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# Genomic and genetic analysis of the wheat race-specific yellow rust resistance gene *Yr5*

Graham R.D. McGrann<sup>1,2</sup>, Philip H. Smith<sup>3</sup>, Christopher Burt<sup>2</sup>, Gabriel Rodriguez Mateos<sup>2</sup>, Turnbull N. Chama<sup>2</sup>, Ruth MacCormack<sup>2</sup>, Elsabet Wessels<sup>4</sup>, Gloudi Agenbag<sup>4</sup> and Lesley A. Boyd<sup>5,6\*</sup>

\*Correspondence: [lesley.boyd@niab.com](mailto:lesley.boyd@niab.com)



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<sup>1</sup>Crop Protection Team, Crop and Soil Systems Group, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK.

<sup>2</sup>Department of Crop Genetics, John Innes Centre, Norwich, NR4 7UH, UK.

<sup>3</sup>Teacher Scientist Network, John Innes Centre, Norwich, NR4 7UH, UK.

<sup>4</sup>CenGen (Pty) Ltd, 78 Fairbairn Street, Worcester, 6850, South Africa.

<sup>5</sup>National Institute for Agricultural Botany, Huntingdon Road, Cambridge, CB3 0LE, UK.

<sup>6</sup>Department of Life and Medical Sciences, University of Hertfordshire, Hatfield, Hertfordshire, AL10 9AB, UK.

## Abstract

Yellow rust, caused by the biotrophic fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a significant foliar disease of wheat (*Triticum aestivum*) and a major target for resistance breeding world-wide. The wheat race-specific yellow rust resistance (R)-gene *Yr5* is a valuable source of resistance, still being effective against the pathogen in most wheat growing regions of the world. Through comparative genomic analyses, differential gene transcription and mutation studies, new genetic and genomic insights into this valuable resistance gene are provided. Co-locating DNA markers have been identified that have the potential to provide informative signatures for marker-based *Yr5* introgression. Utilising the synteny between wheat and other cereal and grass species for which whole genome sequences are available, primer sets were developed for wheat sequences which co-segregate with *Yr5* resistance, located on the long arm of chromosome 2B. Nucleotide and amino acid sequence searches aligned these wheat sequences with both the 5' and 3' ends of rice loci which showed significant homology to NB-LRR type R-genes. These rice loci were located on chromosomes 4 and 7, the rice chromosomes which are syntenic with the wheat group 2 chromosomes. Mutation analysis of the *Yr5* locus indicated that loss of the gene had significant effects on the transcription of a number of genes involved in diverse cellular processes, this differential transcription being independent of the presence of the yellow rust pathogen *Pst*. Mutation analysis also identified loci, unlinked to *Yr5*, that were required for *Yr5*-mediated resistance.

**Keywords:** Wheat, disease resistance, genetics, DNA markers, *Triticum aestivum*, yellow rust, *Yr5* R-gene, mutation, resistance breeding, fungal pathogens, stripe rust, genomics, transcriptomics, *Puccinia striiformis*, cereal synteny

## Introduction

Yellow rust is a significant foliar disease of wheat and a major target for resistance breeding world-wide [1]. The recent discovery of a sexual cycle for *Pst* [2] and the emergence of new, more aggressive races [1] indicate that the impact of this disease on global wheat production will continue to increase. *Yr5*, a yellow rust race-specific R-gene effective at both seedling and adult plant growth stages was first identified in a *T. aestivum* ssp. *spelta* var. *album* accession [3]. Monosomic analysis placed the *Yr5* gene on the long arm of chromosome 2B [4], being allelic to *Yr7*, an R-gene introgressed into hexaploid wheat from the tetraploid wheat *T. turgidum* ssp. *durum* [5]. *Yr5* has not been extensively used in wheat breeding programmes and consequently is still potentially effective against *Pst* on many continents [1].

Previous studies identified two *Yr5*-linked AFLP markers which showed significant sequence similarity to rice homologues of the bacterial blight NB-LRR R-gene *Xa-1* [6]. Despite overlap in the nucleotide sequence genetic mapping indicated distinct

loci, one AFLP marker co-segregating with *Yr5* while the other mapped at a distance of 0.7 cM from the *Yr5* gene. This would suggest that *Yr5* lies within a region of the wheat genome enriched for NB-LRR-type R-gene analogs. A number of resistance genes effective against fungal pathogens have been isolated from hexaploid wheat, including the leaf rust R-genes *Lr1* [7], *Lr10* [8] and *Lr21* [9], the yellow rust R-gene *Yr10* [10], the stem rust R-genes *Sr33* and *Sr35* [11,12] and the powdery mildew R-gene *Pm3b* [13]. All belong to the NB-LRR class of R-genes. Non-race-specific resistance genes cloned from wheat include the *Lr34/Yr18/Pm38* locus which confers durable resistance against leaf rust, yellow rust and powdery mildew and the yellow rust resistance gene *Yr36*. Neither encodes a NB-LRR protein, *Lr34/Yr18/Pm38* encoding an ATP-binding cassette (ABC)-transporter [14], whereas *Yr36* encodes a START-kinase type gene [15].

Numerous genomic and functional genetic tools are now available for cereals. The public release of whole genome sequences of cereals and related grass species has enabled

exploitation of the syntenic relationships between grass species. The co-linearity between wheat and the small genomes of rice (*Oryza sativa*) and the model grass *Brachypodium distachyon* has been successfully used to identify and develop markers linked to genes of interest, aiding in the fine mapping of the powdery mildew resistance gene *Pm6* [16] and *Pch1* for resistance to eyespot [17]. High-density oligonucleotide arrays are now available for many crop species allowing the detection of thousands of polymorphisms in a high-throughput and cost effective manner [18]. Most of these array-based technologies use genomic DNA to identify single nucleotide polymorphisms (SNPs), but transcription profiling of RNA levels can be used to detect expression level polymorphisms (ELPs) and single-feature polymorphisms (SFPs) between genotypes. The 55K Affymetrix Wheat1 GeneChip was used to identify 118 SFP and 91 ELPs between two near-isogenic lines differing at the *Yr5* locus [19]. Microarray technology has also been used for gene discovery, using differences in transcript levels between wild-type and mutant plants to identify candidate transcripts for the gene of interest [20,21].

Here we describe a genomic and genetic analysis of wheat yellow rust resistance conferred by the R-gene *Yr5*. These studies were undertaken to develop tools and resources to understand the functionality of *Yr5* and eventually clone this R-gene. A comparative genomic analysis, which utilised the synteny between wheat and cereal and grass species for which whole genome sequences are available, lead to the identification of NB-LRR-type sequences which co-segregated with *Yr5* resistance. These co-segregating DNA markers have the potential to provide informative signatures for marker-based *Yr5* introgression. Furthermore the genetic complexity underlying the control of *Yr5*-mediated yellow rust resistance in wheat was demonstrated by the identification of unlinked gene sequences differentially transcribed, in the absence of the yellow rust pathogen, in lines from which the *Yr5* gene had been deleted. Similarly, genetic characterisation of EMS mutants compromised in their *Yr5* resistant phenotype identified additional genetic loci required for *Yr5*-mediated resistance.

