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ORIGINAL PAPER

Mapping of powdery mildew resistance gene *Pm53* introgressed from *Aegilops speltoides* into soft red winter wheat

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Abstract

Key message **A powdery mildew resistance gene was introgressed from *Aegilops speltoides* into winter wheat and mapped to chromosome 5BL. Closely linked markers will permit marker-assisted selection for the resistance gene.**

Abstract Powdery mildew of wheat (*Triticum aestivum* L.) is a major fungal disease in many areas of the world, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*). Host plant resistance is the preferred form of disease prevention because it is both economical and environmentally sound. Identification of new resistance sources and closely linked markers enable breeders to utilize these new sources in marker-assisted selection as well as in gene pyramiding. *Aegilops speltoides* ($2n = 2x = 14$, genome SS), has been a valuable disease resistance donor. The powdery mildew resistant wheat germplasm line NC09BGTS16 (NC-S16) was developed by backcrossing an *Ae. speltoides* accession, TAU829, to the susceptible soft red winter wheat cultivar 'Saluda'. NC-S16 was crossed to the susceptible cultivar 'Coker 68-15' to develop $F_{2:3}$ families for gene mapping. Greenhouse and field evaluations of these $F_{2:3}$ families indicated that a single gene, designated *Pm53*, conferred resistance to powdery mildew. Bulked segregant analysis showed that multiple simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers specific to chromosome 5BL segregated with the resistance gene. The gene was flanked by markers *Xgwm499*, *Xwmc759*, *IWA6024* (0.7 cM proximal) and *IWA2454* (1.8 cM distal). *Pm36*, derived from a different wild wheat relative (*T. turgidum* var. *dicoccoides*), had previously been mapped to chromosome 5BL in a durum wheat line. Detached leaf tests revealed that NC-S16 and a genotype carrying *Pm36* differed in their responses to each of three *Bgt* isolates. *Pm53* therefore appears to be a new source of powdery mildew resistance.

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Introduction

Powdery mildew of wheat (*Triticum aestivum* L.) is caused by the obligate, biotrophic fungal pathogen *Blumeria graminis* (syn. *Erysiphe graminis*) DC. f. sp. *tritici* (*Bgt*) Em. Marchal. This ascomycete infects the foliage, stem, and spikes of the wheat host. *Bgt* typically thrives in mild, temperate climates with high relative humidity. Wheat powdery mildew is very common in Europe, parts of Asia, and the southeastern part of the United States, where it occurs annually and can cause significant yield losses. In years with major powdery mildew epidemics, yield losses have ranged from 17 to 34 percent in Maryland and North Carolina (Johnson et al. 1979; Leath and Bowen 1989). Severe powdery mildew infection can cause yield loss by reducing number of tillers, number of grains, kernel weight, and grain protein content (Johnson et al. 1979; Parry 1990; Bowen et al. 1991; Everts et al. 2001).

Host plant resistance is often preferred over alternative disease management methods, such as fungicide application, because it is more consistent, economical, and environmentally sound. The most commonly deployed form

of powdery mildew resistance is race-specific, or qualitative, host resistance (Huang and Roder 2004). Over 70 powdery mildew resistance (*Pm*) alleles at 44 loci have been formally designated (*Pm1–Pm49*) (Cowger et al. 2012; McIntosh et al. 2013). In addition, 18 temporarily designated powdery mildew resistance genes have been identified. These temporarily designated genes usually need to undergo further allelism tests to determine whether they are alleles at novel or previously established *Pm* loci. Several sources of resistance genes have come from wild relatives of wheat (Cowger et al. 2012; McIntosh et al. 2013). One source of resistance genes is *Aegilops speltoides* Tausch, the closest extant relative of the wheat B genome (Luo et al. 2005). To date, two *Pm* genes have been derived from *Ae. speltoides*: *Pm12* (Miller et al. 1988) and *Pm32* (Lapochkina et al. 1996; Hsam et al. 2003). *Pm12* has been mapped to chromosome 6B (Jia et al. 1996), and *Pm32* was mapped to chromosome 1B (Hsam et al. 2003).

As powdery mildew populations are very dynamic and continually change virulence structure, *Pm* genes in current cultivars are constantly challenged and often defeated (McDonald and Linde 2002; Parks et al. 2008). Wheat breeders are therefore persistently searching for new sources of resistance to powdery mildew to incorporate in breeding programs (Miller et al. 1988; Lapochkina et al. 1996; Schneider et al. 2008; Gill et al. 2011).

