

# QTL for resistance to root lesion nematode (*Pratylenchus thornei*) from a synthetic hexaploid wheat source

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## Abstract

**Key message** A whole genome average interval mapping approach identified eight QTL associated with *P. thornei* resistance in a DH population from a cross between the synthetic-derived wheat Sokoll and cultivar Krichauff.

**Abstract** *Pratylenchus thornei* are migratory nematodes that feed and reproduce within the wheat root cortex, causing cell death (lesions) resulting in severe yield reductions globally. Genotypic selection using molecular markers

closely linked to *Pratylenchus* resistance genes will accelerate the development of new resistant cultivars by reducing the need for laborious and expensive resistance phenotyping. A doubled haploid wheat population (150 lines) from a cross between the synthetic-derived cultivar Sokoll (*P. thornei* resistant) and cultivar Krichauff (*P. thornei* moderately susceptible) was used to identify quantitative trait loci (QTL) associated with *P. thornei* resistance. The resistance identified in the glasshouse was validated in a field trial. A genetic map was constructed using Diversity Array Technology and the QTL regions identified were further targeted with simple sequence repeat (SSR) and single-nucleotide polymorphism (SNP) markers. Six significant and two suggestive *P. thornei* resistance QTL were detected using a whole genome average interval mapping approach. Three QTL were identified on chromosome 2B, two on chromosome 6D, and a single QTL on each of chromosomes 2A, 2D and 5D. The QTL on chromosomes 2BS and 6DS mapped to locations previously identified to be associated with *Pratylenchus* resistance. Together, the QTL on 2B (*QRInt.sk-2B.1–2B.3*) and 6D (*QRInt.sk-6D.1* and *6D.2*) explained 30 and 48 % of the genotypic variation, respectively. Flanking PCR-based markers based on SSRs and SNPs were developed for the major QTL on 2B and 6D and provide a cost-effective high-throughput tool for marker-assisted breeding of wheat with improved *P. thornei* resistance.

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## Introduction

The development of resistant wheat cultivars to root lesion nematodes (*Pratylenchus* spp.) has become increasingly important due to the prevalence and wide host range of the nematode, and usage restrictions and inefficacy of

nematicides. Resistant cultivars have the capacity to reduce nematode reproduction and nematode densities in the soil (Rohde 1972). Tolerant wheat cultivars can survive and yield well within nematode-infested soils but allow reproduction, thus leaving nematodes within the soil to attack subsequent crops. Therefore, the use of resistant and tolerant cultivars is now considered the most efficient, economical and environmentally acceptable means for *Pratylenchus* control (Castillo et al. 1998).

*Pratylenchus* spp. are migratory endoparasitic nematodes that feed and migrate within root cortical tissue causing necrosis and reduced root lateral branching at high nematode densities (Vanstone et al. 1998). Yield losses occur due to diminished uptake of water and nutrients in infested plants. Yield losses due to the most economically important and widespread species, *Pratylenchus thornei*, can be very high and losses of 85 % in Australia, 37 % in Mexico, 70 % in Israel, and 50 % in the USA have been reported (Armstrong et al. 1993; Nicol and Ortiz-Monasterio 2004; Smiley et al. 2005).

Considerable research efforts in Australia have led to the identification of several sources of partial *Pratylenchus* spp. resistance, which have been mapped to reveal quantitative trait loci (QTL) for resistance using different mapping populations in wheat. These *Pratylenchus* spp. resistance QTL were identified over 10 years ago (Thompson et al. 1999), but despite this, currently there are no commercially available wheat cultivars with strong resistance to *Pratylenchus* (Sheedy and Thompson 2009; Thompson et al. 2008). The GS50a resistance source identified as single plant selection from a severely affected field of the wheat cultivar Gatcher (Thompson and Clewett 1986), has been used to improve the levels of resistance to some degree in Australian cultivars.

Due to the lack of genetic diversity for *P. thornei* resistance in cultivated modern bread wheat, new sources of resistance are being sought from wild wheat progenitors (Ogbonnaya 2008; Zwart et al. 2010). Hexaploid bread wheat originated in the Fertile Crescent in the Middle East through a few random crossings between wild wheat species (Nesbitt 2001). It has been suggested that in the original hybridization, only a limited number of nematode resistance genes from the diploid *Aegilops tauschii* and the tetraploid *Triticum turgidum* wild progenitors were involved, and thus hexaploid wheat lacks the diverse genetic sources of resistance genes that its ancestors possess (Breiman and Graur 1995). Initially, Thompson and Haak (1997) screened accessions of *Ae. tauschii* from Iran and found *P. thornei* resistance in all taxonomic subgroups of this species. They investigated over 200 accessions and identified many with lower *P. thornei* reproduction than GS50a. Resistance found in *Ae. tauschii* can be transferred to bread wheat by direct crossing (Gill and Raupp 1987) or by developing synthetic

hexaploids through hybridization with a durum, *T. turgidum*, which can then be crossed to bread wheats (Lagudah et al. 1993; Mujeeb-Kazi 1995). In recent years, effective sources of resistance to *P. thornei* and *P. neglectus* have been identified and mapped in synthetic hexaploid wheat lines (Ogbonnaya 2008; Thompson et al. 2008; Toktay et al. 2006; Zwart et al. 2004, 2005, 2010) and Middle Eastern landraces (Schmidt et al. 2005; Sheedy and Thompson 2009; Thompson et al. 2009). QTL for resistance to *P. thornei* have been identified on the B and D genomes on five different chromosomes in the investigated sources (Schmidt et al. 2005; Toktay et al. 2006; Zwart et al. 2005, 2006, 2010). The mode of resistance to *P. thornei* is multigenic and additive (Zwart et al. 2004) and thus makes it a suitable trait for marker-assisted selection (MAS).

