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ORIGINAL PAPER

Stripe rust resistance genes in the UK winter wheat cultivar Claire

N. M. Powell · C. M. Lewis · S. T. Berry · R. MacCormack · L. A. Boyd

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Abstract Stripe rust resistance in the winter wheat cultivar Claire had remained effective in the UK and Europe since its release in 1999 and consequently has been used extensively in wheat breeding programs. However, in 2012, reports indicated that this valuable resistance may now have been compromised. To characterise stripe rust resistance in Claire and determine which genes may still confer effective resistance a cross was made between Claire and the stripe rust susceptible cultivar Lemhi. A genetic linkage map, constructed using SSR, AFLP, DArT and NBS-AFLP markers had a total map length of 1,730 cM. To improve the definition of two quantitative trait loci (QTL) identified on the long arm of chromosome 2D further markers were developed from wheat EST. Stripe rust resistance was evaluated on adult plants under field and glasshouse conditions by measuring the extent of fungal growth and sporulation, percentage infection (Pi) and the necrotic/chlorotic responses of the plant to infection, infection type (IT). Four QTL contributing to stripe rust adult plant resistance (APR) were identified in Claire, *QYr.niab-2D.1*, *QYr.niab-2D.2*,*QYr.niab-2B* and *QYr.niab-7B*. For Pi *QYr.niab-2D.1* explained up to 25.4 % of the phenotypic variation, *QYr.niab-2D.2* up to 28.7 %, *QYr.niab-2B* up to 21.7 % and *QYr.niab-7B* up to 13.0 %. For IT the percentages of phenotypic variation explained were 23.4, 31.8, 17.2 and 12.6 %, respectively. In addition to the four QTL conferring APR in Claire, a race-specific, seedling expressed resistance gene was identified on chromosome 3B.

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N. M. Powell
CSIRO, Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia
e-mail: nicola.powell@csiro.au

C. M. Lewis · R. MacCormack
Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

S. T. Berry
Limagrain UK Ltd, Station Road, Docking, King's Lynn, Norfolk PE31 8LS, UK

L. A. Boyd (✉)
Department of Genetics and Breeding, National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE, UK
e-mail: lesley.boyd@niab.com

Introduction

In a world economy faced with global food insecurity the demand for increased agricultural production has never been greater. The challenge ahead is to supply enough safe and nutritious food to feed a growing population through sustainable agricultural practices. The development of low-chemical input, sustainable agricultural systems is therefore crucial (The Royal Society Report 2009; http://www.royalsociety.org/). Along with rice and maize, wheat provides a substantial proportion of the calorific intake for the human population, either directly, or indirectly through livestock feed (http://faostat.fao.org). By 2030 the demand for wheat is estimated to increase by 40 % (House of Commons Environment, Food and Rural Affairs Committee 2009; http://www.parliament.uk). Disease is a major constraint to sustainable wheat production, with rust pathogens being a significant global problem that

could prevent this target being reached (http://www.globalrust.org).

Stripe rust, caused by the fungus *Puccinia striiformis* f. sp. *tritici* is considered one of the most damaging global diseases of wheat, causing losses in both yield and quality as a consequence of reduced tillering and shrivelled grain (Roelfs et al. 1992). Prevalent in temperate and maritime wheat growing regions, yield losses ranging from 10 to 70 % have been reported (Chen 2005), resulting in estimated world losses of 20 million tonnes per annum (Kosina et al. 2007). Currently the disease is controlled through effective resistance gene deployment and the application of fungicides.

The first genetic characterisation of disease resistance in wheat was to stripe rust in the cv. Rivet (Biffen 1905). Since then many major genes effective against stripe rust have been identified (McIntosh et al. 2008; Wellings et al. 2012). Whilst this approach initially proved effective in controlling stripe rust outbreaks, it soon became apparent that, over time, the resistance in many cultivars was rapidly overcome (Johnson 1983), whereas other cultivars, grown extensively for many years, retained significant levels of resistance. The term 'durable resistance' was subsequently introduced to describe resistance that remained effective in a cultivar during its widespread cultivation, over a long period of time, in an environment favourable to the disease (Johnson and Law 1973; Johnson 1981). The cultivars Cappelle-Deprez (Agenbag et al. 2012), Camp Remy (Boukhatem et al. 2002; Mallard et al. 2005) and Alcedo (Meinel 1997; Jagger et al. 2011a) were described as examples of European cultivars possessing durable stripe rust resistance. The wheat cv. Cappelle-Desprez occupied over 80 % of the UK wheat area for more than 10 years (Parry 1990) and is credited with carrying the durable stripe rust resistance gene *Yr16* (Agenbag et al. 2012). The German cv. Alcedo remained resistant over 15 years of cultivation, occupying 47 % of the German wheat area at its peak in 1981 (Meinel 1997).

In recent years breeding strategies have strived to accumulate more durable sources of resistance, such as *Yr18* and *Yr29*, into modern wheat cultivars (Rosewarne et al. 2008). These genes often express a partial, growth stage specific phenotype referred to as adult plant resistance (APR; Boyd et al. 2006). While individual APR genes may confer partially resistant phenotypes, they can be combined to provide adequate levels of resistance (Singh et al. 2005). It is, therefore, essential to genetically define these quantitative trait loci (QTL) using DNA markers and important that we obtain an understanding of the interaction between such resistance genes (Jagger et al. 2011b; Agenbag et al. 2012).

The winter wheat cv. Claire has had a HGCA rating of nine (complete resistance) for stripe rust on the UK Home Grown Cereals Authority (HGCA) Recommended List (http://www.hgca.com) since its release in 1999. Claire has, therefore, been used extensively as a parent in many wheat breeding programs and stripe rust resistance genes from Claire are believed to be present in many modern cultivars. The stripe rust resistance in Claire represents a valuable source of resistance that needs genetic characterisation.

Materials and methods

Plant materials and mapping populations

The UK winter wheat cv. Claire (pedigree: Flame × Wasp), released in 1999 by Nickerson Seeds Ltd, UK (now Limagrain UK Ltd), was crossed to the American spring wheat cv. Lemhi (pedigree: Dicklow/Federation; Lewis 2006). Claire expressed complete field stripe rust resistance, while Lemhi was susceptible to all UK isolates of *P. s.* f. sp. *tritici*. F_2 and F_3 populations consisting of 183 $F_{2:3}$ families were developed from the cross, as well as a doubled haploid (DH) population, comprising 78 lines, using the wheat-maize technique of Laurie and Bennett (1988) as described in Prins et al. (2005).