Pyramiding several major genes into a single cultivar disrupts directional selection pressure, and therefore is expected to improve the durability of resistance. When a cultivar with one major resistance gene is deployed over large areas, it will cause directional selection, e.g. pathotypes virulent to the resistance gene will increase in frequency, where as avirulent pathotypes will decrease in relative frequency. However, when cultivars with multiple resistance genes are deployed, it should take longer for pathogen populations to achieve virulence because of the increased number of mutations needed to overcome all resistance genes present in the host cultivar (McDonald and Linde 2002; Huang and Roder 2004). Of course, *Bgt* populations undergo frequent sexual recombination, which could facilitate the eventual defeat of pyramided *Pm* genes.

In order to optimize selection for *Pm* resistance genes, breeders can utilize molecular markers in close linkage with the genes. Marker-assisted selection (MAS) can be a faster and less expensive alternative to phenotypic selection in the greenhouse or field, it can be done in the absence of the pathogen, and it facilitates pyramiding of multiple resistance genes. To date, SSR markers have been the most commonly used markers in MAS, but recently SNP markers have become available. SNPs are even more abundant than SSRs and are often present within genes, which makes them functional or perfect (Somers et al. 2004). In the past few years, both a 9,000 SNP chip platform and a 90,000 SNP chip platform have been developed for wheat and linkage maps based on SNP data have been published (Cavanagh et al. 2013; Wang et al. 2014). The development of Kompetitive Allele Specific PCR (KASP) single-tube assays (LGC Genomics, Hoddesdon, UK) suitable for selecting for a single gene or trait on a large number of genotypes (Chen et al. 2010) makes SNPs very useful in gene mapping and MAS. KASP assays are fast to run and easy to interpret, making development of genotyping assays based on KASP technology desirable. So far, only one temporarily designated *Pm* gene, *MlUM15*, has been mapped using SNP genotyping via KASP assays (Worthington et al. 2014).

The objectives of this study were to determine the inheritance, chromosomal location, and molecular markers linked to powdery mildew resistance in the *Ae. speltoides* derived germplasm line NC-S16.

Materials and methods

Plant materials

The soft red winter wheat germplasm line NC09BGTS16 (NC-S16) (PI 669386) is a BC_2F_7-derived germplasm line with the pedigree Saluda × 3/TAU829. TAU829 is an *Ae. speltoides* accession obtained from Gene Bank of Tel Aviv University, Israel. It was collected in Haifa (Technion) in the Mount Carmel region of Israel in 1985. Its accession number on the Tel Aviv University Gene Bank website is AEG-829-15 (http://www2.tau.ac.il/ICCI/default.asp). 'Saluda' (PI 480474) is a soft red winter wheat cultivar developed by Virginia Polytechnic Institute and State University (Starling et al. 1986). Saluda contains *Pm3a*, a defeated major gene for powdery mildew resistance (Niewoehner and Leath 1998), and is now susceptible to the naturally occurring powdery mildew population in North Carolina (Parks et al. 2008).

NC-S16 and Coker 68-15 (CItr 15291), a susceptible cultivar with no known *Pm* genes, were crossed in 2008. The F_1 hybrid was self-pollinated to produce F_2 seeds in the greenhouse during the 2009–2010 winter. F_2 plants were grown in separate pots in the greenhouse and harvested individually without selection in the 2010–2011 winter to produce $F_{2:3}$ families used in subsequent disease evaluations.

Disease evaluations in greenhouse

One hundred and forty $F_{2:3}$ families were evaluated for resistance to powdery mildew in the greenhouse during

November 2011 in a randomized complete block design experiment with two replicates. Each experimental unit consisted of three 10 cm pots planted with a total of fifteen seeds of a given $F_{2:3}$ family. Parent lines Coker 68-15 and NC-S16, as well as susceptible check Saluda, were planted in ten-pot intervals along the greenhouse bench. Greenhouse temperatures were held at 24/18 °C (day/night) and high-intensity 1,000-W discharge lights supplemented natural light.

The powdery mildew *Bgt* isolate 'Arapahoe' was used as the inoculum source. It was obtained from the *Bgt* collection maintained by the USDA-ARS Plant Science Research Unit at North Carolina State University. Arapahoe was virulent to *Pm1c*, *Pm3a*, *Pm3c*, *Pm3e*, *Pm3g*, *Pm4a*, *Pm5a*, *Pm5b*, *Pm5d*, *Pm6*, *Pm7*, *Pm9*, and *Pm20* and avirulent to *Pm1a*, *Pm3b*, *Pm3d*, *Pm3f*, *Pm4b*, *Pm8*, *Pm12*, *Pm16*, *Pm17*, *Pm21*, *Pm25*, *Pm37* (personal observation). The inoculum was increased on Saluda plants under greenhouse conditions prior to setting up the disease evaluation experiment. $F_{2:3}$ seedlings were inoculated 19 and 20 days after planting at Zadoks growth stages 13–20 by gently shaking conidia from leaves of infected Saluda plants onto the foliage of the $F_{2:3}$ families, parent lines, and susceptible checks.

