadle-Davidson L, Cadle-Davidson MM et al. (2010). Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* 330: 1543–1546.

Srichumpa P, Brunner S, Keller B, Yahiaoui N. (2005). Allelic series of four powdery mildew resistance genes at the *Pm3* locus in hexaploid bread wheat. *Plant Physiol* 139: 885–895.

Srnic G, Murphy JP, Lyerly JH, Leath S, Marshall DS. (2005). Inheritance and chromosomal assignment of powdery mildew resistance genes in two winter wheat germplasm lines. *Crop Sci* 45: 1578–1586.

Summers RW and Brown JKM. (2013). Constraints on breeding for disease resistance in commercially competitive wheat. *Plant Pathol* 62 (Suppl. 1): 115–121.

Sun H, Hu J, Song W, Qiu D, Cui L, Wu P, Zhang H, Liu H, Yang L, Qu Y, Li Y, Li T, Cheng W, Zhou Y, Liu Z, Li J, Li H. (2018). *Pm61*: a recessive gene for resistance to powdery mildew in wheat landrace Xuxusanyuehuang identified by comparative genomics analysis. *Theor Appl Genet* 131(10): 2085–2097.

Švec M and Miklovičova M. (1998). Structure of populations of wheat powdery mildew (*Erysiphe graminis* DCf.sp. *tritici* Marchal) in Central Europe in 1993–1996: I. Dynamics of virulence. *Eur J Plant Pathol* 104**:** 537–544.

Takken FLV and Goverse A. (2012). How to build a pathogen detector: structural basis of NB-LRR function. *Curr Opin Plant Biol* 15(4): 375–384.

Tan C, Li G, Cowger C,·Carver BR, Xu X. (2018). Characterization of *Pm59*, a novel powdery mildew resistance gene in Afghanistan wheat landrace PI 181356. *Theor Appl Genet* 131(5): 1145–1152.

Tang D and Innes RW. (2002). Overexpression of a kinase-deficient form of the *EDR1* gene enhances powdery mildew resistance and ethyleneinduced senescence in *Arabidopsis*. *Plant J* 32: 975–983.

Tang D, Christiansen KM, Innes RW. (2005). Regulation of plant disease resistance, stress responses, cell death, and ethylene signaling in *Arabidopsis* by the EDR1 protein kinase. *Plant Physiol* 138: 1018–1026.

Tao W, Liu D, Liu J, Feng Y, Chen P. (2000). Genetic mapping of the powdery mildew resistance gene *Pm*6 in wheat by RFLP analysis. *Theor Appl Genet* 100: 564–568.

Tao Y, Xie Zh, Chen W, Glazebrook J, Chang H-S, Han B, Zhu T, Zou G, Katagiri F. (2003). Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* 15(2): 317–330.

Thind AK, Wicker T, Šimková H, Fossati D, Moullet O, Brabant C, Vrána J, Doležel J, Krattinger SG. (2017). Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. *Nature biotechnol* 35(8): 793.

Thomma BPHJ, Nürnberger T, Joosten MHAJ. (2011). Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* 23: 4–15.

Timonova EM, Leonova IN, Belan IA, Rosseeva LP, Salina EA. (2012). The influence of particular chromosome regions of *Triticum timopheevii* on the formation of resistance to diseases and quantitative traits in common wheat. *Russ J Genet: Appl Res* 2(4): 330–343.

Timonova EM, Leonova IN, Röder MS, Salina EA. (2013). Marker-assisted development and characterization of a set of *Triticum aestivum* lines carrying different introgressions from the *T. timopheevii* genome. *Mol Breeding* 31: 123–136.

Tommasini L, Yahiaoui N, Srichumpa P, Keller B. (2006). Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. *Theor Appl Genet* 114: 165–175.

Torp J and Jùrgensen JH. (1986). Modification of barley powdery mildew resistance gene *Mla12* by induced mutation. *Can J Genet Cytol* 28: 725–731.

Troch V, Audenaert K, Vanheule A, Bekaert B, Höfte M, Haesaert G. (2013). Evaluation of resistance to powdery mildew in triticale seedlings and adult plants. *Plant Dis* 97: 410–417.

Tsilo TJ, Jin Y, Anderson JA. (2008). Diagnostic microsatellte markers for the detection of stem rust resistane gene *Sr36* in diverse genetic backgrounds of wheat. *Crop Sci* 48: 253–261.

Tsõmbalova J, Karafiátová M, Vrána J, Kubaláková M, Peuša H, Jakobson I, Järve M, Valárik M, Doležel J, Järve K. (2016). A haplotype specific to North European wheat (*Triticum aestivum* L.). *Genet Resour Crop Evol* 64(4): 653–664.

Tsunewaki K and Ogihara J. (1983). The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* species. 11. On the origin of polyploid wheat cytoplasms as suggested by chloroplast DNA restriction fragment patterns. *Genetics* 104: 155–171.

Tucker DM, Griffey CA, Liu S, Brown-Guedira G, Marshall DS, Maroof MAS. (2007). Confirmation of three quantitative trait loci conferring adult plant resistance to powdery mildew in two winter wheat populations. *Euphytica* 155: 1–13.

Uauy C. (2017). Wheat genomics comes of age. *Curr Opin Plant Biol* 36: 142–148.

Underwood W and Somerville SC. (2008). Focal accumulation of defences at sites of fungal pathogen attack. *J Exp Bot* 59(13): 3501–3508.

van Loon LC, Rep M, Pieterse CMJ. (2006). Significance of inducible defense-related proteins in infected plants. *Annu Rev Phytopathol* 44: 135–162.

Vanegas CDG, Garvin DF, Kolmer JA. (2008). Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by *Lr34*. *Euphytica* 159: 391–401.

Vergne E, Grand X, Ballini E, Chalvon V, Saindrenan P, Tharreau D, Nottéghem J-L, Morel J-B. (2010). Preformed expression of defense is a hallmark of partial resistance to rice blast fungal pathogen Magnaporthe oryzae. *BMC Plant Biol* 10: 206–223.

Wagenaar EB. (1961). Studies on the genome constitution of *Triticum timopheevii* Zhuk. I. Evidence for genomic control of meiotic irregularities in tetraploid hybrids. *Can J Genet Cytol*. 3: 47–60.

Wang N, Huang H-J, Ren S-T, Li J-J, Sun Y, Sun D-Y, Zhang S-Q. (2012). The rice wall-associated receptor-like kinase gene *OsDEES1* plays a role in female gametophyte development. *Plant Physiol* 160: 696–707.

Wang X, Richards J, Gross T, Druka A, Kleinhofs A, Steffenson B, Acevedo M, Brueggeman R. (2013). The *rpg4*-mediated resistance to wheat stem rust (*Puccinia graminis*) in barley (*Hordeum vulgare*) requires *Rpg5*, a second NBS-LRR gene, and an actin depolymerization factor. *MPMI* 26(4): 407–418.

Wang X-Y, Qi Z-J, Ma Z-Q, Chen P-D, Liu D-L. (2000). Identification of RAPD markers tightly linked to wheat powdery mildew resistance gene *Pm6. Acta Genet Sinica* 27 (12): 1072–1079.

Wang Y and Li J. (2008). Molecular basis of plant architecture. *Annu Rev Plant Biol* 59: 253–279.

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu J. (2014a). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnol* 32(9): 947–951.

Wang Y, Wang C, Quan W,. Jia X, Fu Y, Zhang H, Liu X, Chen C, Ji W. (2016). Identification and mapping of *PmSE5785*, a new recessive powdery mildew resistance locus, in synthetic hexaploid wheat. *Euphytica* 207: 619–626.

Wang ZL, Li LH, He ZH, Duan XY, Zhou YL, Chen XM, Lillemo M, Singh RP, Wang H, Xia XC. (2005). Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. *Plant Dis* 89: 457–463.

Warburton ML, Crossa J, Franco J, Kazi M, Trethowan R, Rajaram S, Pfeiffer W, Zhang P, Dreisigacker S, van Ginkel M. (2006). Bringing wild relatives back into the family: recovering genetic diversity in CIMMYT improved wheat germplasm. *Euphytica* 149: 289–301.

Wei Y, Zhang Z, Andersen CH, Schmelzer E, Gregersen PL, Collinge DB, Smedegaard-Petersen V, Thordal-Christensen H. (1998). An epidermis/papilla-specific oxalate oxidase-like protein in the defence response of barley attacked by the powdery mildew fungus. *Plant Mol Biol* 36(1): 101–112.

Wicker T, Oberhaensli S, Parlange F, Buchmann JP, Shatalina M, Roffler S, Ben-David R, Doležel J, Šimková H, Schulze-Lefert P, Spanu PD, Bruggmann R, Amselem J, Quesneville H, van Themaat EVL, Paape T, Shimizu KK, Keller B. (2013). The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. *Nature Gen* 45(9): 1092–1096.

Wiersma AT, Pulman JA, Brown LK, Cowger C, Olson EL. (2017). Identification of *Pm58* from *Aegilops tauschii*. *Theor Appl Genet* 130: 1123–1133.

Williams SJ, Sohn KH, Wan L, Bernoux M, Sarris PF, Segonzac C, Ve T, Ma Y, Saucet SB, Ericsson DJ, Casey LW, Lonhienne T, Winzor DJ, Zhang X, Coerdt A, Parker JE, Dodds PN, Kobe B, Jones JD. (2014). Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science* 344: 299–303.

Wilson JA and Ross WM. (1962). Male-sterility interaction of the *Triticum aestivum* nucleus and *Triticum timopheevi* cytoplasm. *Wheat Inf Serv* 14: 29–30.

Wolter M, Hollricher K, Salamini F, Schulze-Lefert P. (1993). The mlo resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defense mimic phenotype. *Mol Gen Genet* 239: 122–128.

Worland AJ, Law CN, Li WM. (1989). The location of genes depressing yield associated with the transfer of eyespot resistance from *Aegilops ventricosa*. Annual Report of the AFRC Institute of Plant Science Research, John Innes Institute and Sainsbury Laboratory. Norwich, UK: IPSR and John Innes Institute. 7–8.

Wu JL, Sinha PK, Variar M, Zheng KL, Leach JE, Courtois B, Leung H. (2004). Association between molecular markers and blast resistance in an advanced backcross population of rice. *Theor Appl Genet* 108: 1024–1032.

Wu S, Pumphrey M, Bai G. (2009). Molecular mapping of stem-rust resistance gene *Sr40* in wheat. *Crop Sci* 49: 1681–1686.

Xiao M, Song F, Jiao J, Wang X, Xu H, Li H. (2013). Identification of the gene *Pm47* on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. *Theor Appl Genet* 126 (5): 1397–1403.

Xiao S, Calis O, Patrick E, Zhang G, Charoenwattana P, Muskett P, Parker JE, Turner JG. (2005). The atypical resistance gene, *RPW8*, recruits components of basal defence for powdery mildew resistance in *Arabidopsis*. *Plant J* 42: 95–110.

Xing L, Hu P, Liu J, Cui C, Wang H, Di Z, Zhou S, Xu J, Gao L, Huang Z, Cao A. (2017). NLR1-V, a CC-NBS-LRR encoding gene, is a potential candidate gene of the wheat powdery mildew resistance gene *Pm21*. bioRxiv 114058 (http://dx.doi.org/10.1101/114058).

Xu H, Yao G, Xiong L, Yang L, Jiang Y, Fu B, Zhao W, Zhang Z, Zhang C, Ma Z. (2008). Identification and mapping of *Pm2026*: a recessive powdery mildew resistance gene in an einkorn (*Triticum monococcum* L.) accession. *Theor Appl Genet* 117(4): 471–-477.

Xu HX, Yi YJ, Ma PT, Qie YM, Fu XY, Xu YF, Zhang XT, An DG. (2015). Molecular tagging of a new broad-spectrum powdery mildew resistance allele *Pm2c* in Chinese wheat landrace Niaomai. *Theor Appl Genet* 128: 2077–2084.

Xue F, Wang C, Li C, Duan X, Zhou Y, Zhao N, Wang Y, Ji W. (2012). Molecular mapping of a powdery mildew resistant gene in common wheat landrace Baihulu and its allelism with *Pm24*. *Theor Appl Genet* 125(7): 1425–1432.

Xue F, Zhai W, Duan X, Zhou Y, Ji W. (2009). Microsatellite mapping of a powdery mildew resistance gene in wheat landrace Xiaobaidong. *Acta Agron Sin* 35(10): 1806–1811.

Yahiaoui N, Brunner S, Keller B. (2006). Rapid generation of new powdery mildew resistance genes after wheat domestication. *Plant J* 47: 85–98.

Yahiaoui N, Srichumpa P, Dudler R, Keller B. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. *Plant J* 37:528–538.

Yamamori M. (1994). An N-band marker for gene *Lr18* for resistance to leaf rust in wheat. *Theor Appl Genet* 89: 643–646.

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J. (2004). The wheat *Vrn2* gene is a flowering repressor down-regulated by vernalization. *Science* 303: 1640–1644.

Yang Q, Zhang D, Xu M. (2012). A sequential quantitative trait locus fine-mapping strategy using recombinant-derived progeny. *J Integr Plant Biol* 54(4): 228–237.

Yasuda N, Mitsunaga T, Hayashi K, Koizumi S, Fujita Y. (2015). Effects of pyramiding quantitative resistance genes *pi21 Pi34*, and *Pi35* on rice leaf blastdisease. *Plant Dis* 99(7): 904–909.

Young ND and Tanksley SD. (1989). RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theor Appl Genet* 77: 353–359.

Young ND. (1996). QTL mapping and quantitative disease resistance in plants. *Annu Rev Phytopathol* 34: 479–501.

Youssefian S, Kirby EJM, Gale MD. (1992). Pleiotropic effects of the GA-insensitive *Rht* dwarfing genes in wheat. 2. Effects on leaf, stem, ear and floret growth. *Field Crop Res* 28: 191–210.

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

Yu L, Niu JS, Cheng PD, Ma ZQ, Liu DJ (2005). Cloning, physical mapping and expression analysis of a wheat *Mlo*-like gene. *J Integr Plant Biol* 47: 214–222.

Zeller FJ, Lutz J, Stephan U. (1993). Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.) *Mlk* and other alleles at the *Pm3* locus. *Euphytica* 68: 223–229.

Zhai C, Zhang Y, Yao N, Lin F, Liu Z, Dong Z, Wang L, Pan Q. (2014). Function and interaction of the coupled genes responsible for *Pik-h* encoded rice blast resistance. *PLOS ONE* 9(6): e98067.

Zhang L, Wang J, Wang J, Wang L, Ma B, Zeng L, Qi Y, Li Q, He Z. (2015). Quantitative trait locus analysis and fine mapping of the *qPL6* locus for panicle length in rice. *Theor Appl Genet*128: 1151–1161.

Zhang P, Friebe B, Gill BS. 2002. Variation in the distribution of a genome-specific DNA sequence on chromosomes reveals evolutionary relationships in the Triticum and Aegilops complex. *Plant Syst Evol* 235: 169–179.

Zhang Q, Klindworth DL, Friesen TL, Chao S, Jin Y, Cai X, Xu S. (2012). Development and characterization of wheat lines with *Sr37* for stem rust resistance derived from wild Timopheev's wheat. Abstract Intern. Conf. "Plant and Animal Genome XXI". USA: San Diego, P. 316.

Zhang S, Chen C, Li L, Meng L, Singh J, Jiang N, Xing-Wang Deng X-W, He Z-H, Lemaux PG. (2005). Evolutionary expansion, gene structure, and expression of the rice wall-associated kinase gene family. *Plant Physiol* 139(3): 1107–1124.

Zhang XK, Xiao YG, Zhang Y, Xia XC, Dubcovsky J, He ZH. (2008). Allelic variation at the vernalization genes *Vrn-A1, Vrn-B1, Vrn-D1*, and *Vrn-B3* in Chinese wheat cultivars and their association with growth habit. *Crop Sci* 48: 458–470.

Zhang Y, Bai Y, Wu G, Zou S, Chen Y, Gao C, Tang D. (2017). Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. *Plant J* 91: 714–724.

Zhang ZG, Collinge DB, Thordal-Christensen H. (1995). Germin-like oxalate oxidase, a H2O2-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. *Plant J* 8: 139–145.

Zhang, R, Fan, Y, Kong, L, Wang Z, Wu J, Xing L, Cao A, Feng Y. (2018). *Pm62*, an adult-plant powdery mildew resistance gene introgressed from *Dasypyrum villosum* chromosome arm 2VL into wheat. *Theor Appl Genet* 131(12): 2613–2620.

Zhou FS, Zhang ZG, Gregersen PL, Mikkelsen JD, de Neergaard E, Collinge DB, Thordal-Christensen H. (1998). Molecular characterization of the oxalate oxidase involved in the response of barley to the powdery mildew fungus. *Plant Physiol* 117: 33–41.

Zhou RH, Zhu ZD, Kong XY, Huo NX, Tian QZ, Li P, Jin C, Dong Y, Jia J. (2005). Development of wheat near-isogenic lines for powdery resistance. *Theor Appl Genet* 110: 640–648.

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

Zhukovsky PM. (1928). A new species of wheat. *Bull on Appl Bot Genet Breed* 19: 59–66 (in Russian).

Zhukovsky PM. (1971). Cultivated plants and their wild relatives.systematics, geography, cytogenetics, immunity, origin and use. Kolos, Leningrad, p 121 (in Russian).

Zimmermann G, Bäumlein H, Mock H-P, Himmelbach A, Schweizer P. (2006). The multigene family encoding germin-like proteins of barley. Regulation and function in basal host resistance. *Plant Physiol* 142: 181–192.

Zohary D and Hopf M. (2000). Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Clarendon Press, Oxford.

Zoshchuk S, Badaeva E, Zoshchuk N, Adonina I, Shcherban' A, Salina E. (2007). Intraspecific divergence in wheats of the Timopheevi group as revealed by in situ hybridization with tandem repeats of the Spelt1 and Spelt52 families. *Russ J Genet* 43: 636–645 (in Russian).

Zou SH, Wang H, Li YW, Kong Z S, Tang DZ. (2018). The NBLRR gene *Pm60* confers powdery mildew resistance in wheat. *New Phytologist* 218(1): 298–309.

Zuo W. Chao N, Zhang J, Ye G, Tan, B. Li B, Xing Y, Zhang B, Liu H, Fengler KA, Zhao J, Zao X, Chen Y, Lai J, Yan J, Xu M. (2015). A maize wall-associated kinase confers quantitative resistance to head smut. *Nat Genet* 47(2): 151–157.

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ABSTRACT

Genetic Analysis of Powdery Mildew Resistance in an Introgression Line of Wheat

The common wheat line 8.1 (AABBDD, 2n=42) resistant to powdery mildew (*Blumeria graminis* DC. f. sp. *tritic*i) was obtained from a cross of the spring wheat cv. Tähti and tetraploid wheat *Triticum militinae* (A^tA^tGG, 2n=28). The genome of the line was screened with 220 microsatellite markers, and nine introgressions from *Triticum militinae* were detected (on chromosomes 1A, 1B, 2A, 4A, 5B, 5A and 7A).

QTL analysis of F_2 and DH (doubled haploid) mapping populations of the cross (line 8.1 x cv. Tähti) revealed five *T. militinae*-origin regions (on chromosomes 1A, 4A, 5A, 5B, and 7A affecting seedling resistance to powdery mildew, three of them (on chromosomes 4A, 5A and 7A) also carried a QTL for adult plant resistance (APR). The main QTL for both seedling and adult plant resistance, *QPm.tut-4A,* was located on chromosome 4A and was found to account for on average 35-49% of seedling resistance variance and 33-52% of APR variance. Each of the detected minor QTL was responsible for 10-17% of the resistance variance. With the exception of the minor QTL on chromosome 5B, *QPm.tut-4A* and all minor QTL acted in a non-race-specific manner.

It was shown that the *QPm.tut-4A* locus does not require any other QTL for its activity and is effective in different genetic backgrounds. Near-isogenic lines resistant to powdery mildew carrying the single introgression on chromosome arm 4AL in the background of cvs. Tähti and Mooni were developed.

A highly reduced level of recombination in the region of the *QPm.tut-4A* in line 8.1 hampered precise mapping of the locus, and different approaches to map *QPm.tut-4A* more precisely were applied. A new mapping population was created from crossing the cv. CS and line 8.1, and screening of 8425 F_3-F_6 plants yielded 30 lines with a recombination in the *QPm.tut-4A* region, delimiting the *QPm-tut.4A* locus to 0.012 cM, flanked by markers *owm202* and *owm223*. An additional mapping population of 1225 BC_2F_2 was developed from the cross of the line 8.1 and line *CS ph1b* carrying a deletion in the region of the *Ph1* gene. Inactivation of the *Ph1* gene removes the restriction of homoeolog pairing of hexaploid wheat chromosomes. In plants homozygous for *ph1b* and heterozygous in the region of the *QPm.tut-4A,* recombination level was increased 33-fold, resulting in 155 additional recombinations, four of them within the 0.012 cM *QPm-tut.4A* locus. Genotyping of the recombinant line allowed us to split the 0.012 cM *QPm.tut-4A* region into eight subregions. Phenotyping allowed to map the *QPm-tut.4A* to a limited region flanked by markers *owm169* and *owm228*.