Assessment of stripe rust resistance under field conditions

Stripe rust reactions were assessed on the Claire × Lemhi $F_{2:3}$ and DH populations at independent field trial sites near Norwich, Norfolk. In 2002/03 183 F_2 plants were sown at Newfound Farm. In 2003/04 F_3 lines, derived from the F_2 plants, were sown as rows of 11 plants per $F_{2:3}$ family, in three replicated blocks (a total of 33 F_3 plants representing each $F_{2:3}$ family), at another location on the Newfound Farm site. The parental controls and the World and European stripe rust differential sets were included in both the F_2 and F_3 field trials, while Lemhi was included as a susceptible control and spreader within the trials. In 2004/05 and 2006/07 the DH lines was sown in rows of ten plants, in two replicated blocks, on field plots near JIC. The parents were sown every tenth row, and every fourth row was planted with the stripe rust susceptible cv. Avocet S. The World and European differential sets and the Avocet stripe rust NILs were included in the field trials. All replicated trials followed a complete randomisation design. The World and European differential sets and the Avocet stripe rust NILs were included to monitor the virulence profile of the *P. s.* f. sp. *tritici* population within the field trial for additional, naturally introduced races.

Stripe rust was introduced into the field trials in March of each year using *P. s.* f. sp. *tritici* infected spreader plants of Lemhi or Avocet S. Isolates virulent on Claire and

Lemhi at seedling growth stage (GS) 12–13 (Zadoks et al. 1974) were used in field trials (Supplementary Table 1). Levels of stripe rust infection were scored two or three times during the season, approximately 14 days apart. Individual plants were scored for (1) percentage infection (Pi), taken as the percentage of green leaf tissue with sporulating uredinia, using the modified Cobb scale (Peterson et al. 1948) and (2) infection type (IT), recording the extent of the necrotic and chlorotic response of the plant (Supplementary Table 2; McIntosh et al. 1995). The IT scores were converted to numerical values to give an IT nominal (Melichar et al. 2008; Supplementary Table 2). Scoring began once the 1st flag leaf, on the 1st tiller had emerged on Claire and at least 60 % stripe rust infection was seen on the stripe rust susceptible controls Lemhi and Avocet S.

Assessment of stripe rust resistance in seedlings

Seedlings of Claire, Lemhi and each DH line were grown to GS12–13 in a spore-free glasshouse, containment level 2. Glasshouse conditions were maintained at a 16/8 h, 18/14 °C day-night cycle, and relative humidity (RH) of 60 %. Seedlings were inoculated with one of the *P. s.* f. sp. *tritici* isolates listed in Supplementary Table 1 using the protocol described by Boyd and Minchin (2001). Following inoculation plants were transferred to a 100 % humidity chamber, at 10 °C in total darkness for 24 h. It was previously shown that Lemhi was susceptible to all isolates tested, whereas Claire was resistant. Each test was carried out using five seedlings per line. At 14–16 days post inoculation (dpi) stripe rust phenotypes on seedling leaves were scored using the seedling infection type scale (SIT; Supplementary Table 3; Rodrigues et al. 2004). These qualitative SIT scores were converted to a numerical scale for all statistical and QTL analyses (Supplementary Table 3).

Assessment of stripe rust resistance under glasshouse conditions

Plants of Claire, Lemhi and each DH line were grown to GS22 and GS50, in two independent experiments, in a spore-free glasshouse, containment level 2. Glasshouse conditions were maintained at a 16/8 h, 18/14 °C day-night cycle, with RH at 60 %. Plants at GS22 were inoculated with *P. s.* f. sp. *tritici* isolate WYR80/9 (Supplementary Table 1). Adult plants at GS50 were inoculated with the *P. s.* f. sp. *tritici* isolate WYR96/502 (Supplementary Table 1). Claire and Lemhi were seedling susceptible (GS12–13 SIT = 4) to both isolates. Inoculations were carried out as described by Boyd and Minchin (2001). Following inoculation, plants were transferred to a 100 % humidity chamber, 10 °C, in total darkness for 24 h. Inoculated plants were transferred back to the glasshouse under the growth conditions described above. At GS22 the level of stripe rust infection was measured 14–16 dpi using the scoring scale in Supplementary Table 3. At GS50, when most DH lines had produced four tillers, stripe rust Pi and IT were scored at 18–20 dpi on three plants per line, taking an average measure for the whole plant. Pi scores were also measured on the flag leaf (last emerged leaf) of the 1st, 2nd, 3rd and 4th emerged tillers of each of the three plants.

Marker screening and linkage map construction

Claire and Lemhi were screened with 287 publicly available SSR primers, including BARC (Song et al. 2005), CFD (Guyomarc'h et al. 2002), GWM (Röder et al. 1998), PSP (Bryan et al. 1997), WMC (Edwards et al. 1996) and the D genome specific GDM markers (Pestova et al. 2000), and 126 AFLP primer pair combinations (Vos et al. 1995) using the restriction enzymes *Mse*I and *Sda*I (Lewis 2006). Polymorphic SSR markers and AFLP primer pairs were then screened against the Claire × Lemhi F_2 population.

Polymorphic SSR markers identified using the F_2 population were subsequently mapped on the DH population, along with an additional 39 SSRs (Powell 2010). Preliminary QTL analyses using the $F_{2:3}$ populations identified a QTL for stripe rust resistance associated with two AFLP loci; these loci were also screened in the DH population. A DArT marker screen was performed on the DH population by Diversity Arrays Technology Pty Ltd using the Wheat *Pst*I (*Taq*I) v2.3 array (http://www.triticarte.com.au/; Yarralumla, ACT, Australia). NBS-AFLP loci, polymorphic between Claire and Lemhi, were identified using the restriction enzymes *Mse*I and *Rsa*I in combination with the primer NBS2 (van der Linden et al. 2004) and the cereal-specific primer NBS2cer (Sayar-Turet et al. 2011). Polymorphic NBS-AFLP loci were mapped on the DH population.

Wheat EST (197) which had been physically mapped to the wheat telomeric deletion bin 2DL 0.76–1.00 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) were used to develop 22 genetic markers. A BLASTn pairwise alignment was carried out between low copy number wheat EST and the rice genomic sequence, using the NCBI web interface (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify predicted exon-intron-exon junctions within the wheat EST. Forward and reverse primers were designed to the exon sequences that flanked the exon-intron-exon junction sites, thereby producing a PCR amplicon that spanned the predicted intron (Supplementary Table 4; Powell 2010). Primers were designed using the default settings of Primer 3 (http://primer3.sourceforge.net/; Rozen and Skaletsky

2000). The putative function of each EST was identified by searching against the NCBI non-redundant protein sequence database using the BLASTx algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The corresponding unigene of each EST was identified using the EST identifier to search the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). The corresponding unigene sequence was then subjected to a BLASTx search against the NCBI non-redundant protein sequence database.