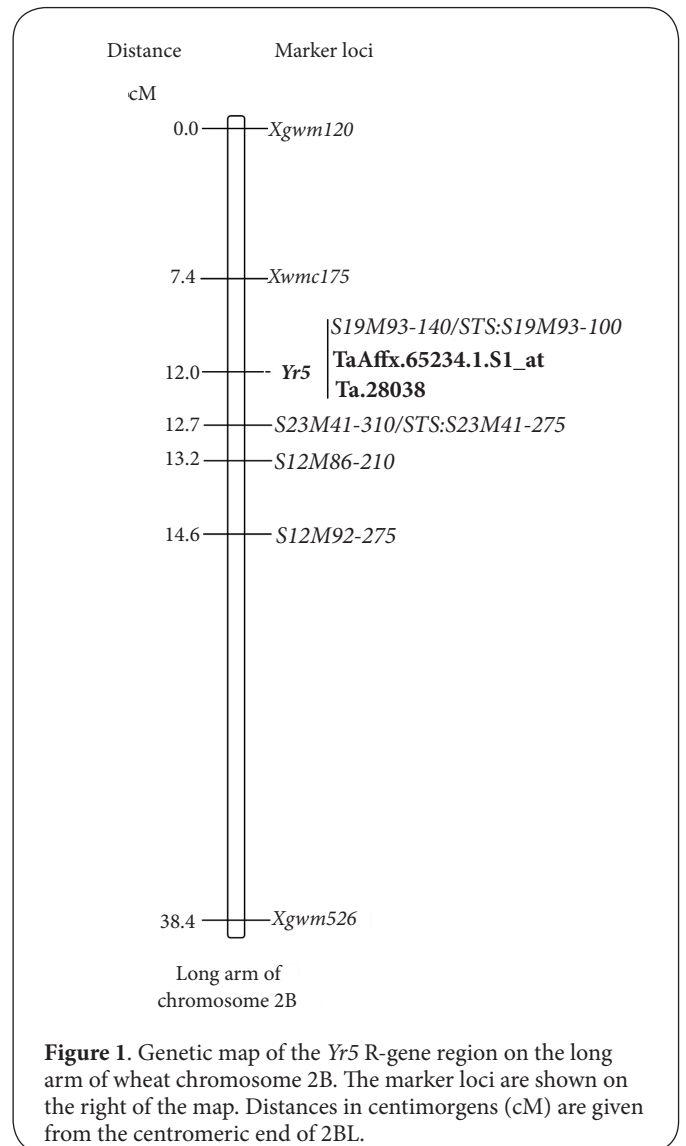
## Materials and methods

### Plant material and mapping populations

A BC1 population [(Lemhi\*8/*Yr5* x Lemhi) x Lemhi] (Figure 1) [6] consisting of 248 lines and the doubled haploid (DH) population of the cross Kariega x Avocet S (250 lines) [22] were used to map wheat gene markers to the *Yr5* region of chromosome 2B. Wheat lines Lemhi, Avocet S, Kariega, Chinese Spring, *T. spelta* and Lemhi\*8/*Yr5* were used as controls.

### *Puccinia striiformis* f. sp. *tritici* infection tests

The *Pst* isolate WYR81/20 (avir *Yr1,5,7,8,9,10,15,17*/vir *Yr2,3,4,6*) was used in all yellow rust infection tests to detect the presence of *Yr5*. Isolate WYR81/20 is virulent on Lemhi, Avocet S and Chinese Spring (Infection Type (IT) - 4) and avirulent on *T. spelta*



**Figure 1.** Genetic map of the *Yr5* R-gene region on the long arm of wheat chromosome 2B. The marker loci are shown on the right of the map. Distances in centimorgans (cM) are given from the centromeric end of 2BL.

and Lemhi\*8/*Yr5* (IT0/;). All yellow rust infection tests were carried out on seedlings at growth stage 12-13 [23] grown under spore-free conditions [24]. Infection reactions were scored 14-16 days after inoculation using the following IT scale:

- 0 no visible symptoms
- ; small necrotic flecks
- n<sup>i</sup> necrotic regions > 1mm in diameter
- 0<sup>n</sup> necrotic regions > 2mm in diameter
- 0<sup>nn</sup> spreading necrotic regions > 4mm in diameter
- 1 small, sporulating uredinia surrounded by necrotic tissue.
- 2 moderately sized, sporulating uredinia surrounded by necrotic tissue.
- 3 moderately sized, sporulating uredinia surrounded by chlorotic tissue.
- 4 large, sporulating uredinia surrounded by green tissue.
- c chlorotic tissue associated with uredinia
- n necrotic tissue associated with uredinia

### Fast-neutron and EMS-mutation lines compromised for *Yr5* resistance

Fast-neutron mutants were generated as previously described [25].  $F_1$  plants monosomic for chromosome 2B:*Yr5* were made by crossing Lemhi\*8/*Yr5* as the male parent to the Chinese Spring 2B monosomic line. The monosomic status of the  $F_1$  plants was confirmed using 2B-specific SSR markers GWM388, GWM120 and GWM526, for which Lemhi\*8/*Yr5* and Chinese Spring carry distinct alleles.  $F_1$  plants were selfed to give  $F_2$  seed. Five thousand  $F_2$  seed were exposed to 3 Gy of fast neutrons. Twenty-five  $F_2$  plants displayed a susceptible disease phenotype (IT4) when inoculated with isolate WYR81/20 which was maintained in the  $F_3$  generation.

EMS mutants were made by soaking 500 seed of Lemhi\*8/*Yr5* in ethyl methanesulfonate (0.5% v/v in water) for 2 hours. Excess liquid was removed and the seed left at room temperature for 16 hours, maintaining high humidity. Seed was washed 3 times in ddH<sub>2</sub>O before sowing in a peat/sand (1:1 v/v) mix. Plants of this M1 generation were allowed to self-pollinate and 20 M2 progeny from each M1 plant tested for *Yr5* resistance. Twelve of the 500 M2 families segregated for a compromised *Yr5* resistant phenotype (Table 1), which was confirmed in the M3 and M4 generations.

Two homozygous M3 plants from each of the 12 *Yr5*EMS mutations were crossed to the yellow rust susceptible variety Avocet S to produce  $F_2$  populations. Lemhi\*8/*Yr5* was also crossed to Avocet S to produce a control population. Approximately 200  $F_2$  seedlings from each cross were screened using isolate WYR81/20.

The 25 *Yr5* fast-neutron and 12 *Yr5*EMS mutants were screened with the *Yr5* linked SSR markers GWM120, WMC175 and GWM526, the STS markers S19M93-100 (S19M93-100F 5'TAATTGGGACCGAGAGACG, S19M93-100R 5'TTCTTGCAGCTCCAAAACCT) and S23M41-275 (S23M41-275F 5'TCAACGGAACCTCCAATTTC, S23M41-275R 5'AGGTAGGTGTTCCAGCTTGC) (Figure 1) [6] and with the primers designed to the wheat sequences TaAffx.65234.1.S1\_at and Ta.28038 developed in this study (Figure 1). The mutants *Yr5del55* and *Yr5del67*, which retained the *T. spelta* alleles for the three SSR markers (the other fast-neutron mutants having null alleles for the three SSR markers), but did not amplify the *Yr5* STS marker alleles, were used in the Affymetrix Wheat1 GeneChip analysis (Table 1).

### Comparative genomic analyses of wheat sequences

The DNA sequence of AFLP markers S19M93-140 and S23M41-310 [6] were used in *BlastN* searches against the GrainGenes wheat sequence database ( $e^{-10}$ ; http://wheat.pw.usda.gov/GG2/blast.shtml), the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/analyses\_search\_blast.shtml) and the *Brachypodium* Genome Sequence (http://www.modelcrop.org/) databases. Rice and *Brachypodium* sequences with significant similarity to the AFLP markers ( $e^{-5}$ ) were used to further interrogate the GrainGenes wheat sequence database.

The selection criteria used to search the rice and *Brachypodium* genome sequence databases were less stringent than that used for the wheat EST analysis to account for cross-species variation. The group 2 wheat sequences from the wheat Whole Chromosome Survey Sequencing database (http://www.wheatgenome.org/Projects/IWGSC-Bread-Wheat-Projects/Sequencing/Whole-Chromosome-Survey-Sequencing) were also interrogated with the wheat gene sequences identified from the comparative genome analysis and the Affymetrix Wheat1 GeneChip transcriptomics analysis. A stringent cut-off level of  $< e^{-50}$  was used to select only highly similar sequences. Nucleotide and amino acid sequences were aligned with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) using the default settings.

### Transcriptome analysis of *Yr5* mutants *Yr5del55* and *Yr5del67*

Seedlings of Lemhi\*8/*Yr5*, *Yr5del55* and *Yr5del67* were grown to growth stage 12-13 under spore-free conditions [24]. Total RNA was extracted and purified from leaf tissue of six seedlings pooled as one replicate, with three replicates per genotype [26]. Affymetrix Wheat1 GeneChip processing, including RNA quality control, microarray hybridisation and data acquisition was performed by the research services of the Genome Laboratory, Norwich, U.K. Data was analysed using GeneSpring GX10 (Agilent) and probe sets annotated as previously described [26]. Lemhi\*8/*Yr5* was compared to *Yr5del55* and *Yr5del67* to identify probe sets that were differentially expressed in the parental line compared to the mutants, having a fold change  $>2$  and a p-value  $<0.05$  (Welch T-Test).

