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ORIGINAL PAPER

Identification of a stem rust resistance locus effective against Ug99 on wheat chromosome 7AL using a RAD-Seq approach

Vincent Pujol[1] · Kerrie L. Forrest[2] · Peng Zhang[3] · Matthew N. Rouse[4] · Matthew J. Hayden[2] · Li Huang[5] · Linda Tabe[1] · Evans Lagudah[1]

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Abstract

Key message **A locus of major effect for stem rust resistance, effective against Ug99 and possibly a target of a suppressor on chromosome arm 7DL in wheat cultivar Canthatch, was mapped to 7AL.**

Abstract Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is responsible for major production losses around the world. The development of resistant cultivars is an effective and environmentally friendly way to manage the disease, but outbreaks can occur when new pathogen races overcome the existing host resistance genes. Ug99 (race TTKSK) and related *Pgt* races are virulent to the majority of existing cultivars, which presents a potential threat to global wheat production. The hexaploid wheat cultivar Canthatch has long been known to carry a suppressor of stem rust resistance on chromosome arm 7DL. Multiple "non-suppressor" mutants of Canthatch are reported to have gained resistance to *Pgt* races, including Ug99 (TTKSK) and related races TTKST and TTTSK. To genetically map the suppressor locus, a mapping population was developed from a cross between the susceptible cultivar Columbus, thought to possess the suppressor, and Columbus-NS766, a resistant, near-isogenic line believed to contain a mutant non-suppressor allele introgressed from Canthatch. Genetic mapping using a 9K SNP genotyping assay and restriction site-associated DNA sequencing (RAD-Seq) on bulked segregants led to the identification of markers linked to a locus of stem rust resistance. Surprisingly, genomic sequence information revealed the markers to be located on 7AL instead of 7DL, indicating that the resistance phenotype was due to a new resistance locus, rather than the inactivated suppressor. We suggest that the 7AL locus of resistance is most likely suppressed by the 7DL suppressor.

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✉ Vincent Pujol
pujol@affrc.go.jp

[1] CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

[2] Department of Environment and Primary Industries, Agribio, Centre for AgriBioscience, Bundoora, VIC 3083, Australia

[3] Plant Breeding Institute, University of Sydney, Cobbitty, NSW 2570, Australia

[4] United States Department of Agriculture-Agricultural Research Service, Cereal Disease Laboratory and Department of Plant Pathology, University of Minnesota, St. Paul 55108, USA

[5] Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, USA

Abbreviations

7DL-Sup	Canthatch 7DL suppressor of stem rust resistance
Col	Columbus
CTH	Canthatch
CSS	Chromosome survey sequencing
KASP	Kompetitive Allele Specific PCR
NS	Non-suppressor
RAD-Seq	Restriction site-associated DNA sequencing
SNP	Single nucleotide polymorphism

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world by acreage (FAOSTAT 2013,

http://faostat.fao.org/). Wheat stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a devastating wheat disease that can quickly destroy an entire crop (Singh et al. 2012). A highly virulent pathotype of *Pgt*, Ug99 (race TTKSK), was first observed in Uganda in 1999 (Pretorius et al. 2000) and has now spread across wheat-growing regions ranging from South Africa to Iran (Wanyera et al. 2006; Nazari et al. 2009; Pretorius et al. 2010). It is estimated that 85–95 % of global wheat cultivars are susceptible to at least one of the races in the Ug99 race group (Singh et al. 2011).

Control of wheat stem rust mostly relies upon the deployment of resistant cultivars. Several resistance genes are effective against Ug99 (Singh et al. 2011), and two of them, *Sr33* and *Sr35*, were recently cloned (Periyannan et al. 2013; Saintenac et al. 2013). However, individual resistance genes can be overcome by new pathogen variants. For example, *Sr31* remained an effective resistance gene for decades, but was overcome by Ug99 (Pretorius et al. 2000). It is generally recognised that multiple sources of resistance should be deployed together to minimise the chance of new pathogen variants emerging that defeat valuable resistance genes. Hence the search for, and characterisation of, new sources of stem rust resistance remains an important feature of wheat improvement programs.