Disease reactions were scored 10 and 11 days after inoculation following the 0–9 rating scale developed by Leath and Heun (1990). Plants scored 0–3 were considered resistant according to this scale, where 0 = an immune reaction with no visible signs of infection, 1 = chlorotic flecks with no necrosis, 2 = chlorotic flecks with some necrosis, and 3 = mild chlorosis and barely detectable mycelium. Rating between 4 and 6 was indicative of an intermediate disease reaction, with the degree of chlorosis and visible mycelium and conidial chains increasing from slight to moderate. Plants rated between 7 and 9 were scored as susceptible with increasing amounts of mycelium and conidia. Each of the fifteen plants within an experimental unit was scored individually and compared with the parental lines and susceptible check. The $F_{2:3}$ families were classified as homozygous resistant, homozygous susceptible or segregating depending on their phenotypic reactions. Chi square tests were performed to test the goodness of fit between observed and expected segregation ratios.

Disease evaluations in field

Each of the 140 $F_{2:3}$ families was planted at the Lake Wheeler Road Field Laboratory south of Raleigh, NC, and the Cunningham Research Station in Kinston, NC. Seeds from each family were planted in a randomized complete block experiment, in which each experimental unit consisted of a single 1.2-m row. Each row was planted with 40–60 seeds from a given $F_{2:3}$ family. The rows were spaced at 30.5 cm intervals. Two replications per family were planted at both locations. Rows of NC-S16, Coker 68-15, and Saluda were included at 60-plot intervals as resistant and susceptible controls. The experiments were bordered by rows of Saluda to promote an even spread of powdery mildew. The planting dates were October 22, 2011 at the Cunningham Research Station site and October 31, 2011 at the Lake Wheeler Road Field Laboratory site. Fertilization, irrigation, and other agronomic treatments followed standard management procedures for North Carolina (Weisz 2013).

Disease ratings were made in the middle of March 2012 when plants had reached Zadoks Growth Stage 39–50 and powdery mildew symptoms had developed uniformly at the two field sites. Each plot was evaluated on the 0–9 rating scale used in previous greenhouse screening (Leath and Heun 1990) and categorized as either homozygous resistant, segregating, or homozygous susceptible. Chi squared tests were performed to test the goodness of fit between the observed segregation ratios in the field and the expected segregation ratios. One $F_{2:3}$ line that was scored as segregating in the greenhouse was resistant in the field, but scores were otherwise consistent among trials. This difference was presumably due to seed contamination, so this line was excluded from the analysis.

A powdery mildew differential test in an adjacent field at the Lake Wheeler Road Field Laboratory showed that wheat lines carrying *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3f*, *Pm4a*, *Pm5a*, *Pm17*, and *Pm20* were susceptible to naturally occurring *Bgt* isolates, and lines carrying *Pm1c*, *Pm3d*, *Pm3e*, *Pm3g*, *Pm5d*, *Pm12*, *Pm13*, *Pm16*, *Pm21*, *Pm25*, *Pm34*, *Pm35*, and *Pm37* were resistant. Wheat lines carrying *Pm4b*, *Pm5b*, *Pm6*, *Pm7*, and *Pm8* displayed adult plant resistance (Cowger, personal observation).

Molecular marker analysis

Genomic DNA was extracted from leaf tissue collected from the F_2 plants from which the $F_{2:3}$ families originated. DNA was extracted according to the CTAB protocol described by Stein et al. (2001). Ten $F_{2:3}$ families with consistently resistant phenotypes and ten families with consistently susceptible phenotypes were chosen for bulk segregant analysis (BSA) (Michelmore et al. 1991). DNA from the ten resistant families was pooled into a resistant bulk, and DNA from the ten susceptible families was pooled into a susceptible bulk. The resistant parent, resistant bulk, susceptible bulk, and susceptible parent were then screened with genome- and chromosome-specific markers from across the wheat genome. Markers polymorphic between the resistant parent and bulk, on the one hand, and the susceptible parent and bulk on the other, were considered tentatively linked to the resistance gene. These markers were

then used to screen the entire F_2 population. Once the chromosome with the resistance gene was identified, additional polymorphic markers specific to that chromosome were evaluated across the population.

The SSR primers *Xwmc537* and *Xwmc759* were ordered according to their sequences in Grain Genes (http://wheat.pw.usda.gov) with M13 sequence tags (5′-CACGACGTTGTAAACGAC-3′) attached to the 5′ end for universal fluorescent labeling (Schuelke 2000). The EST-derived SSR marker *BJ261635* (Blanco et al. 2008) with forward primer (5′-TAGCCTGGTACCATTCTGCC-3′) and reverse primer (5′-TGTAATGGAGGTGCAGCTTG-3′) was also ordered with M13 sequence tags.