Quantitative reverse transcription PCR (qRT-PCR) was used to validate the expression levels of selected probe sets [26]. Total RNA was extracted from leaf samples of Lemhi\*8/*Yr5*, *Yr5del55* and *Yr5del67* using the RNeasy Plant Mini Kit (QIAGEN) and subsequently treated with TURBO DNA-free (Ambion). cDNA was synthesised using SuperScript™ III-RNase H<sup>-</sup> Reverse Transcriptase (Invitrogen CA, USA), according to the manufacturer's recommendations and diluted 20-fold with nuclease-free water prior to use. qRT-PCR validation was carried out using gene specific primers (Supplement Table S1). Only primer sets with an amplification efficiency  $>80\%$  were used for qRT-PCR analysis. The DNA engine Opticon2 Continuous Fluorescence Detector (M.J. Research Inc., Alameda, CA, U.S.A.) was used for PCR amplification with cycling conditions of 95°C for 4 min, followed by 40 cycles of 30 sec at 94°C, 30 sec 60°C and 30 sec at 72°C. Melt curve analysis was used at the end of each reaction to check primer-dimer formation and gene-specific product amplification. Data were analysed using Opticon Monitor™ analysis software v2.02 (M.J. Research Inc.). cDNA was normalised with geNorm (geNorm program v3.5 http://medgen.ugent.be/~jvdesomp/genorm/) [27] using three reference genes, ubiquitin [28], GAPDH [29] and elongation factor-1 $\alpha$  [30] that were all stable

**Table 1. Genetic, marker and phenotypic assessment of mutations compromised for *Yr5*-mediated yellow rust resistance.**

Varieties, <i>Yr5del</i> & <i>Yr5EMS</i> mutants	<i>Yr5EMS</i> IT to WYR 81/20	Yr5EMS mutants x AvocetS: <sup>1</sup> <i>Yr5</i> infection phenotypes					DNA marker analysis: Alleles present <sup>2</sup>							
		F <sub>1</sub> IT	F <sub>2</sub> segregating ITs				STS: S19M93 -100	STS: S23M41 -275	GWM 120	WMC 175	GWM 526	TaAffx. 65234.1 S1_at	Ta28038	
			n <sup>i</sup>	0 <sup>nn</sup>	1/2 <sup>n</sup>	3/4								
<i>Yr5EMS</i> 18	1 <sup>n</sup>	4	0	1	19	214	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 90	4	4	0	6	15	189	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 94	0 <sup>nn</sup>	0 <sup>nn</sup>	27	66	39	84	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 95	4	4	0	0	0	234	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 98	1/2 <sup>n</sup>	4	0	34	66	122	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 99	4	4	0	0	12	218	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 115	4	4	0	0	0	228	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 241	4	4	0	0	0	212	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 287	4	4	0	0	0	216	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 387	4	4	0	0	0	220	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 474	4	4	0	0	0	218	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5EMS</i> 500	4	4	0	0	0	228	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Yr5del55</i>	4	-	-	-	-	-	DEL	DEL	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	DEL	DEL	
<i>Yr5del67</i>	4	-	-	-	-	-	DEL	DEL	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	DEL	DEL	
<b>Controls:</b>														
Lemhi*8/ <i>Yr5</i>	n <sup>i</sup>	0 <sup>n</sup> /1 <sup>n</sup>	52	116	2	64	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
<i>Triticum spelta</i>	;	-	-	-	-	-	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	<i>T.sp</i>	
Lemhi	4	-	-	-	-	-	null	L	L	L	L	L	L	
Avocet S	4	-	-	-	-	-	n/t	n/t	n/t	n/t	n/t	n/t	n/t	
Chinese Spring	3/4	-	-	-	-	-	null	L	CS	CS	CS	n/t	n/t	

<sup>1</sup>Lemhi\*8/*Yr5* and the *Yr5EMS* mutants were crossed to the yellow rust susceptible variety AvocetS. The infection type (IT) scores given to the F1 and F2 progeny follow the scheme set out in Materials and Methods.

<sup>2</sup>Marker alleles are indicated as *T.sp.* (as present in *Triticum aestivum* ssp. *spelta* var. *album*), CS (as present in cultivar Chinese Spring), L (as present in cultivar Lemhi), null (no band detected), or DEL (marker absent), n/t (not tested).

under our experimental conditions. Relative transcript levels of the genes represented by each probe set were calculated between Lemhi\*8/*Yr5* and *Yr5del55* or *Yr5del67*. Where primer sets suitable for qRT-PCR analysis could not be designed end-point RT-PCR was used to assess gene expression. PCR amplification of 50 ng of genomic DNA was used to test for loss of probe set sequences from the mutants *Yr5del55* and *Yr5del67*. Primers of each probe set were also tested against the Chinese Spring wheat group 2 nulli-tetrasomic lines to identify group 2 homoeologous-specific chromosomal sequences.

### Mapping of wheat sequences

Primer pairs were designed to wheat sequences using the PRIMER3 program (<http://www.genome.wi.mit.edu>) and screened for polymorphisms between Lemhi, Lemhi\*8/*Yr5*, Kariaga and Avocet S, and against the *Yr5* mutants *Yr5del55* and *Yr5del67* using Single Strand Confirmation Polymorphism (SSCP) analysis [6]. Markers polymorphic between Lemhi and

Lemhi\*8/*Yr5* were mapped in the BC1 population [(Lemhi\*8/*Yr5* x Lemhi) x Lemhi] using Joinmap, version 3.0 [31,32]. The map was constructed using a LOD threshold of 3.0 and a maximum recombination frequency of 0.45. The recombination values were converted into genetic distances using the Kosambi mapping function [33]. Markers polymorphic between Kariega x Avocet S were mapped in the DH population [22] using the screening and mapping procedures previously described [34].

### Results

#### Identification of gene sequences within the *Yr5* genetic region through comparative genomic analyses

The AFLP markers S19M93-140 and S23M41-310 had previously been mapped to the yellow rust R-gene *Yr5* (Figure 1) [6]. S19M93-140 co-segregated with *Yr5*, while S23M41-310 mapped at a distance of 0.7cM. To specifically identify gene sequences within the region of the wheat genome containing *Yr5* a *BlastN* search was conducted against the GrainGenes

wheat sequence database using the nucleotide sequence of S19M93-140 and S23M41-310 as query sequences. Both S19M93-140 and S23M41-310 showed significant matches ( $e^{-10}$ ) to the same three wheat gene sequences, TaAffx.65234.1.S1\_at, BJ255716 and CJ700174 even though the map locations of the two AFLP markers indicated that they represented distinct loci (**Supplement Table S2**). TaAffx.65234.1.S1\_at, BJ255716 and CJ700174 showed 100% identity to each other at the nucleotide level (data not shown) and will be referred to by the probe set name TaAffx.65234.1.S1\_at.

The AFLP marker sequences were used to interrogate the rice and *Brachypodium* genome databases, exploiting the co-linearity between wheat and other grass species to identify further candidate gene sequences. S23M41-310 showed significant similarity to the rice loci LOC\_Os04g53496 ( $8.6e^{-17}$ ) and LOC\_Os07g04900 ( $1.0e^{-16}$ ), and to the *Brachypodium* sequence Bradi4g09800.1. S19M93-140 showed significant similarity to the rice sequence LOC\_Os11g15670 and to the *Brachypodium* sequences Bradi4g09800.1, Bradi2g36040.1 and Bradi2g36030.1 (**Supplement Table S2**). As the wheat group 2 chromosomes are syntenous with rice chromosomes 4 and 7 [35] LOC\_Os07g04900 and LOC\_Os04g53496 were used as query sequences in BlastN searches of the GrainGenes database to identify orthologous wheat sequences. Due to the lack of reported syntenic relationship between rice chromosome 11 and *Brachypodium* chromosomes 2 and 4 [35,36] with wheat chromosome 2B, LOC\_Os11g15670, Bradi4g09800.1, Bradi2g36040.1 and Bradi2g36030.1 were not pursued as loci for further wheat sequence analysis.