Numerous resistance loci have been described in wheat and related species. However, in some cases the resistance conferred by these genes is not expressed due to the presence of suppressors of resistance (Bai and Knott 1992; Knott et al. 2005). Characterising resistance suppressors is therefore important to prevent their transfer in breeding programs, and to reveal masked resistance genes. The Canthatch 7DL stem rust suppressor locus (7DL-Sup) was discovered more than 30 years ago by Kerber and Green (1980), who observed that the hexaploid wheat cultivar Canthatch (CTH) was susceptible to several *Pgt* races, whereas the extracted tetraploid wheat component derived from CTH, which does not possess the D genome, was resistant. A comparable resistance was also observed in CTH nullisomic 7D (CTH-N7D) and CTH ditelosomic 7DS (CTH-Dt7DS), which respectively lack the whole chromosome 7D or the long arm of 7D (Kerber and Green 1980; Kerber 1991). In contrast, the reaction of CTH ditelosomic 7DL (CTH-Dt7DL) was similar to that of CTH, indicating that a gene(s) located on the long arm of chromosome 7D in CTH suppresses the resistance conferred by other gene(s), most likely on the A or B genome (Kerber and Green 1980). Seventeen independent CTH non-suppressor (NS) mutants, which were resistant to the *Pgt* races to which CTH was susceptible, were developed using EMS mutation; a combination of genetic and cytogenetic analyses indicated that the mutations were located at the 7DL suppressor locus (Kerber 1991; Williams et al. 1992; Kerber and Aung 1995). The CTH 7DL-Sup locus is now of particular interest because the genes it inhibits were recently found to confer resistance against Ug99 (TTKSK) and related races TTKST and TTTSK at the seedling stage (Y. Jin, unpublished data).

Mapping the 7DL-Sup locus using progeny from a cross between CTH and the CTH non-suppressor EMS mutants would likely present difficulties for finding DNA polymorphism. To overcome this constraint, $F_{2:3}$ progeny from a cross between Columbus (Col) and Columbus NS mutant 766 (Col-NS766) were used to generate a mapping population. Like CTH, Col is closely related to Thatcher as it is a Neepawa backcross line (a Thatcher backcross derivative); Col was reported to possess 7DL-Sup (Kerber 1991). Col-NS766 was developed by backcrossing one of the CTH mutants (CTH-NS) into Col (BC_5F_4) to introgress the NS allele into the Col background (Kerber 1991).

In this study, we initially attempted to genetically map the 7DL-Sup in the Col × Col-NS766 population. Unexpectedly, in the course of genetic mapping it became clear that the resistance was not conferred by introgression of the NS allele on chromosome 7DL, but by a major resistance locus on chromosome 7AL. Consequently, we focused on the genetic mapping of the 7AL resistance locus.

Materials and methods

Plant materials

All Canthatch (CTH-K, CTH-NS1 and CTH-NS2) and Columbus (Col, Col-NS765 and Col-NS766) lines/mutants were provided by E. R. Kerber, Agriculture Canada Research Station in Winnipeg, Canada. CTH-K is a single seed derived and purified selection of CTH descent from which the NS EMS mutants originated (Kerber 1991). Col-NS765 and Col-NS766 are near-isogenic lines, derived from independent backcrosses (BC_5F_4) of each of the two CTH-NS mutants with the recurrent parent, Col. The mapping population (196 $F_{2:3}$ families) was developed from three F_1 plants derived from the cross Col × Col-NS766. The isolation of DNA from leaves was carried out as described in Lagudah et al. (1991).

Evaluation of seedling response to stem rust

Resistance to stem rust was evaluated with two *Pgt* races: culture 313 (pathotype 34-1,2,3,5,6,7 according to Australian nomenclature) from the Plant Breeding Institute, University of Sydney, Australia, and Ug99 (race TTKSK isolate 04KEN156/04). Culture 313 is avirulent on *Sr9e* and *Sr21*, but virulent on *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9b*, *Sr9g*, *Sr11*, *Sr15* and *Sr17*. For culture 313, at least two replications of

up to 15 F_3 plants for each $F_{2:3}$ family were scored at the Plant Breeding Institute. Seedlings were grown in a microclimate room (20–22 °C day/night) until the two-leaf stage (approximately 2 weeks). Inoculation was achieved by spraying the plants with urediniospores suspended in light mineral oil. Plants were dried for 30 min and incubated for 2 days in incubation cabinets (20–22 °C day/night, high humidity). Finally, plants were transferred to a microclimate room at lower temperature (18/17 °C day/night) until scoring (approximately 12 days). For race TTKSK, 10–20 F_3 plants for each $F_{2:3}$ family were scored twice, once in 2012 and once in 2013, at the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota, following the method detailed in Rouse et al. (2011). Infection types (ITs) were assessed using the stem rust scoring scale (Stakman et al. 1962; McIntosh et al. 1995). Chi-squared tests were used to evaluate the goodness of fit between observed and expected resistance segregation ratios. The evaluation of quantitative phenotypes was done using data from infection with Ug99 only; phenotypes of F_3 seedlings were divided into three categories, depending on the percentage of susceptibility: resistant (0 %), intermediate (50 %) and susceptible (100 %). The overall percentages of susceptibility (quantitative phenotype) of F_3 families was calculated using the mean of F_3 seedlings.