Yield-related agronomic traits were evaluated in a DH mapping population and *T. militinae*–origin QTL affecting flowering time, plant height, spike length and density, and 1000-kernel-weight were detected. A minor QTL responsible for 6% of the spike length variation was detected on chromosome 4A in line 8.1 As recombination was suppressed in the region, additional efforts will be needed to evaluate the possible link between the resistance locus *QPm.tut-4A* and crop performance. *T. militinae* regions that can positively affect wheat yield were also detected in line 8.1

The results obtained in this work make it feasible to use *QPm.tut-4A* in resistance breeding and provide a basis for cloning of the major QTL for powdery mildew resistance in line 8.1 - *QPm.tut-4A.*

KOKKUVÕTE

Jahukastekindla introgressiivse suvenisuliini geneetiline analüüs

Hariliku nisu (AABBDD, 2n=42) jahukastele (tekitaja *Blumeria graminis* DC. f. sp. *tritic*i) vastupidav liin 8.1 oli varem saadud suvinisusordi 'Tähti' ja tetraploidse nisu *Triticum militinae* (A^tA^tGG, 2n=28) ristamise järglasena. Käesolevat tööd alustati liini 8.1 genoomi skriinimisega 220 mikrosateliitmarkeri abil, liini genoomis tuvastati üheksa *Triticum militinae* genoomist pärinevat introgressiooni (kromosoomidel 1A, 1B, 2A, 4A, 5B, 5A ja 7A).

Liini 8.1 tagasiristamisest sordiga 'Tähti' saadud F_2 ja DH (topelthaploidide) kaardistamispopulatsioonides leiti viis *T. militinae* päritoluga idandite jahukastekindlust suurendavat kvantitatiivset lookust (QTL, kromosoomidel 1A, 4A, 5A, 5B ja 7A), kolm neist (kromosoomidel 4A, 5A ja 7A) kandsid ka täiskasvanud taime resistentsuse (APR) kvantitatiivset lookust (QTL).

Nii idandite kui ka täiskasvanud taimede resistentsuse puhul oli peamiseks tunnuse varieeruvuse eest vastutavaks lookuseks 4A kromosoomil paiknev *QPm.tut-4A* lookus, mis vastutas keskmiselt 35-49% idandite haiguskindluse ja 33-52% APR varieeruvuse eest. Leiti ka minoorsed QTLd, millest igaüks vastutas 10-17% haiguskindluse varieeruvuse eest.

Välja arvatud minoorne QTL 5B kromosoomil, ei sõltunud *QPm.tut-4A* ega ka teiste minoorsete QTLde efektiivsus patogeeni rassist.

Näidati, et lookus *QPm.tut-4A* ei vaja toimimiseks teisi tuvatatud QTLe ja on efektiivne erineva geneetilise tausta korral. Ristamiste teel saadi peaaegu isogeensed haiguskindlad nisuliinid (NIL), mis sortide 'Tähti' ja 'Mooni' geneetilisel taustal kandsid ainult *QPm.tut-4A* lookust.

Liini 8.1 *QPm.tut-4A* lookuse täpset kaardistamist pidurdas äärmisel madal rekombinatsioonisagedus lookuse piirkonnas ning selle takistuse ületamiseks rakendati erinevaid meetmeid. Hiina rahvaselektsioonisordi Chinese Spring ja liini 8.1 ristamise teel loodi esmalt uus kaardistamispopulatsioon, mille 8425 F_3-F_6 taime testimise tulemusena leiti *QPm.tut-4A* piirkonnas 30 rekombinanti, mis piirasid *QPm.tut-4A* lookuse markeritega *owm202* and *owm223* (0.012 cM).

Liini 8.1 ja deleteeritud *Ph1* geeni kandva CS*ph1b* ristamisest saadi 1225 BC_2F_2 taime populatsioon. *Ph1* geeni inaktiveerimisega võetakse heksaploidses nisus maha kromosoomide homeoloogilise paardumise keeld. Rekombinatsioonide arvukus *ph1b* suhtes homosügootsetes ja *QPm.tut-4A* piirkonna suhtes heterosügootsetes taimede populatsioonis oli ligikaudu 33 korda suurem kui (CS*Ph1* x liin 8.1) populatsioonis ning *QPm.tut-4A* piirkonnas tuvastati veel 115 rekombinantset taime, millest neljas oli rekombinatsioon *QPm.tut-4A* lookuse 0.012 cM pikkuses segmendis. Leitud rekombinandid võimaldasid jagada 0.012 cM pikkuse segmendi kaheksaks osaks. Fenotüübi hindamine kaardistas *QPm.tut-4A* markeritega *owm169* ja *owm228* piiratud segmendile (480 kbp).

DH kaardistamise populatsioonis hinnati saagiga seotud agronoomilisi tunnuseid ning leiti õitsemise aega (FT), taime kõrgust, viljapea pikkust ja tihedust ning 1000 tera massi mõjutavaid *T. militinae* päritoluga QTLe. Liini 8.1 4A kromosoomil leiti 6% ulatuses viljapea pikkuse varieeruvust mõjutav QTL. Kuna rekombinatsioon selles

kromosoomipiirkonnas on alla surutud, siis tuleb teha täiendavaid uurimisi *QPm.tut-4A* ja viljapea pikkust mõjutava QTL aheldatuse kohta.

Töö tulemuseks on uudne jahukastekindel doonorliin, milles on markeeritud haiguskindlust tagav kromosoomilõik. Doonorliin on kasutatav aretuses ja sellega ei kaasne saaki vähendavat ebasoovitavat mõju.

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ORIGINAL PAPER

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

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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).

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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.

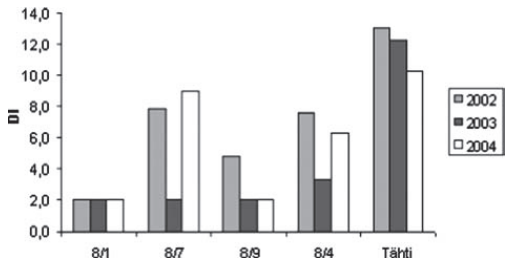

Fig. 1 Phenotypic distribution of four hybrid lines from the cross Tähti × *T. militinae* for APR across 3 years

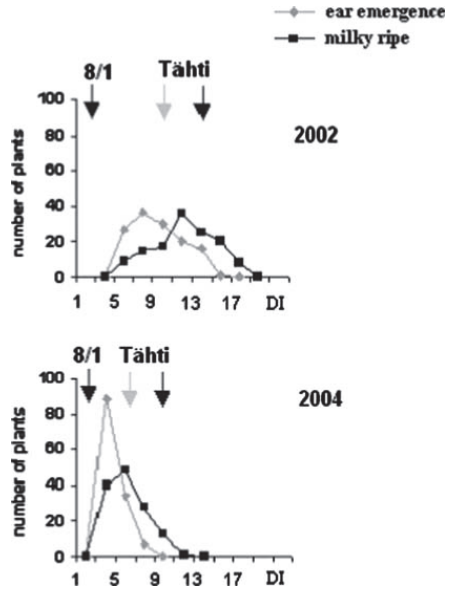

Fig. 2 Distribution of the adult plant powdery mildew DI of F_2 plants (2002) and $F_{2:3}$ families (2004) derived from the cross 8/1 × Tähti in the first and second assessment. The adult plant powdery mildew DI of two parents (8/1 and Tähti) is indicated

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).

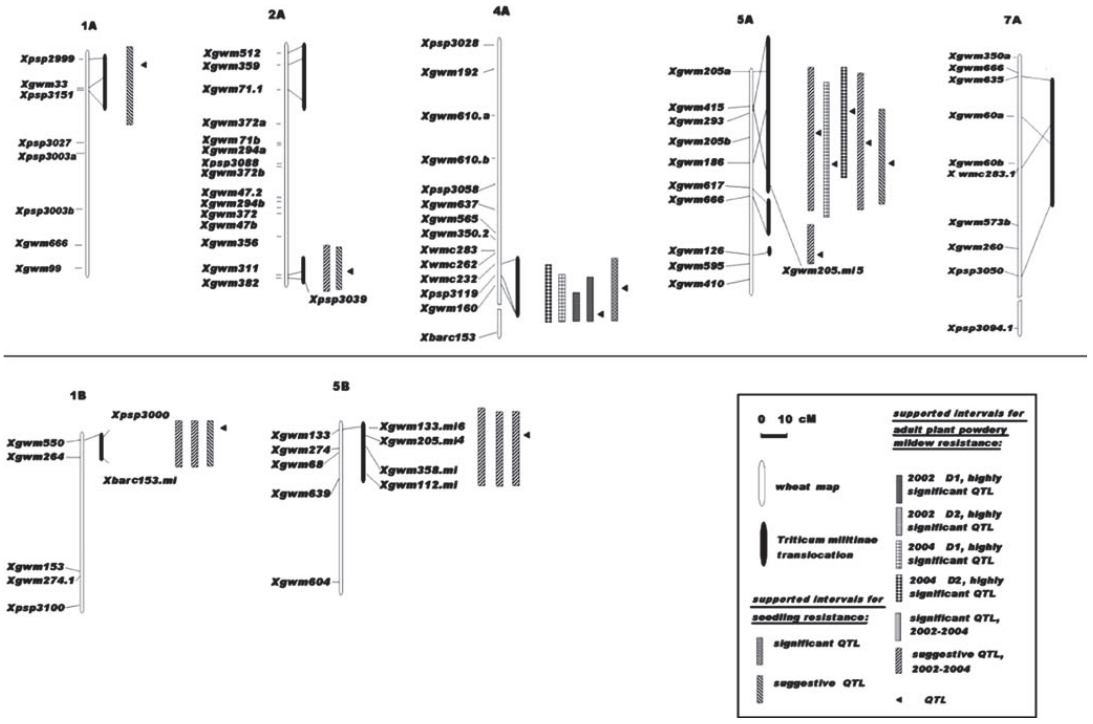

Fig. 3 The map of *T. militinae*-derived translocations and powdery mildew resistance QTLs in the mapping population derived from a cross between the hybrid line 8/1 and cultivar Tähti. Only chromosomes with *T. militinae* translocations are represented. Multiple loci detected by single markers have 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.

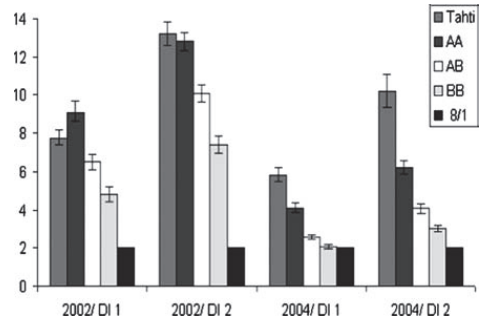

Fig. 4 The effect of alternative alleles in the QTL region of chromosome 4A (*Xgwm160*) on APR in the mapping population for the two assessments, in 2002 and in 2004. *AA* homozygous Tähti allele, *AB* heterozygous, *BB* homozygous *T. militinae*-derived allele

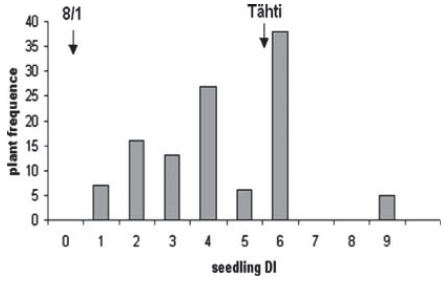

Fig. 5 Distribution of the seedling plant powdery mildew DI for F_2 plants in the mapping population (*0* no visible symptoms; *9* heavy sporulation). The scores for the parents are indicated by *arrows*

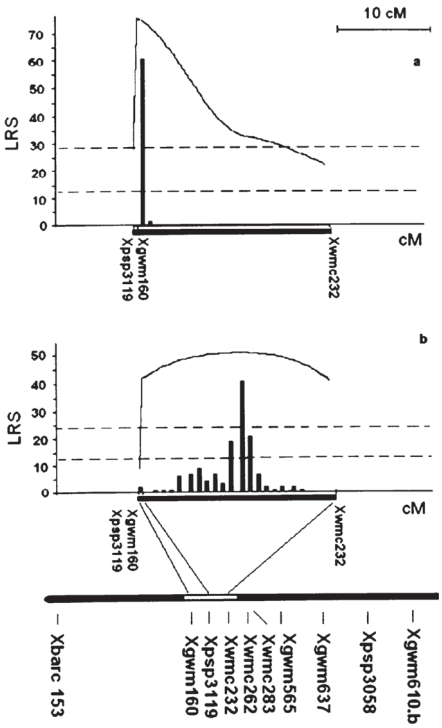

Fig. 6 The likelihood plots of QTLs associated with the adult plant (**a** 2002, DI 1) and seedling resistance (**b** 2002) to powdery mildew on chromosome 4A in the 8/1 × Tähti mapping population. The estimations of confidence intervals by bootstrap resampling are plotted as histograms. The *horizontal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

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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

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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

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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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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 $\mu Em^{-2}\ s^{-1}$). 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 $\mu Em^{-2}\ s^{-1}$), 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 $\times$ 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 $\times$ 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	Väänа	TTÜ	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 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

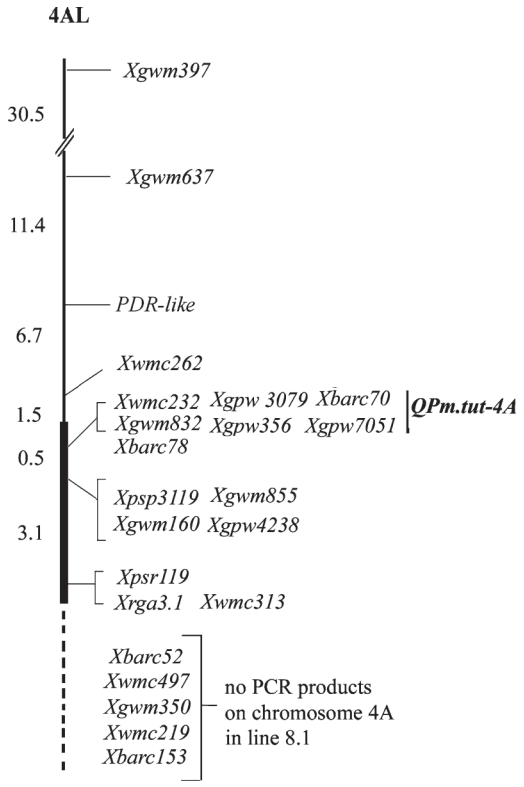

Fig. 1 Genetic map of the region introgressed from *T. militinae* (*bold*) on chromosome 4A of the line 8.1. *QPm.tut-4A* region is marked by a *vertical bar*. Distances of the map are calculated on the basis of segregation in F_2 CS × 8.1 population with the exception of the 3.1 cM distance between 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

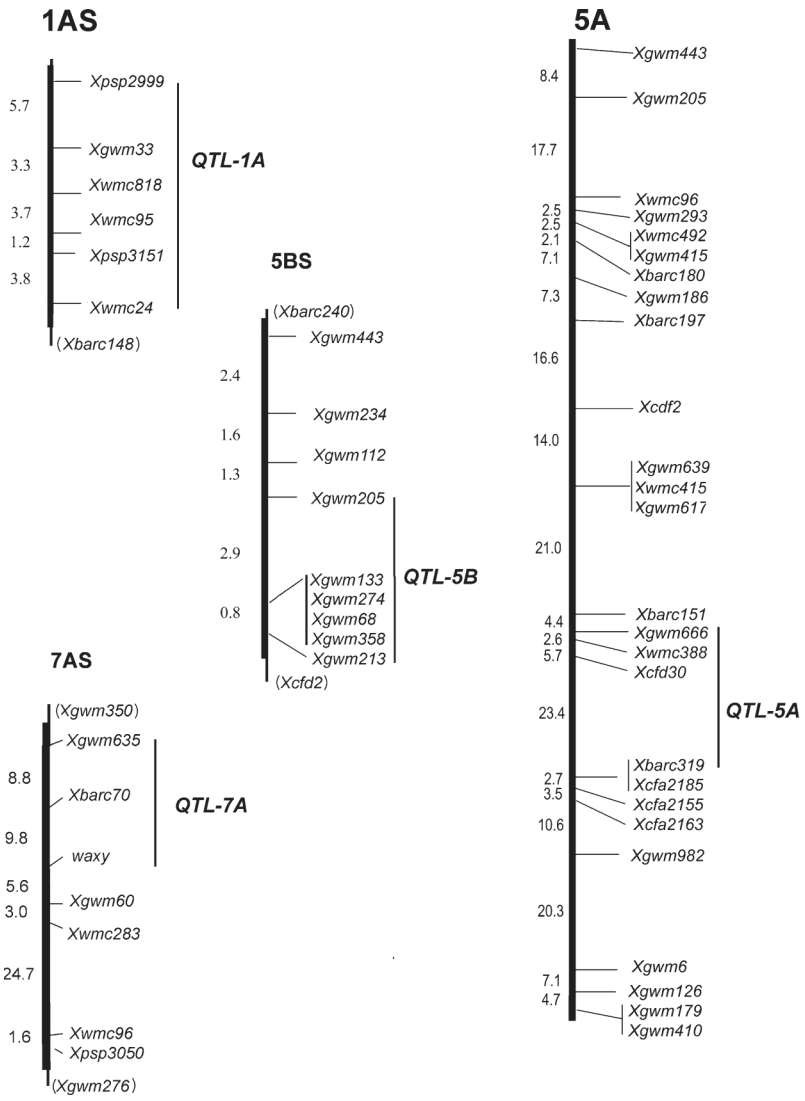

Fig. 2 Genetic maps of the regions introgressed from *T. militinae* (*bold*) on chromosomes 1A, 5A, 7A and 5B. QTL regions are marked by *vertical bars*. Markers are ordered according to the data of the DH population, and distances of the map are calculated on the basis of segregation in the F_2 8.1 × 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.

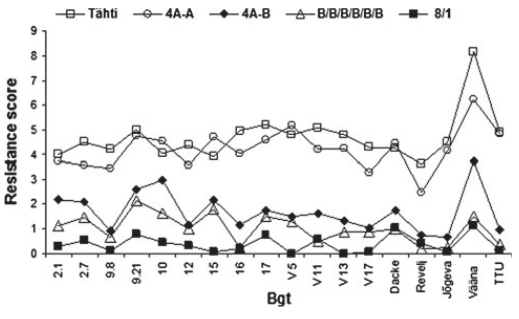

Fig. 3 Mean of seedling resistance scores upon inoculation with different subisolates, isolates and mixtures of *Blumeria graminis* DC. f. sp. *tritici* (Bgt). Data for the parental cv. Tähti, the resistant introgression line 8.1 together with data for the DH subpopulations carrying *T. militinae* allele (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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References

Asíns MJ (2002) Present and future of quantitative trait locus analysis in plant breeding. Plant Breed 121:281–291

Badaeva ED, Budashkina EB, Bilinskaya EN, Pukhalskiy VA (2010) Intergenomic chromosome substitutions in wheat interspecific hybrids and their use in the development of genetic nomenclature of *Triticum timopheevii* chromosomes. Russ J Genet 46:869–886

Bent AF, Mackey D (2007) Elicitors, effectors and R-genes: the new paradigm and a lifetime supply of questions. Annu Rev Phytopathol 45:399–436

Bougot Y, Lemoine J, Pavoine MT, Guyomar'ch H, Gautier V, Muranty H, Barloy D (2006) A major QTL effect controlling resistance to powdery mildew in winter wheat at the adult plant stage. Plant Breed 125:550–556

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

Canady MA, Ji Y, Chetelat RT (2006) Homeologous recombination in *Solanum lycopersicoides* introgression lines of cultivated tomato. Genetics 174(4):1775–1788

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

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, Buffalo

Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913

Devos KM, Dubcovsky J, Dvořák J, Chinoy CN, Gale MD (1995) Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. Theor Appl Genet 91:282–288

Dobrovolskaya OB, Sourdille P, Bernard M, Salina EA (2009) Chromosome synteny of the A genome of two evolutionary wheat lines. Russ J Genet 45:1368–1375

Dorofeyev VF, Jakubtsiner MM, Rudenko MI, Migushova EF, Udachin RA, Merezhko AF, Semenova LV, Novikova MV, Gradchaninova OD, Shitova IP (1976) The wheats of the world. Brezhnev DD, Dorofeyev VF (eds) Kolos Publ., Leningrad, 487 pp (in Russian)

Dvořák J, Zhang HB (1990) Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci USA 87:9640–9644

Dvořák J, di Terlizzi P, Zhang HB, Resta P (1993) The evolution of polyploid wheats: identification of the A genome donor species. Genome 36:21–31

Feuillet C, Langridge P, Waugh R (2008) Cereal breeding takes a walk on the wild side. Trends Genet 24:24–32

Gordeeva EI, Leonova IN, Kalinina NP, Salina EA, Budashkina EB (2009) Comparative cytological and molecular analysis of common wheat introgression lines containing genetic material of *T. timopheevii* Zhuk. Russ J Genet 45:1428–1437

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752

Gustafson GD, Shaner G (1982) Influence of plant age on the expression of slow-mildewing resistance in wheat. Phytopathology 72:746–749

Hossain KG, Kalavacharla V, Lazo GR, Hegstad J, Wentz MJ, Kianian PMA, Simons K, Gehlhar S, Rust JL, Syamala RR, Obeori K, Bhamidimarri S, Karunadharma P, Chao S, Anderson OD, Qi LL, Echalier B, Gill BS, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Akhunov ED, Dvořák J, Miftahudin, Ross K, Gustafson JP, Radhawa HS, Dilbirligi M, Gill KS, Peng JH, Lapitan NLV, Greene RA, Bermudez-Kandianis CE, Sorrells ME, Feril O, Pathan MS, Nguyen HT, Gonzalez-Hernandez JL, Conley EJ, Anderson JA, Choi DW, Fenton D, Close TJ, McGuire PE, Qualset CO, Kianian SF (2004) A chromosome bin map of 2148 expressed sequence tag loci of wheat homoeologous group 7. Genetics 168:687–699