The DH mapping population was screened with the EST-derived primer pairs at the industrial partner Limagrain UK Ltd. The primer pairs were fluorescently labelled and screened using a DNA Analyzer Gene ReadIR 4200, Li-COR sequencing system (Li-Cor Biosciences UK Ltd).

Joinmap (version 3.0 for Windows) was used to construct genetic linkage maps of the Claire × Lemhi F_2 and DH populations (Van Ooijen and Voorrips 2001; Lewis 2006; Powell 2010). A Chi-squared goodness-of-fit analysis (Snedecor and Cochran 1989) was carried out on the marker loci segregation ratios to identify marker loci that deviated significantly from the expected 3:1 and 1:2:1, or 1:1 ratios for F_2 and DH populations, respectively. Linkage groups were constructed using a minimum LOD of 5.0 and a recombination fraction of <0.4. Recombination fractions were converted to map distances (cent-Morgans; cM) using the Kosambi mapping function (Kosambi 1944). Ordering of markers and assignment of linkage groups to chromosomes were checked against published wheat consensus maps (http://wheat.pw.usda.gov and http://shigen.nig.ac.jp/wheat).

Quantitative trait loci analyses

Quantitative trait loci (QTL) analysis was performed using MapQTL version 5 (van Ooijen 2004). Interval mapping (Lander and Botstein 1989) was carried out to locate regions within the genome associated with the resistance phenotypes. MQM mapping was used to confirm the identification of minor QTL (Jansen and Stam 1994) selecting markers associated with major QTL as co-factors, supported by the backward elimination program in the automatic cofactor selection tool ($p = 0.001$). Genome wide and linkage group significant LOD thresholds were obtained for each data set analysed by carrying out a permutation test with 1,000 permutations (Churchill and Doerge 1994).

Pi and IT nominal data sets obtained from the field and glasshouse adult plant trials were used to identify QTL associated with stripe rust resistance in the Claire × Lemhi populations. The SIT nominal scores, for both the 1st and 2nd leaves of seedlings at GS12–13, and for plants at GS22, were analysed to identify genes expressing stripe rust resistance in Claire at these growth stages. For all data sets both untransformed and transformed data and predicted means obtained across replicates were used in the QTL analyses to have confidence in the QTL identified. The results presented are those from the predicted means obtained for each data set.

DH lines were grouped depending on the combinations of QTL they carried. The presence of a QTL was defined by the parental alleles of the QTL-associated markers with the highest LOD values in the QTL interval, using 3–4 marker loci to define each QTL region. The predicted means for Pi and IT were calculated for each QTL group.

Statistical analyses

All analyses were performed using the statistical package Genstat for Windows, release 12 (Genstat 12 Committee 2009; Payne et al. 2009). The Pi and IT nominal scores were transformed to achieve near normality and independence of means and variances. Pi scores were transformed using a LOGIT + transformation adapted to deal with percentage data where the upper and lower limits of 100 and 0 % can be recorded (Arraiano et al. 2006; Lewis 2006).

$$\mathrm{LOGIT}^{+} = \mathrm{Log_n}[(\mathrm{Piscore} + (\mathrm{minimumPi} + 0.25))/((\mathrm{maximumPi} + 0.25) - \mathrm{Piscore})]$$

$\mathrm{Log_n}$ is natural logarithm, Pi score is individual data point score.

Adjusted log transformation was used to achieve near normality for the IT nominal scores (Ramburan et al. 2004).

$$\mathrm{Logtransformation} = \mathrm{In.}(\mathrm{ITnominal} + 1)$$

An analysis of variance (ANOVA) was used on the untransformed and transformed data sets using the general linear regression option in Genstat v. 12. The effects of test replications and genotypes were accounted for in the model. The outputs from the ANOVA provided predicted means of each Pi, IT and SIT nominal data set. The values of variance obtained from the ANOVA were used to calculate narrow sense heritability (Lillemo et al. 2008) using the following formula:

$$\mathrm{Heritability}\ (h^2) = \sigma_g^2/\sigma_p^2$$

where $\sigma_g^2 = (\sigma_l^2 - \sigma_e^2)/r$ and $\sigma_p^2 = \sigma_g^2 + \sigma_e^2/r$, $\sigma_g{}^2$ is genetic variance, $\sigma_p{}^2$ is phenotypic variance, $\sigma_l{}^2$ is variance in DH lines, $\sigma_e{}^2$ is error variance and r is number of replications

The total phenotypic variation explained by the QTL identified by each phenotypic data set was divided by the h^2 value, providing a measure of the total genetic variation explained by the QTLs detected.

For seedling stripe rust resistance tests (GS12–13) segregation ratios were used to postulate gene number by Chi-squared goodness-of-fit. The cumulative probability based on

the Chi-squared statistic was calculated using an online program (http://stattrek.com/online-calculator/Chi-square.aspx).

To test the significance of QTL combinations the general linear regression model compared DH lines between and within QTL groups using *t* test comparisons, with all QTL groups being compared with other groups (Jagger et al. 2011a).

Results

Phenotypic assessment of stripe rust resistance in the Claire × Lemhi cross

To assess stripe rust APR in the Claire × Lemhi cross field trials were undertaken over 4 years on $F_{2:3}$ and DH populations inoculated with isolates of *P. s.* f. sp. *tritici* to which both Claire and Lemhi were susceptible at seedling GS12–13 (Fig. 1). An adult plant trial was also undertaken on the DH population under glasshouse conditions (Fig. 1). The Pi scores, for both the $F_{2:3}$ and DH populations displayed a positively skewed distribution, indicating the presence of a resistance gene of major effect segregating within the Claire × Lemhi cross. There were no stripe rust pustules on adult plants of Claire under field or glasshouse conditions, whereas Lemhi had consistently high Pi scores of 80–100 % in the field and 90 % in the glasshouse adult plant trial. The frequency distributions of IT scores from field and glasshouse adult plant trials showed more continuous distributions (Fig. 1). However, correlation analyses indicted that the Pi and IT nominal scores were correlated, with correlation coefficients (r^2) ranging from 0.75 to 0.90. The highest correlation was seen in the glasshouse trial, with an $r^2 = 0.9$, while in the field trials correlation between the two phenotypic scores was higher at the later scoring dates. Claire exhibited a fleck phenotype (IT nominal = 0.1) under both field and glasshouse conditions, whereas Lemhi had an IT nominal of 0.7–0.8, indicating an MS/S to S phenotype.