Previous *Pratylenchus* spp. resistance mapping studies were conducted on maps with a limited number of restriction fragment length polymorphisms and PCR-based markers, apart from the ITMI (International Triticeae Mapping Initiative) population used by Zwart et al. (2006, 2010). Genetic maps were generated with less than 170 markers and in some cases the intervals of the QTL identified were quite large (Schmidt et al. 2005; Zwart et al. 2005). These studies also utilized traditional phenotyping methods, which quantified nematodes from soil and roots via extraction of live nematodes and direct counting by microscopic assessment. Studies have shown positive correlations between the numbers of *P. thornei* quantified by soil DNA extraction followed by real-time PCR and traditional water extraction techniques followed by microscopic examination (Daniel et al. 2013; Yan et al. 2012). However, higher variation between replicates occurs with traditional techniques due to variable nematode extraction efficiencies, taxonomic misidentifications and sub-sampling errors (Hollaway et al. 2003; Taylor et al. 2002). To successfully implement MAS in a plant breeding program, the identified molecular markers need to be tightly linked, reliable across genotypes and cost-efficient. Several advancements have occurred in marker and QTL analysis technology in the past 10 years since the last reported *Pratylenchus* spp. resistance QTL in wheat. These advancements provide increased marker density across the wheat genome, which improves the chance of detecting QTL and defining their location. Improvements in phenotyping methods through DNA quantification have increased efficiency in terms of time and sample throughput (Min et al. 2012; Ophel-Keller et al. 2008; Yan et al. 2012) and reduced variation (Hollaway et al. 2003; Taylor et al. 2002), which is vital as the quality of QTL mapping relies heavily on the quality of the phenotypic data generated. A recent genetic mapping study has utilized this DNA quantification method for phenotyping to successfully identify QTL and develop molecular markers for *P. thornei* and *P. neglectus* in wheat (Jayatilake et al. 2013).

In this study, we utilized a sensitive quantitative PCR phenotyping method (Ophel-Keller et al. 2008) for the quantification of nematodes in soil to identify QTL and develop molecular markers linked to *P. thornei* resistance in the synthetic-derived wheat Sokoll. The resistance identified in the glasshouse was validated in a field trial. We constructed a genetic map for a doubled haploid population originating from a cross between Sokoll and the South Australian wheat cultivar Krichauff (*P. thornei* moderately susceptible) using Diversity Array Technology (DArT). Targeted mapping of SSRs and SNPs to the QTL identified was used to develop breeder-friendly markers to assist the introgression of the *P. thornei* resistance QTL into breeding germplasm.

## Materials and methods

### Plant material

The mapping population consisted of 150 doubled haploid (DH) lines derived from a cross between the synthetic-derived cultivar Sokoll (pedigree: Pastor/3/Altar84/*Ae.squarrosa*(Taus)//Opata) developed by the International Maize and Wheat Improvement Centre Mexico (CIMMYT), and cultivar Krichauff (pedigree: Wariquam//Kloka/Pitic62/3/Warimek/Halberd/4/3Ag3Aroona) developed by Tony Rathjen and the Waite Institute Wheat Breeding team. The DHs were generated by the South Australian Research and Development Institute (SARDI) doubled haploid and cell culture unit using the method of Howes et al. (2003). DNA was extracted from leaf material of 1- to 2-week-old seedlings according to Williams et al. (2002).

### Nematodes

*Pratylenchus thornei* was obtained from the SARDI culture (originally sourced from wheat at Nunjirkompita, South Australia) maintained on carrot callus as described by Nicol and Vanstone (1993). To collect the nematodes, carrot callus was placed in funnels in a misting chamber under intermittent aqueous mist of 10 s every 10 min for 96 h (Southey 1986) at room temperature (22 °C). Nematodes extracted were counted and diluted with water to the inoculum concentration of 3,000 nematodes per 1 mL.

### Phenotypic screening of *P. thornei* resistance

The resistance response of the 150 DH lines to *P. thornei* was assessed in a glasshouse maintained at 20–23 °C. One pre-germinated seed was sown per tube (55 × 120 mm) in 350 g of steam-pasteurized sand quarried near Taillem Bend, South Australia (Sloans Sand Pty Ltd, Dry Creek,

SA). Tubes were arranged in a random block design (5 × 5 blocks) and placed in trays that were flooded for 4 min every 3 days to a depth of 100 mm. One week after the seedling emerged it was inoculated with 1,500 nematodes per plant via two aliquots into two 50-mm-deep holes on either side of the seedling. Watering was suspended until 3 days after inoculation. Two days after inoculation a slow release fertilizer (Osmocote, Scotts Miracle-Gro, USA) was added (4 g/kg sand) and each tube was covered with plastic beads to reduce evaporation. Eight weeks after inoculation plants were removed and roots were washed free of sand and dried. DNA was extracted from roots and the amount of *P. thornei* reproduction within roots was assessed via DNA quantification through the SARDI Root Disease Testing Service (Haling et al. 2011; Ophel-Keller et al. 2008; Riley et al. 2010). The amount of *P. thornei* DNA was quantified using a real-time TaqMan PCR system with primers specific to the internal transcribed spacer (ITS) region of *P. thornei* (unpublished data). DNA was quantified using a standard curve that was established using known amounts of DNA. The final level of nematodes in the roots was expressed as picograms (pg) *P. thornei* DNA per plant. The controls included the susceptible wheat cultivar Machete, moderately susceptible cultivar Meering, and moderately resistant cultivar Chara. The trial included five replicates of each DH line and controls. The trial was independently replicated twice under the same experimental conditions 1 week apart in August/September 2006 and was conducted by SARDI Nematology staff. A natural log transformation was performed to restore normality. Differences between population individuals were assessed using one-way analysis of variance (ANOVA). Broad-sense heritability ( $H^2$ ) was estimated using the formula:  $H^2 = \text{Var}(G)/\text{Var}(P)$ , where  $\text{Var}(G)$  is the genetic variance for the DH population and  $\text{Var}(P)$  is the phenotypic variance.

### Map construction

Diversity Array Technology (DArT) markers were scored on the DH population by Diversity Array Technology Pty Ltd (Yarralumla, ACT, Australia) using wheat DArT array version 2.6 (5,000 markers) (Akbari et al. 2006). A total of 889 informative markers were identified and 849 were used to construct a genetic linkage map using MapManager QTXb20 (Manly and Olson 1999) with the Kosambi mapping function (Kosambi 1944). Marker order was finalized using RECORD (Van Os et al. 2005). Markers that showed significant segregation distortion from the expected 1:1 ratio, as determined by Chi-square ( $\chi^2$ ) test were excluded from QTL analysis. Following initial QTL detection, the QTL regions on chromosomes 2B and 6D were enriched for marker density using SSRs and SNPs.