LOC\_Os04g53496 as query sequence identified nine wheat ESTs (significance score  $< e^{-35}$ ), all of which represented the wheat unigene Ta.28038 (**Supplement Table S2**). LOC\_Os07g04900 matched ten wheat ESTs, nine of which (significance score  $< e^{-17}$ ) were assigned to unigene Ta.28038 (**Supplement Table S2**). The tenth wheat EST sequence showed sequence similarity to Ta.28038 of  $2e^{-12}$ . Ta.28038 was not detected by either AFLP marker sequence, so represented a new candidate gene for *Yr5* mapping and marker development. TaAffx.65234.1.S1\_at also showed significant similarity to LOC\_Os04g53496 ( $4.4e^{-51}$ ) and LOC\_Os07g04900 ( $1.3e^{-37}$ ). The wheat contig TaAffx.65234.1.S1\_at and unigene Ta.28038 were therefore selected for further study.

Nucleotide and amino acid sequence alignment of TaAffx.65234.1.S1\_at and Ta.28038 indicated that the two wheat sequences aligned to different regions of the rice loci. Ta.28038 aligned to the 5' end of rice loci LOC\_Os04g53496 and LOC\_Os07g04900, whilst TaAffx.65234.1.S1\_at aligned to the 3' end of these two rice genes (**Supplement figure S1**). The two AFLP marker sequences S19M93-140 and S23M41-310, which showed  $2e^{-24}$  nucleotide similarity to each other, aligned with TaAffx.65234.1.S1\_at (**Supplement Table S2**). A BlastN search of the wheat Whole Chromosome Survey Sequencing database located TaAffx.65234.1.S1\_at and Ta.28038 to wheat chromosome 2B ( $e$ -value = 0.0), the location

of *Yr5*, although significant matches ( $e$ -value  $< -60$ ) were also found on chromosomes 2A and 2D, implying the presence of homoeologous sequences (**Supplement Table S3**). While TaAffx.65234.1.S1\_at and Ta.28038 both annotated as NB-LRR disease resistance proteins (http://www.plexdb.org/modules/PD\_probeset/annotation.php) [37] there was a low degree of similarity between TaAffx.65234.1.S1\_at and Ta.28038 at the nucleotide level.

Primers designed to the wheat sequences TaAffx.65234.1.S1\_at and Ta.28038 (**Supplement Table S1**) were used to amplify genomic DNA from *T. spelta*, Lemhi\*8/*Yr5*, Lemhi, *Yr5del55* and *Yr5del67*. No polymorphisms were apparent between *T. spelta*, Lemhi\*8/*Yr5* and Lemhi using agarose gel separation (data not shown), therefore amplicons were screened using SSCP analysis. SSCP bands present in *T. spelta* and Lemhi\*8/*Yr5*, but absent in Lemhi, *Yr5del55* and *Yr5del67* (**Supplement figure S2**) were used to map TaAffx.65234.1.S1\_at and Ta.28038 in the [(Lemhi\*8/*Yr5* x Lemhi) x Lemhi] BC1 population TaAffx.65234.1.S1\_at and Ta.28038 both co-segregated with *Yr5* resistance (**Figure 1**).

#### Identification of gene sequences within the *Yr5* genetic region through transcript profiling of *Yr5* fast-neutron mutants

A transcriptome analysis of Lemhi\*8/*Yr5*, *Yr5del55* and *Yr5del67* was undertaken as an alternative approach to identify wheat gene sequences physically linked to the *Yr5* locus. *Yr5del55* and *Yr5del67* were generated by fast neutron bombardment, displayed a susceptible infection type (IT 4), and had lost the STS markers and wheat gene sequences TaAffx.65234.1.S1\_at and Ta.28038 mapped to *Yr5* (**Table 1**). Compared to the parental line Lemhi\*8/*Yr5*, *Yr5del55* and *Yr5del67* showed significant changes in the transcript levels of 714 and 396 probe sets, respectively (**Supplement Table S4** and **Supplement figure S3**). Only 145 probe sets were in common between the mutants, with 105 probe sets being up-regulated and 40 down-regulated.

Functional classification of the 40 down-regulated probe sets, representing 37 unique gene sequences indicated that the majority were of unknown function (**Supplement figure S3**). Probe sets with predicted roles in plant defence or stress responses, including superoxide dismutase, cystatin and a non-specific lipid transfer protein, as well as transcripts involved in cellular transport, transcription, energy and metabolic processes were identified (**Table 2**). However, none of the probe sets were annotated as NB-LRR-type R-genes and the probe sets representing the sequences TaAffx.65234.1.S1\_at and Ta.28038 did not show differential transcript levels between Lemhi\*8/*Yr5* and either *Yr5* mutant.

Of the 105 up-regulated probe sets, which represented 100 unique gene transcripts, more than half could not be assigned a potential function (**Supplement figure S3**). Those that could be assigned a possible function were involved in processes such as plant defence and stress responses, cellular transport, metabolism, energy, cell component biogenesis, transcription,

**Table 2. Affymetrix Wheat1 GeneChip probe sets showing down-regulated transcript levels in the fast-neutron mutants *Yr5del55* and *Yr5del67* compared to Lemhi\*8/*Yr5*.**

Probe Set ID	<i>Yr5del55</i> v Lemhi*8/ <i>Yr5</i>		<i>Yr5del67</i> v Lemhi*8/ <i>Yr5</i>		Annotation from HarvEST/Plexdb
	p-value	FC	p-value	FC	
Ta.1944.1.S1_at	9.67E-05	-49.4881	4.79E-04	-46.19151	Superoxide dismutase [Mn] 3.1, mitochondrial precursor
Ta.27140.1.S1_at	0.001556	-5.7364397	3.65E-04	-6.0843663	Putative MATE efflux protein family protein
Ta.178.2.S1_at	0.0108303	-8.481019	0.0164578	-7.719493	Cystatin WC-1
Ta.178.2.S1_x_at	0.0063519	-3.0741243	3.94E-04	-3.1455898	Cystatin WC-1
Ta.1688.1.S1_at	0.0172641	-2.4441025	0.0143996	-2.8630302	UDP-glucosyltransferase BX8
Ta.14545.1.S1_at	0.0188015	-6.2296305	0.0337206	-4.38153	Putative O-methyltransferase
Ta.1112.1.S1_at	0.0104491	-13.676553	0.0097063	-11.446178	none
TaAffx.14477.1.S1_at	2.92E-04	-3.4393673	7.88E-04	-3.140847	none
Ta.611.1.A1_at	3.35E-04	-75.71564	8.97E-05	-73.59188	Hypothetical protein
Ta.21290.1.A1_s_at	0.0194118	-3.8935266	0.0315453	-3.4232893	none
Ta.8757.2.S1_at	0.0363228	-2.5777307	0.0382263	-2.3648314	Phosphoserine phosphatase
Ta.21005.1.S1_at	0.0143132	-3.9735072	0.0168927	-3.7059195	none
Ta.14729.1.S1_at	0.0321907	-2.1196191	0.0433241	-2.1017556	none
Ta.30504.1.A1_at	0.0069815	-3.1662958	0.0080916	-2.542934	Nonspecific lipid transfer protein
TaAffx.73741.1.S1_at	0.0066044	-2.3675597	0.0203833	-2.5882344	none
Ta.17028.1.A1_s_at	0.0485578	-2.5108774	0.0117914	-2.0112689	Putative HGA1
Ta.7149.1.A1_s_at	0.0011034	-3.3122602	0.0024801	-2.7918813	none
TaAffx.616.2.S1_s_at	0.0392292	-2.034222	0.0124056	-2.1673586	Hypothetical protein
TaAffx.66205.2.S1_s_at	0.0011056	-3.7338786	7.50E-04	-4.0016356	Intracellular protease, PfpI family protein
TaAffx.100436.1.S1_at	0.0086405	-11.848047	0.0050554	-13.363708	none
Ta.16298.1.S1_at	0.0120654	-2.2416117	0.0070407	-4.001226	none
Ta.16582.1.S1_at	0.0380914	-4.0853386	0.0334678	-4.1695147	none
Ta.8552.1.A1_at	0.0082964	-8.785303	0.0076009	-10.224292	none
Ta.16476.1.S1_at	0.016985	-2.2019472	0.041713	-2.0130389	none
TaAffx.43914.1.S1_s_at	0.0482835	-2.589973	0.0170548	-2.3887548	Expressed protein
Ta.17295.1.S1_at	0.0014027	-6.2927947	6.71E-04	-6.619841	none
Ta.9039.2.S1_x_at	3.03E-05	-4.570216	1.28E-04	-4.4122486	Intracellular protease, PfpI family protein
TaAffx.53602.1.S1_at	0.0419488	-4.863748	0.0042042	-2.164832	NAD(P)H-quinone oxidoreductase chain 4, chloroplast
TaAffx.57738.1.S1_at	6.15E-04	-2.2537112	3.81E-04	-2.5442169	aspartic proteinase nepenthesin-1 precursor
TaAffx.51578.1.S1_at	0.0383779	-2.1451643	0.0222281	-2.5058053	none
TaAffx.7104.1.S1_at	0.0041442	-3.927811	0.0064122	-3.9704103	Putative DNA-directed RNA polymerase III subunit 22.9 kDa polypeptide
TaAffx.7104.1.S1_x_at	0.0319099	-2.7847736	0.0351291	-2.5745335	Putative DNA-directed RNA polymerase III subunit 22.9 kDa polypeptide
TaAffx.74512.2.S1_s_at	0.0128684	-2.3639889	0.0182374	-2.343101	flavonol 4-sulfotransferase
Ta.611.2.S1_at	2.18E-04	-17.154543	9.36E-05	-15.461136	none
TaAffx.21788.1.S1_at	1.99E-04	-5.1074266	4.92E-04	-4.860779	none
Ta.19222.1.S1_at	7.20E-04	-10.351834	0.0032261	-11.462584	none
Ta.19222.1.S1_x_at	0.0026437	-3.3132606	0.0040692	-3.4967139	none
Ta.20262.2.S1_at	0.0120548	-6.4605107	0.0153281	-5.864731	none
Ta.23203.3.S1_at	9.05E-06	-9.235376	3.23E-04	-9.367666	none
TaAffx.22603.1.S1_at	0.0068985	-2.319124	0.0109216	-2.5344298	none