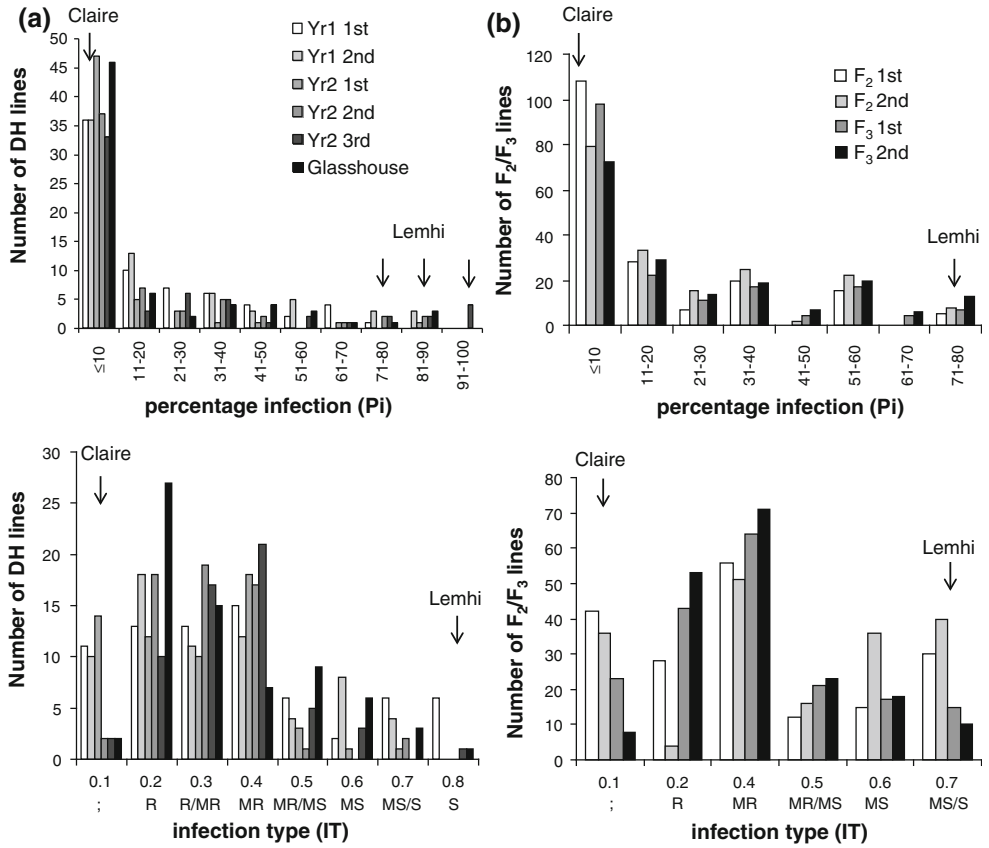

Fig. 1 Phenotypic variation in stripe rust adult plant resistance in the Claire × Lemhi cross. Distribution of percentage infection (Pi) and infection type (IT) phenotypes in the Claire × Lemhi cross **a** doubled haploid (DH) and **b** $F_{2:3}$ populations, recorded over 2 years of field trials and in a glasshouse adult plant trial in the case of the DH population. For the DH population Pi and IT scores are shown for two scoring dates in year 1 (Yr1), three scoring dates in year 2 (Yr2) and a single score taken in the glasshouse trial. For the $F_{2:3}$ populations two scoring dates are shown. IT are fleck (;), resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Parental scores are indicated by *arrows*

For both Pi and IT highly significant differences were found between $F_{2:3}$ families and DH lines across all years of field data and in the glasshouse adult plant trial (*F*-value $P < 0.001$). Differences between field replicates for the first Pi scores on the DH population (Yr1Pi-1st and Yr2Pi-1st) and in the glasshouse trial (Table 1) were significant, probably reflecting uneven disease establishment across the field trials early in the season, and uneven inoculation and infection in the glasshouse trial. High narrow sense heritabilities (h^2) in the DH population for both Pi and IT indicated a strong genetic component affecting both stripe rust phenotypes (Table 1). Correlations between Pi and IT values over the two scoring dates in year 1 ($r^2 = 0.89$ and 0.81, respectively) were higher than over the three scoring dates in year 2 ($r^2 = 0.71$ and 0.74, respectively). This suggested that in year 2 the disease did not progress at the same rate in all DH lines.

Table 1 Analysis of variance of the adult plant stripe rust infection phenotypes in the Claire × Lemhi DH population

Infection phenotype	Source	F-prob.[a]	*Her*[b]	*R2*[c]	*p*[d]
Yr1Pi-1st	DH line	<0.001	0.899	45.1	50.17
	Rep	0.001			
Yr1IT-1st	DH line	<0.001	0.889	70.8	79.64
	Rep	0.56			
Yr1Pi-2nd	DH line	<0.001	0.886	77.1	87.02
	Rep	0.010			
Yr1IT-2nd	DH line	<0.001	0.855	76.5	89.47
	Rep	0.926			
Yr2Pi-1st	DH line	<0.001	0.902	55.1	61.09
	Rep	<0.001			
Yr2IT-1st	DH line	<0.001	0.823	27.4	33.29
	Rep	0.025			
Yr2Pi-2nd	DH line	<0.001	0.925	44.4	48.00
	Rep	0.044			
Yr2IT-2nd	DH line	<0.001	0.871	41.9	48.11
	Rep	0.008			
Yr2Pi-3rd	DH line	<0.001	0.896	54.4	60.72
	Rep	0.005			
Yr2IT-3rd	DH line	<0.001	0.858	40.8	47.55
	Rep	0.174			
Pi-Glasshouse	DH line	<0.001	0.905	61.8	68.29
	Rep	<0.001			
IT-Glasshouse	DH line	<0.001	0.868	48.0	55.30
	Rep	0.135			

[a] F-value probabilities for Pi and IT nominal data sets in years 1 (Yr1) and 2 (Yr2) for the first (1st), second (2nd) and third (3rd) scoring dates, and the glasshouse adult plant trial

[b] *Her* narrow sense heritability estimates

[c] R^2 total phenotypic variation (%) explained by the QTL identified with each data set

[d] *p* total genotypic variation (%) explained by the QTL identified with each data set

Although Claire is seedling susceptible to current UK races of *P. s.* f. sp. *tritici,* tests with older isolates indicated that Claire possessed seedling expressed, race-specific stripe rust resistance. Two *P. s.* f. sp. *tritici* isolates (Supplementary Table 1), to which Claire exhibited a resistant SIT (SIT = ; to 0^n; Supplementary Table 3), were used to test the DH population for seedling resistance in glasshouse tests. A single gene in Claire conferred resistance to isolates WYR92/1 ($\chi^2_{1:1} = 0.07$, $P_{1\ df} = 0.80$) and WYR76/10 ($\chi^2_{1:1} = 0.3$, $P_{1\ df} = 0.60$). Subsequent QTL analyses confirmed the presence of a single gene in Claire conferring resistance to both isolates.