SSR markers selected for known map location were assayed in a 9.5  $\mu$ L reaction mixture containing 0.2 mM dNTP, 1x PCR buffer, 1.5 mM MgCl<sub>2</sub>, 0.2  $\mu$ M each of forward and reverse primer, and 0.25 U Taq DNA polymerase (Qiagen). PCR cycling was performed using a touchdown profile comprising an initial denaturation step of 94 °C for 2 min, followed by a total of 37 cycles of 94 °C for 30 s, an annealing step for 30 s and 72 °C for 30 s. The initial annealing temperature was 59 °C and was reduced by 0.5 °C for each of the next 8 cycles. The remaining 29 cycles had an annealing temperature of 55 °C, the program ended with a 5-min extension at 72 °C. The SSR marker-amplified products were separated on 8 % non-denaturing polyacrylamide gels (Sigma Aldrich, Australia) at constant 300 V for 180 min, and visualized with ethidium bromide staining.

Gene-based markers assayed were specific for homoeologous copies of the vernalization gene *Vrn1* (Fu et al. 2005), the *P. neglectus* resistance gene *Rlnn1* [marker *uat0001*, Australian Wheat and Barley Molecular Marker Program (AWBMMP), University of Adelaide] and height genes *Rht1* and *Rht2* (Ellis et al. 2002).

SNP markers linked to the 2B and 6D QTL regions were identified using bulk segregant analysis (Michelmore et al. 1991) and a custom iSelect bead chip targeting 90,000 gene-associated wheat SNPs (Wang et al. 2013). DNA bulks were constructed for the presence or absence of the QTL: the bulk for the 6D resistance QTL contained 39 DH lines, the bulk for the 2B QTL contained 23 DH lines, and the contrasting bulk contained 20 DH lines carrying susceptible alleles at both the 2B and 6D QTL. Each bulk was prepared by pooling equal amounts of DNA from each DH line. The bulked DNA samples and Sokoll and Krichauff were assayed using the bead chip assay on the iScan instrument following the manufacturer's instructions (Illumina Ltd). Genotypic analysis was performed using GenomeStudio v2011.1 software (Illumina Ltd). The SNPs were assessed for linkage by comparing the normalized theta values for each sample (Hyten et al. 2009). Polymorphism was considered to be putatively linked when the normalized theta values for the resistant bulk and Sokoll, and susceptible bulk and Krichauff were similar. Linked SNPs were converted into KASP™ markers according to the manufacturer's manual (LGC Genomics, [www.lgcgenomics.com](http://www.lgcgenomics.com)) and as described by Trick et al. (2012), and mapped in the DH population. The KASP marker names and sequences as well as the 90 K SNP assay identifiers are provided in Supplementary Table 1.

Selected DArT markers that mapped to the 2B or 6D QTL region were converted to sequence-tagged site (STS) markers. Sequences obtained from Diversity Array Technology Pty Ltd for eight DArT markers were used

as BlastN queries against EST databases at NCBI (<http://blast.ncbi.nlm.nih.gov>). Resulting overlapping ESTs were assembled into larger contigs using the ContigExpress function implemented in VectorNTI 11 (Invitrogen, Victoria, Australia). PCR primers were designed to amplify the largest contig. Six of the converted DArT markers were polymorphic and were mapped in the Sokoll  $\times$  Krichauff population (Supplementary Table 2). STS markers were amplified as described above but using an initial denaturation step of 94 °C for 2 min, followed by 94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s. Amplicons were separated on 2 % agarose gels for 90 min at 100 V and visualized by ethidium bromide staining.

The marker allele distributions among the 150 DH lines were integrated into the DArT map using the Links report function in MapManager QTXb20. In conjunction with the ripple function and published maps, an order of markers was established and finalized using RECORD (Van Os et al. 2005).

#### Phenotypic modeling and whole genome QTL analysis

Using the complete linkage map, QTL analysis of the log-measured nematode traits was performed using the package whole genome average interval mapping (WGAIM) (Taylor and Verbyla 2011) implemented in the statistical computing environment R (R Development Core Team 2013). The package is a computational implementation of the whole genome interval mapping techniques derived in Verbyla et al. (2007, 2012) and uses ASReml-R (Butler et al. 2009) to perform all statistical modeling analysis. In this approach, an initial linear mixed model is developed that incorporates non-genetic and genetic sources of variation relevant for the nematode experiment conducted. The complete set of constructed inferred interval markers is then added to the linear mixed model as a contiguous block of random effects with a single variance parameter. This variance parameter is then formally tested and if found significant a QTL is selected using an outlier detection method. Once selected the QTL is removed from the contiguous block of inferred interval markers and placed as a single random covariate in the model. This process is then repeated until the variance parameter is found to be not significant. The final set of putative QTL appear additively as random covariates in the final model and are summarized using the methods of Verbyla et al. (2007, 2012). The summary includes flanking markers in the significant interval, the magnitude of the QTL effect, the logarithm of odds (LOD) score and the percentage contribution of the QTL to the genetic variance. QTL were considered significant when LOD  $\geq$  3 and suggestive when LOD < 3 but > 1.4.

## Field evaluation of selected DH lines for *P. thornei* resistance

Ten lines from each of the four genotypic groups [i.e., contained both resistance QTL (both 6D and all three 2B QTL), only 2B QTL, only 6D QTL, and neither QTL] were selected for field evaluation. The ten lines from each genotypic group were pooled and the resulting four bulked lines were screened for *P. thornei* resistance at a site on the Minnipa Research Centre in South Australia in 2011, along with 29 South Australian common wheat cultivars. The site was selected to primarily contain *P. thornei* with minimal numbers of other soil pathogens (nematodes and fungi). A large, split-plot trial design with 5 replicates was used. Cultivars were sown to pairs of adjacent plots within replicates. High and low *P. thornei* densities were established in 2010 in the two plots within each pair of plots through planting resistant field pea and susceptible Narbon bean cultivars. Total DNA was extracted from the soil (Haling et al. 2011; Ophel-Keller et al. 2008; Riley et al. 2010) and the amount of *P. thornei* DNA within roots was measured through the SARDI Root Disease Testing Service using a real-time TaqMan PCR system with *P. thornei* specific primers (unpublished data). For soil sampling, 20 cores from a depth of 10 cm (Taylor and Evans 1998) were collected from each plot. Initial nematode numbers (Pi) were recorded before sowing and the final number of nematodes (Pf) was recorded after harvest (6 months post initial sampling). A multi-environment trial (MET) linear mixed model analysis (Smith et al. 2001) of the log e-transformed Pf numbers was then undertaken. This analysis approach includes the multiplicative modeling of the site