Hsam SLK, Zeller FJ (1997) Evidence of allelism between genes *Pm8* and *Pm17* and chromosomal location of powdery mildew and leaf rust resistance genes in the common wheat cultivar Amigo. Plant Breed 116:119–122

Hsam SLK, Zeller FJ (2002) Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In: Bélanger RR, Bushnell WR, Dick AJ, Carver TLW (eds) The powdery mildews: a comprehensive treatise. American Phytopathological Society Press, St. Paul, pp 219–238

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

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

Ishikawa G, Nakamura T, Ashida T, Saito M, Nsuda S, Endo TR (2009) Localization of anchor loci representing five hundred annotated rice genes to wheat chromosomes using PLUG markers. Theor Appl Genet 118:499–514

Jakobson I, Peusha H, Timofejeva L, Järve K (2006) Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line. Theor Appl Genet 112:760–769

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

Jiang J, Gill BS (1994a) Different species-specific chromosome translocation in *Triticum timopheevii* and *T. turgidum* support diphyletic origin of polyploid wheats. Chromosome Res 2:59–64

Jiang J, Gill BS (1994b) New 18S-26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma 103:179-185. Chromosoma 103:179–185

Jorgensen JH, Jensen CJ (1973) Gene *Pm6* for resistance to powdery mildew. Euphytica 22:423

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

Khlestkina EK, Kumar U, Röder MS (2010) *Ent*-kaurenoic acid oxidase genes in wheat. Mol Breed 25:251–258

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 232:1360–1363

Krattinger SG, Lagudah ES, Wicker T, Risk JM, Ashton AR, Selter LL, Matsumoto T, Keller B (2011) Lr34 multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. Plant J 65:392–403

Kubaláková M, Valárik M, Barto J, Vrána J, Číhalíková J, Molnár-Láng M, Dolezel J (2003) Analysis and sorting of rye (*Secale cereale* L.) chromosomes using flow cytometry. Genome 46(5):893–905

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theor Appl Genet 114:21–30

Lan CX, Liang SS, Wang ZL, Yan J, Zhang Y, Xia XC, He ZH (2009) Quantitative trait loci mapping for adult-plant resistance to powdery mildew in Chinese wheat cultivar Bainong 64. Phytopathology 99:1121–1126

Lan CX, Ni X, Yan J, Zhang Y, Xia XC, Chen X, He ZH (2010) Quantitative trait loci mapping of adult-plant resistance to powdery mildew in Chinese wheat cultivar Lumai 21. Mol Breed 25:615–622

Large EC (1954) Growth stages in cereals. Illustrations of the Feeke's scale. Plant Pathol 3:129

Lillemo M, Skinnes H, Brown JKM (2010) Race specific resistance to powdery mildew in Scandinavian wheat cultivars, breeding lines and introduced genotypes with partial resistance. Plant Breed 129:297–303

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

Maestra B, Naranjo T (1999) Structural chromosome differentiation between *Triticum timopheevii* and *T. turgidum* and *T. aestivum*. Theor Appl Genet 98:744–750

Maxwell JJ, Lyerly JH, Cowger C, Marshall D, Brown-Guedira G, Murphy JP (2009) MlAG12: a *Triticum timopheevii*-derived powdery mildew resistance gene in common wheat on chromosome 7AL. Theor Appl Genet 119:1489–1495

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

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

Muranty H, Pavoine M-T, Jaudeau B, Radek W, Doussinault G, Barloy D (2009) Two stable QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 are expressed at different times along the growing season. Mol Breed 23:445–461

Naranjo T, Roca A, Goicoechea PG, Giraldez R (1987) Arm homeology of wheat and rye chromosomes. Genome 29:873–882

Perugini LD, Murphy JP, Marshall D, Brown-Guedira G (2008) *Pm37*, a new broadly effective powdery mildew resistance gene from *Triticum timopheevii*. Theor Appl Genet 116:417–425

Peusha H, Enno T, Jakobson I, Tsõmbalova J, Ingver A, Järve K (2008) Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia. Acta Agric Scand Sect B Plant Soil Sci 54:289–296

Robe P, Pavoine MT, Doussinault G (1996) Early assessment of adult plant reaction of wheat (*Triticum aestivum* L) to powdery mildew (*Erysiphe graminis* f sp *tritici*) at the five-leaf stage. Agronomie 16:441–451

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

Rodriguez S, Perera E, Maestra B, Díez M, Naranjo T (2000) Chromosome structure of *Triticum timopheevii* relative to *T. turgidum*. Genome 43:923–930

Salina EA, Leonova IN, Efremova TT, Röder MS (2006) Wheat genome structure: translocations during the course of polyploidization. Funct Integr Genomics 6:71–80

Šimková H, Svensson JT, Condamine P, Hribová E, Suchánková P, Bhat PR, Bartos J, Safár J, Close TJ, Dolezel J (2008) Coupling amplified DNA from flow-sorted chromosomes to high-density SNP mapping in barley. BMC Genomics 9:294

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor Appl Genet 111:731–735

Srnić G, Murphy JP, Lyerly JH, Leath S, Marshall DS (2005) Inheritance and chromosomal assignment of powdery mildew resistance genes in two winter wheat germplasm lines. Crop Sci 45:1578

Tommasini L, Yahiaoui N, Srichumpa P, Keller B (2006) Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. Theor Appl Genet 114:165–175

Touraev A, Indrianto A, Wratschko I, Vicente O, Heberle-Bors E (1996) Efficient microspore embryogenesis in wheat (*Triticum aestivum* L.) induced by starvation at high temperature. Sex Plant Reprod 9:209–215

Tucker DM, Griffey CA, Liu S, Maroof MAS (2006) Potential for effective marker-assisted selection of three quantitative trait loci conferring adult plant resistance to powdery mildew in elite wheat breeding populations. Plant Breed 125:430–436

Tucker DM, Griffey CA, Liu S, Brown-Guedira G, Marshall DS, Saghai Maroof MA (2007) Confirmation of three quantitative trait loci conferring adult plant resistance to powdery mildew in two winter wheat populations. Euphytica 155:1–13

Vrána J, Kubaláková M, Šimková H, Číhalíková J, Lysák MA, Dolezel J (2000) Flow sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). Genetics 156(4):2033–2041

Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 37:528–538

Yu DZ, Yang XJ, Yang LJ, Jeger MJ, Brown JKM (2001) Assessment of partial resistance to powdery mildew in Chinese wheat varieties. Plant Breed 120:279–284

PUBLICATION III

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Research Paper

Characterization of new allele influencing flowering time in bread wheat introgressed from *Triticum militinae*

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Flowering time variation was identified within a mapping population of doubled haploid lines developed from a cross between the introgressive line 8.1 and spring bread wheat cv. Tähti. The line 8.1 carried introgressions from tetraploid *Triticum militinae* in the cv. Tähti genetic background on chromosomes 1A, 2A, 4A, 5A, 7A, 1B and 5B. The most significant QTL for the flowering time variation was identified within the introgressed region on chromosome 5A and its largest effect was associated with the *VRN-A1* locus, accounting for up to 70% of phenotypic variance. The allele of *T. militinae* origin was designated as *VRN-A1f-like*. The effect of the *VRN-A1f-like* allele was verified in two other mapping populations. QTL analysis identified that in cv. Tähti and cv. Mooni genetic background, *VRN-A1f-like* allele incurred a delay of 1.9–18.6 days in flowering time, depending on growing conditions. Sequence comparison of the *VRN-A1f-like* and *VRN-A1a* alleles from the parental lines of the mapping populations revealed major mutations in the promoter region as well as in the first intron, including insertion of a MITE element and a large deletion. The sequence variation allowed construction of specific diagnostic PCR markers for *VRN-A1f-like* allele determination. Identification and quantification of the effect of the *VRN-A1f-like* allele offers a useful tool for wheat breeding and for studying fine-scale regulation of flowering pathways in wheat.

Introduction

Bread wheat (*Triticum aestivum* L.) is a staple food for 40% of the human population [1] and is one of the three most important crops worldwide. It is an allohexaploid species with a large and complex genome (1C = 17 Gb, $2n = 6x = 42$, AABBDD genome) which is composed of more than 80% of repetitive elements [2]. It is a relatively young species and its emergence, spread and adaptation to a wide range of environmental conditions are closely connected to the development of settled human society. The fast growth of the human population and changes in diet preferences have significantly increased demands for higher wheat production. It was estimated that the annual yield increase should reach about 1.7% to satisfy the needs. However, the current increase is only about 1% a year [3].

Changing environmental conditions pose a challenge to breed varieties with high and stable yield under the pressure of biotic and abiotic stress. Furthermore, intensive breeding has led to a narrowed gene pool in current elite wheat cultivars [4]. An attractive source of new genes and alleles for wheat improvement are related species, wild wheat ancestors and landraces which have not experienced bottlenecks connected to the emergence of modern cultivars [5]. One of the most important traits affecting yield is flowering time. Optimal timing of flowering is critical not only for successful flower development, but also for grain filling and for

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GLOSSARY

Vernalization The original term 'jarovizacia' by Lysenko was replaced by the currently used term 'vernalization' by Chouard [47]. Vernalization is defined as the importance of prolonged cold exposure for initiating the flowering process. On the basis of their vernalization requirement, bread wheat varieties are classified as winter, spring and facultative varieties. Winter wheat is sown in winter and needs cold exposure for flowering, spring wheat (sown in spring) will flower without any cold period. Facultative wheat varieties can be sown either in autumn or in spring with a safe transition to generative stage.

MITE transposable element Miniature inverted-repeat transposable elements (MITEs) are non-autonomous transposable elements often associated with genes. Unlike autonomous DNA transposons, the internal sequences of MITEs are short and devoid of coding sequences. MITE sequences are frequently transcribed with plant genes.

MADS box The name refers to four of the originally identified members: MCM1, AG, DEFA and SRF of the MADS box proteins. MADS box is a conserved motif found within DNA binding protein domains. It consists of 56 amino acids, 9 of which are identical in all MADS box family members described so far. The N-terminal part is the major determinant of DNA-binding specificity whilst the C-terminal half is necessary for dimerisation. The majority of MADS-box proteins bind similar sites based on the consensus sequence $CC(A/T)_6GG$, although each protein apparently possesses a distinct binding specificity. Moreover, several MADS-box proteins specifically recruit other transcription factors into multi-component regulatory complexes. Such interactions with other proteins appear to be a common theme within this family and play a pivotal role in the regulation of target genes.

survival of plants *sensu lato*, especially for annual species. The importance of this trait is expressed in the large number of genes involved in its regulation. However, only some of these genes have been cloned, well characterized and used in breeding (reviewed in [6]).

A complex genome and wide range of environmental conditions affecting wheat during its evolution have led to the development of a robust flowering regulation which allows adaptation to various macro- and micro-environmental conditions. Many genes working in a redundant manner are involved in the regulation of flowering and, at present, three key pathways are considered [7]. The vernalization pathway is responsible for avoiding flowering before or during the cold period (*VRN* genes). The photoperiodic pathway is responsible for perception of day length (*PPD* genes) and the earliness per se (*EPS*) genes are responsible for fine tuning of flowering time in reaction to specific climatic conditions. Even a minor variation in major photoperiod and vernalization genes is connected to large differences in flowering time and thus fast adaptability of wheat to diverse locations [8]. Currently, the vernalization pathway is the most characterized and its three major genes *VRN1*, *VRN2*, and *VRN3* have been cloned [9–11]. The *VRN3* gene is orthologous to *Arabidopsis thaliana FLOWERING LOCUS T* (*FT*) [11] and seems to be the major integrator of environmental signals from cold, photoperiod, and *EPS* genes in leaves [12]. The VRN3 protein is transported through phloem to apical meristem where it triggers transition from vegetative to generative stage by inducing the expression of the *VRN1* gene [13]. The *VRN2* gene, originally believed to be the vernalization gene [9], is the most likely mediator between vernalization and photoperiodic pathways and is involved in the repression of the *VRN3* gene [14]. The *VRN1* gene is homologous to *A. thaliana* flower meristem identity gene *APETALA1* (*AP1*) [15]. It seems that *VRN1* has pleiotropic function and besides affecting the floral meristem identity in apex, it is involved in the integration of cold signals in leaves. Cold treatment leads to *VRN1* upregulation which inhibits expression of *VRN2* and releases expression of *VRN3* [14]. Additionally, *VRN1* expression has been linked to altered frost tolerance [16] and yield traits regulation [17]. *VRN1* belongs to the family of MADS box transcription factors [14,18,19] with a CArG regulatory box in promoter region [10] and an additional regulatory region in the first intron [20]. Even though it has been determined that *VRN1* is not absolutely essential for flowering in polyploids [14], its altered expression can significantly affect the flowering pathway. The significance of this gene was demonstrated by the discovery that a mutation in the first intron or promoter of the gene leads to spring growth habit [19,20]. Flowering time modulation through allelic variation of the *VRN1* gene [10,13,19–22] is a promising tool for breeding new varieties better adapted to local environmental conditions.

Attractive sources of genetic variability are wild wheat ancestors and landraces. One of them is *Triticum militinae* (Zhuk. &Migush) ($2n = 4x = 28$, A^tA^tGG genome) which originated from a hybridization event separate from emmer wheats and belongs to the *T. timopheevii* (Zhuk.) Zhuk. group. Timopheevii wheats have been used as a source of new resistance genes: four genes for resistance to leaf rust (*LrTt1*, *LrTt2*, *Lr18* and *Lr50*), three genes for resistance to stem rust (*Sr36*, *Sr37*, and *Sr40*) and four genes for resistance to powdery mildew (*Pm6*, *Pm27*, and *Pm37*) [23] have been introduced into common wheat. Recently, an introgression line 8.1 was created from a cross of *T. militinae* and elite Finnish cv. Tähti [24]. In the line, *T. militinae* introgressions were identified on seven chromosomes (1AS, 2AS, 2AL, 4AL, 5AL, 7AS, 1BS, and 5BS), and improved resistance to powdery mildew in seedlings and adult plants was found to cosegregate with the locus *QPm-tut-4A* on chromosome 4A [25]. The *QPm-tut-4A* gene is a subject of cloning, however, the analyzed mapping population showed a significant variability in flowering time. In this study, we report identification, quantitative trait loci (QTL) mapping, verification and sequence characterization of a *VRN-A1* allele introgressed into bread wheat from *T. militinae* that affects flowering time of elite spring bread wheat varieties.

Material and methods

Plant material

A mapping population of 314 doubled haploid (DH) lines was developed from a cross between introgression spring line 8.1 and

spring *T. aestivum* cv. Tähti as described by Jakobson *et al.* [24,25]. The line 8.1 contains *T. militinae* introgressions on seven chromosomes (1AS, 2AS, 2AL, 4AL, 5AL, 7AS, 1BS, and 5BS). Thus, the DH lines vary in the presence of different *T. militinae* segments. Seeds of spring wheat *T. militinae* (accession no. K-46007) were obtained from the N.I. Vavilov Institute of Plant Industry (St. Petersburg, Russia). Two verification mapping populations (VMP1 and VMP2) were developed to estimate and confirm the influence of *T. militinae* introgressions on flowering time in different genetic backgrounds. To simplify *T. militinae* based traits segregation analysis, the DH81 line was derived by dihaploidization of the original introgression line 8.1. The DH81 line contains all the introgression segments except the 2AL and 1BS introgressions and introgression on 5A was slightly shortened. To specifically address the effect of *VRN1–VRN3* genes, the DH397 line was selected from the DH mapping population described above. In the DH397 line, introgressions on 1A, 2AL, and 7A chromosomes are not present and introgressions on 5A and 5B were shorter as compared to DH81. The introgression loss or shortening included loci of the *VRN1–VRN3* genes (data not shown). VMP1 comprises 194 F_2 lines derived from a cross of the doubled haploid lines DH81 and DH397 (98 F_2 plants with DH397 as the female parent, 96 F_2 plants with DH81 as the female parent). VMP2 was developed by crossing of the DH81 line with *T. aestivum* cv. Mooni as female and comprises 94 F_2 lines. Seeds of spring bread wheat cv. Mooni were provided by Estonian Crop Research Institute (Jõgeva, Estonia).

DNA extraction and PCR amplification

Genomic DNA was extracted from 100 mg of lyophilized and homogenized leaf tissue. Homogenization was done using Tissue Lyser (Qiagen, Germany) in 96-well deep well plates with two glass beads (5 mm) in each well for 4 min at 27 Hz. DNA was purified either using the NucleoSpin® 96 Plant II kit (Macherey-Nagel, Germany) according to manufacturer's instructions, or with a modified Agencourt® Genefind v2 kit (Beckman Coulter, USA). The Genefind v2 kit was used in 96-well plate format and pipetting was done by automatic pipetting workstations Biomek NX^P (Beckman Coulter, USA). Homogenized leaf tissue was incubated in 1 ml of Lysis Buffer [0.5% sodium bisulfite; 0.1% ascorbic acid; 1% β-mercaptoethanol; 0.5 M NaCl; 0.1 M Tris–HCl; 0.05 M EDTA, pH 7.2; and 2.1 U of RNase A (Sigma–Aldrich, USA)] at 65°C for 45 min. 10 µl of Genefind v2 magnetic beads (Beckman Coulter, Beverley, USA) was mixed with 100 µl of lysate and 80 µl of isopropanol and incubated for 10 min at room temperature to bind DNA to the beads. The mixture was incubated on 96 S Super Magnet Plate (Alpaqua, USA) for 3 min to settle the beads. Supernatant was removed and the beads were washed four times with 100 µl of 70% ethanol. DNA was diluted in 50 µl of redistilled water after evaporation of the residual ethanol. PCR consisted of template DNA (~10 ng), 1× PCR buffer (10 mM Tris–HCl, pH 8.8 at 25°C; 1.5 mM $MgCl_2$; 50 mM KCl; 0.1% Triton X-100); 200 mM dNTPs; 0.5 µM of each primer; 0.5 U of HOT FIREPol® DNA Polymerase (Solis BioDyne, Estonia). PCRs were performed in the C1000 Touch™ Thermal cycler (BIO-RAD, USA). Annealing temperatures of SSR markers followed information published at Graingenes 2.0 (http://wheat.pw.usda.gov/). Amplicons were separated either on 3.5%/6% non-denaturing polyacrylamide gel using Mega-gel apparatus (C.B.S. SCIENTIFIC, USA) or on 1.5% agarose gel stained with ethidium bromide or as described by Jakobson *et al.* [22] and visualized under UV light using the InGenius imaging system (Syngene, UK).

Genotyping and sequencing

Individual plants of mapping populations were genotyped using a set of 57 polymorphic markers (Supplementary file 1, Tab. S1). Since the original mapping population (line 8.1 × Tähti) was created as near-isogenic lines (NILs), the markers used were chosen with a focus on the introgressed regions, only. In addition, markers for major flowering time genes (*VRN1*, *VRN3* and *PPD-A1*) were added. *VRN-A1* was mapped with publicly available markers [26] and with VRN-1A_F4 and VRN-1A_R4 primers developed in this study (Supplementary file 1, Tab. S2). *PPD-A1* and *VRN3* were mapped by genotyping-by-sequencing with markers developed in this study (Supplementary file 1, Tab. S1 and S2). A subset of markers specific for introgressions were used to determine the genomic composition of VMP lines (Supplementary file 1, Tab. S1). To identify sequence variation of the *VRN-A1* gene, a set of 25 primer pairs (Supplementary file 1, Tab. S2) covering the whole length of the *VRN-A1* gene locus (~13,000 bp) and ~ 600 bp of the promoter sequence were designed and used for amplification in all three parental lines (cv. Tähti, cv. Mooni and DH81). *VRN-A1* specific primers were designed manually using publicly available sequences of the *VRN1* gene for wheat A, B, D genome and A genome of *T. timopheevi* (GenBank accession nos. AY747601.1, AY747604.1, AY747606.1, GQ451751). Additionally, cv. Chinese Spring chromosome-derived survey sequences were used [27]. All sequences were aligned using the Geneious 5.6.4 software (http://www.geneious.com) [28]. The designed primer pairs were considered specific when at least one primer had two or more genome specific SNPs/indels in its sequence. Melting temperature was determined by Primer-BLAST [29]. DNA amplification was carried out by touchdown PCR: initial denaturation for 15 min at 95°C followed by 14 cycles at 95°C for 40 s, 65°C for 40 s (increment −0.7°C/per cycle) and 72°C for 2 min. Twenty six more cycles were performed at 95°C for 40 s, 55°C for 40 s and 72°C for 2 min. Amplicons were separated as described above. Before sequencing, 5–20 ng of PCR products were purified in a mixture of 1 U Exonuclease I/0.5 U FastAP™ Thermosensitive Alkaline Phosphatase (Thermoscientific, USA) in 7 µl of 1× PCR buffer for 20 min at 37°C. Enzymes were inactivated by incubation for 30 min at 95°C.

The sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and purified using the Agencourt Clean SEQ kit (Beckmann Coulter, USA). The reactions were analyzed on the ABI 3730xl DNA analyzer (Applied Biosystems, USA). The resultant sequences were trimmed and assembled using Geneious 5.6.4 (http://www.geneious.com) [28]. Assemblies were verified by alignment with the reference sequence of Triple Dirk D *VRN-A1* gene (AY747601.1). Regulatory elements were delimited according to publicly available sequences [30]. Transposable elements were identified using the TriAnnot pipeline [31]. Polymorphisms in the *VRN-A1* gene between parental lines of mapping populations used (cv. Tähti, cv. Mooni, and DH81) were identified by alignment using the Geneious 5.6.4 software (http://www.geneious.com) [28].