SSR primers *Xgwm499*, *Xgwm408, Xcfd7,* and *Xwmc75* were direct-labeled with NED, FAM, PET, or VIC. PCR conditions were performed as described by Miranda et al. (2006). Products from markers with M13 sequence-tagged primers were run on polyacrylamide gels following procedures described in Miranda et al. (2006). Direct-labeled SSR products were run on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems) and scored with Peak Scanner 2 (Life Technologies, Carlsbad, CA).

SNP markers were selected from the Illumina Infinium 9 K SNP array (Cavanagh et al. 2013) based on map location and polymorphism between Coker 68-15 and NC-S16, with the NC-S16 alleles at low frequencies among 558 eastern winter wheat lines genotyped with the 9 K array. Source sequences of the selected SNP markers were used for development of KASP assays. Primers were designed using Primer Picker software with default settings (LGC Genomics, Hoddesdon, UK). KASP assays included two allele-specific forward primers with tail sequences and one common reverse primer (Table 1). Assay mix preparation and PCR protocols were conducted according to LGC Genomics protocols. Fluorescent endpoint genotyping was done using a LightCycler 480 (Roche Applied Sciences, Indianapolis, IN).

All markers were checked for distortion from expected segregation ratios using Chi squared tests. Linkage analysis was performed using MAPMAKER/EXP 3.0 (Lincoln et al. 1993). The Kosambi mapping function estimated centimorgan (cM) distances between markers (Komsambi 1944). The chosen marker order was selected based on maximum likelihood estimates, and compared to SNP and SSR consensus map positions (Somers et al. 2004; Cavanagh et al. 2013) and physical location of markers on the 5B wheat deletion bin map. Genetic linkage maps were drawn using MapChart 2.1 software (Voorrips 2002).

Deletion bin mapping

The chromosomal location of each linked marker was confirmed using Chinese Spring (CS) and chromosome

Table 1 Primers for KASP assays used to screen the NC09BGTS16 (NC-S16) × Coker 68-15 population

SNP no.	SNP name	Primer A1[a]	Primer A2[a]	Primer C1
IWA1441	wsnp_Ex_c11131_18036595	TTCTTGCCCGTTCTTAGTGAGAGA	CTTGCCCGTTCTTAGTGAGAGC	TGAACTTCTCAAACTGAACGGTTCAGTTA
IWA2454	wsnp_Ex_c19724_28721128	GGAACCTTGAATGTTTCCCATTCCA	GAACCTTGAATGTTTCCCATTCCG	CCTCCCGGCAATATGCCTCCAA
IWA2698	wsnp_Ex_c2224_4171424	CATCATTAGCTTCAGGTAGTTCACC	CATCATTAGCTTCAGGTAGTTCACT	GCCATATGCAGAAATATCAGCCGCTA
IWA5669	wsnp_Ex_rep_c70120_69069699	GTTTCCCAACCTGTTCCCAGTCA	TCCCAACCTGTTCCCAGTCG	AGATCCAGAAAACCAGTCGCTGGAA
IWA6024	wsnp_JD_c37023_27225840	CGACAACATCAACCCTCCTCTAT	CGACAACATCAACCCTCCTCTAC	GTACTTTAGTCGATGAACTGCTAGCTTTA
IWA6516	wsnp_Ku_c14202_22436656	CCTCAAGCACCACAACGCCG	ACCTCAAGCACCACAACGCCA	TTCCTACACTTCCCTAGTTACAGAGAAA

The prefix *IWA* is followed by an index number

SNP single nucleotide polymorphism

[a] Primer sequences are given without the FAM (A1) and VIC (A2) tails

5BL aneuploid lines (provided by the Kansas State University Wheat Genetics Resource Center). The aneuploids tested included nullisomic5B-tetrasomic5A (N5BT5A), ditelosomic5BL (Dt5BL), and deletion lines 5BL-2 (FL 0.26), 5BL-6 (FL 0.29), 5BL-1 (FL 0.55), 5BL-14 (FL 0.75), and 5BL-16 (FL0.79). The name specifies the chromosome arm carrying the deletion followed by its arbitrary line number designation and the fraction length of the arm present in brackets.

Detached leaf evaluations

Detached leaf segments were used to test for differential response to 80 *Bgt* isolates between NC-S16 and '5BIL-29', a durum wheat line containing *Pm36*, located on chromosome 5BL. *Pm36* was introgressed from wild emmer, *T. turgidum* var. *dicoccoides* ($2n = 4x = 28$, AABB) into durum wheat (Blanco et al. 2008). About half a million years ago hybridization between the two diploid species *T. urartu* Tumanian ($2n = 2x = 14$, AA) and *Ae. speltoides* gave rise to the tetraploid wheat *T. turgidum* var. *dicoccoides* (Huang et al. 2002), which is considered the wild progenitor of tetraploid and hexaploid wheats.