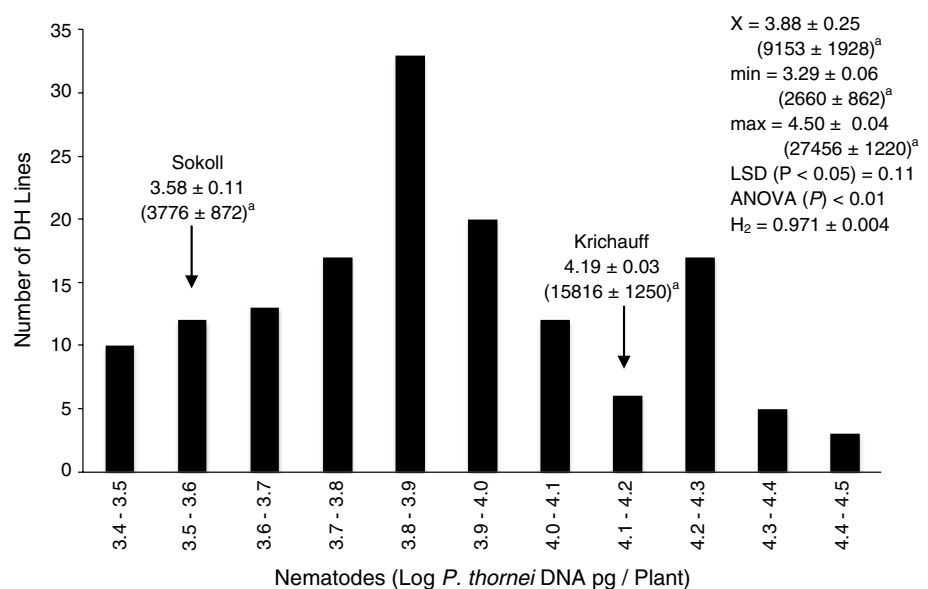
by treatment (high and low) by cultivar interaction effects (Smith et al. 2005), as well as accounts for non-genetic effects through the appropriate spatial modeling of the environmental variation possibly existing at each site. The log e-transformed Pi numbers for each treatment were also included as a covariate in the analysis. The Pf was predicted on the transformed scale and at the average Pi (avgPi) for each treatment which were 4.794 and 2.695 for high and low, respectively. Predicted multiplication rates for each treatment were derived by re-exponentiating the difference between the predicted Pf and avgPi for that treatment, that is, as  $\exp(\text{Pf} - \text{avgPi})$ . The MET data were analyzed using ASReml-R (Butler et al. 2009). The Sokoll  $\times$  Krichauff lines and the South Australian wheat cultivars were ranked according to their low and high plot multiplication rates.

## Results

### Phenotypic assessment

The final nematode-DNA quantifications showed a regression significantly skewed  $1.27 \pm 0.2013$  and thus were log transformed to ensure statistical modeling assumptions were adequately met. There was a high correlation ( $R^2 = 0.7$ ) between the phenotypic data of the two trials. The population displayed a continuous distribution with an average nematode count of 9,153 pg (log average = 3.88) *P. thornei* DNA/plant for the population and 3,766 pg (log mean = 3.58) and 15,816 pg (log mean = 4.19) for the resistant parent Sokoll and moderately susceptible parent Krichauff, respectively (Fig. 1). An ANOVA showed highly

**Fig. 1** Frequency distribution of *Pratylenchus thornei* DNA quantification data (average of trials 1 and 2) for the 150 doubled haploid lines of the Sokoll  $\times$  Krichauff wheat cross. The figure shows the log mean and standard error values of *P. thornei* DNA pg/plant in the parents, the population mean ( $X$ ), the minimum ( $\min$ ) and the maximum ( $\max$ ) values and standard error, least significant difference ( $LSD$ ), analysis of variance ( $ANOVA$ ) and the broad-sense heritability ( $H^2$ ). The numbers in brackets are the back-transformed *P. thornei* DNA pg/plant values



<sup>a</sup> Back-transformed values

significant differences between individual genotypes for *P. thornei* DNA pg/plant (average of trials 1 and 2). The broad-sense heritability ( $H^2$ ) was high (0.971), indicating that resistance was largely controlled by genetic variation.

#### Genetic map construction

The final Sokoll  $\times$  Krichauff map contained 849 DArT markers, 102 SSR and 14 SNP markers arranged across 24 linkage groups. Chromosomes 5B, 7A and 7D are represented by two linkage groups due to poor marker coverage across the chromosomes. The total map length was 3,463 centiMorgans (cM), with an average marker density of 3.6 cM. Segregation distortion from the expected 1:1 ratio was observed for 61 (6.3 %) of the mapped loci ( $P < 0.05$ ). Markers linked to traits that could be relevant to *P. thornei* resistance were integrated into the genetic map and included *Vrn1A*, *Vrn1B*, *Vrn1D* and the *P. neglectus* resistance gene *Rlnn1*. The height genes *Rht1* and *Rht2* did not segregate in this population. Neither *Rlnn1* on chromosome 7AL nor *Vrn1A* and *Vrn1B* on chromosomes 5A and 5B, respectively, were linked to *P. thornei* resistance. Nevertheless, the gene-specific marker *Vrn1D* was flanking resistance QTL *QRlnt.sk-5D*.

The coefficient of parentage between Sokoll and Krichauff is 0.056 using the International Crop Information System (ICIS) as described in Cane et al. (2013), indicating that they are only remotely related. Like almost all semi-dwarfs, Norin 10/Brevor Selection 14 is part of their pedigree and a very small contribution comes from Gabo.

#### Marker regression and QTL analysis

Significant QTL ( $\text{LOD} \geq 3$ ) were detected on three chromosomes and in all cases, except *QRlnt.sk-2B.3*, were inherited from the synthetic-derived parent Sokoll (Fig. 2; Table 1). Two suggestive QTL were identified on chromosomes 2A and 2D and both inherited from the moderately susceptible parent Krichauff. The QTL on chromosomes 2B and 6D explained the highest percentage of the genotypic variation and mapped to locations previously associated with *Pratylenchus* resistance (Table 2).