Construction of a genetic linkage map for the Claire × Lemhi cross

A genetic map was constructed for the Claire × Lemhi cross using SSR, DArT, AFLP, NBS-AFLP and EST-derived markers. The genetic map was first created using the F_2 population. However, low levels of polymorphism between Claire and Lemhi and subsequent limited identification of stripe rust resistance QTL using the $F_{2:3}$ populations resulted in transfer of SSR and AFLP markers to the DH population. The genetic map was then improved by the addition of DArT, NBS-AFLP and EST-derived markers to the DH population.

Of 287 SSR markers screened against Claire and Lemhi only 82 (29 %) were polymorphic, and of these, only 43 (52 %) could be mapped in the Claire × Lemhi F_2 population (Lewis 2006). Thirty-six of these SSR markers were subsequently mapped in the DH population. From 126 AFLP primer pair combinations screened 66 polymorphic bands were identified. Forty-eight AFLP loci were mapped in the Claire × Lemhi F_2 population (Lewis 2006). Preliminary QTL analyses of the $F_{2:3}$ populations identified a stripe rust APR QTL associated with AFLP loci S15M32-155 and S15M45-190. These AFLP primer pairs were, therefore, screened on the Claire × Lemhi DH mapping population. To improve the density of DNA markers DArT markers were added to the DH population, and NBS-AFLP markers were used to target regions of the genome containing potential NBS-type disease resistance genes (van der Linden et al. 2004). Additional chromosome 2D SSR markers were added to the DH population after initial QTL analyses identified QTL on that chromosome.

Joinmap version 3.0 for windows was used to create a linkage map of the Claire × Lemhi cross. Any marker loci with more than 10 % missing data, or which exhibited segregation distortion ($p < 0.01$), were removed from the linkage analysis. A total of 320 marker loci were finally used to construct a map (251 DArT, 54 SSRs, 10 EST-

derived, 3 AFLP and 2 NBS-AFLP marker loci). This produced 49 linkage groups, representing all 21 wheat chromosomes and a total map length of 1,730 cM (Supplementary Fig. 1). Three linkage groups could not be assigned to a chromosome due to the absence of an SSR or DArT marker locus with a known chromosomal location.

The CMAP programme (http://wheat.pw.usda.gov) was used to compare the locations of SSR marker loci in the Claire × Lemhi genetic map with their locations in other published genetic maps of hexaploid wheat, including the wheat consensus map (Somers et al. 2004), the wheat composite map (http://shigen.nig.ac.jp/wheat/komugi/maps/markermap.jsp) and the ITMI map (Song et al. 2005). The majority of SSR marker loci mapped to their predicted chromosomes, the exceptions being *Xwmc*154 (mapped to 3BS) and *Xgwm*357 (mapped to the 5BL/7BL translocation), where the expected locations were 2BS and 1AL, respectively. A high proportion of DArT markers tended to cluster to the same locus, thereby reducing the amount of unique information on the genetic map. Of the clustered DArT markers 184 (42.2 % of mapped DArT loci) were removed, leaving 251 DArT loci in the map.

The AFLP locus *S15M32-155,* associated with stripe rust APR in the Claire × Lemhi $F_{2:3}$ populations, was linked to the SSR locus *Xgwm301*, placing the AFLP locus within the telomeric deletion bin 0.76–1.00 on chromosome 2DL. Wheat EST (197) physically mapped to this bin (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) were used to develop EST-derived markers (Supplementary Table 5). Ninety-four EST, that represented low copy number genes and hybridised specifically to group 2 chromosomes, were selected. A BLASTn pairwise alignment was carried out between the 94 wheat EST and the rice genomic sequence, using the NCBI web interface (http://blast.ncbi.nlm.nih.gov/Blast.cgi), to identify predicted exon-intron-exon junctions within the wheat EST (Supplementary Table 5). Thirty-five (37.2 %) wheat EST were predicted to have no introns, 15 (16 %) had introns >500 bp in length and eight (8.5 %) had introns of <50 bp in length. Two EST (2.1 %) had no significant pairwise alignments with the rice genomic sequence and 12 (12.8 %) had low sequence similarities to rice, having e-values greater than $1e \times 10^{-15}$. For the remaining 22 EST, forward and reverse primers were designed to the exon sequences that flanked the exon-intron-exon junction sites, thereby producing PCR amplicons that spanned the predicted intron (Supplementary Table 4; Powell 2010).

All 22 primer pairs generated multiple PCR products, potentially identifying homoeologous loci from each of the three wheat genomes. Eleven EST-derived primer pairs were polymorphic between Claire and Lemhi, and except for EST18, amplified a single polymorphic locus. EST18 amplified three polymorphic loci and each locus was given a lower case letter (a, b and c) for identification. All 13 EST-derived marker loci were screened in the DH mapping population with ten mapping to linkage groups. Six mapped to the expected location on the long arm of chromosome 2D, three mapped to chromosome 2AL and one, EST18c, to the 5BL/7BL translocation (Supplementary Fig. 1).

The 22 wheat EST selected for primer design and their corresponding unigenes were subjected to BLASTx similarity searches to assign putative functions (Supplementary Table 5). All 22 EST showed high levels of similarity to sequences with predicted functions. These functions included hydrolase and transferase activity, transporters, elongation factors and protein kinases. Two EST mapped to regions where QTL for stripe rust resistance were identified in the Claire × Lemhi cross. EST22 mapped within the *QYr.niab-2D.1* region; it resembled a group of aminotransferases which are involved in a diverse variety of processes, including metabolism, photorespiration and plant stress responses (Liepman and Olsen 2004). EST18a mapped close to *QYr.niab-2D.2* and resembled a GDP-mannose transporter (Linka and Weber 2010).

QTL analyses of stripe rust adult plant resistance in the Claire × Lemhi cross

Interval mapping identified four QTL in Claire explaining up to 88.8 % of the Pi and 85.0 % of the IT phenotypic variation in the Claire × Lemhi cross (Table 2). Two QTL located on chromosome 2DL, *QYr.niab-2D.1* and *QYr.niab-2D.2*, accounted for a large portion of the stripe rust APR in Claire (Fig. 2). *QYr.niab-2D.1* was consistently expressed in all adult plant trials. However, examination of flag leaves showed resistance conferred by *QYr.niab-2D.1* to increase with flag leaf age (Supplementary Table 6). The expression of *QYr.niab-2D.2* was more inconsistent, being more evident in the 2004/05 (year 1) and the glasshouse adult plant trials, than in the 2006/07 (year 2) field trial (Table 2). Resistance conferred by *QYr.niab-2D.2* was, however, equally effective in the flag leaves of all four tillers examined (Supplementary Table 6).