The lines NC-S16 and 5BIL-29 were included in detached leaf tests. Seeds of 5BIL-29 are maintained by the USDA-ARS at North Carolina State University. Cultivars Coker 68-15, Jagalene, Saluda, and Chancellor (CItr 12333) were used as susceptible controls.

The *Bgt* isolates used in this test were from powdery mildew samples collected in different years at various locations in the US and abroad, and are maintained by the USDA-ARS Plant Science Research Unit at North Carolina State University. The isolates were chosen due to their broad virulence spectra.

Several 1.5 cm leaf segments of each of the wheat lines under study were placed on the surface of each Petri dish containing 0.5 % water agar supplemented with 50 mg/L benzimidazole. Each Petri dish was inoculated with conidia from a single *Bgt* isolate propagated on leaf segments of Chancellor. Two replicate plates were inoculated with each isolate. Four additional replicate plates were inoculated with isolates PRA-A-3-1, MOB-B-1-2, and C1-4 in a follow-up test because these were the only isolates for which NC-S16 showed an intermediate reaction in the original test. The Petri dishes were placed in a growth chamber at 85 % humidity and 18 °C temperature with a 12 h photoperiod. Disease reactions were scored 10 days later according to Leath and Heun (1990), where 0 = an immune reactions with no visible signs of infection, 1 = chlorotic flecks with no necrosis, 2 = chlorotic lesions, 3 = necrotic lesions, 4 = first signs of mycelium, 5 = 1–2 pustules per leaf segment, 6 = less than 20 % of leaf area covered by pustules, 7 = 20–50 % of leaf area covered in pustules, 8 = 50–90 % of leaf area covered in pustules, 9 = 100 % of leaf area covered in pustules. Lines with an average score of 3 or lower were considered resistant (R), while lines with an average score over 3 and under 6 were considered intermediate (I), and lines with an average score greater than 6 were considered susceptible (S).

Analyses of variance (ANOVA) were conducted using PROC GLM in SAS 9.3 (SAS 2011) using genotype as a fixed variable and replication as a random variable. Least significant differences (LSD) were also calculated using SAS 9.3.

Results

Inheritance of resistance

Coker 68-15 and Saluda were consistently susceptible to *Bgt* isolate Arapahoe in the greenhouse, with a mean disease score of 8. NC-S16 was resistant with mean disease score of 0.2. The 140 $F_{2:3}$ families were resistant, segregating, and susceptible at a 1:2:1 ratio ($\chi^2 = 3.66$; $P = 0.16$) (Table 2) as expected for a trait controlled by a single gene. The pooled numbers of resistant and susceptible plants within segregating lines did not fit a 3:1 ratio (1,223 resistant: 464 susceptible, $\chi^2 = 5.64$; $P = 0.02$), suggesting the resistance in NC-S16 might be conferred by a single, partially dominant allele. NC-S16 was also highly resistant (mean disease score 0.5) to powdery mildew infection by naturally occurring *Bgt* isolates at Cunningham Research Station and Lake Wheeler Field Laboratory in the spring of 2012. Coker 68-15 (mean disease score of 8) and Saluda (mean disease score of 8) were both

Table 2 Segregation ratios for disease reaction of $F_{2:3}$ families from the NC-S16 × Coker 68-15 population evaluated in the greenhouse and two field sites in North Carolina in spring 2012

Location	Number of $F_{2:3}$ families					
	Resistant	Segregating	Susceptible	Total	χ^2 (1:2:1)	*P* value
Greenhouse	43	70	27	140	3.66	0.16
Cunningham	44	69	27	140	4.16	0.13
Lake Wheeler	44	69	27	140	4.16	0.13

susceptible to *Bgt* populations at both field locations. The $F_{2:3}$ families at both Lake Wheeler and Cunningham field sites were resistant, segregating, and susceptible in a 1:2:1 ratio ($\chi^2 = 4.16$; $P = 0.13$) (Table 2). The single partially dominant resistance gene identified in NC-S16 was designated *Pm53*.