Two significant resistance QTL, *QRlnt.sk-6D.1* and *QRlnt.sk-6D.2*, were identified on chromosome 6DS. The *QRlnt.sk-6D.1* was flanked by SSR markers *barc183* and *cf135*, and *QRlnt.sk-6D.2* was flanked by converted DArT marker *DPT1519* and by SSR marker *cf49* (Fig. 2; Table 1). Together, the 6D QTL explained 47 % of the genotypic variation for *P. thornei* resistance.

Three significant QTL mapped to chromosome 2B. *QRlnt.sk-2B.1* was flanked by SSR marker *wmc382* and converted DArT marker *DPT3390*. *QRlnt.sk-2B.2* was flanked by SSR markers *barc297* and *gwm614*. A

suggestive QTL ( $\text{LOD} < 3$ ), *QRlnt.sk-2B.3*, was flanked by DArT markers *wPt-1646* and *wPt-9736* (Fig. 2; Table 1). The combined 2B QTL explained 32 % of the genotypic variation for *P. thornei* resistance.

Another significant QTL, *QRlnt.sk-5D*, was located on the long arm of chromosome 5D and was linked to the vernalization *Vrn1D* marker and SSR marker *gpw320*. This QTL explained 4.3 % of the genetic variation and had a large interval (35.03 cM). Two suggestive QTL were identified on chromosomes 2A and 2D (Fig. 2; Table 1) but only after iteration 7 (Supplementary Fig. 1).

*Rlnn1* marker *uat0001*, mapped to 7A, where the resistance locus to *P. neglectus* had originally been mapped (Williams et al. 2002). The *Rlnn1* QTL, donated by Krichauff, did not contribute to improved *P. thornei* resistance.

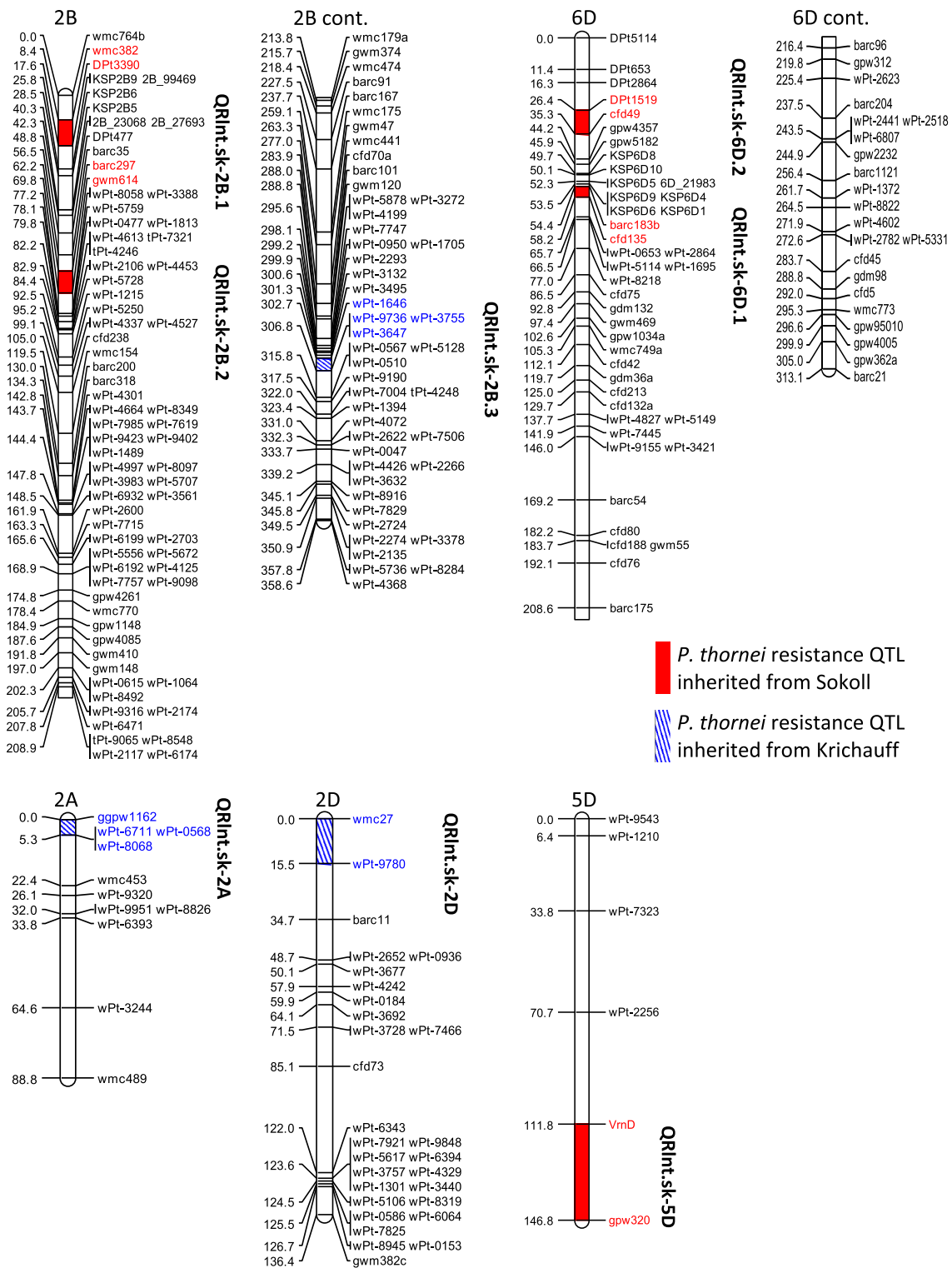
#### Field evaluation of selected DH lines for *P. thornei* resistance

All genotypes reduced *P. thornei* numbers below the initial nematode level in both, the plots with high and low nematode numbers. Lines with both resistance QTL supported the least amount of nematode reproduction followed by the lines with 6D and 2B QTL only (Table 3). All of these had significantly less *P. thornei* reproduction than lines with neither QTL. The lines with both QTL ranked 4th and 6th (out of 33 cultivars and lines tested) for the low and high plots, respectively, indicating they have high resistance levels in comparison to most of common South Australian cultivars.