SNP genotyping

9K SNP genotyping assay

DNA samples of Col and Col-NS766 were genotyped for 8632 gene-based single nucleotide polymorphisms (SNPs) using the wheat iSelect 9K SNP genotyping array (Cavanagh et al. 2013), on the Illumina iScan instrument following the manufacturer's instructions. Genotypic analysis was performed using R v3 (http://www.R-project.org) and loci were considered present if signal intensity (R) $\geq$ 0.14. This threshold was obtained by evaluating the standard deviation of allele frequencies (theta) of four technical replicates of Col and Col-NS766, which were highly dissimilar in low R values. Polymorphism was found by comparing the normalised theta values or R values. Genotypes were considered significantly different if the confidence intervals (95 %) did not overlap and if there was a difference between the means of at least 0.05 (theta only) or 10 % of the max R mean (R only). SNP chromosomal locations were determined using the published maps for the 9K SNP genotyping assay (Cavanagh et al. 2013).

RAD-Seq on bulked segregants

Thirty-six homozygous resistant and 34 homozygous susceptible F_2 individuals (inferred from phenotyping of F_3 families) were selected to compose the two bulks. The genome complexity for sequencing of Col, Col-NS766 and the bulks was reduced through the construction of restriction site-associated DNA (RAD)-tag libraries (Baird et al. 2008). Briefly, genomic DNA was first digested using methylation-sensitive restriction enzymes *Aat*II or *Pst*I and an adapter (PE2), specific to the restriction site, was ligated to each end. DNA restriction fragments were then randomly sheared using a Covaris S220 Focused-ultrasonicator and new ends were ligated to another adapter (PE1). Fragments of about 450 bp (excluding adapters) were selected on 1.5 % agarose gels and purified using the QIAquick Gel Extraction Kit (Qiagen Pty Ltd, VIC, Australia). Libraries were amplified using PCR with primers specific to each adapter, and then size distributions were assessed on an Agilent 2100 Bioanalyzer before pooling to a final concentration of 20 nM. The library pool was quantified using the KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, MA) and sequenced on the Illumina HiSeq 2000 instrument to generate paired-end 100 bp reads. The sequence reads were quality filtered using custom perl scripts. The PE2 reads (corresponding to the restriction site ends) were adapter trimmed to remove an internal barcode introduced during library preparation, which reduced their length to 95 bp (*Aat*II) and 97 bp (*Pst*I). Reads over 50 bp were aligned against all flow-sorted chromosome survey sequence (CSS) contigs of cultivar Chinese Spring (The International Wheat Genome Sequencing Consortium (IWGSC) et al. 2014) using BWA v0.5.9-r16 (Li and Durbin 2009) with two mismatches allowed. Pileup files were generated using SAMtools v0.1.14 (Li et al. 2009) and SNPs were discovered using custom perl scripts. Alignment of at least seven reads at a sequence variant position was required to call a genotype, and SNP genotypes were deemed to be homozygous if they had an alternate allele frequency <5 %. SNPs were considered putatively linked to the resistance locus if they were polymorphic between the bulks and with expected genotype phasing with Col and Col-NS766.

Genetic mapping

PCR-based SNP markers were developed using reference sequences of the 9K SNP assay and CSS contigs. Primers were designed for the Kompetitive Allele Specific PCR (KASP) genotyping assays, and markers were mapped following the manufacturer's protocol (KBioScience, Hoddesdon, UK). Following PCR, end-point fluorescence was measured using a BioRad CFX96 real-time instrument and genotypes were determined using CFX Manager v3.2. Genetic mapping and QTL analysis were performed using the software package QTL IciMapping v3.2 (Wang et al. 2012). Distances were calculated using the Kosambi