In both years of field trials a QTL was detected in Claire on chromosome 2BL. *QYr.niab-2B* explained up to 21.7 % of the phenotypic variation in Pi and 17.2 % of the variation in IT (Table 2). Although *QYr.niab-2B* was not detected in the glasshouse adult plant trial using the whole plant Pi and IT datasets, it was detected using the individual flag leaf Pi scores, resistance being stronger in the flag leaves of the older tillers (Supplementary Table 6).

A fourth QTL, *QYr.niab-7B,* was detected on chromosome 7BL in both the field and glasshouse adult plant trials (Table 1), but not when individual flag leaves were

Table 2 Stripe rust adult plant resistance QTL detected in the Claire × Lemhi cross

Dataset[a]	Chrom.[b]	Locus[c]	Peak LOD[d]	Explained variance[e]	Phenotypic means[f]	
					C	*L*
PiYr1-1st	2D.1	*EST22*	2.6	18.3	10.6	28.1
	2D.2	*S15M32-155*	4.7	26.8	2.8	23.6
PiYr1-2nd	2B	*wPt-9190*	1.7	10.5	10.0	25.6
	2D.1	*EST22*	3.6	25.4	9.2	32.5
	2D.2	*S15M32-155*	5.1	28.7	8.7	39.5
	7B	*wPt-1069*	2.0	12.5	11.5	29.5
		wPt-4057				
PiYr2-1st	2B	*wPt-9190*	2.4	18.7	6.9	21.8
	2D.1	*EST22*	1.6	11.9	3.9	15.3
	2D.2	*S15M32-155*	1.9	13.7	4.0	16.7
	7B	*wPt-1069*	1.6	10.8	6.6	17.5
PiYr2-2nd	2B	*wPt-9190*	1.8	12.9	6.2	21.8
		wPt-0950				
	2D.1	*EST22*	2.1	18.5	7.3	29.2
		wPt-4413				
	7B	*wPt-1069*	1.8	13.0	8.3	26.6
PiYr2-3rd	2B	*wPt-9190*	2.9	21.7	11.2	41.7
	2D.1	*EST22*	2.4	20.1	9.8	39.7
		wPt-4413				
	7B	*wPt-1069*	1.7	12.6	12.6	35.3
		wPt-4057				
Pi- Glasshouse	2D.1	*EST22*	3.2	21.6	3.0	18.0
	2D.2	*S15M32-155*	4.1	28.4	2.0	16.5
	7B	*wPt-1069*	1.8	11.8	2.8	11.0
ITYr1-1st	2B	*wPt-9190*	1.8	11.1	0.36	0.49
	2D.1	*EST22*	2.1	16.7	0.35	0.54
	2D.2	*S15M32-155*	5.8	31.8	0.32	0.60
	7B	*wPt-1069*	1.8	11.2	0.35	0.50
ITYr1-2nd	2B	*wPt-9190*	1.6	10.4	0.37	0.51
	2D.1	*EST22*	3.4	23.4	0.35	0.57
	2D.2	*S15M32-155*	5.3	30.7	0.33	0.59
	7B	*wPt-1069*	1.9	12.0	0.36	0.53
		wPt-3190				
ITYr2-1st	2B	*wPt-9190*	2.3	17.2	0.20	0.35
	2D.1	*EST22*	1.4	10.2	0.22	0.35
ITYr2-2nd	2B	*wPt-9190*	1.7	13.4	0.27	0.40
	2D.1	*EST22*	2.1	16.6	0.29	0.44
		wPt-4413				
	7B	*wPt-3190*	1.7	11.9	0.28	0.39
		wPt-4057				
ITYr2-3rd	2B	*wPt-9190*	1.5	12.1	0.32	0.42
	2D.1	*EST22*	1.8	16.1	0.30	0.42
		wPt-4413				
	7B	*wPt-3190*	1.7	12.6	0.31	0.42
		wPt-4057				

Table 2 continued

Dataset[a]	Chrom.[b]	Locus[c]	Peak LOD[d]	Explained variance[e]	Phenotypic means[f]	
					C	*L*
IT-Glasshouse	2D.1	*EST22*	2.9	21.4	0.3	0.5
	2D.2	*S15M32-155*	4.5	26.6	0.3	0.5

[a] The datasets used are the predicted means of the 1st, 2nd and 3rd score dates of Pi and IT measured in field trials in year 1 (Yr1) and year 2 (Yr2) and in a glasshouse adult plant trial

[b] Chromosomal location of QTL identified by interval mapping, using MapQTL v. 5.0

[c] Marker loci associated with QTL peak

[d] Maximum LOD score associated with QTL peak

[e] Percentage phenotypic variance explained

[f] Phenotypic means of allelic classes at QTL. LOD thresholds, based on a *p* value of 0.05, ranged from 1.4 to 1.6. C, cv. Claire. L, cv. Lemhi