protein synthesis and binding interactions. The transcripts that were found to be down-regulated in both *Yr5del55* and *Yr5del67* were selected for further analysis.

Probe set transcript levels were validated using qRT-PCR analysis, comparing transcript levels in Lemhi\*8/*Yr5* to those in *Yr5del55* and *Yr5del67*. Primer sets suitable for qRT-PCR analysis could only be designed for 20 of the 37 unique transcripts (**Supplement Table S1**). Those primer sets that were not suitable for qRT-PCR had either low amplification efficiency or produced primer dimers, preventing robust assay design. qRT-PCR analysis confirmed down-regulation of 12 of the 20 probe set transcripts (63%) in both *Yr5del55* and *Yr5del67* (**Table 3; Supplement figure S4**). For a further five probe sets qRT-PCR demonstrated transcript repression in only one of the mutants (**Table 3**). End point RT-PCR was used to detect transcripts for 14 of the probe sets for which qRT-PCR suitable primers could not be designed. For five of these probe sets no transcript was detected in either mutant (**Table 3; Supplement figure S5**). A transcript for probe set Ta.16582.1.S1\_at was absent only from *Yr5del67*, while the remaining eight probe sets tested by end point RT-PCR produced transcripts in both *Yr5del55* and *Yr5del67*. Two products were observed for probe set TaAffx.21788.1.S1\_at in Lemhi\*8/*Yr5*, suggesting that these primers may amplify homoeologous or alternative spliced transcripts produced by this gene.

PCR amplification of genomic DNA was carried out to determine whether probe set sequences had been lost from the genomes of *Yr5del55* and *Yr5del67*. Thirty-three primer sets amplified from the genomes of Lemhi\*8/*Yr5* and Lemhi, with 28 probe set sequences also being detected in the genomes of the two *Yr5* mutants (**Table 3**). Primers for the probe sets TaAffx.74512.2.S1\_s\_at; Ta.178.2.S1\_at/Ta.178.2.S1\_x\_at; TaAffx.100436.1.S1\_at and Ta.17295.1.S1\_at failed to amplify from genomic DNA of either mutant, while probe set Ta.30504.1.A1\_at amplified from genomic DNA of *Yr5del55*, but not from *Yr5del67* (**Table 3; Supplement figure S6**).

### Mapping of the down-regulated wheat transcripts to the wheat genome

The syntenic relationship between the genomes of wheat and other cereal and grass species was used to assign the genetic loci represented by the 37 down-regulated probe sets to a chromosomal location through *in silico* mapping. The sequences of the 37 probe sets were used to investigate the wheat Whole Chromosome Survey Sequencing database, the mapped wheat EST database, and the rice and *Brachypodium* genome sequences. Nineteen of the transcripts (~51%) had highly significant matches ( $<e^{-50}$ ) to sequences located on wheat group 2 chromosomes (**Supplement Table S3**). Of these, 11 (~30%) transcripts had strong matches on all three homoeologous chromosomes, four (~11%) had matches on 2A and 2B, two (~5%) had matches on 2A, one on 2B and one on 2D. Seven probe sets produced significant hits ( $e$ -value  $< e^{-10}$ )

against the mapped wheat EST database, with two mapping to the short arm of wheat chromosome 2B (Ta.27140.1.S1\_at and Ta.8757.2.S1\_at), and one, Ta.1944.1.S1\_at mapping to the long arm of 2B (**Supplement Table S3**). Twenty-one of the probe set sequences had significant matches on the rice genome, whereas 18 had significant hits to *Brachypodium* (**Supplement Table S3**). Ta.1112.1.S1\_at showed the best hit to rice chromosome 4, while Ta.27140.1.S1\_at, Ta.21290.1.A1\_s\_at, TaAffx.74512.2.S1\_s\_at and TaAffx.7104.1.S1\_at/TaAffx.7104.1.S1\_x\_at produced significant hits on rice chromosome 7. Ta.21290.1.A1\_s\_at and TaAffx.7104.1.S1\_at/TaAffx.7104.1.S1\_x\_at also produced significant hits to sequences located on *Brachypodium* chromosome 5.

Genomic DNA of the Chinese Spring wheat group 2 nulli-tetrasomic lines were screened with the 33 primer probe sets by PCR. Only Ta.1944.1.S1\_at was assigned solely to wheat chromosome 2B. Ta.30504.1.A1\_at, TaAffx.100436.1.S1\_at, Ta.17295.1.S1\_at, TaAffx.74512.2.S1\_s\_at and Ta.178.2.S1\_at/Ta.178.2.S1\_x\_at could not be amplified from any of the Chinese Spring nulli-tetrasomic group 2 lines, while the remaining probe sets amplified from genomic DNA in all the group 2 null-tetrasomic lines (**Supplement Table S3**).

SSCP analysis failed to identify polymorphisms between Lemhi\*8/*Yr5* and Lemhi for any of the 33 probe set primers (data not shown). An alternative mapping population, Karioga x Avocet S [22] was therefore used in an attempt to map the probe set transcripts to the wheat genome. While SSCP analysis identified polymorphic bands between Karioga and Avocet S for seven probe sets (Ta.178.2.S1\_at, Ta.1112.1.S1\_at, Ta.8757.2.S1\_at, TaAffx.616.2.S1\_s\_at, TaAffx.21788.1.S1\_at, Ta.611.2.S1\_at and Ta.Affx.7104.1.S1\_at) only Ta.611.2.S1\_at could be mapped in the Karioga x Avocet S population. However, Ta.611.2.S1\_at did not map to chromosome 2BL, but to the long arm of chromosome 4A, near to an adult plant, slow rusting QTL for yellow rust resistance (Gloudi Agenbag, unpublished data). *In silico* mapping identified highly significant hits for Ta.611.2.S1\_at on both the group 2 and group 4 chromosomes of the Chinese Spring Whole Chromosome Survey Sequencing database, supporting the map location of Ta.611.2.S1\_at in the Karioga x Avocet S population.

### Genetic and molecular characterisation of EMS-derived mutations compromised for the *Yr5* resistance phenotype

As a resource to genetically define the chromosomal region containing the *Yr5* locus and subsequently confirm *Yr5* candidate genes, EMS mutants were selected that were compromised for *Yr5* resistance. Twelve mutants were identified of which nine were fully susceptible to Pst isolate WYR81/20, displaying IT4, while three had partially susceptible phenotypes ranging from IT0<sup>nn</sup> to 1/2<sup>n</sup> (**Table 1; Supplement figure S7**).