Phenotyping of developed mapping populations

Phenotyping experiments were performed during three seasons (2009/2010, 2013 and 2014) in three different locations (Jõgeva and Tallinn – Estonia, and Olomouc – the Czech Republic) in the field as well as under controlled environmental conditions. Each phenotype experiment was designed as follows:

*Experiment 1: evaluation of the effect of **T. militinae** introgressions on flowering time variation in the DH mapping population (cv. Tähti genetic background)*

To confirm the effect of the introgressions on flowering time, experiments were established in two different environmental conditions. Experiment 1a: the full mapping population of 314 DH lines was tested in the Czech Republic. Experiment 1b: a random subset of 113 DH lines was tested in Estonia. Each plant in the experiments was genotyped using the set of 55 markers (Supplementary file 1, Tab. S1).

1a. Olomouc, the Czech Republic, season 2013

Seed germination of 314 DH lines was synchronized by soaking the seeds in tap water for 24 hours at room temperature, followed by incubation of the seeds on wet filter paper for two days at 4°C and one day at 25°C in plastic Petri dishes. Four seedlings per line were planted in jiffy pots (6 cm × 6 cm) and grown in a greenhouse at 20°C under natural light. Seedlings at the stage of three leaves were transplanted to the field in a randomized manner. Flowering time (days to emergence of half of the spike on the main stem) of each individual plant was recorded daily at the same time of a day. Mean flowering time of individual lines was used for QTL mapping.

1b. Jõgeva, Estonia, winter 2009/2010

Six seeds per line of 113 DH lines, cv. Tähti and line 8.1 were planted directly into the soil in a plastic container (35 cm × 65 cm) and grown under controlled environmental conditions (16/8 hours day/night, light intensity 168 $\mu E\, m^{-2}\, s^{-1}$, high pressure sodium lamps, temperature 20–23°C) in a greenhouse. The day when at least half of spike of at least half of plants of every line emerged was considered to be the flowering time.

Experiment 2: assignment of the flowering time variation to particular introgressions (Olomouc, 2014)

Verification of introgression effect on flowering time was done using 194 F_2 plants of VMP1 derived from reciprocal crosses of lines DH397 and DH81. Seeds were germinated, plants were grown and flowering time was recorded as described in Experiment 1a. The presence of *T. militinae* introgressions was verified by genotyping using at least one marker per introgression (Supplementary file 1, Tab. S1).

*Experiment 3: verification of the effect of **T. militinae** introgressions on flowering time variation in cv. Mooni genetic background*

F_3 plants from cross of DH81 x Mooni (VMP2, 94 lines), developed in previous experiments (Jakobson and Järve, unpublished data), were employed using $F_{2:3}$ approach described at Austin and Lee [32]. F_3 plants from the VMP2 lines were screened for flowering time in two different locations. Experiments 3a and 3b were performed in Olomouc, the Czech Republic and Tallinn, Estonia, respectively. Mean flowering time of each line was used to reconstruct the phenotype of F_2 lines. A subset of 24 markers (Supplementary file 1, Tab. S1) was used for introgression segments mapping.

3a. Olomouc, the Czech Republic, season 2014

Ten F_3 plants from 80 lines of the VMP2 were evaluated for flowering time in field conditions in Olomouc. Seeds were germinated and grown as described in Experiment 1a. Flowering time data of 80 F_3 lines was collected. Every individual F_3 plant was genotyped for 14 markers (one to five markers for every introgression (Supplementary file 1, Tab. S1) to confirm identity to the original F_2 plant.

3b. Tallinn, Estonia, season 2014

Four F_3 plants from 94 families of the VMP2 were grown in a room with controlled environmental conditions at 22–23°C, 16/8 hours day/night, humidity 50–70%, fluorescent light with intensity 150 $\mu E\, m^{-2}\, s^{-1}$. The seeds were sown directly into 13 cm × 13 cm cm pots. Plants were not ordered, but grown in a randomized manner. For each of the F_3 plants, flowering time was recorded as described in Exp. 1b.

QTL mapping and statistical analysis

QTL analysis was performed using the statistic software Map Manager QTX Version b16 [33]. Genetic distances were established using the Kosambi mapping function. The threshold value for Likelihood ratio statistic (LRS) for suggestive ($P < 0.05$), significant ($P < 0.001$) and highly significant ($P < 0.0001$) loci was calculated for each experiment using permutation test at 1000 iterations. Single marker regression was used to associate phenotype with marker in each experiment. The position of detected QTLs was determined using simple interval mapping. The confidence interval was established by bootstrap analysis with 1000 iterations using the same software. Statistical significance was determined using single factor analysis of variance (ANOVA).

Results

Identification of QTLs controlling flowering time

Using gene-specific primers (Supplementary file 1, Tab. S2) and genotyping-by-sequencing, *VRN-A1*, *VRN3* and *PPD-A1* genes were mapped within the *T. militinae* introgressions in line 8.1. *VRN-A1* was located 16.2 cM distal to *Xcfd30* and 26 cM proximal to *Xpsp3001* on chromosome 5A (Fig. 1). *VRN3* was positioned 8.8 cM distal to *Xwmc283* on chromosome 7A. *PPD-A1* was mapped in close proximity to *Xgwm71.1* loci (4.1 cM distant) on chromosome 2A (data not shown). In total, 314 and 113 DH lines generated from the progeny of the cross between line 8.1 and cv. Tähti were screened for flowering time in Olomouc (Experiment 1a) and Jõgeva (Experiment 1b). The core population analyzed in Jõgeva was included in the population tested in Olomouc. A moderate phenotypic correlation ($r = 0.46$, $P < 0.001$) between the two experiments was observed. Flowering time of line 8.1 and cv. Tähti differed by 3.5 days (89.3 versus 92.8) in Olomouc and by 5 days in Jõgeva (72 versus 77 days). In both experiments, line 8.1 flowered later than cv. Tähti. Flowering time of individual plants in mapping populations ranged from 81 to 98 and from 65 to 87 days in

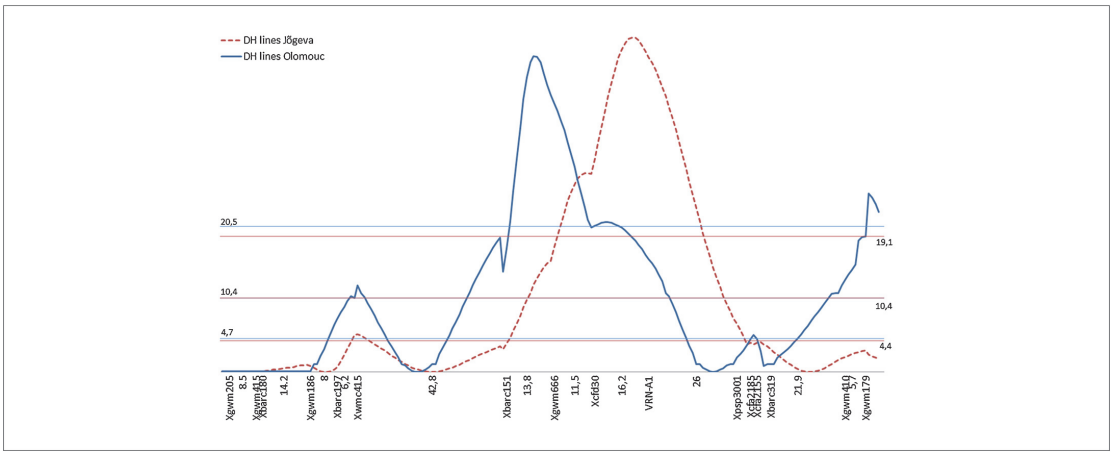

FIGURE 1

Location of the flowering time QTL on chromosome 5A. Simple interval mapping analyses of the DH mapping population based on heading time data from Experiment 1a (introgression line 8.1 × cv. Tähti, Olomouc, solid line) and Experiment 1b (introgression line 8.1 × cv. Tähti, Jõgeva, dashed line) localised the flowering time QTL in the region between markers *Xbarc151* and *Xcfa2155*, with the peak at the *Xgwm666/VRN-A1* locus. The suggestive threshold line (likelihood ratio statistic LRS: 4.7/4.4), the significant threshold line (LRS: 10.4/10.4) and highly significant threshold line (LRS: 20.5/19.1) are displayed for Experiments 1a/1b, respectively. Threshold values are shown on the left (Experiment 1a) and on the right side (Experiment 1b) of the graph. The threshold lines of respective QTL lines are marked with the same colour. The distance in cM between markers is shown on *x* axis.

Experiment 1a and Experiment 1b, respectively (Table 1). Marker regression analysis showed that at least 29 markers were associated with phenotypic variation on a suggestive level in at least one experiment (Supplementary file 1, Tab. S3). Significant QTLs were identified on five chromosomes: 1A, 2A, 4A, 5A and 7A; however, highly significant loci responsible for variation in at least one experiment were determined only on chromosomes 5A and 7A. The QTL localised on 7A, delimited by the *Xbarc70*–*Xpsp3050* markers and peaking at *Xwmc283* was associated with *VRN3* [34,35] and was significant only in Olomouc (Supplementary file 1, Tab. S3). The QTL localised on 5A detected in both experiments was delimited by the *Xbarc151*–*Xcfa2155* markers and overlapped with the *VRN-A1* gene locus (Fig. 1). The *T. militinae* allele of the *VRN-A1* gene accounted for 1.9 and 5.8 days delay in flowering time in Experiment 1a and 1b, respectively. Large differences in the number of significant QTLs and flowering time suggested additional environmental and genetic influences in Experiments 1a and 1b, which necessitated further verification.

Confirmation of flowering time difference using verification mapping populations (VMPs)

Quantitative trait loci responsible for phenotypic variance in the primary mapping population often do not operate in the same manner in a different genetic background [36]. To verify loci identified in previous experiments, VMP1 and VMP2 were created and used. A difference of 6.5 days ($P < 0.001$) was identified between the parental lines of VMP1 (DH397 × DH81) in Experiment 2 (Tab. 1). Marker regression analysis showed highly significant contribution of the *VRN-A1* locus on chromosome 5A (Supplementary file 1, Tab. S4) to flowering time variation.

TABLE 1

Statistical analysis of phenotyping data.Phenotyping experiments were performed under controlled environmental conditions (greenhouse; 1b, 3b), or in field conditions (1a, 2, 3a). The experiment 1 was conducted on the DH mapping population generated from the cross between the introgression line 8.1 and cv. Tähti; the experiment 2 was performed on VMP1 population (DH397 x DH81) and experiment 3 was performed on VMP2 population (Mooni x DH81). Data are given for all lines of the mapping populations. FT - mean heading time in days. MIN - minimum value; MAX - maximum value; SD - standard deviation, *VRN1-A1f-like* contribution - contribution of the *VRN1-A1f*-like allele to the phenotype variation, FT difference between parental lines. Note that in all experiments the *T. militinae* allele caused a delay in flowering time. All parameters are in days.

Experiment	Parental lines	Background	Location	Greenhouse/ field	FT	MIN	MAX	SD	VRN1-A1f-like contribution	FT difference between parents
1a	8.1/Tähti	Tähti	O	f	90	81	98	3	+1.9	+3.5
1b	8.1/Tähti	Tähti	J	g	76	65	87	5	+5.8	+5. 5
2	DH397/DH81	Tähti	O	f	86	82	94	2	+1.7	+6.5
3a	DH81/Mooni	Mooni	O	f	84	78	90	2	+3.3	+9.8
3b	DH81/Mooni	Mooni	T	g	66	52	89	9	+18.6	+29.9

g-greenhouse; f – field; O – Olomouc; T – Tallinn; J - Jõgeva

The locus was delimited by markers *Xgwm639*-*Xcfd2155* (Supplementary file 2, Fig. S1) and responsible for 9% of the trait variation. The effect of replacing the Tähti allele with the *T. militinae* allele in the *VRN-A1* locus was almost the same as in Experiment 1a. The phenotypic analysis of the parental genotypes of VMP2 (DH81 × cv. Mooni) revealed an approximately 10 days and 30 days difference in flowering time in Experiment 3a and Experiment 3b, respectively (Tab.1). A moderate phenotypic correlation ($r = 0.63$, $P < 0.001$) was detected between these two experiments. Flowering time analysis of the F_3 generation was used for reconstruction of the F_2 phenotype followed by QTL analysis. The analysis identified the same loci for both experimental locations (Supplementary file 1, Tab. S5; Supplementary file 2, Fig. S5). The QTL with highest significance was located on chromosome 5A in the region delimited by markers *Xgwm666*–*Xgwm982*, with the peak in the position of the *VRN-A1* gene (Supplementary file 2, Fig. S2). The *VRN-A1* locus was responsible for 38% and 70% of flowering time variation in Experiment 3a and Experiment 3b, respectively (Supplementary file 1, Tab. S5; Supplementary file 2, Fig. S5). In different locations, the *T. militinae VRN-A1* allele caused a significant delay in flowering time in both backgrounds. However, the trait variance in tested populations was highly affected by growth conditions (greenhouse/field), and to a lesser extent, by the background of the *VRN-A1* allele.

Sequence analysis of VRN-A1 *alleles*

QTL analyses of the DH mapping population as well as VMPs indicated that different *VRN-A1* alleles were responsible for the major part of flowering time variation, and therefore the variability of the gene sequence in the parental lines (cv. Tähti, DH81 and cv. Mooni) was examined. The gene was amplified using 25 primer pairs (Supplementary file 1, Tab. S2) that cover the whole length of the *VRN-A1* gene (~13,000 bp), including approximately 600 bp of the promoter sequence. Comparison of the obtained sequences with the sequence of *VRN-A1* allele of the Triple Drk D (TDD) (GenBank accession no. AY747601.1), which was taken as reference, confirmed the completeness of the gene sequences. The sequences of *VRN-A1* alleles (cv. Mooni, cv. Tähti and DH81) have been deposited in the NCBI database (as KT696535, KT696536, KT696537, respectively) and contain the whole gene sequence, including the 340 bp and 625 bp segments of the promoter region in line DH81 and cvs. Tähti and Mooni, respectively, and a 276 bp segment downstream of the stop codon. The acquired sequences were aligned with the sequence of TDD and three types of mutations were identified which could be classified as small indels (1–10 bp), large indels (>10 bp), and SNPs. The promoter region of the *T. militinae VRN-A1* gene (from line DH81) was highly similar to the previously published *VRN-A1f* promoter (GQ451751) from *Triticum timopheevii* [37]. Only one 1 bp indel difference was identified (Supplementary file 2, Fig. S3) between these sequences in the HDD region as defined in [19] and hence the *T. militinae VRN1* allele from line DH81 was named *VRN-A1f-like*. No sequence variation in the *VRN-A1* locus was identified between the wheat cultivars Tähti and Mooni and they differ from TDD only by two single-base indels in the first intron and one SNP in intron 2 (Supplementary file 2, Fig. S4). On the other hand, *VRN-A1f-like* showed 38 SNPs, 6 small indels and 3 large indels as compared to TDD. In the promoter region, 5 SNPs were identified accompanied by a small (8 bp) deletion in the position −128 bp, and a large (50 bp) deletion in the position −63 bp, both from the start codon, compared to TDD (Fig. 2; Supplementary file 2, Fig. S3). In contrast to TDD, the Spring foldback transposable element (SFE) insertion [19] is absent in the *T. militinae VRN-A1f-like* allele (Fig. 2; Supplementary file 2, Fig. S3). None of these mutations is present in the known regulatory regions (CArG box, TATA box) of the *VRN-A1* alleles.

A majority of remaining mutations were identified in the first intron. The mutations included 24 SNPs, 2 small indels, a large deletion (2753 bp) and a large insertion (433 bp) of the miniature inverted-repeats transposable element (MITE) (Fig. 3; Supplementary file 2, Fig. S4). The newly identified MITE consisted of 424 bp including 53 bp inverted repeats at the ends and 9 bp host duplication (TAAAAAATA). The sequence was found to be 98% identical to DXX-MITE_3bSeqIt2-B-G2821-Map7. Six insertions of MITE

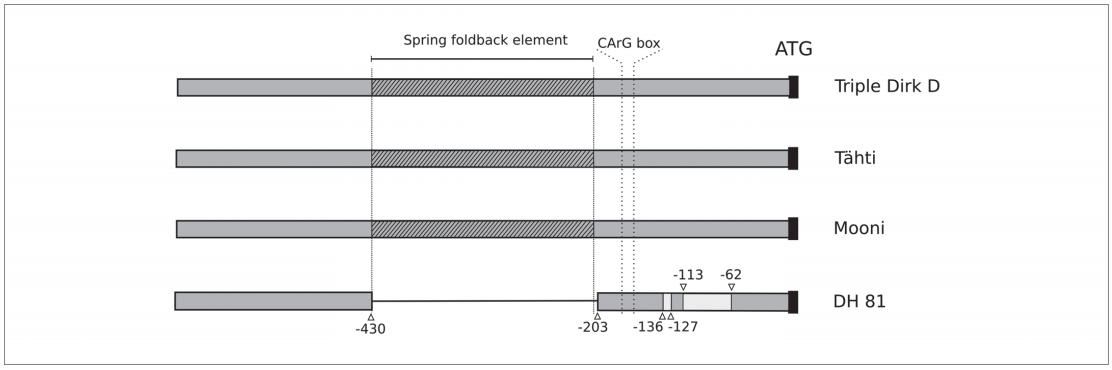

FIGURE 2

Comparison of the variation in VRN-A1 promoter region among parental lines. Sequencing the promoter region in parental lines of mapping populations showed sequence variation of *T. militinae VRN-A1f-like* allele from DH81 line in comparison with reference sequence of *VRN-A1a* promotor of Triple dirk D (GenBank accession no. AY747601.1). The differences comprised the absence of Spring fold element (SFE) insertion and two deletions (8 bp and 50 bp) located downstream of the CArG box. The exact position of indels is marked by white triangles and numbers represent flanking bases counted upstream from the START codon. Light grey boxes represent deletions, and the box with diagonal stripes represents the insertion of SFE. For sequence details see alignment (Supplementary file 2, Fig S3).

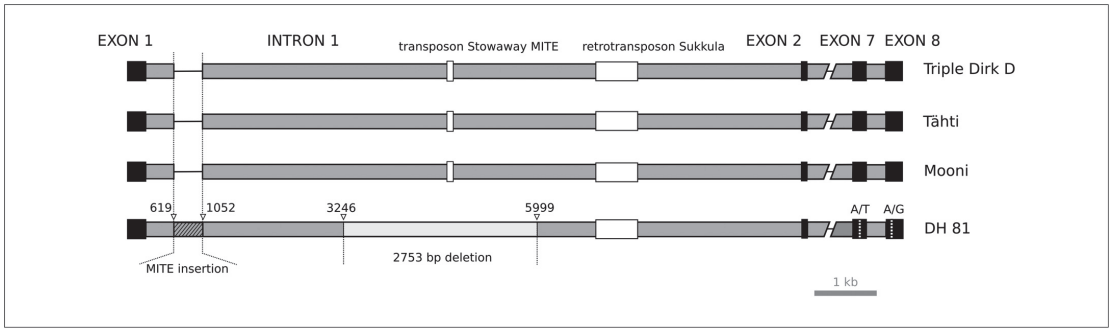

FIGURE 3

Comparison of the variation in *VRN-A1* allele among parental lines. Sequencing the *VRN-A1* gene from parental lines of mapping populations, and comparisons with the reference sequence of the *VRN-A1a* gene of Triple dirk D (AY747601.1) revealed insertion of MITE transposon (424 bp) with (9 bp) host duplication and large deletion (2753 bp) within the first intron of the *VRN-A1f-like* allele from DH81 line. Moreover, two synonymous SNPs were found in exon 7 and exon 8. The positions of indels are marked by white inverted triangles. Light grey box represents deletion; black boxes represent exons; the box with diagonal stripes represents MITE insertion and white boxes represent known retroelement insertions. Dashed vertical line shows single nucleotide substitution in exons only.

were identified on wheat chromosome 3B using the TRIANNOT pipeline [31]. In all six cases, the host duplications resemble TATA-box sequence. The identified MITE is homologous to four sequences in barley deposited at NCBI (www.ncbi.nlm.nih.gov) where it has been detected in close proximity to or directly associated with genes (either promoters or introns, data not shown).

In addition, six and one SNPs were identified in introns 2 and 4, respectively. In introns 4 and 8, the remaining 2 and 1 small indels were found, respectively. Of all the exons, only exons 7 and 8 had one SNP each. The SNP in exon 7 causes no amino acid change. The SNP in exon 8 causes a neutral amino acid substitution (A**A**C→A**G**C; ASN→SER; Fig. 3; Supplementary file 2, Fig. S4). Besides sequence polymorphisms, differences in amplicon size can be used as markers. Ten of the 25 primer pairs can be used as markers without sequencing (Supplementary file 1, Tab. S2). Three of them (VRN1AF/VRN_R1_2a, VRN1A_F3/R3 and F4/R4) are codominant markers showing size difference between the *VRN-A1a* and *VRN-A1f-like* alleles. Another five markers (VRN1_F2a/R2, F6/R6, F7/R7, F8/R8, and F9/R9a) are dominant for cvs. Tähti and Mooni, and two (VRN1_F2/R2 and F6/R9a) are dominant for *T. militinae*. The VRN1A_primer pairs F3/R3 and F4/R4 detect a 433 bp size variation caused by insertion of the MITE to the first intron of the *VRN-A1f-like* allele. VRN1 markers _F2a/R2 and F2/R2 detect the presence/absence of the 50 bp deletion in the promoter of the *VRN-A1f-like* allele. The remaining dominant markers detect the 2753 bp deletion in the first intron of the *VRN-A1f-like* allele (Supplementary file 1, Tab. S2).