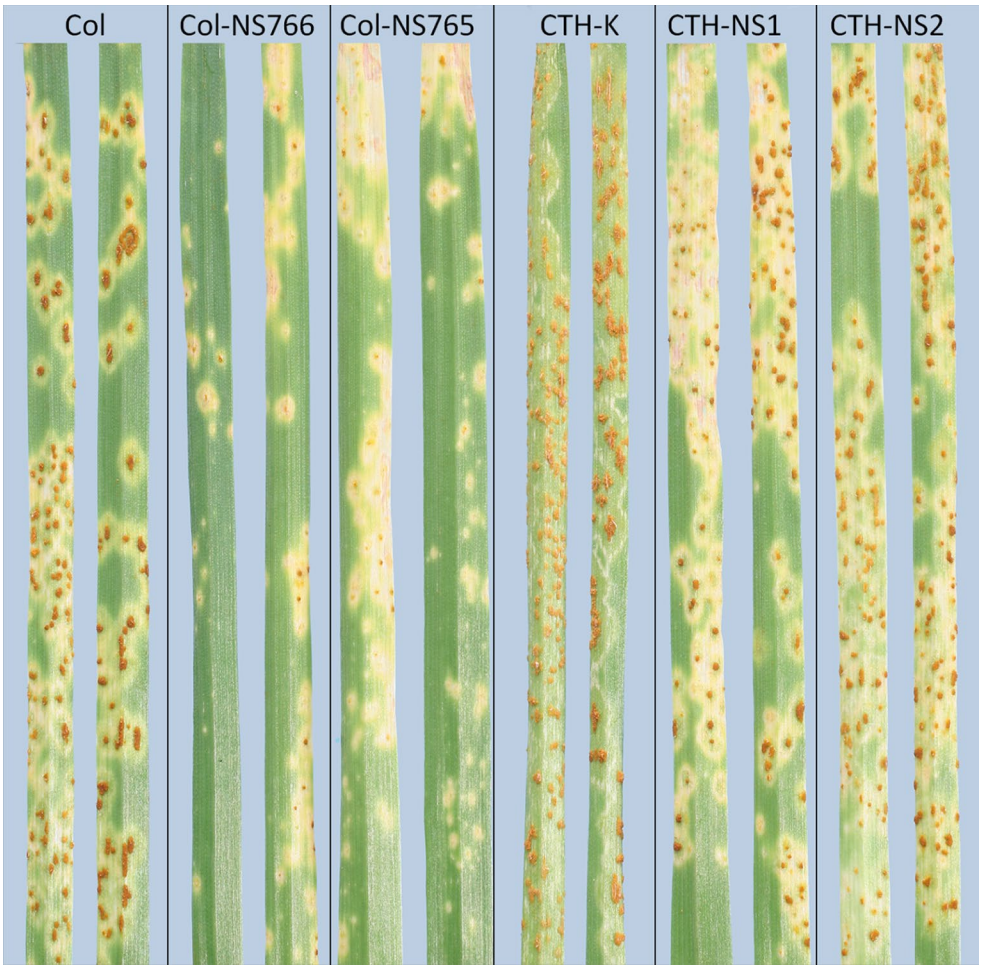

Fig. 1 Phenotypes of parental lines and Canthatch derivatives inoculated with *Pgt* culture 313 on seedlings, 15 days post-inoculation on seedlings. Col was moderately susceptible ($2^{+}3$), Col-NS766 and Col-NS765 were resistant (;1), CTH-K was susceptible ($3^{+}4$) and CTH-NS1 and CTH-NS2 were resistant (22^{+})

mapping function (Kosambi 1943) and LOD scores were calculated using the interval mapping for additive QTL (IM-ADD) method for bi-parental populations.

Results

Inheritance of resistance against stem rust

Resistance against *Pgt* was assessed using two distinct races: (1) culture 313, tested at the University of Sydney, Australia, and (2) Ug99 (race TTKSK), tested at the USDA-ARS, Minnesota, USA. Because the use of Ug99 is prohibited in Australia, culture 313 was used as a surrogate race. Col was moderately susceptible with an infection type (IT) of $2^{+}3$ when infected with rust culture 313 and IT of 3 with Ug99, whereas Col-NS766 and Col-NS765 were resistant (IT ;1 [culture 313 and Ug99]) (Fig. 1). In the case of the CTH lines, CTH-K was susceptible (IT $3^{+}4$ [culture 313]), whereas CTH-NS1 and CTH-NS2 were scored as resistant (IT 22^{+} [culture 313]). F_3 plants from the cross between Col and Col-NS766 exhibited phenotypes similar to those of the two parents, as well as a range of intermediate phenotypes. Phenotypic observations with both *Pgt* races were similar for most F_3 families, indicating that the genetic basis of the resistance conferred against Ug99 and culture 313 races was common to both, and that culture 313 could be used as a surrogate for Ug99. The few instances where phenotypes were different between races were considered to be due to the potential environmental conditions, notably temperature, which were observed to greatly affect the infection type of the stem rust resistance, independently of the *Pgt* races used.

Using the results from both races, 159 $F_{2:3}$ families were confidently scored, of which 33 (21 %) were resistant, 86 (54 %) segregating and 40 (25 %) susceptible. These observations fitted a single-gene segregation model ($\chi^2_{1:2:1} = 1.679$, $df = 2$, $P = 0.4319$). However, variations

in the infection types of individual F_3 plants were observed from families scored as homozygous susceptible or resistant. Furthermore, segregation ratios in F_3 families scored as segregating for resistance greatly varied between each other (based on infection with Ug99 only). When pooling all F_3 phenotypes from segregating $F_{2:3}$ families, more were susceptible (1277) than resistant (860) or intermediate (776). Statistical analyses using individual F_3 phenotypes were not performed, as the distinction of the intermediate phenotype from resistance or susceptibility was often difficult, which could bias the results. Thus, while a single locus of major effect was inferred from the broad categories of phenotypic classes used in our assessment, the complexity of the intermediate phenotype categories suggests additional genes of minor effect are present in the population. It is also possible that some phenotypic variations were due to varying environmental conditions.