The *Yr5*EMS mutants were crossed to the yellow rust susceptible variety Avocet S to determine whether the mutation responsible for the altered *Yr5* resistance phenotype



**Table 3. PCR analysis of Affymetrix Wheat1 GeneChip probe sets down-regulated in *Yr5del55* and *Yr5del67* compared to *Lemhi\*8/Yr5*.**

Probe Set ID	qRT-PCR <sup>a</sup>		End point RT-PCR		PCR from genomic DNA	
	<i>Yr5del55</i>	<i>Yr5del67</i>	<i>Yr5del55</i>	<i>Yr5del67</i>	<i>Yr5del55</i>	<i>Yr5del67</i>
Ta.1944.1.S1_at	33.5	<u>-23.7</u>	+ <sup>b</sup>	+	+	+
Ta.27140.1.S1_at	nt <sup>d</sup>	nt	-	-	+	+
Ta.178.2.S1_at/ Ta.178.2.S1_x_at	<u>-2194.5</u>	<u>-8364.0</u>	-	-	-	-
Ta.1688.1.S1_at	<u>-106.7</u>	<u>-56.4</u>	+	+	+	+
Ta.14545.1.S1_at	<u>-1294.9</u>	<u>-1956.8</u>	-	-	+	+
Ta.1112.1.S1_at	<u>-3.1</u>	-0.9	+	+	+	+
TaAffx.14477.1.S1_at	nt	nt	+	+	+	+
Ta.611.1.A1_at	<u>-42912.3</u>	<u>-21240</u>	-	-	+	+
Ta.21290.1.A1_s_at	<u>-4.3</u>	<u>-2.7</u>	+	+	+	+
Ta.8757.2.S1_at	<u>-4.4</u>	<u>-4.6</u>	+	+	+	+
Ta.21005.1.S1_at	<u>-83.8</u>	<u>-271.3</u>	-	-	+	+
Ta.14729.1.S1_at	2.8	-1.0	+	+	+	+
Ta.30504.1.A1_at	<u>-584.8</u>	<u>-253.0</u>	-	-	+	-
TaAffx.73741.1.S1_at	nt	nt	-	-	+	+
Ta.17028.1.A1_s_at	nt	nt	+	+	+	+
Ta.7149.1.A1_s_at	nt	nt	+	+	+	+
TaAffx.616.2.S1_s_at	0.0	<u>-2.2</u>	+	+	+	+
TaAffx.66205.2.S1_s_at	nt	nt	+	+	+	+
TaAffx.100436.1.S1_at	nt	nt	-	-	-	-
Ta.16298.1.S1_at	nt	nt	+	+	+	+
Ta.16582.1.S1_at	nt	nt	+	-	+	+
Ta.8552.1.A1_at	<u>-231.5</u>	<u>-154.1</u>	-	-	+	+
Ta.16476.1.S1_at	<u>-2.3</u>	-0.7	+	+	+	+
TaAffx.43914.1.S1_s_at	nt	nt	-	-	+	+
Ta.17295.1.S1_at	nt	nt	-	-	-	-
Ta.9039.2.S1_x_at	<u>-13.0</u>	<u>-11.9</u>	+	+	+	+
TaAffx.53602.1.S1_at	nt	nt	nt	nt	nt	nt
TaAffx.57738.1.S1_at	nt	nt	+	+	+	+
TaAffx.51578.1.S1_at	nt	nt	nt	nt	nt	nt
TaAffx.7104.1.S1_at/ TaAffx.7104.1.S1_x_at	nt	nt	nt	nt	nt	nt
TaAffx.74512.2.S1_s_at	<u>-530.9</u>	<u>-300.0</u>	-	-	-	-
Ta.611.2.S1_at	<u>-28521.9</u>	<u>-10866.1</u>	-	-	+	+
TaAffx.21788.1.S1_at	nt	nt	-	-	+	+
Ta.19222.1.S1_at/ Ta.19222.1.S1_x_at	-1.1	<u>-10.8</u>	+	+	+	+
Ta.20262.2.S1_at	nt	nt	+	+	+	+
Ta.23203.3.S1_at	nt	nt	nt	nt	nt	nt
TaAffx.22603.1.S1_at	0.0	-1.3	+	+	+	+

<sup>a</sup>Transcript levels expressed as fold change differences in deletion mutant relative to *Lemhi\*8/Yr5*. Underlined expression values highlight those probe sets where qRT-PCR analysis was used to validated the microarray data.

<sup>b</sup>+ = PCR amplicon detected in mutant

<sup>c</sup>- = PCR amplicon absent from mutant; <sup>d</sup>nt = not tested

was within the *Yr5* gene itself, or at a second, independent locus required for the expression of *Yr5* resistance. Seven mutants produced only yellow rust susceptible  $F_2$  progeny, indicating that the mutation event directly affected the *Yr5* locus (Table 1). All seven mutants had a fully susceptible infection type (IT4) reaction to isolate WYR81/20. These seven *Yr5*EMS mutants retained not only the linked STS and SSR markers mapped to *Yr5*, but also TaAffx.65234.1.S1\_at and Ta.28038 (Table 1). Primers for TaAffx.65234.1.S1\_at and Ta.28038 produced amplicons with identical SSCP band patterns in Lemhi\*8/*Yr5* and all 12 *Yr5*EMS mutants (Table 1).

The remaining five *Yr5*EMS mutants segregated  $F_2$  individuals expressing a more resistant phenotype than that shown by the parental mutation (Table 1). This would indicate a mutation within a second locus, required for *Yr5*-mediated resistance. In the control cross, Lemhi\*8/*Yr5* (ITn) x Avocet S (IT4), *Yr5* expressed as a semi-dominant gene, the heterozygous  $F_1$  progeny (IT 0<sup>nm/n</sup>) appearing less resistant to isolate WYR81/20 than the *Yr5* donor parent Lemhi\*8/*Yr5* (Table 1). This made it difficult to confidently predict the number of additional mutant loci affecting the *Yr5* resistance phenotype based on an analysis of segregation ratios. Similar segregation ratios were obtained in both populations made from each *Yr5*EMS mutant M3 family (data not shown).

## Discussion

To date, virulence for the wheat race-specific yellow rust R-gene *Yr5* has only been confirmed in Australia [38]. *Yr5* is therefore a valuable resource, which used in combination with other R-genes, as well as quantitative sources of yellow rust resistance, would support breeding for durable yellow rust control in wheat. Deploying multiple R-genes in combination with more durable, but partial resistance genes such as the *Lr34/Yr18/Pm38* locus would provide a more sustainable approach to disease protection in regions of the world where yellow rust disease pressure is high [39,40]. Here we successfully use comparative genomics analyses between wheat and related cereal and grass genomes, transcriptomics analysis and *in silico* mapping to locate candidate wheat genes linked to the *Yr5* locus. Two previously described AFLP markers that map to *Yr5* [6] were initially used in cross-species comparative sequence analyses. This led to the identification of two rice NB-LRR-type genes, one on rice chromosome 4 and the other on chromosome 7, which subsequently supported the identification of wheat gene sequences that were shown to co-segregate with *Yr5* resistance.

The co-linearity between wheat and other cereal and grass species for which a full genomic sequence is available provides a valuable resource by which to interrogate the genomic region around a gene of interest. Co-linearity provides a way to identify additional wheat sequences, flanking the gene of interest, which can be used for additional marker development [41], with the use of more than one genomic sequence providing greater confidence in the inferred syntenic relationships [16,17].

Previously, two genetically distinct AFLP markers linked to *Yr5* resistance were shown to have significant sequence similarity to rice homologues of the rice bacterial blight NB-LRR R-gene *Xa-1* [6]. In this study, comparative genomic analysis with rice further places the *Yr5* gene within a region of the wheat genome enriched with NB-LRR-type R-gene sequences. While two wheat gene sequences annotated as NB-LRR-type R-genes, TaAffx.65234.1.S1\_at and Ta.28038 were shown to co-locate with *Yr5*. *Yr5* may therefore represent another NB-LRR-type R-gene similar to many of the R-genes already cloned from wheat [8-13]. It is not uncommon for NB-LRR-type R-genes to occur in clusters within plant genomes [42] and the development of resistance gene-analog polymorphism (RGAP) markers which co-segregated with *Yr5* also support these observations [43].