Discussion

Allelic variation of vernalization (*VRN)* and photoperiod (*PPD)* genes is considered to be the main source of flowering time plasticity which enables wheat to grow and successfully flower at different altitudes and latitudes. The variability in flowering time is of high economic importance as it affects wheat reproductive success and in elite breeding material strongly affects yield components. Variability in the *VRN* and *PPD* genes can be defined either as copy number variation [38,39] or mutations in coding regions, promoters, or regulatory elements [19,20]. Extensive studies in cultivated and wild wheats were performed to characterize mutations in the promoter region or the first intron of the *VRN1* gene and to identify new *VRN1* alleles [13,19,30,37,40]. Moreover, the contribution of *VRN1* homoeologous alleles in hexaploid wheat and their effect on vigor and yield components has been estimated.

Tetraploid spring wheat *T. militinae* belongs to the Timopheevi group of wheat originating from a hybridization event separate from emmer wheats [41]. *T. militinae* is a promising source of new alleles useful in breeding [24], including new functional alleles of genes affecting flowering time. Diagnostic markers specific for these alleles are a valuable addition to the toolkit available for optimizing flowering time. Here we report interspecific introgression of *T. militinae* alleles of vernalization genes *VRN1, VRN3* and possibly *VRN2*, as well as the photoperiod-related gene *PPD1*, into the genome of spring wheat cv. Tähti (line 8.1). Using gene-specific primers and genotyping-by-sequencing, *VRN-A1, VRN-A3* and *PPD-A1* genes were mapped in the line 8.1 within the regions of *T. militinae* introgressions on chromosomes 5A, 7A and 2A, respectively. Furthermore, in at least one experiment, QTL analysis detected significant QTLs in the regions of these genes, indicating that the *T. militinae* alleles of *VRN-A1, VRN3, PPD-A1*, and possibly also *VRN-A2* may affect flowering time in *T. aestivum*.

Flowering time QTL analysis identified several minor QTLs at a significant level. A highly significant major QTL was identified in the locus of *VRN-A1* gene on chromosome 5A. The effect of the major QTL was confirmed in two additional mapping populations and, depending on genetic background and environmental conditions, it explained 5–70% of phenotype variability (Fig. 1, Supplementary file 1, Tab. S3–5). The *T. militinae VRN-A1* allele was designated *VRN-A1f-like* as its promoter region resembles the *VRN-A1f* allele from *T. timopheevii* [37]. The replacement of the Tähti *VRN-A1* allele with the *VRN-A1f-like* allele in the DH mapping population delayed flowering time by 5.8 and 1.9 days under controlled and field conditions, respectively. A similar effect was observed during subsequent verification of the effect (Experiment 2), where sister doubled haploid introgression lines DH81

and DH397 (VMP1) differed in flowering time by 6.5 days ($P < 0.001$) (Tab. 1). Line DH81 carries the *VRN-A1f-like* allele while DH397 does not, and the allele contributed to flowering time delay by 1.7 days (Tab. 1; Supplementary file 1, Tab. S4). Replacement of the allele in cv. Mooni caused an 18.6 and a 3.3 day flowering time delay under controlled and field conditions, respectively. Thus, the *VRN-A1f-like* allele can cause a delay of approximately 3 days in flowering time in field conditions. The approximately 3–6 fold increase in flowering time delay in controlled environmental conditions as compared to the field conditions could be explained by the effect of lower light quality and quantity, and/or limited root space. The controlled environmental conditions also caused slight alteration from normal distribution of flowering time within lines of mapping populations as compared to field conditions (Supplementary file 2, Fig. S5). Significant alteration of phenotype between field and greenhouse conditions has been described before, but the causes of this phenomenon remain largely unknown [42–44].

Nevertheless, a stable difference in flowering time due to the *VRN-A1* allele in spring wheat cultivars was not described before. The fact that no recombination suppression in the *VRN-A1f-like* locus was observed and that the *VRN-A1f-like* allele provides difference in flowering time in different genetic backgrounds of spring wheat, could be used as additional source of flowering time variation in breeding with negligible linkage drag. Such phenotypic variability is often associated with alteration in gene sequence, which facilitates development of markers tightly linked with to the trait and suitable for marker assisted selection.

To identify the possible causes of phenotypic variation on DNA sequence level, *VRN-A1* genes of *T. militinae* (in DH81), cv. Tähti and Mooni were sequenced. The promoter region of *T. militinae VRN-A1f-like* allele was found almost identical to the previously described *VRN-A1f* allele from *T. timopheevii* [37]. The only difference between the two sequences was one 1 bp indel in the HDD region of the *VRN-A1* promoter (Supplementary file 2, Fig. S3). However, the incomplete sequence of the *VRN-A1f* allele did not permit precise sequence comparison to confirm that these two alleles were the same. Comparison of the *VRN-A1* promoter region from spring wheat cvs. Mooni and Tähti using TDD as a reference revealed that cvs. Tähti and Mooni carry a *VRN-A1a* promoter region identical to that of the reference sequence. The *T. militinae VRN-A1f-like* allele differed mainly by three indels, one small and two large (Fig. 2). The main difference in the *VRN-A1f-like* allele was an absence of a Spring foldback element (SFE) insertion, which was observed in the promoter of *VRN-A1a* in spring wheat cultivars [19]. The element was also not found in the *VRN-A1b*–*VRN-A1h* alleles of hexaploid wheat [19,37]. A similar situation was also observed in related species *T. monococcum*, *T. boeticum*, and *T. urartu* [13] and *T. dicoccoides* [21]. In the *VRN-A1a* allele, the SFE insertion is often accompanied by promoter duplication [19]. Additionally, duplication of the whole *VRN1* gene causing a delay in flowering has been reported [30,39], however, no obvious duplication in the *VRN-A1f-like* allele was observed in the present study. The second large indel was a 50 bp deletion close to the start codon (Fig. 2). The deletion was reported only in the promoter of the *VRN-A1f* allele [37]. The small indel of 8 bp found between this deletion and the CArG-box (Fig. 2, Supplementary file 2, Fig. S3) is more frequent and common for the promoters of the *VRN-A1f, g, h, VRN-B1a* and *VRN-G1a* alleles [37]. On the basis of currently available data, it seems that the 50 bp deletion is only found in *T. timopheevii* group.

Comparison of the *VRN-A1* exon and intron regions from spring wheat cvs. Mooni and Tähti to TDD as the reference revealed that cvs. Tähti and Mooni are highly similar to the reference and the only differences in intronic regions involved two single base indels and one SNP. These mutations are outside the known regulatory regions and probably have no effect on the *VRN-A1a* gene expression. The *VRN-A1f-like* sequence differs from cvs. Mooni, Tähti, and TDD more significantly. The majority of the variability comprising 24 SNPs, 2 small indels, one large deletion (2753 bp) and one large insertion (433 bp) located in the first intron. This observation is not surprising since the first intron represents 64.4% of the gene. Only two SNPs were found in exon sequence: one causing no amino acid change and one causing a neutral amino acid change. The major mutation of the first intron comprised a 2753 bp deletion (Fig. 3, Supplementary file 2, Fig. S4). The second large indel was insertion of a MITE transposon in 5′ direction from the deletion (Fig. 3, Supplementary file 2, Fig. S4). The MITE has not been described before, however its homologue (98% identical) was identified on wheat chromosome 3B and designated as DXX-MITE_3bSeqIt2-B-G2821-Map7 [45]. The insertion of a repetitive element close to the 5′ end of the first intron was described in the *VRN-D1a* allele [46]. However, the effect of such an insertion on gene expression is unknown and requires further examination.

Despite the fact that the introgression line 8.1 flowered later than cvs. Tähti and Mooni in all experiments, not all *T. militinae* alleles in the detected QTLs increased flowering time. While the *VRN-A1f-like* allele as well as (to a lesser extent) the *T. militinae* allele of *VRN3* delayed flowering in the common wheat background, the *T. militinae* alleles of *PPD-A1* as well as the significant QTLs flanking the major QTL of *VRN-A1* on chromosome 5A (Fig. 1), showed an opposite effect, accelerating flowering by 1-2 days each (Supplementary file 1, Tab. S3). However, the minor QTLs accelerating flowering were only detected in the DH population. Further investigations involving larger populations and/or other genetic backgrounds are needed to prove these QTLs which, if verified, could find application in wheat breeding.

Conclusions

QTL analysis of a doubled haploid mapping population led to the identification of five loci originating from *T. militinae* and affecting flowering time variation. The QTL with the most significant effect was located on chromosome 5A in the region of *VRN-A1* gene. Sequence analysis of the *T. militinae VRN-A1* allele (designated as *VRN-A1f-like*) revealed significant variation in the promoter and regulatory regions of the first intron. Moreover, a new insertion of a transposable element MITE to the first intron was identified. The phenotypic effect of the allele was tested and verified in two elite spring cultivars of bread wheat, and in both backgrounds, the *VRN-A1f-like* allele significantly delayed flowering. Moreover, significant variation in flowering time of spring cultivars caused by *VRN-A1* allele has not been reported before. This new finding opens additional way to manipulate flowering time and breed cultivars better adapted to specific environmental niches. Identification of the novel *VRN-A1f-like* allele with the MITE insertion

will allow future studies to quantify its effect on fine tuning of the flowering time. The specific set of eleven PCR markers developed here for the *VRN-A1f-like* allele will enable its tracking in future studies and broadens the toolkit of diagnostic molecular assays available for *VRN1* alleles in temperate cereal species. Characterization of the variation in flowering time loci together with improved knowledge of molecular mechanisms controlling flowering time will facilitate breeding improved varieties better adapted to changing environmental conditions and human needs.

Author's contributions

Z.I. carried out genotyping and phenotyping in Olomouc, sequencing and sequence processing and drafted the manuscript. I.J. performed genotyping, development of mapping populations, QTL and statistical analysis, participated in study design and drafted the manuscript. D.R. carried out phenotyping and genotyping in Tallinn. J.S. contributed to design of the study and drafting of the manuscript. Z.M. participated in drafting the manuscript and sequence processing. M.A. contributed to sequence analysis annotation and bioinformatics analysis. J.D. participated in the design and coordination of study and preparation of the manuscript. K.J. participated in the design and coordination of the study. M.V. conceived the study participated in its design and coordination and drafted the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nbt.2016.01.008.

References

[1] Bushuk W. Wheat breeding for end-product use. Euphytica 1998;100:137–45.
[2] Devos KM, Doležel J, Feuillet C. Genome organization and comparative genomics. In: Carver BF, editor. Wheat sci. trade. Oxford: Wiley–Blackwell; 2009. p. 327–67. http://dx.doi.org/10.1002/9780813818832.ch15.
[3] Gill BS, Appels R, Botha-Oberholster AM, Buell CR, Bennetzen JL, Chalhoub B, et al. A workshop report on wheat genome sequencing: international genome research on wheat consortium. Genetics 2004;168:1087–96. http://dx.doi.org/10.1534/genetics.104.034769.
[4] Feuillet C, Langridge P, Waugh R. Cereal breeding takes a walk on the wild side. Trends Genet 2008;24:24–32. http://dx.doi.org/10.1016/j.tig.2007.11.001.
[5] Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, et al. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci U S A 2013;110:8057–62. http://dx.doi.org/10.1073/pnas.1217133110.
[6] Wang M, Wang S, Xia G. From genome to gene: a new epoch for wheat research? Trends Plant Sci 2015;1–8. http://dx.doi.org/10.1016/j.tplants.2015.03.010.
[7] Milec Z, Valárik M, Bartoš J, Šafář J. Can a late bloomer become an early bird? Tools for flowering time adjustment. Biotechnol Adv 2014;32:200–14. http://dx.doi.org/10.1016/j.biotechadv.2013.09.008.
[8] Gororo N. Photoperiod and vernalization responses in *Triticum turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann Bot 2001;88:947–52. http://dx.doi.org/10.1006/anbo.2001.1531.
[9] Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, et al. The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 2004;303:1640–4. http://dx.doi.org/10.1126/science.1094305.
[10] Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J. Positional cloning of the wheat vernalization gene VRN1. Proc Natl Acad Sci U S A 2003;100:6263–8. http://dx.doi.org/10.1073/pnas.0937399100.
[11] Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, et al. The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc Natl Acad Sci U S A 2006;103:19581–86. http://dx.doi.org/10.1073/pnas.0607142103.
[12] Li C, Distelfeld A, Comis A, Dubcovsky J. Wheat flowering repressor VRN2 and promoter CO_2 compete for interactions with NUCLEAR FACTOR-Y complexes. Plant J 2011;67:763–73. http://dx.doi.org/10.1111/j.1365-313X.2011.04630.x.
[13] Dubcovsky J, Loukoianov A, Fu D, Valarik M, Sanchez A, Yan L. Effect of photoperiod on the regulation of wheat vernalization genes VRN1 and VRN2. Plant Mol Biol 2006;60:469–80. http://dx.doi.org/10.1007/s11103-005-4814-2.
[14] Chen A, Dubcovsky J. Wheat TILLING mutants show that the vernalization gene VRN1 down-regulates the flowering repressor VRN2 in leaves but is not essential for flowering. PLoS Genet 2012;8. http://dx.doi.org/10.1371/journal.pgen.1003134.
[15] Li C, Dubcovsky J. Wheat FT protein regulates VRN1 transcription through interactions with FDL2. Plant J 2008;55:543–54. http://dx.doi.org/10.1111/j.1365-313X.2008.03526.x.
[16] Dhillon T, Pearce SP, Stockinger EJ, Distelfeld A, Li C, Knox AK, et al. Regulation of freezing tolerance and flowering in temperate cereals: the VRN-1 connection. Plant Physiol 2010;153:1846–58. http://dx.doi.org/10.1104/pp.110.159079.
[17] Kamran A, Iqbal M, Spaner D. Flowering time in wheat (*Triticum aestivum* L.): a key factor for global adaptability. Euphytica 2014;197:1–26. http://dx.doi.org/10.1007/s10681-014-1075-7.
[18] Loukoianov A, Yan L, Blechl A, Sanchez A, Dubcovsky J. Regulation of VRN-1 vernalization genes in normal and transgenic polyploid wheat. Plant Physiol 2005;138:2364–73. http://dx.doi.org/10.1104/pp.105.064287.
[19] Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J. Allelic variation at the VRN-1 promoter region in polyploid wheat. Theor Appl Genet 2004;109:1677–86. http://dx.doi.org/10.1007/s00122-004-1796-4.
[20] Fu D, Szucs P, Yan L, Helguera M, Skinner JS, Von Zitzewitz J, et al. Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. Mol Genet Genomics 2005;273:54–65. http://dx.doi.org/10.1007/s00438-004-1095-4.
[21] Shcherban AB, Strygina KV, Salina Ea. VRN-1 gene- associated prerequisites of spring growth habit in wild tetraploid wheat *T. dicoccoides* and the diploid A genome species. BMC Plant Biol 2015;15:1–13. http://dx.doi.org/10.1186/s12870-015-0473-x.
[22] Santra DK, Santra M, Allan RE, Campbell KG, Kidwell KK. Genetic and molecular characterization of vernalization genes Vrn-A1, Vrn-B1, and Vrn-D1 in spring wheat germplasm from the pacific northwest region of the U.S.A. Plant Breed 2009;128:576–84. http://dx.doi.org/10.1111/j.1439-0523.2009.01681.x.
[23] Leonova IN, Budashkina EB, Kalinina NP, Salina EA. *Triticum aestivum* – *Triticum timopheevii* introgression lines as a source of pathogen resistance genes. Czech J Genet Plant Breed 2011;49–55.
[24] Jakobson I, Peusha H, Timofejeva L, Järve K. Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line. Theor Appl Genet 2006;112:760–9. http://dx.doi.org/10.1007/s00122-005-0181-2.
[25] Jakobson I, Reis D, Tiidema A, Peusha H, Timofejeva L, Valárik M, et al. Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat–*Triticum militinae* introgression line. Theor Appl Genet 2012;125:609–23. http://dx.doi.org/10.1007/s00122-012-1856-0.
[26] Zhang XK, Xiao YG, Zhang Y, Xia XC, Dubcovsky J, He ZH. Allelic variation at the vernalization genes Vrn-A1, Vrn-B1, Vrn-D1, and Vrn-B3 in Chinese wheat cultivars and their association with growth habit. Crop Sci 2008;48:458–70. http://dx.doi.org/10.2135/cropsci2007.06.0355.
[27] IWGSC. A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 2014;345:1251788. http://dx.doi.org/10.1126/science.1251788.
[28] Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 2012;28:1647–9. http://dx.doi.org/10.1093/bioinformatics/bts199.
[29] Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 2012;13:134. http://dx.doi.org/10.1186/1471-2105-13-134.
[30] Muterko a, Balashova F, Fayt Ia, Sivolap VIYM. Molecular-genetic mechanisms of regulation of growth habit in wheat. Cytol Genet 2015;49:58–71. http://dx.doi.org/10.3103/S0095452715010089.
[31] Leroy P, Guilhot N, Sakai H, Bernard A, Choulet F, Theil S, et al. TriAnnot: a versatile and high performance pipeline for the automated annotation of plant genomes. Front Plant Sci 2012;3:1–14. http://dx.doi.org/10.3389/fpls.2012.00005.
[32] Austin DF, Lee M. Comparative mapping in F2:3 and F6:7 generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet 1996;92:817–26. http://dx.doi.org/10.1007/BF00221893.
[33] Manly KF, Cudmore RH, Meer JM. Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 2001;12:930–2. http://dx.doi.org/10.1007/s00335-001-1016-3.

[34] Somers DJ, Isaac P, Edwards K. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 2004;109:1105–14. http://dx.doi.org/10.1007/s00122-004-1740-7.
[35] Bonnin I, Rousset M, Madur D, Sourdille P, Dupuits C, Brunel D, et al. FT genome A and D polymorphisms are associated with the variation of earliness components in hexaploid wheat. Theor Appl Genet 2008;116:383–94. http://dx.doi.org/10.1007/s00122-007-0676-0.
[36] Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 2005;142:169–96. http://dx.doi.org/10.1007/s10681-005-1681-5.
[37] Golovnina Ka, Kondratenko EY, Blinov AG, Goncharov NP. Molecular characterization of vernalization loci VRN1 in wild and cultivated wheats. BMC Plant Biol 2010;10:168. http://dx.doi.org/10.1186/1471-2229-10-168.
[38] Würschum T, Boeven PHG, Langer SM, Longin CFH, Leiser WL. Multiply to conquer: copy number variations at Ppd-B1 and Vrn-A1 facilitate global adaptation in wheat. BMC Genet 2015;16:96. http://dx.doi.org/10.1186/s12863-015-0258-0.
[39] Díaz A, Zikhali M, Turner AS, Isaac P, Laurie Da. Copy number variation affecting the photoperiod-B1 and vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). PLoS One 2012;7. http://dx.doi.org/10.1371/journal.pone.0033234.
[40] Milec Z, Tomková L, Šumíková T, Pánková K. A new multiplex PCR test for the determination of Vrn-B1 alleles in bread wheat (*Triticum aestivum* L.). Mol Breed 2012;30:317–23. http://dx.doi.org/10.1007/s11032-011-9621-7.
[41] Gill BS, Chen PD. Role of cytoplasm-specific introgression in the evolution of the polyploid wheats. Proc Natl Acad Sci U S A 1987;84:6800–4. http://dx.doi.org/10.1073/pnas.84.19.6800.
[42] Zeller SL, Kalinina O, Brunner S, Keller B, Schmid B. Transgene × environment interactions in genetically modified wheat. PLoS One 2010;5:e11405. http://dx.doi.org/10.1371/journal.pone.0011405.
[43] Liu Y, Song L, Yu W, Hu Y, Ma X. Light quality modifies camptothecin production and gene expression of biosynthesis in *Camptotheca acuminata* Decne seedlings. Ind Crop Prod 2015;66:137–43. http://dx.doi.org/10.1016/j.indcrop.2014.12.046.
[44] Park Y, Muneer S, Jeong B. Morphogenesis, flowering, and gene expression of *Dendranthema grandiflorum* in response to shift in light quality of night interruption. Int J Mol Sci 2015;16:16497–513. http://dx.doi.org/10.3390/ijms160716497.
[45] Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, et al. Structural and functional partitioning of bread wheat chromosome 3B. Science 2014;345:1249721. http://dx.doi.org/10.1126/science.1249721.
[46] Muterko A, Balashova I, Cockram J, Kalendar R, Sivolap Y. The new wheat vernalization response allele Vrn-D1s is caused by DNA transposon insertion in the first intron. Plant Mol Biol Rep 2014;294–303. http://dx.doi.org/10.1007/s11105-014-0750-0.
[47] Chouard P. Vernalization and its relations to dormancy. Annu Rev Plant Physiol 1960;11:191–238. http://dx.doi.org/10.1146/annurev.pp.11.060160.001203.