#### Discussion

In this study, three of the QTL identified on chromosomes 2B and 6D co-located with genomic regions previously linked to *P. thornei* resistance in wheat. This finding further validates the robustness of these QTL as useful sources of resistance in different genetic backgrounds. In six out of the seven previous *P. thornei* resistance mapping studies both using Middle Eastern landraces and synthetic hexaploid wheats, significant resistance QTL were identified on chromosome 6D and in four studies also on chromosome 2B (Schmidt et al. 2005; Thompson et al. 1999; Zwart et al. 2005, 2006, 2010) demonstrating that these resistance loci appear to be widespread.

Through the comparison of common markers in published genetic maps, the 6DS QTL identified in this study, *QRlnt.sk-6D.1*, mapped to the same location identified by Zwart et al. (2005, 2006, 2010). *QRlnt.sk-6D.1* and the 6DS QTL identified by Zwart et al. (2006) in the synthetic hexaploid ITMI population, derived from a cross between the synthetic W-7984 and the spring wheat Opata 85, share



**Fig. 2** Genetic linkage groups of Sokoll × Krichauff doubled haploid wheat population showing QTL for *Pratylenchus thornei* resistance. The length of bars indicates the QTL position and the color

indicates donor parent. Map distances are shown in centiMorgans. STS and SNP markers are denoted by the prefix ‘DPl’ and ‘KSP’, respectively, and DaT markers by the prefix ‘tPt’ or ‘wPt’

**Table 1** Description of QTL associated with *Pratylenchus thornei* resistance in the Sokoll × Krichauff wheat population

QTL	Parental allele	Effect size	Chr	Left flanking marker	Right flanking marker	P value	% Var <sup>a</sup>	LOD <sup>b</sup>	QTL interval <sup>c</sup>
<i>QRInt.sk-2A</i>	Krichauff	0.055	2A	<i>gpw1162</i>	<i>wPt-7211</i> <i>wPt-0568</i>	0.01	1.2	1.45	5.34
<i>QRInt.sk-2B.1</i>	Sokoll	0.214	2B	<i>wmc382</i>	<i>DPt3390</i>	0.0001	17.8	10.56	9.13
<i>QRInt.sk-2B.2</i>	Sokoll	0.165	2B	<i>barc297</i>	<i>gwm614</i>	0.0001	10.6	6.13	7.59
<i>QRInt.sk-2B.3</i>	Krichauff	0.092	2B	<i>wPt-1646</i>	<i>wPt-9736</i> <i>wPt-3755</i> <i>wPt-3647</i>	0.0001	3.3	3.83	4.12
<i>QRInt.sk-2D</i>	Krichauff	0.059	2D	<i>wmc27</i>	<i>wPt-9780</i>	0.009	1.3	1.47	15.51
<i>QRInt.sk-5D</i>	Sokoll	0.105	5D	<i>VrmD</i>	<i>gpw320</i>	0.0001	4.3	3.44	35.03
<i>QRInt.sk-6D.2</i>	Sokoll	0.136	6D	<i>DPt1519</i>	<i>cf49</i>	0.0001	7.2	3.66	8.96
<i>QRInt.sk-6D.1</i>	Sokoll	0.320	6D	<i>barc183</i>	<i>cf135</i>	0.0001	39.5	20.38	3.81

<sup>a</sup> Genotypic variation

<sup>b</sup> Logarithm of odds

<sup>c</sup> centiMorgan

the same flanking SSR marker *barc183* (Zwart et al. 2006) (Tables 1, 2). The major *P. thornei* resistance QTL on 6DS identified by Zwart et al. (2005, 2010) in a synthetic hexaploid wheat population derived from a cross between CPI133872 and the susceptible Australian bread wheat cultivar Janz was also linked to *barc183*. In this cross, the resistance QTL to *P. thornei* was also associated with *P. neglectus* resistance but donated from the other parent, Janz (Tables 1, 2). Resistance assays in crushed root suspensions and root exudate from lines with the *QRInt.sk-6D.1* showed suppression of hatching and motility of *P. thornei*, however, no suppression was observed for *P. neglectus* (Linsell et al. 2014). These findings imply that the *P. neglectus* resistance allele or gene near *barc183* found in Janz (Zwart et al. 2005) is not present in Sokoll or Krichauff. A second *P. thornei* resistance QTL reported by Zwart et al. (2005) on the long arm of 6D that explained up to 14 % of the genotypic variation, was not detected in the Sokoll × Krichauff population.

The second significant 6D QTL identified in this study *QRInt.sk-6D.2*, mapped to the same location identified by Zwart et al. (2005, 2010). Flanking marker *cf49* was also reported to flank this 6D QTL in the synthetic CPI133872. SSR marker *cf49* also mapped closely to restriction fragment length polymorphism markers *psr964* and *psr889* (Somers et al. 2004), which were linked to the 6DS QTL identified by Zwart et al. (2006). Multiple alleles of the same gene or different genes clustered at the *QRInt.sk-6D* QTL locus may be associated with resistance in the different resistance sources.

Previous mapping studies identified *P. thornei* resistance QTL on the short arm of chromosome 2B from three different sources. *QRInt.sk-2B.2* identified in this study co-located with the 2BS QTL identified by Zwart et al.

(2006) when the locations of common markers were compared. In the synthetic W-7984 × Opata85 (ITMI) population, a 2BS QTL explaining 19 % of the genetic variation, enclosed restriction fragment length polymorphism marker *cdo447*, which in this investigation maps within 3 cM of the *QRInt.sk-2B.2* flanking marker *gwm614* (Appels 2003) (Tables 1, 2). Toktay et al. (2006) showed the presence of this 2BS QTL in a cross between the Middle Eastern synthetic AUS4930 and Pastor with linkage to *gwm614*. *QRInt.sk-2B.1* and *QRInt.sk-2B.3* identified in the present study do not co-locate to any previously mapped *Pratylenchus* resistance. Similarly, the QTL detected on chromosomes 2A, 2D and 5D have not been previously linked to *Pratylenchus* resistance. The identification of multiple QTL contributing to the resistance phenotype further confirms the complexity of *Pratylenchus* resistance.