Microarray-based transcription profiling has shown potential as a tool to assist in the cloning of genes of agronomically important traits, successfully identifying genes involved in symbiotic interactions in *Medicago truncatula* [20] and defence responses against stem rust in barley [21]. The Affymetrix Wheat1 GeneChip was therefore used to identify gene transcripts down-regulated in two *Yr5* fast-neutron mutants, *Yr5del55* and *Yr5del67* compared to the parental line Lemhi\*8/*Yr5*. However, while microarray-based transcription profiling identified a number of wheat gene transcripts which located to chromosome 2B, the polyploidy nature of hexaploid wheat limited the potential of this approach as a tool to identify wheat gene transcripts that lay within the genomic region of the *Yr5* locus. Of the 37 unique gene transcripts down-regulated 28 amplified from genomic DNA in both *Yr5del55* and *Yr5del67*. *In silico* mapping to the wheat group 2 chromosomes indicated that 12 of these gene transcripts had homoeologous sequences on more than one of the group 2 chromosomes, while Ta.611.2.S1\_at was also shown to have homoeologous sequences on the group 4 chromosomes. The probe sets representing the wheat sequences TaAffx.65234.1.S1\_at and Ta.28038, which had been shown to co-locate with *Yr5*, did not show differential transcript levels between Lemhi\*8/*Yr5* and either *Yr5del55* and *Yr5del67*. Again this observation was supported by the presence of homoeologues on chromosomes 2A and 2D, transcripts from which may have masked the loss of the loci on chromosome 2B in *Yr5del55* and *Yr5del67*.

While 28 probe sets amplified from genomic DNA in both *Yr5del55* and *Yr5del67*, down-regulation was confirmed for 12 transcripts by qRT-PCR in both mutants, and for a further five probe sets in one of the mutants. The function of many of the genes represented by these probe sets is unknown, but defence related proteins, including superoxide dismutase, cystatin and a non-specific lipid transfer protein were confirmed as down-regulated.

Interestingly, primers for five probe sets failed to amplify from genomic DNA in one or both *Yr5* fast-neutron mutants, while successfully amplifying DNA from Lemhi and Lemhi\*8/

*Yr5*. These five probe sets also failed to amplify from genomic DNA of the Chinese Spring nulli-tetrasomic group 2 lines and did not show hits against the wheat Whole Chromosome Survey Sequencing database. This would suggest that these genes, while present in Lemhi and Lemhi\*8/*Yr5* are not present in Chinese Spring, from which the wheat Whole Chromosome Survey Sequence was generated.

EMS mutagenesis of Lemhi\*8/*Yr5* identified twelve mutants, seven of which were within the *Yr5* locus and five in a second gene required for *Yr5* resistance. A number of genes have been identified which are required for the full expression of NB-LRR-type R-gene-mediated resistance. *RAR1*, *SGT1* and the cytosolic molecular chaperone *HSP90* have all been shown to be required for *Lr21* wheat leaf rust resistance [44], while *Lr10*-mediated leaf rust resistance requires the presence of a second Resistance Gene Analogue [45]. Mutations to genes such as *RAR1*, *SGT1*, *HSP90* or other currently unknown regulators of R-gene function may be responsible for the loss of *Yr5* resistance in these five EMS-derived *Yr5* mutants.

## Conclusions

*Yr5* still has potential as an effective source of yellow rust resistance if deployed in combination with other R-genes and sources of partial, but potentially more durable yellow rust resistance. Two approaches were undertaken to identify gene sequences within the genetic region defining the *Yr5* gene, demonstrating the power of comparative genomic analyses and *in silico* mapping to identify and locate genes. While DNA markers have been published for *Yr5* there are no guarantees that a given marker will be polymorphic between the wheat genotypes within a breeding program, so the DNA sequences shown here to co-locate with *Yr5* provide a potential new resource of informative DNA sequences for *Yr5* marker introgression. The ideal marker is always within the gene itself, and results for this study and others [43] would indicate that *Yr5* lies within a gene region of the wheat genome rich in NB-LRR-type sequences, potentially being a NB-LRR-type R-gene. The genetic and transcriptomic analyses of the mutants compromised for *Yr5*-mediated resistance indicates the complexity of this yellow rust R-gene, and the dependence of *Yr5* resistance on additional genes for normal function. The mutants described also provide a valuable resource for further work on the functional genomics of *Yr5* resistance.

## Additional files

Supplement Table S1  
 Supplement Table S2  
 Supplement Table S3  
 Supplement Table S4  
 Supplement figure S1  
 Supplement figure S2  
 Supplement figure S3  
 Supplement figure S4  
 Supplement figure S5  
 Supplement figure S6  
 Supplement figure S7

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

Authors' contributions	GRDM	PHS	CB	GRM	TNC	RM	EW	GA	LAB
Research concept and design	✓	--	--	--	--	--	--	--	✓
Collection and/or assembly of data	✓	✓	✓	✓	✓	✓	✓	✓	✓
Data analysis and interpretation	✓	✓	✓	✓	✓	✓	✓	✓	✓
Writing the article	✓	--	✓	--	--	--	--	--	✓
Critical revision of the article	✓	--	✓	--	--	--	--	--	✓
Final approval of article	✓	✓	✓	✓	✓	✓	✓	✓	✓
Statistical analysis	✓	--	--	--	--	--	--	--	✓

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## References

- Wellings C, Boyd LA and Chen X. **Resistance to stripe rust in wheat: pathogen biology driving resistance breeding.** In: Sharma I (Ed), *Disease resistance in wheat*. CABI Plant Protection Series. Wallingford, CABI, 2012; 63-83.
- Jin Y, Szabo LJ and Carson M. **Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host.** *Phytopathology*. 2010; **100**:432-5. | [Article](#) | [PubMed](#)
- Macer RCF. **The formal and monosomic genetic analysis of stripe rust (*Puccinia striiformis*) resistance in wheat.** *Proc 2<sup>nd</sup> Int Wheat Genet Symp, Lund, Hereditas Suppl.* 1963; **2**:127-142.
- Law CN. **Genetic control of yellow rust resistance in *Triticum spelta* album.** *Plant Breeding Institute, Cambridge, Annual Report*, 1976; 108-109.
- Zhang P, McIntosh RA, Hoxha S and Dong C. **Wheat stripe rust resistance genes *Yr5* and *Yr7* are allelic.** *Theor Appl Genet.* 2009; **120**:25-9. | [Article](#) | [PubMed](#)
- Smith PH, Hadfield J, Hart NJ, Koebner RM and Boyd LA. **STS markers for the wheat yellow rust resistance gene *Yr5* suggest a NBS-LRR-type resistance gene cluster.** *Genome.* 2007; **50**:259-65. | [Article](#) | [PubMed](#)
- Cloutier S, McCallum BD, Loutre C, Banks TW, Wicker T, Feuillet C, Keller B and Jordan MC. **Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family.** *Plant Mol Biol.* 2007; **65**:93-106. | [Article](#) | [PubMed](#)
- Feuillet C, Travella S, Stein N, Albar L, Nublal A and Keller B. **Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome.** *Proc Natl Acad Sci U S A.* 2003; **100**:15253-8. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
- Huang L, Brooks SA, Li W, Fellers JP, Trick HN and Gill BS. **Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat.** *Genetics.* 2003; **164**:655-64. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)