PUBLICATION IV

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ORIGINAL ARTICLE

Divergence between bread wheat and *Triticum militinae* in the powdery mildew resistance *QPm.tut-4A* locus and its implications for cloning of the resistance gene

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Abstract
A segment of *Triticum militinae* chromosome 7G harbors a gene(s) conferring powdery mildew resistance which is effective at both the seedling and the adult plant stages when transferred into bread wheat (*T. aestivum*). The introgressed segment replaces a piece of wheat chromosome arm 4AL. An analysis of segregating materials generated to positionally clone the gene highlighted that in a plant heterozygous for the introgression segment, only limited recombination occurs between the introgressed region and bread wheat 4A. Nevertheless, 75 genetic markers were successfully placed within the region, thereby confining the gene to a 0.012 cM window along the 4AL arm. In a background lacking the *Ph1* locus, the localized rate of recombination was raised 33-fold, enabling the reduction in the length of the region containing the resistance gene to a 480 kbp stretch harboring 12 predicted genes. The substituted segment in the reference sequence of bread wheat cv. Chinese Spring is longer (640 kbp) and harbors 16 genes. A comparison of the segments' sequences revealed a high degree of divergence with respect to both their gene content and nucleotide sequence. Of the 12 *T. militinae* genes, only four have a homolog in cv. Chinese Spring. Possible candidate genes for the resistance have been identified based on function predicted from their sequence.

Introduction

Disease imposes an important constraint on crop productivity and is most effectively and sustainably managed by breeding cultivars harboring genetic resistance. The ability of many pathogens to overcome host resistance means that the discovery of additional sources of resistance is a must for any crop improvement program. Since the gene pool of bread wheat (*Triticum aestivum*) has been so extensively narrowed by more than a century of intensive breeding (Feuillet et al. 2008), the search for novel resistance genes needs to be extended to older materials such as landraces and even to related cultivated and wild species (Mondal et al. 2016; Zamir 2001). Over many years, wheat cytogeneticists and breeders have succeeded in developing a diverse collection of germplasm harboring introgression segments of variable length, many of which have targeted the introduction of genes conditioning resistance to leaf pathogens (King et al. 2017; Valkoun 2001). Advances in genomic technologies have enabled a much greater precision than has been possible hitherto in the characterization of these materials (Abrouk et al. 2017; Tiwari et al. 2014; Winfield et al. 2016).

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A number of introgressed genes conferring resistance to a fungal pathogen have been successfully isolated in hexaploid wheat: these currently comprise two conferring resistance to *Puccinia triticina* (Huang et al. 2003; Thind et al. 2017), five to *P. graminis* (Mago et al. 2015; Periyannan et al. 2013; Saintenac et al. 2013; Steuernagel et al. 2016) and one to *Blumeria graminis* (Hurni et al. 2013). *B. graminis* (commonly referred to as powdery mildew) epiphytotics can induce significant yield losses (Conner et al. 2003; Leath and Bowen 1989). A number of major resistance genes (*Pm* genes) have been identified (www.wheat.pw.usda.gov/cgi-bin/GG3/browse.cgi?class=gene); as most of these confer race-specific resistance, they are prone to being overcome by the rapidly evolving pathogen. Often durable form of resistance, referred to as adult plant resistance (APR), is typically conditioned by multiple genes, each conferring a small, but cumulative effect. As a result, uncovering its genetic basis normally requires quantitative trait locus (QTL) analysis. A meta-analysis (Lillemo and Lu 2015) has revealed that such genes are dispersed over 24 QTL-harboring regions, located on 18 of the 21 wheat chromosomes.

The tetraploid bread wheat relative *T. militinae* (genome formula A^tG) is generally considered to be a spontaneous mutant of *T. timopheevii* (Dorofeyev et al. 1976), although it has also been suggested to have arisen from an introgressive hybridization between *T. timopheevii* and the BA^u tetraploid *T. carthlicum* (Järve et al. 2002). Derivatives of a wide cross between the bread wheat cultivar (cv.) Tähti (genome formula BA^uD) and *T. militinae* include a selection (line 8.1) which harbors *T. militinae* segments incorporated within chromosomes 1A, 1B, 2A, 4A, 5A, 5B and 7A (Jakobson et al. 2006). Among other traits introduced from *T. militinae,* line 8.1 exhibits a marked improvement in the level of powdery mildew resistance expressed at both the seedling and adult plant stages. According to a QTL analysis, the genetic basis of this resistance is dominated (respectively, 33% and 54% of the variance shown by seedlings and adult plants) by a genes within a segment located near the distal end of chromosome arm 4AL, referred to as *QPm.tut-4A* (Jakobson et al. 2006). When present in a cv. Tähti background, the resistance decreases the number of secondary haustoria formed by the pathogen and enhances host cell apoptosis (Islamov et al. 2015). The gene (or genes) present within *QPm.tut-4A*, which acts in a race non-specific manner (Jakobson et al. 2012), is located in *T. militinae* itself on chromosome 7G (Abrouk et al. 2017). In a hybrid between wild-type bread wheat and the introgression line, little recombination occurs between the *T. militinae* segment and the segment of chromosome 4AL which has been replaced in line 8.1. However, it has been possible to define the genetic length and position of the segment to a 2.5 cM interval (Jakobson et al. 2012). Here, the focus was to isolate the gene(s) which determine the *QPm.tut-4A* resistance. By deploying a range of genetic and genomic strategies, it has proved possible to identify a small number of candidate genes and to characterize and contrast the sequences which originated from chromosome 7G with those which they replaced on 4A.

Materials and methods

Plant materials and mapping populations

The Jakobson et al. (2012) mapping population comprised 98 F_2 progeny bred from the cross cv. Chinese Spring (CS) × line 8.1; this was extended for the purpose of increasing the level of resolution by self-pollinating F_2 individuals which were heterozygous for *QPm.tut-4A* through to the F_5. In addition, a second mapping population (hereafter referred to as the "*ph1* population") was created by crossing the CS *ph1b* mutant (Sears 1977) with T312.30.38.16, a derivative of introgressive line 8.1 in which the only *T. militinae* segment present was the one harboring *QPm.tut-4A*. In order to derive a parental plant which was both homozygous for the *ph1b* allele and heterozygous for the segment harboring *QPm.tut-4A*, the resulting F_1 hybrid was back-crossed to the *ph1b* mutant. Marker-assisted selected BC_1F_1 individuals were then allowed to self-pollinate. Selection for the *ph1b* allele was performed using a multiplex PCR assay involving the markers AWJL3, PSR128, PSR2120 and PSR574 (Roberts et al. 1999), while the *QPm.tut-4A* harboring segment was marked by *owm76* and *owm96*. The subsequent generations (BC_1F_3 and further) were not employed for this study due to their low viability and fertility presumably caused by extensive chromosomal rearrangements associated with the action of *ph1*. Doubled haploid line DH397 containing the *T. militinae* resistance locus only on 4AL was derived from cross Tähti × 8.1 (Jakobson et al. 2012). A bacterial artificial chromosome (BAC) library was constructed from the DNA of 4AL telosomic chromosome flow sorted from 4AL ditelosomic line carrying the 4A *T. militinae* introgression (Jakobson et al. 2012). Grain of the CS aneuploid stocks nullisomic 4A-tetrasomic 4B and nullisomic 4A-tetrasomic 4D, required to validate the chromosome specificity of newly developed markers, was provided by the National BioResource Centre (Kyoto, Japan). A doubled haploid line (DH81) carrying the same *T. militinae* translocations which determined the powdery mildew resistance as line 8.1 (Jakobson et al. 2012) served as a further control. Finally, the bread wheat cv. Kanzler was used a susceptible host in experiments involving powdery mildew inoculations.

Phenotyping for powdery mildew resistance

Seedling resistance to powdery mildew was scored using an assay based on detached first seedling leaves of 10-day-old plants. Each leaf was cut into four segments, each of which was then laid in a Petri dish containing 0.6% agar supplemented with 0.35% *w/v* benzimidazol. The leaf segments were inoculated with four different isolates (2.1, 9.8, 13 and 14), as described by Jakobson et al. (2012). The Petri dishes were held at 17.5 °C, and the response was evaluated after 10 days using the 0–9 scale devised by Lutz et al. (1992). For each self-pollinated recombinant line selected from F_2-derived F_{3-5} families, up to 30 progeny homozygous in the *QPm.tut-4A* region were phenotyped. The resistance status of each recombinant line was verified by comparing its progeny scores with scores of 12–16 progeny of homozygous nonrecombinant sister line selected from the same self-pollination.

The development of markers used for high-density genetic mapping

The informativeness of a potential marker was first assessed via an *in silico* inspection of the chromosome-specific survey sequences of 4AL-7G (Abrouk et al. 2017) and the chromosome survey sequences (CSS) of $4AL^{CS}$ generated by IWGSC (2014). The choice of sequences was governed by the need to saturate the genetic map of the *QPm.tut-4A* region, so it was based on a virtual gene order of the chromosome 4A represented by GenomeZippers (Abrouk et al. 2017, Hernandez et al. 2012). 4AL CSS scaffolds obtained from the GenomeZipper delimited by the markers flanking the *QPm.tut-4A* segment were aligned with 4AL-7G sequence scaffolds using the BlastN algorithm (Altschul et al. 1990). Only low-copy sequences associated with a nucleotide identity of > 95% were considered for marker development, and those containing short indels were preferred. Where no indel was identifiable, markers were based on single-nucleotide polymorphisms using the cleaved amplified polymorphic sequence approach (Michaels and Amasino 1999). PCR primers were designed using Primer3 software (Untergasser et al. 2012). To ensure specificity for 4AL, given the presence of homoeologous sequence on 7AS and 7DS, primers were positioned to ensure the presence of a variant nucleotide close to 3′ end in the homoeologous sequences. Finally, the specificity of the putative amplicons was verified by a BlastN search against the whole wheat genome CSS (IWGSC 2014). A second marker discovery strategy profited from a BAC library-based, established CS 4AL-specific physical map (IWGSC 2018, URGI; urgi.versailles.inra.fr/). BAC clones making up the set of contigs which covered the *QPm.tut-4A* region were selected from the minimal tiling path and sequenced. Subsequently, sequence scaffolds positioned at the target location were employed for marker development based on the first strategy. A final strategy designed to extend the saturated portion of the *QPm.tut-4A* region beyond what was achievable using the first two strategies was based on the sequence of the *T. dicoccoides* 7AS region (Avni et al. 2017) and on the CS reference sequence WGA v0.4 (www.wheatgenome.org); this also was informative for identifying additional $4AL^{CS}$ scaffolds for targeted marker development. All primer pairs were tested on a template of CS, DH81, a CS/line 8.1 derivative heterozygous for *QPm.tut-4A*, nullisomic 4A-tetrasomic 4B and nullisomic 4A-tetrasomic 4D and DNA from flow-sorted chromosome arms $4AL^{CS}$ and 4AL-7G amplified according to Šimková et al. (2008). The methods used for PCR amplification and electrophoretic separation are given in the following section, and the primer sequences and associated information are summarized in Table S1. Where the physical position of a marker was uncertain, it was determined by screening 62 three-dimensional pools prepared from the minimal tiling path of the CS 4AL-specific physical map.

Genetic mapping

DNA was extracted using Agencourt® Genfind® v2 magnetic beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA) on a Beckman Coulter® Biomek® NX^P workstation, as described by Ivaničová et al. (2016). Markers *owm82* and *Xgwm160* (Röder et al. 1998) were used to select lines in which a recombination event had occurred in the region of the introgression segment in the mapping populations. The full set of markers was applied to genotype those arising from the CS × line 8.1 population, while a subset of 19 selected markers was applied to those arising from the *ph1* population. Each 15 µL PCR contained 0.01% (*w/v*) o-cresolsulphonephtalein, 1.5% (*w/v*) sucrose, 0.2 mM of each dNTP, 0.6 U Taq DNA polymerase, 1 µM of each primer, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.1% (*v/v*) Triton X-100. The template comprised either 10–20 ng genomic DNA or 5 ng DNA amplified from $4AL^{CS}$ or 4AL-7G. The reaction conditions consisted of an initial denaturation step of 95 °C/5 min, followed by 40 cycles of 95 °C/30 s, an optimized annealing temperature (Table S1) for 30 s and 72 °C for 30 s per 500 bp amplicon length; the reactions were completed with an elongation step of 72 °C/5 min. Cleaved amplified polymorphic sequence assays were completed with a digestion using the appropriate restriction endonuclease. The amplicons were electrophoretically separated through 4% non-denaturing polyacrylamide gels and visualized by ethidium bromide staining.

Construction of the physical map

Sequence scaffolds taken from IWGSC RefSeq v1.0 (IWGSC 2018) were used to span the genomic region of CS 4AL replaced by the *QPm.tut-4A* segment in line 8.1. To obtain the corresponding sequence from *T. militinae*, a chromosome walking approach was initiated. A chromosome-specific BAC library designated TaaP-mt4ALhA (www.olomouc.ueb.cas.cz/dna-libraries/cereals) was constructed from flow-sorted 4AL-7G chromosome arms according to the Šimková et al. (2011) protocol. BAC library plate pools were generated by mixing 40 µL of the bacterial culture from each well of a given plate, centrifuging (10 min at 2700 g), suspending the precipitate in 0.5 mL TE and boiling for 30 min. The suspension was then re-centrifuged (60 min at 2700 g), and a 450-µL aliquot of the supernatant was diluted 100-fold. The subsequent PCRs used as template a 1.5-µL aliquot of the diluted plate pool DNA. The plate pools were screened with the markers *owm169* and *owm228,* together with other codominant markers located within the CS 4AL segment replaced by *QPm.tut-4A* (*owm156*, *owm136*, *owm221*, *owm209*, *owm227*, *owm236*, *owm139* and *owm235*). Row and column pools of positive plates were prepared and used in the same way as plate pools. Selected BAC clones were sequenced on a MiSeq instrument (Illumina Inc., San Diego, CA, USA) using a Nextera DNA Library Prep Kit (Illumina) according to the manufacturer's protocol. Paired-end reads were assembled by Ray software (Boisvert et al. 2010). Insertion site-based polymorphism or site-specific presence/absence variation markers were developed from the ends of sequenced BAC clones and used for a further round of BAC library screening. The procedure was iterated until the physical map had been assembled.

The identification and sequence analysis of candidate genes

The gene content along the *QPm.tut-4A* segment on 4AL-7G was annotated using the TriAnnot pipeline (Leroy et al. 2011, www6.inra.fr/decodage/TriAnnot). Predicted genes were subjected to a BlastP search against the set of non-redundant protein sequences (www.ncbi.nlm.nih.gov/BLAST), and conserved domains of putative proteins were annotated using the NCBI Conserved Domain Database (Marchler-Bauer et al. 2017) and the MOTIF search tool (www.genome.jp/tools/motif/) based on the Pfam database (Finn et al. 2016). Searches for homologs and the comparison of candidate gene sequences were based on the BlastN algorithm, applying a threshold of 90% identity and 60% coverage.

Reverse transcription PCR

Segments of the first leaf of 10-day-old seedlings of the doubled haploid line DH397 and line T312.30.38.16 were collected 0-, 24- and 48-h post-inoculation with powdery mildew and snap frozen in liquid nitrogen. Leaf segments from three independent inoculations were bulked for the purpose of RNA extraction, and negative control samples were formulated from non-inoculated leaf segments. The leaf tissue was homogenized using ball mill MM 301 (Retsch, Haan, Germany) with three 3 mm tungsten beads at 30 Hz for 45 s, and total RNA was extracted using a miRNeasy Mini Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol. The synthesis of the first cDNA strand was achieved using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche Life Sciences, Indianapolis, IN, USA) based on an anchored-oligo$(dT)_{18}$ primer. Gene-specific primers were designed for all 12 candidate genes such that the amplicons derived from gDNA differed in length from those derived from cDNA (Table S2). A portion of the *Actin* gene (GenBank accession number AB181991.1) was used as the reference sequence. The procedures used for PCR and electrophoretic separation were as described above.

Results

High-resolution genetic mapping around QPm.tut-4A

A set of 102 new genetic markers was developed to saturate the genetic map in the region of *QPm.tut-4A* (Figs. 1, 2, Table S1). Majority of these were developed using GenomeZipper and 4AL-specific BAC clones. Besides markers *owm16* and *owm39*, polymorphism was inferred using the 4AL-7G and $4AL^{CS}$ survey sequences (Abrouk et al. 2017, IWGSC 2014). Segregation patterns observed in the extended CS × 8.1 mapping population were used to establish marker order. The genotyping of 8425 individuals with respect to *owm82* and *Xgwm160* revealed 30 new recombination events. Testing these recombinant individuals for their reaction to the four powdery mildew isolates showed that the resistance phenotype was fully correlated with the presence of the *T. militinae* segment—the disease scores for the four isolates were, respectively, 0.5, 0.4, 0.1, 1.1 in the presence of the segment, and 3.3, 2.5, 2.6, 3.8 in its absence. The net effect of the mapping was reducing the genetic length of the segment harboring *QPm.tut-4A* to 0.012 cM (Fig. 1). The *ph1* population was derived from the self-pollination of 22 (out of 107 screened) BC_1F_1 ([line 8.1 × *ph1b*] × *ph1b*) selections which were simultaneously homozygous for the *ph1b* allele and heterozygous for the *QPm.tut-4A* segment. The genotyping of the resulting 1255

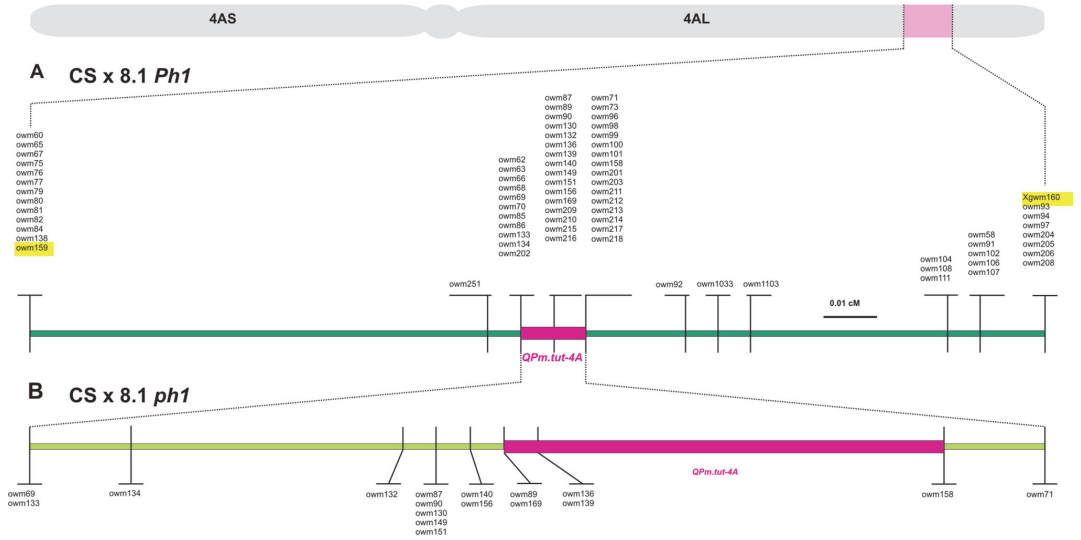

Fig. 1 A high-density genetic map of the region harboring *QPm.tut-4A*. **a** Marker segregation displayed by 8519 progeny bred from the CS × 8.1 mapping population (including the F_2 generation described at Jakobson et al. 2012) defines the genetic length of the *QPm.tut-4A* segment (marked in red) to 0.012 cM, lying within a 0.18 cM window within chromosome 4AL. The flanking markers used for identification of recombination events within the segment are highlighted in yellow. **b** The level of mapping resolution achieved was enhanced through the use of the *ph1* mapping population, which provided an additional 30 new recombination events, thereby splitting the *QPm.tut-4A* region into eight subregions

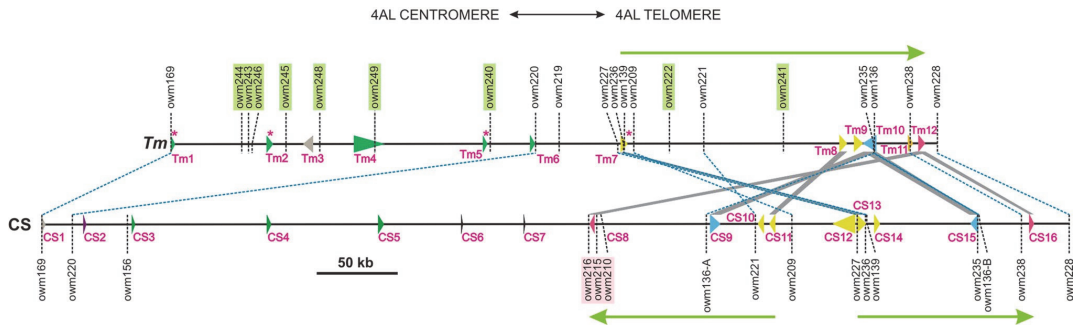

Fig. 2 Physical maps showing the gene content of the 480.2 kbp *QPm.tut-4A* segment inherited from *T. militinae* and the 640.8 kbp segment of chromosome 4AL replaced by the introgression in line 8.1. The predicted genes (*Tm1–Tm12* and *CS1–CS16*) are depicted by arrowheads indicating their orientation. The size of the arrowheads is proportional to the length of the gene, and their color reports the presence of conserved domains: green: CC-(LRR)-NB-ARC; yellow: PGG; orange: ANK and PGG; blue: LRR/MAL/PK; red: patatin-like; black: PMEI-like; and gray: uncharacterized protein. *Tm* and *CS* genes linked by gray lines share sequence identity in their coding region of at least 90% at a coverage of ≥ 60% (a coverage < 80% applied only to the putative pseudogene *CS15*). *Tm* genes which produced a detectable transcript are indicated by a red asterisk. Shared markers are linked by a blue dashed line, those marking *T. militinae* but not CS genomic sequence, or *vice versa,* are highlighted in, respectively, light green and light red. The *T. militinae* region bounded by *owm227* and *owm228* was present in CS as two tandemly arranged, tail-to-tail orientated copies, as indicated by the green arrows

BC_1F_2 progeny revealed 155 recombination events between *owm82* and *Xgwm160*, equivalent to a 33-fold increase in recombination as compared to the rate observed in the presence of *Ph1*. The 0.012 cM *QPm.tut-4A* region was thereby split into eight subregions (Fig. 1b).