Molecular mapping of powdery mildew gene in NC-S16

Over 325 genome- and chromosome-specific molecular markers were initially used to screen for polymorphisms between resistant and susceptible parents and bulks. SSR marker *Xwmc537*, previously mapped to the long arm of chromosome 5B (Somers et al. 2004), was polymorphic between the resistant and susceptible bulks. Therefore, additional markers specific to chromosome 5BL were tested for polymorphism. The SSR markers *Xwmc759*, *Xgwm499*, and *Xgwm408* were polymorphic between resistant and susceptible parents and bulks. All four polymorphic SSR markers were used to screen the F_2 population. Other SSR markers previously linked to *Pm36* (Blanco et al. 2008) on chromosome 5BL (*Xcfd7*, *BJ261635*, and *Xwmc75*) were tested for polymorphism, but were not polymorphic between the resistant and susceptible parents. Six SNPs previously mapped to chromosome 5BL (Cavanagh et al. 2013) were polymorphic between NC-S16 and Coker 68-15 (*IWA2698*, *IWA1441*, *IWA6024*, *IWA2454*, *IWA5669*, and *IWA6516*). These six SNPs were also evaluated across the entire F_2 population.

Xwmc537, *IWA1441*, *Xwmc759*, *IWA6024*, *IWA2454*, and *IWA5669* were dominant markers linked to the powdery mildew resistance gene. Markers *IWA2698*, *Xgwm499*, *Xgwm408*, and *IWA6516* were scored as co-dominant. All co-dominant SSR and SNP markers segregated in the expected 1:2:1 ratio, while the dominant markers segregated in the expected 3:1 ratio. The most likely order of the linked markers (Fig. 1) was generally in agreement with the SNP (Cavanagh et al. 2013) and the SSR (Somers et al. 2004) consensus maps.

The powdery mildew resistance gene *Pm53* was putatively assigned to the long arm of chromosome 5B, based on the reported chromosomal locations of the four linked SSR markers and the six linked SNP markers (Somers et al. 2004; Cavanagh et al. 2013) (Fig. 1). Using Chinese Spring deletion bin lines, its physical location on chromosome 5BL was confirmed by all ten markers. Five Chinese Spring 5BL deletion lines were used to identify the sub-chromosomal location of the loci amplified by each of the markers. *Xwmc537* was the only marker that amplified the Chinese Spring fragment in the 5BL-6 deletion line, indicating that it was physically located in the bin between fraction length 0.00 and 0.29. The other markers did not amplify any fragments in the 5BL-6 deletion line, but amplified fragments

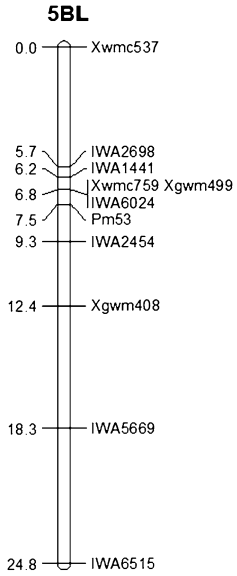

Fig. 1 Genetic linkage map of the powdery mildew resistance gene *Pm53* on the introgressed *Ae. speltoides* segment in line NC-S16. *Xwmc537*, *Xwmc759*, *Xgwm499*, and *Xgwm408* are SSR markers; *IWA2698*, *IWA1441*, *IWA6024*, *IWA5669*, and *IWA6516* are SNP markers. Kosambi map distances (cM) are shown on the *left side* of the map. The software program MapChart 2.1 was used to produce this figure

in deletion lines 5BL-1, 5BL-14, and 5BL-16, which meant they mapped to the bin 5BL-6 (FL 0.29–0.55). Due to the tight linkage between the powdery mildew resistance gene and several SSR and SNP markers, we postulated that the *Pm53* gene is located in bin 5BL-6 (FL 0.29–0.55).

The *Triticum turgidum* var. *dicoccoides* derived *Pm* gene *Pm36* was previously mapped to bin 5BL-6 (FL 0.29–0.76) (Blanco et al. 2008). Of the SSR markers closely linked to *Pm36*, three (*Xwmc75*, *Xcfd7*, and *BJ261635*) were not polymorphic between NC-S16 and Coker 68-15, so these could not be placed on the linkage map for the NC-S16/Coker 68-15 mapping population. *Xgwm408* was polymorphic, however, and mapped 4.9 cM distal from *Pm53*.

Marker *BJ261635* was tested with NC-S16, Coker 68-15, Saluda, and 5BIL-29 (with *Pm36*). The marker produced one fragment of size 241 bp in NC-S16, Coker 68-15, and Saluda. In 5BIL-29, two fragments of sizes 241 and 248 bp were produced. This 7 bp difference between the two fragments from 5BIL-29 was consistent with results from Blanco et al. (2008) for the resistant parent, as well as homozygous resistant and heterozygous plants from their segregating population. They found the smaller fragment to be ubiquitous across samples, and the larger fragment to be co-segregating with *Pm36* (mapped 0.3 cM from the gene), matching the results observed in our test.