All of the previously identified 6DS and 2BS QTL regions mapped by Zwart et al. (2005, 2006) and Schmidt et al. (2005) explained less than 25 % of the genotypic variation, whereas *QRInt.sk-6D.1*, identified here, alone explained over 40 % of the variation. In addition, the QTL widths of the previously mapped 6D and 2B QTL range between 10 and more than 100 cM, however, in this study the QTL span less than 9 cM. The increased percent of genotypic variations explained by the apparent same loci could be attributed to several genomic and phenotypic advancements utilized in this study. Firstly, a more efficient and sensitive phenotyping method for the quantification of nematodes in soil, in comparison to traditional extraction and manual counting methods, was utilized via DNA quantification (Hollaway et al. 2003). Real-time quantitative PCR assays measured the amount of *P. thornei* DNA of each line to serve as an estimate of its resistance level (Ophel-Keller et al. 2008). The QTL interval



**Table 2** Previously mapped *Pratylenchus* resistance QTL in wheat

Reference	Inherited origin	Chr/QTL	Spp.	Flanking markers	% Var <sup>a</sup>	LOD <sup>b</sup>	QTL interval <sup>c</sup>
Thompson et al. (1999)	GS50a	6D	<i>Pt</i>	np	np	np	
Williams et al. (2002)	Excalibur	7A	<i>Pn</i>	<i>AGC/CCT179</i> <i>cdo347</i> <i>psr121</i> <i>psr680</i> <i>schfc3</i>	8	4.5	
Zwart et al. (2005)	CPI133872	<i>QRlnt.lrc-6D.1</i>	<i>Pt</i>	<i>barc183</i> <i>barc173</i>	22–24	5.2–9.2	19
	Janz	<i>QRlnt.lrc-6D.1</i>	<i>Pn</i>	<i>barc183</i> <i>barc173</i>	11–14	3.8–3.9	19
	CPI133872	<i>QRlnt.lrc-2B.1<sup>d</sup></i>	<i>Pt</i>	<i>wmc25</i> <i>wmc154</i>	7	3.3	16
	CPI133872	<i>QRlnt.lrc-2B.1<sup>d</sup></i>	<i>Pn</i>	<i>wmc25</i> <i>wmc154</i>	7–11	2.0–2.7	16
	CPI133872	<i>QRlnt.lrc-6D.2<sup>d</sup></i>	<i>Pt</i>	<i>gdm98</i> <i>gpw95010</i> <i>barc21</i>	8–13	3.6–5.3	15
	CPI133872	<i>QRlnt.lrc-6A.1<sup>d</sup></i>	<i>Pt</i>	<i>psp3029</i> <i>gwm459</i>	9	2.4–2.5	51
	CPI133872	<i>QRlnt.lrc-3D.1<sup>d</sup></i>	<i>Pn</i>	<i>gwm161</i> <i>gwm183</i> <i>gwm664</i>	11	2.7	52
	CPI133872	<i>QRlnt.lrc-4B.1<sup>d</sup></i>	<i>Pn</i>	<i>gwm66</i> <i>wmc47</i>	10	3.7	6
	Janz	<i>QRlnt.lrc-4D.1</i>	<i>Pn</i>	<i>wmc52</i> <i>wmc331</i> <i>barc98</i>	10–15	2.5–3.4	13
Schmidt et al. (2005)	AUS13124	2B	<i>Pt</i>	<i>gwm319</i> <i>gwm494.2</i> <i>ACT/CTC.1</i> <i>gwm191.2</i>	6–13	2.0–2.9	66
	AUS13124	6D <sup>d</sup>	<i>Pt</i>	<i>gwm469</i> <i>gdm36</i> <i>gwm518</i> <i>gdm132</i>	4–6	1.1–2.0	181
	AUS13124	3B <sup>d</sup>	<i>Pt</i>	<i>gwm133</i> <i>gwm340.2</i>	7–24	1.1–4.4	38
	AUS13124	1B <sup>d</sup>	<i>Pt</i>	<i>gwm153.1</i> <i>gwm153.2</i>	3–6	0.7–0.8	7
	AUS4926	3B	<i>Pt</i>	<i>gwm112.2</i> <i>gwm66.1</i> <i>gwm213.2</i> <i>gwm133.1</i> <i>AGC/CAT.1</i>	36	2.2	173
	AUS4926	2B <sup>d</sup>	<i>Pt</i>	<i>wmc25.5</i> <i>wmc25.4</i> <i>gwm428</i>	2–3	0.6–0.7	36
	AUS4926	6D <sup>d</sup>	<i>Pt</i>	<i>gdm98.1</i> <i>gdm98.2</i> <i>gdm132</i>	2–2	0.5–0.6	38
Zwart et al. (2006)	W-7984	6DS	<i>Pt</i>	<i>psr964</i> <i>psr889</i> <i>barc183</i>	11–26	1.0–1.8	22
	W-7984	2BS	<i>Pt</i>	<i>cdo447</i> <i>bcd348</i> <i>gwm210</i>	19–5	0.4–1.6	n/a

**Table 2** continued

Reference	Inherited origin	Chr/QTL	Spp.	Flanking markers	% Var <sup>a</sup>	LOD <sup>b</sup>	QTL interval <sup>c</sup>
Zwart et al. (2010)	CPI133872	6DS	<i>Pt</i>	<i>barc183</i> <i>cf49</i> <i>cf135</i>	18–27	6.1–10.1	11
	Janz	6DS	<i>Pn</i>	<i>barc183</i> <i>cf49</i> <i>cf135</i>	9	2.3–3.9	11
	CPI133872	6DL	<i>Pt</i>	<i>gdm98</i> <i>gpw95010</i> <i>barc21</i>	6–15	2.4–5.1	14
	CPI133872	2B	<i>Pt</i>	<i>wPt-2410</i> <i>wPt-6706</i> <i>wPt-6311</i> <i>wPt-8737</i>	13–22	4.3–7.9	7
	CPI133872	2B	<i>Pn</i>	<i>wPt-2410</i> <i>wPt-6706</i> <i>wPt-6311</i> <i>wPt-8737</i>	11–16	2.7–6.0	16
	CPI133872	3D	<i>Pn</i>	<i>gwm2</i> <i>gwm664</i> <i>gwm314</i>	8	3.4	4
	CPI133872	4B	<i>Pn</i>	<i>gwm368</i> <i>gwm66</i> <i>wmc47</i>	13	5.5	5
	CPI133872	4D	<i>Pn</i>	<i>wPt-5809</i> <i>wPt-431</i>	13	5.3	6