The physical map of the QPm.tut-4A segment

Six additional markers for the *QPm.tut-4A* region were developed by inspection of the three CS reference sequence IWGSC WGA v0.4 scaffolds (134864, 108602 and 47761) (www.wheatgenome.org) selected on the basis of markers lying within the *owm169–owm158* interval (Table S1). The *owm228* marker was derived from same scaffold (134864) which contained the *QPm.tut-4A* flanking marker *owm169*. The application of these markers to the set of recombinant segregants defined the physical length of the CS sequence replaced by the *QPm.tut-4A* introgression to 640.8 kbp (Fig. 2). A 4AL-7G-specific BAC library was constructed in an effort to acquire sequences in the *QPm.tut-4A* region of line 8.1; the mean insert size harbored by the 43,008 clones generated provided a 6.8-fold coverage of the 4AL-7G chromosome arm (www.olomouc.ueb.cas.cz/dnalib/taapmt4alha). Pools of the set of 112 plates were assembled to perform a PCR screen based on the flanking markers *owm169* and *owm228*, along with eight codominant markers (*owm156*, *owm136*, *owm221*, *owm209*, *owm227*, *owm236*, *owm139* and *owm235*) mapping within the region (Fig. 2). One to seven positive BAC clones per marker were sequenced so that their end sequences could be used for the next round of chromosome walking-based marker development: 17 new markers were designed in this way (Table S1). In total, 26 BAC clones were sequenced to provide a complete sequence of the *QPm.tut-4A* region in line 8.1: The physical length of the segment bounded by *owm169* and *owm228* was 480.2 kbp.

Annotation and comparative analysis of the QPm.tut-4A segment

The *T. militinae* region was 25% shorter than the CS one (480.2 vs. 640.8 kbp). Gene annotation suggested that the *QPm.tut-4A* segment harbored 12 high-confidence (HC) protein-encoding genes (denominated *Tm1–Tm12*, Fig. 2, Table 1), while the 4AL region of CS harbored 16 HC genes (*CS1–CS16*, Table 2). The intergenic regions displayed no sequence similarity. A comparison of the *T. militinae* and *CS* genes based on a threshold of 90% identity and 60% coverage implied that only four of the *T. militinae* genes (*Tm7*, *Tm8*, *Tm10* and *Tm12*) shared an appreciable level of homology with members of the *CS* set (*CS8*, *CS9*, *CS11*, *CS13*, *CS15* and *CS16*) (Table 1, Fig. 2). The sequence of

Table 1 Predicted candidate genes present in the *QPm.tut-4A* segment introgressed from *T. militinae* into line 8.1

No.	Putative conserved protein domains	No. of exons	Length of protein sequence (aa)	CS homologous genes in the QPm.tut-4A region[a]	Identity (%)	Coverage (%)	RNA expression[b]
Tm1	CC; NB-ARC	2	225	–	–	–	+
Tm2	CC; LRR; NB-ARC	2	753	–	–	–	+
Tm3	–	3	253	–	–	–	–
Tm4	CC; LRR; NB-ARC	7	894	–	–	–	–
Tm5	CC; LRR; NB-ARC	2	557	–	–	–	+
Tm6	CC; LRR; NB-ARC	2	1017	–	–	–	–
Tm7	5× PGG	2	963	TraesCS4A01G450100.2 (CS13)	97	99	+
Tm8	5× PGG	3	764	TraesCS4A01G449900 (CS11)	90	98	–
Tm9	4× PGG	2	849	–	–	–	–
Tm10	LRR; Malectin; Pkinase	23	913	TraesCS4A01G449700 (CS9) TraesCS4A01G450300 (CS15)	97 98	84 60	–
Tm11	Ankyrin repeats; PGG	4	636	–	–	–	–
Tm12	Patatin-like phospholipase	5	424	TraesCS4A01G449600 (CS8) TraesCS4A01G450400 (CS16)	98 97	100 100	–

CC coiled coil, *NB-ARC* nucleotide-binding adaptor shared by APAF-1, certain *R* gene products and CED4, *LRR* leucine-rich repeat, *PGG* proline-glycine-glycine (domain named for this highly conserved sequence motif found at its start), *Pkinase* protein kinase

[a]Acronym for each gene used in the body of this manuscript and Fig. 2 is provided in the parentheses

[b]Detected in first leaves of DH397 and T312.30.38.16 seedlings

Table 2 Gene content of the segment of CS chromosome arm 4AL replaced by the *QPm.tut-4A* segment introgressed from *T. militinae* into line 8.1

No.	Official designation[a]	Putative conserved protein domains	No. of exons	Length of protein sequence (aa)
CS1	TraesCS4A01G448900	CC	3	313
CS2	TraesCS4A01G449000	Transferase	1	472
CS3	TraesCS4A01G449100	CC; NB-ARC	2	353
CS4	TraesCS4A01G449200	NB-ARC; LRR	2	838
CS5	TraesCS4A01G449300	CC; LRR; NB-ARC	3	1008
CS6	TraesCS4A01G449400	Plant invertase/pectin methylesterase inhibitor	1	203
CS7	TraesCS4A01G449500	Plant invertase/pectin methylesterase inhibitor	1	206
CS8	TraesCS4A01G449600	Patatin-like phospholipase	5	424
CS9	TraesCS4A01G449700	LRR; Malectin; Pkinase	21	867
CS10	TraesCS4A01G449800	5× PGG	2	993
CS11	TraesCS4A01G449900	5× PGG	2	1020
CS12	TraesCS4A01G450000	4× PGG	3	761
CS13	TraesCS4A01G450100.2	5× PGG	3	984
CS14	TraesCS4A01G450200	5× PGG	2	1024
CS15	TraesCS4A01G450300	LRR; Malectin	15	549
CS16	TraesCS4A01G450400	Patatin-like phospholipase	5	424

[a]The International Wheat Genome Sequencing Consortium RefSeq v1.0 annotation (IWGSC 2018)

the proximal ends of the CS and line 8.1 regions (beyond *owm169*) were quite distinct with respect to both their sequence and gene content: The segment from *owm169* to *owm220* was 228 kbp in line 8.1, but only 19 kbp in CS, and they contained, respectively, six (*Tm1–Tm6*) and one (*CS1*) genes. The central part of the segment (*owm220–owm227* in line 8.1 and *owm220–owm216* in CS) was longer in CS (322 vs. 51 kbp) and contained no predicted gene in line 8.1 and *CS2–CS7* in CS. The distal region (*owm227–owm228* in line 8.1 and *owm216–owm228* in CS) was, respectively, of length 200 kbp (genes *Tm7–Tm12*) and 299 kbp (*CS8–CS16*) (Fig. 2). The proximal segment harboring *Tm1–Tm6* was not represented on CS chromosome 4A. However, four of these genes (*Tm1*, *Tm2, Tm5* and *Tm6*) each have a strong homoeolog (Table 3) lying in reverse orientation within a ~ 410 kbp region of chromosome arm 7AS situated about 17.4 Mbp from the telomere. The position of the *QPm.tut-4A* region is 41.05 Mbp distant from the 4AL telomere. The distal part of the region (*owm227–owm228*) has been duplicated in CS, and the gene content has been differentiated. The two copies are arranged tandemly in a tail-to-tail orientation, resulting in the non-syntenic location of the *owm227* marker (Fig. 2).

Functional characterization of the candidate genes for powdery mildew resistance

The five genes *Tm1*, *Tm2* and *Tm4–Tm6* each encoded a coiled-coil (CC) domain and a nucleotide-binding (NB) domain, thereby being members of the NB-ARC family (van der Biezen and Jones 1998). *Tm2*, *Tm4* and *Tm6* also encode a leucine-rich repeat (LRR) domain, indicating them as members of the disease resistance-associated NLR gene family (Ye and Ting 2008). The shorter length of both *Tm1* and *Tm5* (Table 1) implies that both are incomplete

Table 3 Homoeologs on CS chromosome arm 7AS of the NLR family genes present in the *QPm.tut-4A* segment introgressed from *T. militinae* into line 8.1

No.	CS homoeolog on 7AS	Identity (%)	Coverage (%)	Putative conserved protein domains of homoeologs	Length of protein sequence (aa)	Length difference relative to *T. militinae* homoeolog (aa)
Tm1	TraesCS7A01G038800	95	100	CC; NB-ARC	452	+227
Tm2	TraesCS7A01G039300	97	100	CC; LRR; NB-ARC	972	+219
Tm4	–	–	–	–	–	–
Tm5	TraesCS7A01G03910	98	100	CC; LRR; NB-ARC	1074	+517
Tm6	TraesCS7A01G039400	93	79	CC; LRR; NB-ARC	875	−142

genes. *Tm10* is predicted to encode a protein harboring an LRR/malectin/protein kinase (LRR/MAL/PK) domain, also shared by a number of plant disease resistance gene products (Sekhwal et al. 2015); this gene lies in the distal part of the introgression segment and shares homology with both *CS9* and the truncated *CS15* (Fig. 2). The sequences of the other six *Tm* genes have little or no connection with disease resistance: *Tm7*–*Tm9* each encode multiple PGG domains, *Tm11* features ankyrin repeats, while *Tm12* encodes a protein belonging to the patatin-like phospholipase family (Table 1). Four of the 12 *Tm* genes (*Tm1*, *Tm2*, *Tm5* and *Tm7*) produced a detectable level of mRNA, and three of them (*Tm1*, *Tm2* and *Tm5*) encode NLR proteins: *Tm2* appears to be the only one of these which is intact.

Discussion

The introduction into the bread wheat gene pool of genes harbored by species belonging to its secondary and tertiary gene pools represents an attractive strategy for broadening the genetic diversity of a crop which has been intensively bred for over a century. However, the success of the strategy has been not infrequently limited by the simultaneous introgression of linked genes which are deleterious to either productivity and/or product quality. This phenomenon of linkage drag results from the suppression of recombination around an introgressed segment, a phenomenon which also hampers positional cloning, since it magnifies the ratio between physical (in bp) and genetic (in cM) distance and prevents efficient high-density mapping. The *T. militinae* segment harboring *QPm.tut-4A* suffers from exactly this problem. Recent significant progress in the next-generation sequencing technologies allowed development of two new gene cloning approaches called MutRenSeq (Steuernagel et al. 2016) and MutChromSeq (Sánchez-Martín et al. 2016) which can bypass the high-density map construction in gene cloning process. The MutRenSeq approach employing exome capture and sequencing focusses on identification of simultaneous deleterious mutations in single NB-LRR-like gene within mutants with lost resistance. The MutChromSeq uses similar approach consisting in identification of knockout mutations in single gene within mutant lines, but it requires flow sorting of respective chromosomes from all mutant lines and their sequencing. This means that only single dominant major-effect genes can be identified by these approaches. However, the *QPm.tut-4A* locus is associated with race non-specificity and incomplete resistance which suggests the resistance may be encoded by gene different from major-effect *R* genes (predominantly NB-LRR-like genes) or by more than one gene (Jakobson et al. 2006, 2012). The MutRenSeq and MutChromSeq approaches are therefore not feasible for the *QPm.tut-4A* gene/genes cloning. Fortunately, recent advances in DNA technology and the development of sophisticated genomic resources have substantially eased the processes of constructing a high-density map and of acquiring relevant sequence from both the donor and the recipient genomes.

High-density mapping

According to the Wheat-Composite2004-4A map (wheat.pw.usda.gov/GG3), the markers flanking *QPm.tut-4A* (*Xwmc232* and *Xgwm160*) are separated by ~9 cM, which led Jakobson et al. (2012) to suggest that a plant heterozygous for the introgression experiences suppression of recombination in the region, as has been documented for a number of other bread wheat introgression segments (Bariana et al. 2001; Järve et al. 2000; Jia et al. 1996; Lukaszewski 2015). Several genomic resources developed in CS were exploited here to saturate the region with markers in order to fine map the resistance locus, and these greatly enhanced the efficiency with which such markers could be elaborated, especially compared to conventional approaches to marker discovery such as microsatellite screening (Röder et al. 1998) or searching for sequence polymorphism in expressed sequence (e.g. Valárik et al. 2006). The GenomeZipper and chromosome-specific survey sequences (Abrouk et al. 2017, Hernandez et al. 2012, IWGSC 2014) were particularly effective in this context, but use was also made of sequence scaffolds developed in the tetraploid wheat *T. dicoccoides* (Avni et al. 2017) and the BAC contigs defining the chromosome 4AL physical map (IWGSC 2018). Initially, these resources allowed the genetic length of the target to be narrowed to just 0.012 cM (Fig. 1). In a fully homologous situation, such as occurs on wheat chromosome 3B, the mean ratio between cM and Mbp in distal regions varies from 0.60 to 0.96 (Choulet et al. 2014). If the recombination between the introgressed segment and the unaltered bread wheat region was unimpeded, the physical length of the segment would have been estimated to be no longer than 20 kbp. The fact that the segment's length was measured in hundreds of kbp demonstrated that there was a substantial localized suppression of recombination.

Structural divergence and recombination in the QPm.tut-4A segment

The pairing of homoeologs in hexaploid wheat is strongly restricted by the action of the *Ph1* locus (Riley and Chapman 1958). The intention of creating the *ph1* population was to induce a higher rate of recombination between the introgression containing *QPm.tut-4A* and its presumed homoeologous segment of CS 4AL, since the rate of recombination achieved in the presence of *Ph1* was < 0.4% (31 out of 8519) within the *owm82-Xgwm160*

region (Fig. 1): Although this represented a very small genetic distance (0.18 cM), the suppression of pairing/recombination meant that the physical distance involved was potentially rather large. The effect was enhancing the rate of recombination by 33-fold. A comparable elevation in the recombination rate induced by removal of the *Ph1* locus has been reported in a variety of interspecific hybrids (Lukaszewski 1995, Luo et al. 2000). The availability of the genomic sequence of CS (IWGSC 2018) made it possible to identify that the length of the 4AL segment (0.012 cM) replaced by the *owm169-owm228 QPm.tut-4A* locus in line 8.1 was 640 kbp (Figs. 1, 2) and that it harbors 16 predicted genes (Table 2). The inferred relationship between genetic and physical distance in the segment was therefore only 0.019 cM per Mbp, which is much lower than the ratio (0.60–0.96 cM per Mbp) obtained in the distal region of chromosome 3B and is even below the ratio associated with the 3B centromeric region (0.05 cM per Mbp) in which recombination is known to be repressed (Choulet et al. 2014). This major suppression of recombination reflects the lack of homology between the native wheat and the introgressed *QPm.tut-4A* locus. The length of the introgressed *QPm.tut-4A* locus (*owm169–owm228*) was 480 kbp (Fig. 2), and it harbored 12 predicted genes (Table 1), eight of which lacked a homolog in the CS segment (Table 1, Fig. 2). The CS homologs of the other four genes (*Tm7*, *Tm8*, *Tm10* and *Tm12*) lay in a duplicated segment, and one of the duplicated copies was present in inverted orientation. The region was further disrupted by a number of indels, and all these changes suggest a high level of evolutionary dynamics of the wheat genome. While loss of synteny and sequence divergence are commonplace between homoeologous genomes (Saintenac et al. 2013; Wicker et al. 2003), they can also feature in comparisons made between homologous genomes of different hexaploid wheat cultivars (Mago et al. 2014; Tsõmbalova et al. 2016).

The introgressed *QPm.tut-4A* locus included three full-length and two truncated NLR family genes (Table 1). This class of genes is frequently arranged in clusters, which is thought to reflect the outcome of duplication events followed by sequence divergence (Michelmore and Meyers 1998). The coding sequences of the *T. militinae* NLR-like genes shared only a moderate to a high (73–89%) level of identity at the nucleotide level. Their putative homoeologs present on chromosome arm 7AS of CS displayed a similar level of sequence relatedness (71–85%). This chromosomal location confirms the conclusion of Abrouk et al. (2017) that in *T. militinae* itself, the *QPm.tut-4A* segment is present on chromosome 7G. The G genome donor is thought to be a member of the *Sitopsis* section of the genus *Aegilops,* as is also the donor of the bread wheat B genome (Gornicki et al. 2014). The bread wheat chromosome 4A itself is a restructured chromosome composed of a mosaic of segments derived from 4AL, 5AL and 7BS (Devos et al. 1995, Hernandez et al. 2012), and the *QPm.tut-4A* introgression appears to lie within a part of this region which originated from 7BS, consistent with its transfer following meiotic pairing.

The potential function of the QPm.tut-4A candidates

The resistance to powdery mildew associated with the presence of the *QPm.tut-4A* segment was race non-specific and in a cv. Tähti background, accounted for 40% of the variation in resistance (Jakobson et al. 2012). To date, only few wheat genes associated with APR have been isolated, so unlike the case for race-specific seedling resistance genes, many of which belong to the NLR family, it is not clear what functionality the product of such a gene might have. The *Lr34/Yr18/Pm38* gene, which provides protection against three distinct foliar pathogens, has been shown to encode an ABC transporter (Krattinger et al. 2009), while *Lr22a*, which confers broad-spectrum APR to leaf rust, encodes an NLR-like protein (Thind et al. 2017), as does the rice NLR family *Pb1* gene against panicle blast (Hayashi et al. 2010). Assuming that one of the three NLR family genes *Tm2*, *Tm4* and *Tm6* represents the most likely candidate for the *QPm.tut-4A* resistance, the possible basis of its resistance being race non-specific needs to be explored. In one scenario, it may be that either two or even all three of the *Tm* genes, which are each individually race-specific, act together to confer apparent race non-specificity. Alternatively, it is possible that one of the three genes is a "defeated" major resistance gene which has retained some residual broad-spectrum effect, as has been demonstrated for some other defeated major resistance genes (Li et al. 1999). The latter hypothesis is probably the more plausible, given that the only gene for which a mRNA was detected was *Tm2,* although surprisingly it was possible to detect transcript of the two NLR-like pseudogenes *Tm1* and *Tm5*. However, the lack of an LRR domain in the *Tm2*-encoded protein implies that it would be difficult for its product to recognize the pathogen's avirulence signal. An additional candidate is represented by *Tm10* which encodes a protein containing an LRR, a MAL and a PK domain. A barley protein of this domain composition (HvLEMK1) is known to mediate non-host resistance to powdery mildew, while its wheat ortholog acts to enhance the level of wheat host resistance to powdery mildew (Rajaraman et al. 2016). A comparison of the TaLEMK1 and the *Tm10* protein sequences showed that they share only a 34% level of identity; this rather low level of homology, combined with the observations that the wheat *LEMK1* homoeologs map to the group 5 chromosomes and that no *Tm10* transcript was detected, rules out the possibility that the *QPm.tut-4A* resistance is conferred by *Tm10*. None of the other *Tm* genes

encode a product which has been directly associated with disease resistance to date. However, *Tm12*—which encodes a protein belonging to the patatin-like phospholipase family—remains a candidate since the patatin-like protein AtPLP2 has been shown to represent a component of the cell machinery delivering apoptosis, and therefore makes a contribution toward resistance against an obligate biotroph (La Camera et al. 2009).

Author contribution statement KJ, JD and MV designed the study; MV, EJ, MŠ, HŠ, JŠ and IJ were responsible for marker development, genotyping, BAC library construction, chromosome sorting, data analysis and the construction of the mapping populations. HP performed the phenotypic evaluation and IJ the statistical analysis. The bioinformatics analyses were conducted by EJ and MA, who also contributed to data interpretation. Other experiments were conducted by EJ. The manuscript was drafted by EJ and MV, and all the authors contributed to its editing and proofreading.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author confirms that no conflict of interest applies.

Data availability The DNA sequence of the *T. militinae*-derived *QPm.tut-4A* introgression segment has been deposited in GenBank (Accession No. MG672525). Supporting data are available from the corresponding author upon reasonable request.