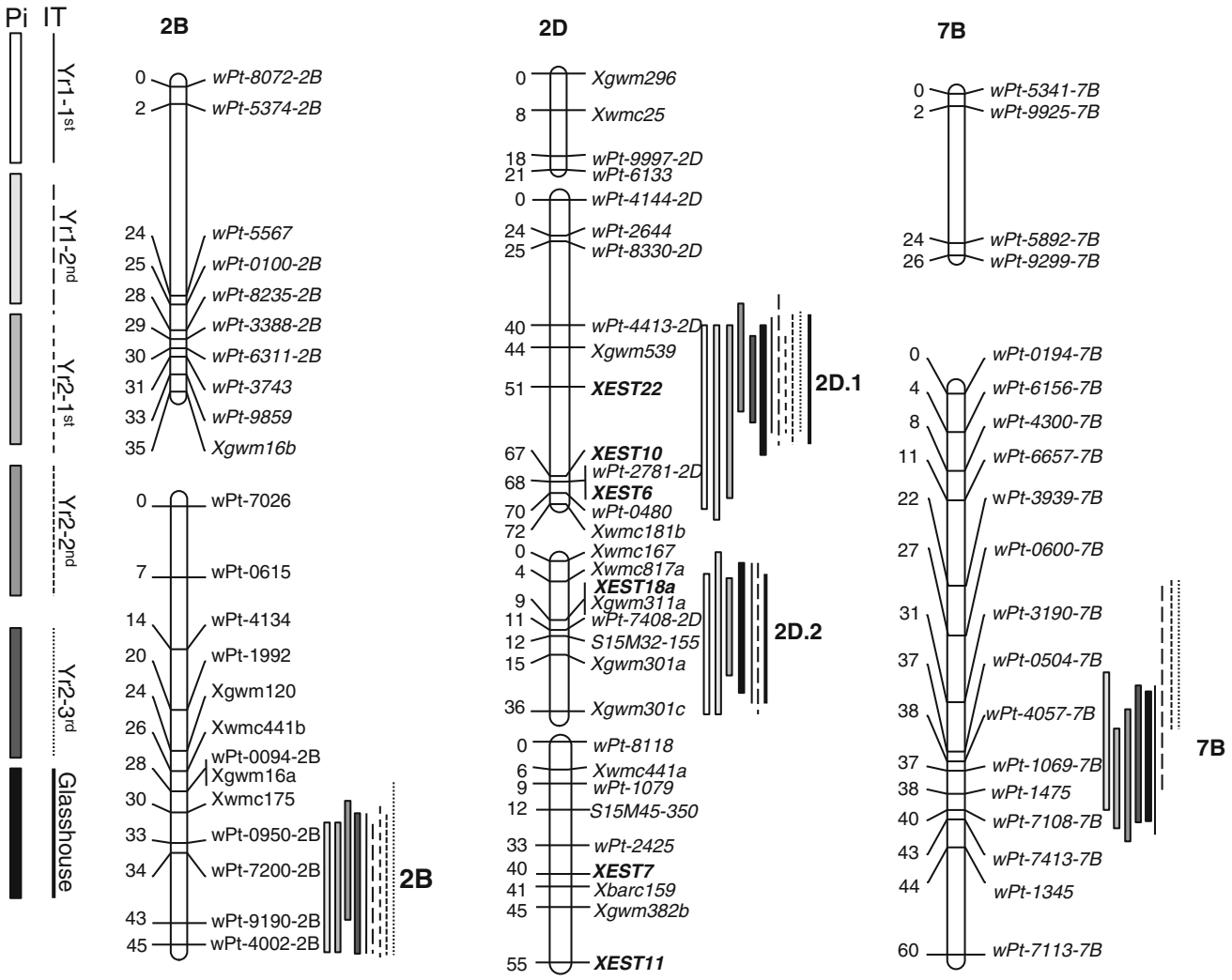

Fig. 2 QTL for stripe rust adult plant resistance identified in the Claire × Lemhi cross. The chromosomal locations of adult plant resistance (APR) QTL, identified using percentage infection (Pi) and infection type (IT) nominal scores, recorded over 2 years of field trials (Yr1 and Yr2) and in a glasshouse adult plant trial. Pi and IT nominal score data sets are shown for two scoring dates in year 1, three scoring dates in year 2 and a single score taken in the glasshouse trial

assessed (Supplementary Table 6). No additional QTL for stripe rust resistance were found using MQM and a Kruskal–Wallis single marker regression analysis did not identify any unlinked markers associated with stripe rust resistance in the Claire × Lemhi cross.

The DH lines were grouped into 16 genotypes depending on which of the four QTL were present. The parental alleles of the marker loci closest to each QTL were used to define the presence/absence of the QTL. The marker loci used to detect each QTL were as follows: *QYr.niab-2B*: marker loci *wPt-4002*, *wPt-9190* and *wPt-7200*; *QYr.niab-2D.1*: marker loci *Xgwm539*, *wPt-4413* and *EST22*; *QYr.niab-2D.2*: marker loci *wPt-7408*, *S15M32-155* and *Xgwm301a* and *QYr.niab-7B*: marker loci *wPt-4057*, *wPt-1069*, *wPt-1475* and *wPt-1708*. The predicted Pi and IT means for each DH line were used to calculate an average score for each QTL genotype group (Supplementary Fig. 2).

In year 1 there was a significant reduction in disease (Pi values) when *QYr.niab-2D.1* was combined with *QYr.niab-2D.2* or *QYr.niab-2B* (*t* test $P < 0.01$), but not when combined with *QYr.niab-7B*. However, the difference in Pi values between these genotypic groups was not significantly different with the year 2 data sets. When *QYr.niab-2D.2* was combined with *QYr.niab-2B* or *QYr.niab-7B* there was a reduction in stripe rust Pi values, but this was not significant (*t* test $P = 0.021$–0.222 and 0.072–0.488, respectively). The interaction between *QYr.niab-2B* and *QYr.niab-7B* was not tested, as no DH line with only these two QTL was identified. A similar effect of combining QTL was seen with the IT phenotypic data sets (Supplementary Fig. 2).

QTL analyses of stripe rust resistance at growth stages (GS) 12–13 and 22

To examine the earliest growth stage at which APR could be detected, the DH population, Claire and Lemhi were inoculated at GS22 (2-tiller stage) with the *P. s.* f. sp. *tritici* isolate WYR80/91 (Supplementary Table 1). WYR80/91 was virulent on seedlings (GS 12–13) of Claire (SIT = 3) and Lemhi (SIT = 4). By GS 22 Claire exhibited IT = 1^{C}, whereas Lemhi maintained an IT = 4. Interval mapping identified a stripe rust resistance QTL on chromosome 2DL, in the same location as *QYr.niab-2D.2* (Table 3).

Although Claire and Lemhi were susceptible as seedlings (GS12–13) to the prevalent UK *P. s.* f. sp. *tritici* races, two *P. s.* f. sp. *tritici* isolates, WYR76/10 and WYR92/1 (Supplementary Table 1) were avirulent on Claire at GS12–13 (SIT = ; to 0^{n}), but virulent on Lemhi (SIT = 4). With both isolates a single QTL on chromosome 3BL was identified (Table 3; Supplementary Fig. 1).

Table 3 Stripe rust resistance QTL detected in the Claire × Lemhi cross at growth stages (GS) 12–13 and 22

Dataset	Chrom[a]	Locus[b]	Peak LOD[c]	Explained variance[d]	Phenotypic means[e]	
					C	*L*
GS 12–13						
Isolate WYR76/10	3BL	*wPt-8845* *wPt-4412*	35.4	96.5	1.3	5.9
Isolate WYR92/1	3BL	*wPt-8845* *wPt-4412*	40.8	96.8	1.5	6.0
GS 22						
Isolate WYR80/9	2DL	*S15M32-155* *Xgwm301a*	5.37	36.1	3.3	5.1

[a] Chromosomal location of QTL identified by interval mapping, using MapQTL v. 5.0

[b] Marker loci associated with QTL peak

[c] Maximum LOD score associated with QTL peak

[d] Percentage phenotypic variance explained

[e] Phenotypic means of allelic classes at QTL. LOD thresholds, based on a *p* value of 0.05, ranged from 1.4 to 1.6. C, cv. Claire. L, cv Lemhi