10. LaRoche A, Nykiforuk CL, Huel R, Frick MM, Conner RL, Kuzyk A, Eudes F, Acharya S and Jordan M. **Identification of a candidate gene for the wheat stripe rust resistance locus *Yr10***. *International Plant Molecular Biology Meeting*, Quebec, Canada, June 2000; 22-55.
11. Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, Deal K, Luo M, Kong X, Bariana H, Mago R, McIntosh R, Dodds P, Dvorak J and Lagudah E. **The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99**. *Science*. 2013; **341**:786-8. | [Article](#) | [PubMed](#)
12. Sainetnac C, Zhang W, Salcedo A, Rouse MN, Trick HN, Akhunov E and Dubcovsky J. **Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group**. *Science*. 2013; **341**:783-6. | [Article](#) | [PubMed](#)
13. Yahiaoui N, Srichumpa P, Dudler R and Keller B. **Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat**. *Plant J*. 2004; **37**:528-38. | [Article](#) | [PubMed](#)
14. Krattinger SG, Lagudah ES, Spielmeier W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL and Keller B. **A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat**. *Science*. 2009; **323**:1360-3. | [Article](#) | [PubMed](#)
15. Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T and Dubcovsky J. **A kinase-START gene confers temperature-dependent resistance to wheat stripe rust**. *Science*. 2009; **323**:1357-60. | [Article](#) | [PubMed](#)
16. Qin B, Chen T, Cao A, Wang H, Xing L, Ling H, Wang D, Yu C, Xiao J, Ji J, Chen X, Chen P, Liu D and Wang X. **Cloning of a conserved receptor-like protein kinase gene and its use as a functional marker for homoeologous group-2 chromosomes of the triticeae species**. *PLoS One*. 2012; **7**:e49718. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
17. Burt C and Nicholson P. **Exploiting co-linearity among grass species to map the *Aegilops ventricosa*-derived *Pch1* eyespot resistance in wheat and establish its relationship to *Pch2***. *Theor Appl Genet*. 2011; **123**:1387-400. | [Article](#) | [PubMed](#)
18. Gupta PK, Rustgi S and Mir RR. **Array-based high-throughput DNA markers for crop improvement**. *Heredity (Edinb)*. 2008; **101**:5-18. | [Article](#) | [PubMed](#)
19. Coram TE, Settles ML, Wang M and Chen X. **Surveying expression level polymorphism and single-feature polymorphism in near-isogenic wheat lines differing for the *Yr5* stripe rust resistance locus**. *Theor Appl Genet*. 2008; **117**:401-11. | [Article](#) | [PubMed](#)
20. Mitra RM, Gleason CA, Edwards A, Hadfield J, Downie JA, Oldroyd GE and Long SR. **A Ca<sup>2+</sup>/calmodulin-dependent protein kinase required for symbiotic nodule development: Gene identification by transcript-based cloning**. *Proc Natl Acad Sci U S A*. 2004; **101**:4701-5. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
21. Zhang L, Lavery L, Gill U, Gill K, Steffenson B, Yan G, Chen X and Kleinbols A. **A cation/proton-exchanging protein is a candidate for the barley *NecS1* gene controlling necrosis and enhanced defense response to stem rust**. *Theor Appl Genet*. 2009; **118**:385-97. | [Article](#) | [PubMed](#)
22. Ramburan VP, Pretorius ZA, Louw JH, Boyd LA, Smith PH, Boshoff WH and Prins R. **A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar *Kariega***. *Theor Appl Genet*. 2004; **108**:1426-33. | [Article](#) | [PubMed](#)
23. Zadoks JC, Chang TT and Konzak CF. **Decimal code for growth stages in cereals**. *Weed Res*. 1974; **14**:415-421. | [Article](#)
24. Boyd LA, Smith PH, Wilson AH and Minchin PN. **Mutations in wheat showing altered field resistance to yellow and brown rust**. *Genome*. 2002; **45**:1035-40. | [Article](#) | [PubMed](#)
25. Koebner R and Hadfield J. **Large-scale mutagenesis directed at specific chromosomes in wheat**. *Genome*. 2001; **44**:45-9. | [PubMed](#)
26. Bozkurt TO, McGrann GR, MacCormack R, Boyd LA and Akkaya MS. **Cellular and transcriptional responses of wheat during compatible and incompatible race-specific interactions with *Puccinia striiformis* f. sp. *tritici***. *Mol Plant Pathol*. 2010; **11**:625-40. | [Article](#) | [PubMed](#)
27. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A and Speleman F. **Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes**. *Genome Biol*. 2002; **3**:RESEARCH0034. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
28. Van Riet L, Nagaraj V, Van den Ende W, Clerens S, Wiemken A and Van Laere A. **Purification, cloning and functional characterization of a fructan 6-exohydrolase from wheat (*Triticum aestivum* L.)**. *J Exp Bot*. 2006; **57**:213-23. | [Article](#) | [PubMed](#)
29. McGrann GRD, Townsend BJ, Antoniw JF, Asher MJC and Mutasa-Göttgens ES. **Barley elicits a similar early basal defence response during host and non-host interactions with *Polymyxa* root parasites**. *Eur J Plant Pathol*. 2009; **123**:5-15. | [Article](#)
30. Coram TE, Wang M and Chen X. **Transcriptome analysis of the wheat-*Puccinia striiformis* f. sp. *tritici* interaction**. *Mol Plant Pathol*. 2008; **9**:157-69. | [Article](#) | [PubMed](#)
31. Stam P. **Construction of intergrated genetic linkage maps by means of a new computer package: Joinmap**. *Plant J*. 1993; **3**:739-744. | [Article](#)
32. Stam P and van Ooijen JW. **Joinmap version 2.0: software for the calculation of genetic linkage maps**. 1995. CPRO-DLO, Wageningen.
33. Kosambi DD. **The estimation of map distances from recombination values**. *Ann Eugen*. 1944; **12**:173-175. | [Article](#)
34. Agenbag GM, Pretorius ZA, Boyd LA, Bender CM and Prins R. **Identification of adult plant resistance to stripe rust in the wheat cultivar *Cappelle-Desprez***. *Theor Appl Genet*. 2012; **125**:109-20. | [Article](#) | [PubMed](#)
35. Sorrells ME, La Rota M, Bermudez-Kandianis CE, Greene RA, Kantety R, Munkvold JD, Miftahudin, Mahmoud A, Ma X, Gustafson PJ and Qi LL et al. **Comparative DNA sequence analysis of wheat and rice genomes**. *Genome Res*. 2003; **13**:1818-27. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
36. **Genome sequencing and analysis of the model grass *Brachypodium distachyon***. *Nature*. 2010; **463**:763-8. | [Article](#) | [PubMed](#)
37. Dash S, Van Hemert J, Hong L, Wise RP and Dickerson JA. **PLEXdb: gene expression resources for plants and plant pathogens**. *Nucleic Acids Res*. 2012; **40**:D1194-201. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
38. McIntosh RA, Wellings CR and Park RF. (Eds) **Wheat rusts: An atlas of resistance genes**. 1995. East Melbourne, Aust.: CSIRO.
39. Boyd LA, Ridout C, O'Sullivan DM, Leach JE and Leung H. **Plant-pathogen interactions: disease resistance in modern agriculture**. *Trends Genet*. 2013; **29**:233-40. | [Article](#) | [PubMed](#)
40. Michelmore RW, Christopoulou M and Caldwell KS. **Impacts of resistance gene genetics, function, and evolution on a durable future**. *Annu Rev Phytopathol*. 2013; **51**:291-319. | [Article](#) | [PubMed](#)
41. Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I and Moore G. **Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat**. *Nature*. 2006; **439**:749-52. | [Article](#) | [PubMed](#)
42. Hulbert SH, Webb CA, Smith SM and Sun Q. **Resistance gene complexes: evolution and utilization**. *Annu Rev Phytopathol*. 2001; **39**:285-312. | [Article](#) | [PubMed](#)
43. Yan GP, Chen XM, Line RF and Wellings CR. **Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust**. *Theor Appl Genet*. 2003; **106**:636-43. | [PubMed](#)
44. Scofield SR, Huang L, Brandt AS and Gill BS. **Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the *Lr21*-mediated leaf rust resistance pathway**. *Plant Physiol*. 2005; **138**:2165-73. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
45. Loutre C, Wicker T, Travella S, Galli P, Scofield S, Fahima T, Feuillet C and Keller B. **Two different CC-NBS-LRR genes are required for *Lr10*-mediated leaf rust resistance in tetraploid and hexaploid wheat**. *Plant J*. 2009; **60**:1043-54. | [Article](#) | [PubMed](#)

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McGrann GRD, Smith PH, Burt C, Mateos GR, Chama TN, MacCormack R, Wessels E, Agenbag G and Boyd LA. **Genomic and genetic analysis of the wheat race-specific yellow rust resistance gene *Yr5***. *J Plant Sci Mol Breed*. 2014; **3**:2.  
<http://dx.doi.org/10.7243/2050-2389-3-2>