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References

Abrouk M, Balcárková B, Šimková H, Komínková E, Martis M, Jakobson I, Timofejeva L, Rey E, Vrána J, Kilian A, Järve K, Doležel J, Valárik M (2017) The *in silico* identification and characterization of a bread wheat/*Triticum militinae* introgression line. Plant Biotechnol J 15:249–256

Altschul SA, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, Hale I, Mascher M, Spannagl M, Wiebe K, Jordan KW, Golan G, Deek J, Ben-Zvi B, Ben-Zvi G, Himmelbach A, MacLachlan RP, Sharpe AG, Fritz A, Ben-Davis R, Budak H, Fahima T, Korol A, Faris JD, Hernandez A, Mikel MA, Levy AA, Steffenson B, Maccaferri M, Tuberosa R, Cattivelli L, Faccioli P, Ceriotti A, Kashkush K, Pourkheirandish M, Komatsuda T, Eilam T, Sela H, Sharon A, Ohad N, Chamovitz DA, Mayer KFX, Stein N, Ronen G, Peleg Z, Pozniak CJ, Akhunov ED, Distelfeld A (2017) Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science 357:93–97

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA (2001) Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust J Agric Res 52:1247–1255

Boisvert S, Laviolette F, Corbeil J (2010) Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. J Comput Biol 17:1519–1533

Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, Pingault L, Sourdille P, Couloux A, Paux E, Leroy P, Mangenot S, Guilhot N, Le Gouis J, Balfourier F, Alaux M, Jamilloux V, Poulain J, Durand C, Bellec A, Gaspin C, Safar J, Dolezel J, Rogers J, Vandepoele K, Aury JM, Mayer K, Berges H, Quesneville H, Wincker P, Feuillet C (2014) Structural and functional partitioning of bread wheat chromosome 3B. Science 345:1249721

Conner RL, Kuzyk AD, Su H (2003) Impact of powdery mildew on the yield of soft white spring wheat cultivars. Can J Plant Sci 83:725–728

Devos KM, Dubcovsky J, Dvorak J, Chinoy CN, Gale MD (1995) Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. Theor Appl Genet 91:282–288

Dorofeyev VF, Jakubtsiner MM, Rudenko MI, Migushova EF, Udachin RA, Merezhko AF, Semenova LV, Novikova MV, Gradchaninova OD, Shitova IP (1976) The wheats of the world. In: Brezhnev DD, Dorofeyev VF (eds), Kolos Publication, Leningrad, 487 pp. (**in Russian**)

Feuillet C, Langridge P, Waugh R (2008) Cereal breeding takes a walk on the wild side. Trends Genet 24:24–32

Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A (2016) The Pfam protein database: towards a more sustainable future. Nucleic Acids Res 44:D279–D285

Gornicki P, Zhu H, Wang J, Challa GS, Zhang Z, Gill BS, Li W (2014) The chloroplast view of the evolution of polyploid wheat. New Phytol 204:704–714

Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, Kanamori H, Yamane H, Hayano-Saito Y, Matsumoto T, Yano M, Takatsuji H (2010) Durable panicle blast-resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. Plant J 64:498–510

Hernandez P, Martis M, Dorado G, Pfeifer M, Gálvez S, Schaaf S, Jouve N, Šimková H, Valárik M, Doležel J, Mayer KFX (2012) Next-generation sequencing and syntenic integration of flow-sorted arms of wheat chromosome 4A exposes the chromosome structure and gene content. Plant J 69:377–386

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:664–665

Hurni S, Brunner S, Buchmann G, Herren G, Jordan T, Krukowski P, Wicker T, Yahiaoui N, Mago R, Keller B (2013) Rye *Pm8* and wheat *Pm3* are orthologous genes and show evolutionary conservation of resistance function against powdery mildew. Plant J 76:957–969

Islamov B, Peusha H, Jakobson I, Järve K (2015) *Triticum militinae* introgressions into bread wheat affect host responses to powdery mildew challenge. In: Poster session presented at 14th international cereal rusts and powdery mildews conference, Helsingør, Denmark, 5–8 July 2015

Ivaničová Z, Jakobson I, Reis D, Šafář J, Milec Z, Abrouk M, Doležel J, Järve K, Valárik M (2016) Characterization of new allele influencing flowering time in bread wheat introgressed from *Triticum militinae*. New Biotechnol 33:718–727

IWGSC (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345:1251788

IWGSC (2018) Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. Science 361:eaar7191

Jakobson I, Peusha H, Timofejeva L, Järve K (2006) Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line. Theor Appl Genet 112:760–769

Jakobson I, Reis D, Tiidema A, Peusha H, Timofejeva L, Valárik M, Kladivová M, Šimková H, Doležel J, Järve K (2012) Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat-*Triticum militinae* introgression line. Theor Appl Genet 125:609–623

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

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

Jia J, Devos KM, Chao S, Miller TE, Reader SM, Gale MD (1996) RFLP-based maps of the homoeologous group-6 chromosomes of wheat and their application in the tagging of *Pm12*, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. Theor Appl Genet 92:559–565

King J, Grewal S, Yang C, Hubbart-Edwards S, Scholefield D, Ashling S, Broomfield D, Howells C, King IP (2017) A step change in the transfer of interspecific variation into wheat from its wild relatives. In: Buerstmayr H, Lang-Mladek C, Steiner B, Michel S, Buerstmayr M, Lemmens M, Vollmann J, Grausgruber H (eds) Proceedings of the 13th international wheat genetics symposium, Tulln, Austria, 23–28 April 2017. BOKU-University of Natural Resources and Life Sciences, Vienna, p. 40

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

La Camera S, Balagué C, Geoffroy P, Legrand M, Feussner I, Roby D, Heitz T (2009) The Arabidopsis patatin-like protein 2 (PLP2) plays an essential role in cell death execution and differentially affects biosynthesis of oxylipins and resistance to pathogens. Mol Plant Microbe Interaction 22:469–481

Leath S, Bowen KL (1989) Effects of powdery mildew, triadimenol seed treatment and triadimenol foliar sprays on yield of winter wheat in North Carolina. Phytopathology 79:152–155

Leroy P, Guilhot N, Sakai H, Bernard A, Choulet F, Theil S, Reboux S, Amano N, Flutre T, Pelegrin C, Ohyanagi H, Seidel M, Giacomoni F, Reichstadt M, Alaux M, Gicquello E, Legeai F, Cerutti L, Numa H, Tanaka T, Mayer K, Itoh T, Quesneville H, Feuillet C (2011) TriAnnot: a versatile and high performance pipeline for the automated annotation of plant genomes. Front Plant Sci 3:1–14

Li ZK, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Mol Gen Genet 261:58–63

Lillemo M, Lu Q (2015) A meta analysis of partial resistance loci to powdery mildew in wheat. In: Paper presented at 14th international cereal rusts and powdery mildews conference, Helsingør, Denmark, 5–8 July 2015

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90:714–719

Lukaszewski AJ (2015) Introgressions between wheat and rye. In: Molnár-Láng M, Ceoloni C, Doležel J (eds) Alien introgression in wheat. Springer, New York, pp 163–190

Luo MC, Yang ZL, Kota RS, Dvořák J (2000) Recombination of chromosome $3A^m$ and $5A^m$ of *Triticum monococcum* with homeologous chromosomes *3A* and *5A* of wheat: the distribution of recombination across chromosomes. Genetics 154:1301–1308

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

Mago R, Tabe L, Vautrin S, Šimková H, Kubaláková M, Upadhyaya N, Berges H, Kong X, Breen J, Doležel J, Appels R, Ellis JG, Spielmeyer W (2014) Major haplotype divergence including multiple germin-like protein genes, at the wheat *Sr2* adult plant stem rust resistance locus. BMC Plant Biol 14:379

Mago R, Vautrin S, Šimková H, Bansal U, Luo MC, Rouse M, Karaoglu H, Periyannan S, Kolmer J, Jin Y, Ayliffe MA, Bariana H, Park RF, McIntosh R, Doležel J, Bergès H, Spielmeyer W, Lagudah ES, Ellis JG, Dodds PN (2015) The wheat Sr50 gene reveals rich diversity at a cereal disease resistance locus. Nat Plants 1:15186

Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH (2017) CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res 45:D200–D203

Michaels SD, Amasino RM (1999) A robust method for detecting single-nucleotide changes as polymorphic markers by PCR. Plant J 14:381–385

Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130

Mondal S, Rutkoski JE, Velu G, Singh PK, Crespo-Herrera LA, Guzmán C, Bhavani S, Lan C, He X, Singh RP (2016) Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. Front Plant Sci 7:991

Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, Deal K, Luo M, Kong X, Bariana H, Mago R, McIntosh R, Dodds P, Dvorak J, Lagudah E (2013) The gene *Sr33*, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. Science 341:786–788

Rajaraman J, Douchkov D, Hensel G, Stefanoto FL, Gordon A, Ereful N, Caldararu OF, Petrescu AJ, Kumlehn J, Boyd LA, Schweizer P (2016) An LRR/malectin receptor-like kinase mediates resistance to non-adapted and adapted powdery mildew fungi in barley and wheat. Front Plant Sci 7:1836

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behavior of hexaploid wheat. Nature 182:713–715

Roberts MA, Reader SM, Dalgliesh C, Miller TE, Foote TN, Fish LJ, Snape JW, Moore G (1999) Induction and characterization of *Ph1* wheat mutants. Genetics 153:1909–1918

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

Saintenac C, Zhang W, Salcedo A, Rouse MN, Trick HN, Akhunov E, Dubcovsky J (2013) Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science 341:783–786

Sánchez-Martín J, Steuernagel B, Ghosh S, Herren G, Hurni S, Adamski N, Vrána J, Kubaláková M, Krattinger SG, Wicker T, Doležel J, Keller B, Wulff BBH (2016) Rapid gene isolation in barley and wheat by mutant chromosome sequencing. Genome Biol 17:221

Sears ER (1977) Induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Sekhwal MK, Li PC, Lam I, Wang XE, Cloutier S, You FM (2015) Disease resistance gene analogs in plants. Int J Mol Sci 16:19248–19290

Šimková H, Svensson JT, Condamine P, Hřibová E, Suchánková P, Bhat PR, Bartoš J, Šafář J, Close TJ, Doležel J (2008) Coupling amplified DNA from flow-sorted chromosomes to high-density SNP mapping in barley. BMC Genom 9:294

Šimková H, Šafář J, Kubaláková M, Suchánková P, Číhalíková J, Robert-Quatre H, Azhaguvel P, Weng Y, Peng J, Lapitan NLV, Ma Y, You FM, Luo M, Bartoš J, Doležel J (2011) BAC libraries from wheat chromosome 7D: efficient tool for positional cloning of aphid resistance genes. J Biomed Biotechnol 2011:302543

Steuernagel B, Periyannan SK, Hernández-Pinzón I, Witek K, Rouse MN, Yu G, Hatta A, Ayliffe M, Bariana H, Jones JDG, Lagudah ES, Wulff BBH (2016) Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nat Biotechnol 34:652–655

Thind AK, Wicker T, Šimková H, Fossati D, Moullet O, Brabant C, Vrána J, Doležel J, Krattinger S (2017) Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. Nat Biotechnol 35:793–796

Tiwari VK, Wang S, Sehgal S, Vrána J, Friebe B, Kubaláková M, Chhuneja P, Doležel J, Akhunov E, Kalia B, Sabir J, Gill BS (2014) SNP discovery for mapping alien introgressions in wheat. BMC Genom 15:273

Tsõmbalova J, Karafiátová M, Vrána J, Kubaláková M, Peusha H, Jakobson I, Järve M, Valárik M, Doležel J, Järve K (2016) A haplotype specific to North European wheat (*Triticum aestivum* L.). Genet Resour Crop Evol 64:653–664

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3: new capabilities and interfaces. Nucleic Acids Res 40:e115

Valárik M, Linkiewicz AM, Dubcovsky J (2006) A microcolinearity study at the earliness per se gene Eps-Am 1 region reveals an ancient duplication that preceded the wheat-rice divergence. Theor Appl Genet 112:945–957

Valkoun JJ (2001) Wheat pre-breeding using wild progenitors. Euphytica 119:17–23

van der Biezen EA, Jones JDG (1998) The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. Curr Biol 8:R226–R227

Wicker T, Yahiaoui N, Guyot R, Schlagenhauf E, Liu ZD, Dubcovsky J, Keller B (2003) Rapid genome divergence at orthologous low molecular weight glutenin loci of the A and A^m genomes of wheat. Plant Cell 15:1186–1197

Winfield MO, Allen AM, Burridge AJ, Barker GLA, Benbow HR, Wilkinson PA, Coghill J, Waterfall C, Davassi A, Scopes G, Pirani A, Webster T, Brew F, Bloor C, King J, West C, Griffiths S, King I, Bentley AR, Edwards KJ (2016) High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. Plant Biotechnol J 14:1195–1206

Ye ZM, Ting JPY (2008) NLR, the nucleotide-binding domain leucine-rich repeat containing gene family. Curr Opin Immunol 20:3–9

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989

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July 5–8 2015, Helsingør, Denmark. The 14th International Cereal Rusts and Powdery Mildews Conference. Poster presentation: "*Triticum militinae* introgressions into bread wheat affect host responses to powdery mildew challenge." P153.

June 29–July 4, 2014 , Wernigerode, Germany. The Joint EUCARPIA Cereal Section and ITMI Conference. Poster presentation: ''In silico identification and characterization of wheat 4AL - *Triticum militinae* introgression.'' P303.

February 18–19, 2013, Vienna, Austria. The International Conference "Plant Genetics and Breeding Technologies". Poster presentation: "Efficiency of the *Triticum militinae*-derived powdery mildew resistance gene *QPm.tut-4A* on different genetic backgrounds." P52.

February 20–22, 2013, Vienna, Austria. The International Conference "Plant Diseases and Resistance Mechanisms". Poster presentation: "Efficiency of the *Triticum militinae*-derived powdery mildew resistance gene *QPm.tut-4A* on different genetic backgrounds." P37.

August 28–September 1, 2011, Novosibirsk, Russia. The International Conference "Wheat genetic resources and genomics". Poster presentation: "Resistance to powdery mildew in an introgressive wheat line derived from *Triticum militinae*."

August 31–September 4, 2009, Clermont-Ferrand, France. The 19th International Triticeae Mapping Initiative - 3rd COST Tritigen. Poster presentation: "QTL analysis of yield-related morphological traits and powdery mildew resistance in an introgressive line of bread wheat."

February 3–6, 2008, Vienna, Austria. Conference "Molecular Mapping and Marker Assisted Selection in Plants." Poster presentation:
"Resistance to powdery mildew in a *Triicum aestivum x Triticum militinae* hybrid line." P83.

Supervised dissertations

Milana Ussatšova, MSc, 2015:
"Genetic analysis of growth rate in a wheat hybrid line." Tallinn University of Technology Faculty of Science, Department of Gene Technology. Supervised by Kadri Järve and Irena Jakobson

Aleksandra Pahhomova, BSc, 2014:
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Diana Reis, Master's Degree, 2006:
"Mapping yield – related traits in resistant hybrid line of common wheat (*Triticum aestivum* L.)." Tallinn University of Technology Faculty of Science, Department of Gene Technology. Supervised by Kadri Järve and Irena Jakobson

Publications

Janáková E, Jakobson I, Peusha H, Abrouk M, Škopová M, Šimková H, Šafář J, Vrána J, Doležel J, Järve K, Valarik M. (2018). Divergence between bread wheat and *Triticum militinae* in the powdery mildew resistance *QPm.tut-4A* locus and its implications for cloning of the resistance gene. *Theor Appl Genet* https://doi.org/10.1007/s00122-018-3259-3

Abrouk M, Balcárková B, Šimková H, Komínkova E, Martis MM, Jakobson I, Timofejeva L, Rey E, Vrána J, Kilian A, Järve K, Doležel J, Valárik M. (2017). The in silico identification and characterization of a bread wheat/*Triticum militinae* introgression line. *Plant Biotechnol J* 15(2): 249–256.

Tsõmbalova J, Karafiátová M, Vrána J, Kubaláková M, Peuša H, Jakobson I, Järve M, Valárik M, Doležel J, Järve K. (2017). A haplotype specific to North European wheat (*Triticum aestivum* L.). *Genet Resour Crop Evol* 64(4): 653–664.

Ivaničová Z, Jakobson I, Reis D, Šafář J, Milec Z, Abrouk M, Doležel J, Järve K, Valárik M. (2016). Characterization of new allele influencing flowering time in bread wheat introgressed from *Triticum militinae*. *New Biotechnology* 33: 718–727.

Jakobson I, Reis D, Tiidema A, Peusha H, Timofejeva L, Valarik M, Kladivova M, Simkova H, Dolezel J, Järve K. (2012). Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat–*Triticum militinae* introgression line. *Theor Appl Genet* 125: 609–623.

Jakobson I, Peusha H, Timofejeva L, Järve K. (2006). Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* x *Triticum militinae* hybrid line. *Theor Appl Genet* 112: 760–769.

Peusha H, Enno T, Jakobson I, Tsõmbalova J, Ingver A, Järve K. (2008). Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia. *Acta Agriculturae Scandinavica*, Section B - *Plant Soil Science* 58(4): 289–296.

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.

Enno T, Peuša H, Timofejeva L, Tohver M, Jakobson I, Priilinn O. (1998). Identification of chromosomal translocations in common wheat, derivative of *Triticum timopheevii*. *Acta Agron Hung* 46: 209–216.

Jakobson I. (1995). Experimental populations of *Drosophila melanogaster* originated from flies different types of reaction norm. *Proceed Latvian Acad Sci* 5/6:pA39.

Kuznetsova OV, Tishkin VV, Ivanchenko I (Jakobson). (1984). The line of *Drosophila melanogaster* used for estimation of fitness components of population. *Tsitologiya & Genetika* 4: 290–294 (in Russian).

Yacobson I (Jakobson), Glotov NV. (1989). The influence of ecological past and environment microfluctuations of the variability structure of quantitative characters in *Drosophila*. *Genetika* 25(7): 1218–1222 (in Russian).

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Kursused ja konverentsid

July 5–8 2015, Helsingør, Taani. "The 14th International Cereal Rusts and Powdery Mildews Conference". Poster teemal: "*Triticum militinae* introgressions into bread wheat affect host responses to powdery mildew challenge." P153.

June 29–July 4, 2014 , Wernigerode, Saksamaa. "The Joint EUCARPIA Cereal Section and ITMI Conference". Poster teemal: ''In silico identification and characterization of wheat 4AL - *Triticum militinae* introgression.'' P303.

February 18–19, 2013, Viin, Austria. "The International Conference "Plant Genetics and Breeding Technologies". Poster teemal: "Efficiency of the *Triticum militinae*-derived powdery mildew resistance gene *QPm.tut-4A* on different genetic backgrounds." P52.

February 20–22, 2013, Viin, Austria. "The International Conference "Plant Diseases and Resistance Mechanisms". Poster teemal: "Efficiency of the *Triticum militinae*-derived powdery mildew resistance gene *QPm.tut-4A* on different genetic backgrounds." P37.

August 28–September 1, 2011, Novosibirsk, Venemaa. "The International Conference "Wheat genetic resources and genomics". Poster teemal: "Resistance to powdery mildew in an introgressive wheat line derived from *Triticum militinae*."

August 31–September 4, 2009, Clermont-Ferrand, Prantsusmaa "The 19th International Triticeae Mapping Initiative - 3rd COST Tritigen". Poster teemal: "QTL analysis of yield-related morphological traits and powdery mildew resistance in an introgressive line of bread wheat."

February 3–6, 2008, Viin, Austria. Konverents "Molecular Mapping and Marker Assisted Selection in Plants." Poster teemal: "Resistance to powdery mildew in a *Triicum aestivum x Triticum militinae* hybrid line." P83.

Juhendatud diplomitööd

Milana Ussatšova, MSc, 2015,
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Diana Reis, MSc (teaduskraad), 2006,
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Publikatsioonid

Janáková E, Jakobson I, Peusha H, Abrouk M, Škopová M, Šimková H, Šafář J, Vrána J, Doležel J, Järve K, Valarik M. (2019). Divergence between bread wheat and *Triticum militinae* in the powdery mildew resistance *QPm.tut-4A* locus and its implications for cloning of the resistance gene. *Theor Appl Genet* 132: 1061–1072.

Abrouk M, Balcárková B, Šimková H, Komínkova E, Martis MM, Jakobson I, Timofejeva L, Rey E, Vrána J, Kilian A, Järve K, Doležel J, Valárik M. (2017). The in silico identification and characterization of a bread wheat/*Triticum militinae* introgression line. *Plant Biotechnol J* 15(2): 249–256.

Tsõmbalova J, Karafiátová M, Vrána J, Kubaláková M, Peuša H, Jakobson I, Järve M, Valárik M, Doležel J, Järve K. (2017). A haplotype specific to North European wheat (*Triticum aestivum* L.). *Genet Resour Crop Evol* 64(4): 653–664.

Ivaničová Z, Jakobson I, Reis D, Šafář J, Milec Z, Abrouk M, Doležel J, Järve K, Valárik M. (2016). Characterization of new allele influencing flowering time in bread wheat introgressed from *Triticum militinae*. *New Biotechnology* 33: 718–727.

Jakobson I, Reis D, Tiidema A, Peusha H, Timofejeva L, Valarik M, Kladivova M, Simkova H, Dolezel J, Järve K. (2012). Fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat–*Triticum militinae* introgression line. *Theor Appl Genet* 125: 609–623.

Jakobson I, Peusha H, Timofejeva L, Järve K. (2006). Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* x *Triticum militinae* hybrid line. *Theor Appl Genet* 112: 760–769.

Peusha H, Enno T, Jakobson I, Tsõmbalova J, Ingver A, Järve K. (2008). Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia. *Acta Agriculturae Scandinavica*, Section B - *Plant Soil Science* 58(4): 289–296.

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.

Enno T, Peuša H, Timofejeva L, Tohver M, Jakobson I, Priilinn O. (1998). Identification of chromosomal translocations in common wheat, derivative of *Triticum timopheevii*. *Acta Agron Hung* 46: 209–216.

Jakobson I. (1995). Experimental populations of *Drosophila melanogaster* originated from flies different types of reaction norm. *Proceed Latvian Acad Sci* 5/6:pA39.

Kuznetsova OV, Tishkin VV, Ivanchenko I (Jakobson). (1984). The line of *Drosophila melanogaster* used for estimation of fitness components of population. *Tsitologiya & Genetika* 4: 290–294 (in Russian).

Yacobson I (Jakobson), Glotov NV. (1989). The influence of ecological past and environment microfluctuations of the variability structure of quantitative characters in *Drosophila*. *Genetika* 25(7): 1218–1222 (in Russian).