*np* not provided, *Pt* *Pratylenchus thornei*, *Pn* *Pratylenchus neglectus*

<sup>a</sup> Genotypic variation

<sup>b</sup> Logarithm of odds

<sup>c</sup> centiMorgan

<sup>d</sup> below the significance threshold

**Table 3** *Pratylenchus thornei* resistance validation of the Sokoll × Krichauff lines from each of the four genotypic groups based on whether they contained both resistant QTL (both 6D and all three 2B QTL), only 2B QTL, only 6D QTL and neither QTL at a South Australian field trial at Minnipa

Genotype	Final Pt <sup>a</sup> high	Final Pt <sup>a</sup> low	Pt mrate <sup>b</sup> high	Pt mrate low	Rank <sup>c</sup> high	Rank <sup>c</sup> low
Sokoll × Krichauff QTL-2B+6D	3.549	2.014	0.288	0.506	4	6
Sokoll × Krichauff QTL-2B	3.622	2.272	0.31	0.655	7	9
Sokoll × Krichauff QTL-6D	3.83	2.356	0.382	0.712	11	11
Sokoll × Krichauff QTL-0	4.004	2.816	0.454	1.129	13	16

The final number of *P. thornei* (Pf) after harvest in plots with high and low initial nematode numbers was predicted on the log *e*-transformed scale and at the average initial nematode numbers (avgPi) for each treatment which were 4.794 and 2.695 for high and low, respectively. Predicted multiplication rates (ratio of final to initial levels) for plots with high and low initial nematode numbers were derived by re-exponentiating the difference between the predicted Pf and avgPi for that treatment, that is, as exp(Pf–avgPi). The resistance ranking in comparison to the 33 cultivars and lines tested are reported for plots with high and low initial nematode numbers

<sup>a</sup> *P. thornei* numbers after harvest (log *e*-transformed) in plots with high and low initial nematode numbers

<sup>b</sup> *P. thornei* multiplication rates in plots with high and low initial nematode numbers

<sup>c</sup> Resistance ranking of the lines in plots with high and low initial nematode numbers

was better resolved than previous studies due to targeted mapping of QTL regions but also due to increased map marker density. The Sokoll × Krichauff map has 2.5 times

more markers than maps analyzed by Zwart et al. (2005, 2006, 2010) and Schmidt et al. (2005) and was achieved through the high-throughput and highly polymorphic

Diversity Array Technology (Akbari et al. 2006; Jaccoud et al. 2001; Semagn et al. 2006) and targeted QTL regions mapping with SSR and custom iSelect bead chip targeting 90,000 gene-associated wheat SNPs (Wang et al. 2013). In addition, the genetic resistance was characterized using a linear mixed model extension of interval mapping, whole genome average interval mapping (WGAIM), to perform whole genome QTL analysis (Verbyla et al. 2007). The WGAIM approach allows the simultaneous modeling of non-genetic and genetic variation by incorporating the whole genome marker set into the linear mixed model (Taylor and Verbyla 2011). This approach outperforms the standard composite interval mapping techniques that were utilized in previous *P. thornei* mapping analyses by providing an increased power of QTL detection (Verbyla et al. 2007). However, despite these technologies that increase QTL resolution, the presence of different QTL alleles in the various crosses cannot be ruled out to explain the differences in size and genotypic variation explained by the previously mapped 6D and 2B QTL and the QTL identified in this study.

To date, all *P. thornei* resistance mapping studies have generated phenotypic data from glasshouse trials (Schmidt et al. 2005; Thompson et al. 1999; Toktay et al. 2006; Zwart et al. 2006, 2010) due to difficulties associated with field trials such as inconsistent nematode numbers across plots and other environmental implications such as the effects of other diseases and pests and soil type changes, which greatly influence the phenotypic assessments. In this study, field evaluations were used to phenotype a selected subset of the Sokoll  $\times$  Krichauff DH lines for resistance from each of the four genotypic groups based on whether they contained both resistant QTL (both 6D and all three 2B QTL), only 2B QTL, only 6D QTL and lines with neither resistance QTL. The mean amount of *P. thornei* DNA was quantified from the field soil using the SARDI Root Disease Testing Service. All tested lines reduced *P. thornei* numbers below the initial nematode level but lines with both resistance QTL supported the least amount of nematode reproduction followed by the lines with 6D and 2B QTL only and lastly neither resistance QTL. These results show that the resistance of Sokoll  $\times$  Krichauff observed in the glasshouse trials is upheld in the field, further validating the usefulness of the identified resistance QTL and associated markers (highlighted in Fig. 2 and Table 1) for use in MAS.

The DNA markers linked to the major 2B and 6D resistance QTL will provide an efficient way to select lines with enhanced resistance in wheat breeding programs and to pyramid different *P. thornei* resistance genes. These markers will also be useful for pre-screening wheat accessions in studies aimed at identifying new sources of resistance in different germplasm pools. MAS using the flanking

markers developed in this study for the 2B and 6D QTL will help to minimize the linkage drag for introgressing improved *P. thornei* resistance in new wheat cultivars.

In summary, six significant *P. thornei* resistance QTL were identified on chromosomes 2B, 5D and 6D. The QTL on chromosomes 2BS and 6DS, *QRInt.sk-6D.1-2* and *QRInt.sk-2B.2*, mapped to locations previously identified to be associated with *Pratylenchus* resistance (Schmidt et al. 2005; Zwart et al. 2005, 2006, 2010). Our study showed that improvements in DNA quantification of nematodes in roots and soil and or marker development and QTL analysis allowed for better characterization of the magnitude of QTL effects and QTL delinearization in comparison to previous studies representing a sound basis for future map-based cloning of the underlying resistance genes. The 2B and 6D QTL combined accounted for more than 75 % of the observed resistance under glasshouse conditions, and strong *Pratylenchus* resistance under field conditions.

**Author contribution** KL and KHO designed the research (project conception and development of overall research plan). KL, MR, ST and KHO conducted the research (hands-on conduct of the experiments and data collection). RD conducted the field evaluations. HW provided essential plant materials necessary for the research. MH and KF performed the SNP bead chip analysis. KL, JT and BG performed statistical analysis. KL and KHO wrote the paper and had primary responsibility for the final content.

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

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