Identification of SNP markers using the 9K SNP genotyping assay

To discover DNA sequence polymorphism between Col and Col-NS766, the 9K SNP genotyping assay was performed with DNA from both genotypes. Of the 8632 SNPs, 247 were found to be polymorphic between Col and Col-NS766. Of these, 87 % were most likely due to nucleotide variation rather than presence-absence. While it was expected that the majority of the 247 SNPs would be located on chromosome 7D, only 1 % were genetically mapped to this chromosome (Cavanagh et al. 2013), suggesting this region was not associated with stem rust resistance. More than half (55 %) of the polymorphic SNPs were genetically mapped on chromosome 6A, while 13 % were mapped on chromosome 7A (Cavanagh et al. 2013).These results suggested the co-introgression of genomic fragments on chromosomes 6A and 7A from CTH into Col-NS766 over five generations of backcrossing.

KASP markers for 23 of these SNPs were developed and genotyped in the Col × Col-NS766 $F_{2:3}$ mapping population. Markers were first genotyped on a subset of 45 F_2 plants that were homozygous for either resistance or susceptibility to both *Pgt* races. No association was found between phenotype and the markers mapped by Cavanagh et al. (2013) on chromosome 7D (IWA304) or 6A (IWA3527, IWA6247). However, 13 of the markers mapped by Cavanagh et al. (2013) on chromosome 7A showed association with the phenotype (Supplementary File 1, "Population subset"). Those with the strongest association were tested on an additional set of 109 F_2 plants. The most closely linked (IWA3371 and IWA4887) were found to co-segregate with each other, but were separated from the rust phenotype by 8.3 % of recombination (Fig. 2). To further refine the interval, additional markers (IWA737, IWA794 and IWA866) were mapped on additional F_2 plants (Fig. 2).

Identification of SNPs linked to the 7AL locus using RAD-Seq on bulked segregants

To increase mapping resolution around the 7A locus, bulked segregant analysis, an efficient mapping technique for rapidly identifying genomic regions affecting a trait of interest (Michelmore et al. 1991), was performed using RAD-Seq. Two bulked segregants from the Col × Col-NS766 mapping population were generated for resistance and susceptibility. For each bulked segregant and parental line, two RAD-tag libraries were constructed using either the methylation-sensitive restriction enzyme *Aat*II or *Pst*I. These enzymes were selected to focus the sequence analysis on hypo-methylated, gene-rich regions, an approach ideal for species with complex genomes such as wheat. Sequencing of the eight libraries on an Illumina HiSeq 2000 (100 bp paired-end reads) generated an average of 37 million paired-end reads per library after quality trimming and debarcoding.

Reads were aligned against the flow-sorted CSS contigs of cultivar Chinese Spring (CS), yielding an average of 79 % read alignment to about 1.4 million contigs per library. An average of 865,000 SNPs was discovered per library, of which 378 (from *Aat*II libraries) and 588 (from *Pst*I libraries) were putatively associated with the stem rust resistance phenotype. These SNPs were contained in 242 and 318 CSS contigs, respectively. 367 (71 %) of the 518 unique contigs were from chromosome arm 7AL, while four were from 7DL and the remainder were evenly spread throughout the genome.

Of the 966 SNPs associated with stem rust resistance, 32 were selected for genetic mapping using KASP assays based on several criteria (predominantly on 7AL, one SNP per contig, contigs longer than 5 kbp, no repetitive regions and syntenic with rice and *Brachypodium*). The markers were first tested on a subset of 45 F_2 plants that were homozygous for either resistance or susceptibility to stem rust to ascertain which were more strongly associated with the phenotype, before being tested on the entire population. Of the 32 markers, 28 gave scorable genotypes, of which 26 were associated with contigs from 7AL and showed close linkage with the phenotype in the subset of 45 homozygous individuals (Supplementary File 1, "Population subset").