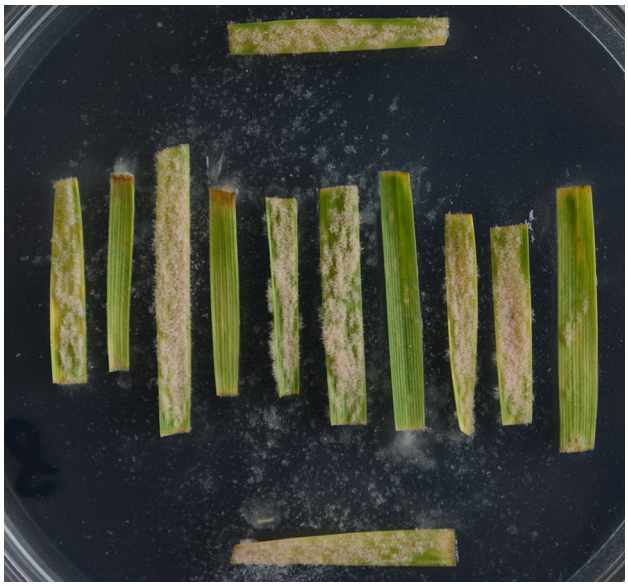

Fig. 2 Virulence test with randomly placed detached leaf segments of genotypes (*from left*) Jagalene, 5BIL-29, Coker 68-15, 5BIL-29, Saluda, Coker 68-15, NC-S16, Jagalene, Saluda, and NC-S16. The segments at the *top* and *bottom* are susceptible check Chancellor. Image shows the responses to inoculation with *Bgt* isolate MOB-B-1-2 9 days after inoculation

Table 3 Differential reactions of NC-S16, Coker 68-15, and 5BIL-29 possessing *Pm36* previously mapped to chromosome 5BL to isolates of *Bgt* evaluated in a detached leaf test

Genotype	*Pm* gene	Isolate[a]		
		C1-4	MOB-B-1-2	PRA-A-3-1
NC-S16	*Pm53*	4.1^{A}	4.2^{A}	3.1^{A}
5BIL-29	*Pm36*	0.1^{B}	0.2^{B}	0.2^{B}
Coker 68-15	None	8.1^{C}	8.1^{C}	8.0^{C}
Chancellor[b]	*Pm10, 15*	7.8^{D}	7.9^{C}	7.7^{C}
No. of replications	–	14	18	12
LSD	–	0.32	0.53	0.82

[a] Disease reactions were scored on a 0–9 scale according to Leath and Heun (1990)

[b] Chancellor = susceptible check; *Pm10* and *Pm15* are universally susceptible (McIntosh et al. 1995a)

[A,B,C,D] Values within a column followed by different letters are significantly different at $P < 0.05$

Detached leaf test

Following inoculation with 3*Bgt* isolates, the disease response pattern of NC-S16 differed from the pattern of 5BIL-29 with *Pm36*, and those of the susceptible checks Coker 68-15, Saluda, Jagalene, and Chancellor (Fig. 2). Across 12–18 replications, NC-S16 consistently showed intermediate responses to isolates C1-4, PRA-A-3-1, and MOB-B-1-2, with scattered sparse pustules and minor chlorosis, whereas 5BIL-29 consistently showed resistant responses to these three isolates (Table 3). Random placement of the various genotypes on a plate confirmed that inoculation across the plate had been even (i.e., no escapes) and reaction type was independent of proximity to a susceptible or resistant genotype (Fig. 2). The differential responses of 5BIL-29 and NC-S16 to isolate MOB-B-1-2 illustrated in Fig. 2 were similar for isolates C1-4 and PRA-A-3-1 as well. The mean disease score for NC-S16 was significantly different from that of 5BIL-29 for all three isolates at $P < 0.05$. Both NC-S16 and 5BIL-29 were resistant to all other *Bgt* isolates in the test.

In addition to the three isolates shown in Table 3, recent tests showed that several *Bgt* isolates from Missouri and at least one isolate from Alabama produced intermediate reactions in NC-S16, while 5BIL-29 showed an immune response (unpub. data).

Discussion

In the present study, a powdery mildew resistance gene was introgressed from *Ae. speltoides* into soft red winter wheat. Greenhouse and field experiments confirmed that resistance was conferred by a single gene. The resistance gene was mapped to chromosome 5BL, in bin 5BL-6 (0.29–0.55), and tightly-linked SSR and SNP markers were identified. The resistance gene was flanked by markers *IWA6024*, *Xgwm499*, and *Xwmc759* (0.7 cM proximally) and *IWA2454* (1.8 cM distally). *Xwmc759* was scored as a dominant marker. *Xgwm499* might therefore be the preferred SSR marker to use in a genotyping assay. NC-S16 is the first soft red winter wheat line with an introgressed *Pm* gene on chromosome 5BL. It should provide breeders with an effective new source of resistance that can be tracked with SNPs and/or SSRs in MAS. Furthermore, it will allow for pyramiding of *Pm53* with *Pm* genes at other loci.