To confirm that the linked SNPs were located on chromosome 7AL, each marker was physically mapped using CS and three CS nullisomic-tetrasomic lines, each missing one pair of the group 7 chromosomes: CS-N7AT7B, CS-N7BT7A and CS-N7DT7B (Sears 1966). Twenty RAD-Seq and three 9K SNP assay SNP markers, which showed linkage with the stem rust phenotype, were tested on the

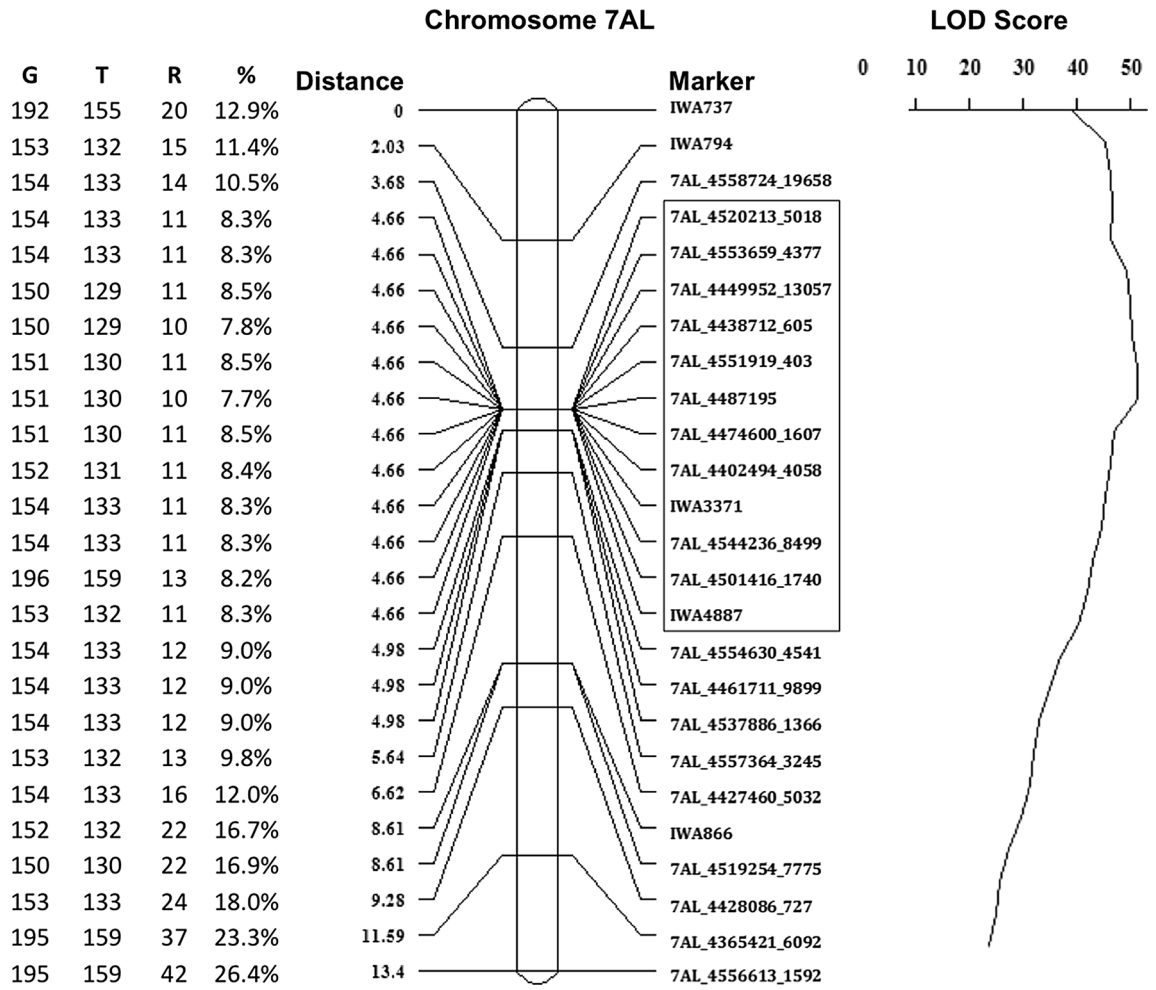

Fig. 2 Mapping of 7AL locus of stem rust resistance. Relative distances to marker IWA737 are in cM according to Kosambi mapping function. The cluster of co-segregating markers, enclosed in the *rectangle*, coincides with the LOD score peak and indicates the most likely position of the 7AL locus of stem rust resistance. *Numbers on the left panel* represent the number of genotyped F_2 individuals (*G*), the number of genotyped F_2 individuals with qualitative phenotypes from culture 313 and Ug99 (*T*), and the number and percentage of recombinants between genotype and phenotype (*R* and %, respectively)

nullisomic-tetrasomic lines. Twenty-one markers were unambiguously assigned to chromosome 7A (Supplementary File 1, "Physical").This result confirmed that the rust resistance locus was located on 7A in the Col × Col-NS766 population, and did not correspond to the 7DL-Sup locus.

Genetic mapping

A 13.4 cM genetic map around the 7AL resistance locus was constructed for markers linked to the phenotype (Fig. 2), including 5 from the 9K SNP assay and 20 from RAD-Seq. Twelve co-segregating markers showed the closest linkage with the phenotype, with less than 9 % recombinants. This genetic map is inconsistent with the observation of at least 11 $F_{2:3}$ families showing recombination between the phenotype and all markers, and points to other loci with an effect on the phenotype. In nine of the "non-conforming" families, the families were homozygous for the marker allele inherited from the resistant parent, yet the phenotype was scored as segregating (Supplementary File 1, "Recombinants"). In the remaining four "non-conforming" families, marker alleles were inherited from both parents (heterozygous), but the corresponding phenotypes were susceptible. To confirm the accuracy of the results obtained from these recombining families, 12 additional F_3 plants from each family were individually phenotyped with culture 313 and genotyped with marker 7AL_4501416_1740. Results confirmed that the genotypes and phenotypes shown for these families were correct (data not shown).

Because the "non-conforming" recombinant families showed the same allele origin for all markers

(Supplementary File 1, "Recombinants"), the 7AL resistance locus could not be positioned on the genetic map using a single-gene segregation model. These unexpected results are a further indication that more than one gene for resistance was segregating in the $F_{2:3}$ population. For this reason, QTL analysis was performed using the overall percentage of susceptibility of F_3 families infected with Ug99. Phenotypic data from infection with culture 313 were not included as infection types from individual F_3 plants were not recorded, thus precluding any quantitative analysis. Most markers on 7AL showed an LOD score greater than 30, and an LOD score peak of 50 was observed for the 12 co-segregating markers (Fig. 2). This result indicated that the major effect locus on 7AL locus of stem rust resistance was most likely co-segregating with this cluster of markers in the Col × Col-NS766 $F_{2:3}$ mapping population. LOD scores for markers on 6A were nearly 0 (data not shown).

Discussion

A locus of stem rust resistance on chromosome arm 7AL

The initial aim of this work was to map a suppressor of stem rust resistance on chromosome 7DL using a mapping population derived from a cross between near-isogenic genotypes segregating for wild-type or mutant NS alleles of the gene. To compensate for the expected low level of polymorphism between the parents, new genotyping methods were employed to develop markers linked to the phenotype. Unexpectedly, results from the 9K SNP assay and RAD-Seq of bulked segregants clearly indicated that the main segregating stem rust resistance locus in the Col × Col-NS766 population was on 7AL, rather than on 7DL. Tests on nullisomic–tetrasomic stocks confirmed this result. This unexpected observation emphasised the power of these methods for finding linked markers, even without preliminary mapping knowledge.

Col-NS766 was developed through backcrossing one of the original two CTH-NS mutants with Col (Kerber 1991). It is not known whether CTH-NS1 or CTH-NS2 was the parent of Col-NS765 or Col-NS766. As expected, Col-NS766, CTH-K, CTH-NS1 and CTH-NS2 all showed the same allele for all the markers shown in Fig. 2 (data not shown), confirming that the 7AL locus in Col-NS766 was derived from the CTH background. Furthermore, tests of eight markers, including three from the cluster of co-segregating markers (most closely linked) in Fig. 2, suggested that the 7AL locus in Col-NS766 and CTH originated from Thatcher, as all loci showed the same allele in all three wheat genotypes (data not shown). Moreover, the independent backcrossed line, Col-NS765, contained the same marker alleles as Col-NS766 and CTH, indicating that the same 7AL locus was introgressed into Col-NS765 from the independent CTH mutant used in this cross. Thus in two independent cases, the same 7AL locus was co-selected with the stem rust resistance through five generations of backcrossing to Col, suggesting that this 7AL locus is responsible for resistance against stem rust.

Two hypotheses could explain the mapping of the resistance locus to chromosome 7A. Firstly, the 7DL-Sup might actually be located on 7AL, and the resistance in Col-NS766 (and Col-NS765) is due to the introgression of NS alleles on 7AL from the CTH-NS mutants. However, this hypothesis is inconsistent with the published evidence for the location of the original CTH suppressor in numerous independent CTH-NS mutants to chromosome 7DL (Kerber 1983, 1991; Williams et al. 1992; Kerber and Aung 1995). Alternatively, the mapping data presented here could indicate the introgression of a previously unrecognised stem rust resistance locus on 7AL from CTH into Col. The expression of resistance in CTH-NS, but not in CTH, suggests that the locus is usually suppressed by the 7DL-Sup in the wild-type CTH. The expression of the stem rust resistance from the CTH-NS mutants only after introgression into the Col genetic background suggests that Col lacks both the 7DL-Sup and the 7AL resistance locus. This would be surprising since, like CTH, Col is closely related to Thatcher, which is thought to be the source of the 7DL-Sup (Kerber 1991). Furthermore, it was reported that 7DL-Sup is not uncommon in wheat and may originate from *Aegilops tauschii* (Kerber 1983). However, the original paper describing the presence of the 7DL-Sup in Columbus (Kerber 1991) lacks the appropriate experiment to confirm this assumption. The presence of the 7DL-Sup in Col (stem rust susceptible) could be tested by crossing Col with CTH nullisomic 7D (stem rust resistant), in a similar approach to that initially used by Kerber (1983) to demonstrate the presence of the 7DL-Sup in several wheat cultivars. If the 7DL-Sup is present in Col, full resistance should not be observed in the progeny that inherits one copy of the Col chromosome 7DL.