In addition to providing powdery mildew resistance, *Ae. speltoides* has served as a source of resistance to leaf rust, including *Lr28* (McIntosh et al. 1982), *Lr35* (Kerber and Dyck 1990), *Lr36* (Dvorak and Knott 1990), *Lr47* (Dubcovsky et al. 1998), and *Lr51* (Dvorak 1977; Dvorak and Knott 1980; Helguera et al. 2005); green bug resistance (Dubcovsky et al. 1998); and stem rust resistance, including *Sr32* (McIntosh et al. 1995a, b; Friebe et al. 1996), *Sr39* (Kerber and Dyck 1990; McIntosh et al. 1995b), *Sr47* (Faris et al. 2008), and *SrAes7t* (Klindworth et al. 2012). Out of nearly 200 *Aegilops speltoides* accessions collected from five regions of Israel, almost all accessions were highly resistant to leaf rust, stripe rust, and stem rust when tested in both Israel and Minnesota, USA (Anikster et al. 2005). It is therefore likely that additional genes for disease resistance for use in wheat breeding could be found in accessions of *Ae. speltoides*.

Only one other known *Pm* gene, *Pm36*, has been mapped to the long arm of chromosome 5B (Blanco et al. 2008). *Pm36* was introgressed from *T. turgidum* var. *dicoccoides* into durum wheat ($2n = 4x = 28$). It is unlikely that the same gene is shared by *T. turgidum* var. *dicoccoides* and *Ae. speltoides* given the amount of time that each of the resistance genes has evolved independently since the polyploidy formation events hundreds of thousands years ago (Huang et al. 2002; Jauhar 2007). No example has been reported of the same resistance gene being found in wild emmer and an *Aegilops* species, except for *Ae. tauschii* (DD genome) (McIntosh, personal communication). Because *Pm36* was introgressed to tetraploid durum wheat and NC-S16 is hexaploid soft red winter wheat, performing a classic allelism test was not considered practical.

Blanco et al. (2008) reported that the EST-derived SSR marker *BJ261635* was in close linkage with *Pm36* (0.3 cM). Generally, EST-SSR markers show high transferability between alien species of wheat (Gupta et al. 2003). We found that NC-S16 did not amplify the fragment linked to resistance contributed by *Pm36*, indicating that the resistance gene in NC-S16 is different from *Pm36*. Also, while marker *Xgwm408* mapped 14.8 cM distal from *Pm36* according to Blanco et al. (2008), it was mapped only 4.9 cM distal from *Pm53*.

In the detached leaf test, NC-S16 consistently showed intermediate reactions to three *Bgt* isolates across 12–18 replications, while 5BIL-29 showed immune responses to the same isolates. The fact that additional *Bgt* isolates from a certain geographical region conferred intermediate responses in NC-S16, but not in 5BIL-29, could be a sign of mutation to partial virulence against *Pm53*.

Taken together, the genotypic and phenotypic data lead us to conclude that *Pm53* is different from *Pm36*.

Extensive production of wheat cultivars with only one *Pm* gene places strong directional selection pressure on the pathogen population, and copious spore production provides ample potential for mutations to virulence, resulting in rapid appearance and increase of adapted isolates. The high evolutionary potential of the *Bgt* pathogen contributes to the low durability of race-specific resistance, forcing breeders and pathologists to constantly search for new sources of resistance and improved resistance deployment strategies. The identification of this new *Pm* gene and closely linked SNPs will be useful for breeders, especially if it can be used in combination with *Pm* genes at other loci and/or quantitative sources of resistance. NC-S16 is currently being used as a parent in the wheat breeding program at North Carolina State University, and breeding lines with *Pm53* and overall good agronomic performance are being tested in the field. Further development of these materials should result in new, powdery mildew resistant cultivars for growers in the southeastern US.

Author contribution statement S. Petersen: Collected phenotypic and genotypic data, analyzed data, performed gene mapping. J.H. Lyerly: Assisted in collection of genotypic data. M.L. Worthington: Assisted in collection of phenotypic data. W.R. Parks: Provided *Bgt* isolates for experiments; assisted with differential leaf test. C. Cowger: Provided *Bgt* isolates for experiments; assisted with differential leaf test; provided guidance throughout this study. D.S. Marshall: Provided guidance throughout the study. G. Brown-Guedira: Provided guidance in collection of genotypic data. J. P. Murphy: Developed the germplasm line NC-S16; provided guidance throughout this study.

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Conflict of interest The authors declare that they have no conflict of interest.

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