Two stem rust resistance genes, *Sr15* and *Sr22*, are known to be located on chromosome arm 7AL (Watson and Luig 1966; Kerber and Dyck 1973). *Sr15* has been reported to be ineffective against both Ug99 and stem rust culture 313 (Jin et al. 2007); thus the resistance we report cannot be attributed to *Sr15*. *Sr22*, which was introgressed into bread wheat from diploid wheat *T. monococcum* (Kerber and Dyck 1973) is effective against Ug99 (Jin et al. 2007). However, the marker csIH81 (Periyannan et al. 2011), which is diagnostic for the *T. monococcum* introgression carrying *Sr22*, was absent in CTH, Col and Col-NS766 (data not shown), excluding the involvement of *Sr22* in the resistance reported here. However, we cannot exclude the

possibility that the resistance mapped in this study is allelic to one of these genes.

The mapping of a stem rust resistance locus to 7AL raises the possibility that the gene conferring resistance on 7AL could be a homoeologous allele of the 7DL-Sup. This type of homoeologous gene suppression has recently been demonstrated in the case of *Pm8*, which confers resistance to powdery mildew in rye. *Pm8* was introgressed into wheat from rye chromosome 1RS, replacing a segment of homoeologous chromosome 1BS, and was found to be suppressed by the orthologous wheat gene *Pm3* on chromosome 1AS (McIntosh et al. 2011; Hurni et al. 2013). If this is the mechanism of suppression for the 7DL-Sup, cloning the 7AL resistance gene may help to isolate the 7DL-Sup.

Multigenic resistance

Although phenotypic data pointed to the segregation of a gene of major effect in the Col × Col-NS766 mapping population, phenotypic anomalies, the presence of a large introgression on chromosome 6A in Col-NS766, and the inability to map the 7AL locus as a single locus, strongly suggest that the 7AL locus is necessary, as it explained most (>90 %) of the phenotypic variation, but not sufficient for full expression of the resistance to stem rust; that is, the resistance is not conferred by a single gene. This would not be surprising as Williams et al. (1992) suggested that the 7DL-Sup might inhibit the expression of three or more genes for resistance in CTH. Similarly, a recent study identified at least three loci conferring adult plant resistance in Thatcher, which is the parent and supposed stem rust resistance donor to CTH (Rouse et al. 2014). Suppressors of the resistance other than the 7DL-Sup could also mask the resistance provided by the 7AL locus.

The introgressed segment from chromosome 6A of CTH in Col-NS766 suggests that one or several genes on this segment may also be involved in resistance. Providing further support for this hypothesis, the three markers on 6A (6AL_5832880_5088, IWA3527 and IWA6247) showed the same alleles in Col-NS766 and CTH, which is consistent with the presence of an introgression from CTH, as well as in Col-NS765 (data not shown).Because both Col-NS765 and Col-NS766 were descended from five backcrosses (BC_5F_4) of two independent CTH mutants into Col, any shared introgressed regions between these two lines strongly suggests they were actively selected. This is consistent with the possibility of a functional connection between regions on 6A and the stem rust resistance phenotype. However, we did not find any statistical evidence for an association between the markers on 6A and the resistance in our mapping population. Nevertheless, as the 7AL locus seemed necessary for resistance and explained most of the phenotypic variation, it is expected that the effects of other genes on the resistance would be small and thus might be difficult to detect in this mapping population. New mapping populations in which the 7AL locus is fixed could help in mapping these genes and understanding their role in resistance.

To conclude, this study led to the discovery of a new major locus on 7AL for wheat stem rust resistance effective against Ug99. We infer that this locus is most likely part of the resistance complex suppressed by the CTH suppressor of stem rust resistance on chromosome 7DL. Molecular markers that identify the 7AL stem rust resistance locus will be useful for further analysis of the trait and may be directly applicable in breeding varieties resistant to Ug99.

Author contribution statement VP was involved in all aspects of this study. KLF assisted in the bioinformatics analysis of RAD-Seq. PZ coordinated phenotyping with *Pgt* culture 313. MNR completed phenotyping with Ug99. MJH contributed to data analysis of 9K SNP assay and RAD-Seq. LT, LH and EL supervised this study.

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

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