A potential suppressor of *QYr.niab-2D.2*

Thirty-seven Claire × Lemhi DH lines contained *QYr.niab-2D.2*; however, contrasting phenotypes were observed among those lines carrying the same combinations of QTL. Eighteen of the *QYr.niab-2D.2* containing lines had a partial stripe rust resistance phenotype, while 19 were fully resistant (Fig. 3). Only two DH lines (11 and 53) carried *QYr.niab-2D.2* alone and both exhibited partial resistance. However, two (42 and 49) of the six lines containing both *QYr.niab-2D.1* and *QYr.niab-2D.2* were fully resistant, with IT to 0R, whereas lines 6, 27, 33 and 66 showed intermediate resistance, with final stripe rust scores in years 1 and 2 of 22.5MS and 52.5MR, 15MR and 30MR, 2.5R/MR and 70MR/MS, and 7.5R/MR and 82.5MS, respectively. Comparing the DH lines lacking *QYr.niab-2D.2*, but containing the same QTL complement, resulted in lines with similar phenotypes, thus excluding the possibility of an unidentified QTL. These results suggest the presence of a suppressor effect, presumably originating from Lemhi and acting preferentially on *QYr.niab-2D.2*. Further statistical and QTL analyses could not locate genetic loci responsible for these phenotypic differences. The phenotypic data were adjusted by an analysis of covariance to remove all phenotypic variation ascribed to *QYr.niab-2B*, *QYr.niab-2D.1* and *QYr.niab-7B*, but subsequent QTL analyses, using all the DH lines, identified only *QYr.niab-2D.2*. Similarly, QTL Cartographer v. 1.16 (Basten et al. 2002) confirmed the locations of *QYr.niab-2B*, *QYr.niab-2D.1*, *QYr.niab-2D.2* and *QYr.niab-7B*, but again

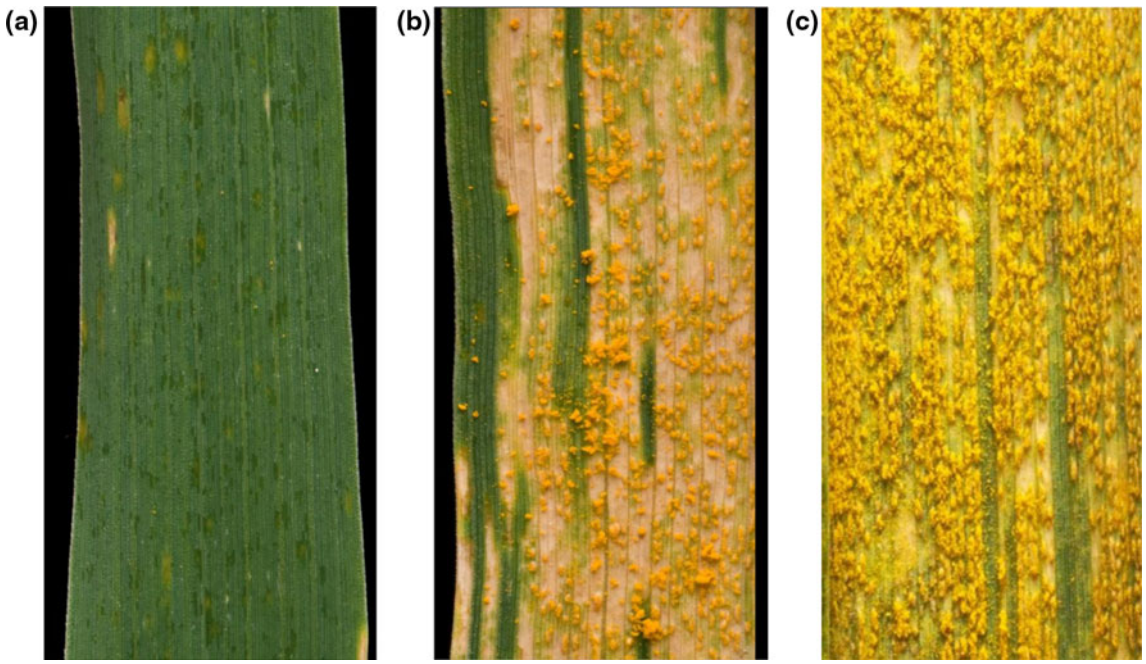

Fig. 3 Stripe rust infection phenotypes on flag leaves of DH lines from the Claire × Lemhi cross. Stripe rust infection phenotypes observed under glasshouse conditions, **a** DH51 (0R) and **b** DH16 (45MR). Both lines carry QTL *QYr.niab-2B*, *QYr.niab-2D.1* and *QYr.niab-2D.2*, **c** cultivar Lemhi

did not locate additional genetic regions that could account for the differences in phenotypes.

Discussion

Breeding for wheat stripe rust resistance in the UK has relied heavily on a small number of race-specific resistance (R−) genes. This has resulted in resistance breakdown often affecting more than one cultivar at the same time. In 1997 the HGCA stripe rust resistance ratings of several UK winter wheat cultivars were downgraded when the stripe rust resistance gene *Yr17* was overcome. In 2009, a widely used, but uncharacterised stripe rust resistance gene/s became ineffective in a number of the top rated UK cultivars (Impey 2009), and in 2011/12 it became evident that at least part of the stripe rust resistance in the cv. Claire had become ineffective (Abel 2012; HGCA Topic Sheet 117) due to the appearance of a new virulent UK *P. s.* f. sp. *tritici* race.

Claire has been extensively used as a parent in wheat breeding and is in the pedigree of many newer cultivars. Four QTL for stripe rust APR were detected, which together explained 85.0–88.8 % of the phenotypic variation in the Claire × Lemhi cross. Neither MQM or Kruskal–Wallis analysis indicated the presence of additional QTL for stripe rust APR, and while some variation in expression of resistance was seen across score dates and environments, all QTL consistently mapped to the same region of the wheat genome.

Two QTL, *QYr.niab-2D.1* and *QYr.niab-2D.2*, were detected on chromosome 2DL. *QYr.niab-2D.1* mapped to a region where the partial APR gene *Yr16* had previously been identified (Worland and Law 1986; Mallard et al. 2005; Agenbag et al. 2012). This resistance is present in the cv. Cappelle-Desprez (Worland and Law 1986), a cultivar which was used extensively in UK wheat breeding (Angus 2001). *QYr.niab-2D.1* was consistently detected with all adult plant data sets, explaining between 10.2 and 25.4 % of the phenotypic variation in the Claire × Lemhi cross, slightly more than reported for *QYr.ufs-2D* (Agenbag et al. 2012). *Yr16* is considered to represent a durable source of stripe rust resistance (Johnson and Law 1973), so it will be interesting to determine the effectiveness of *QYr.niab-2D.1* against the new races of *P. s.* f. sp. *tritici* isolated from Claire in 2011.

The expression of *QYr.niab-2D.2* was more variable and was not detected with most of the field trial data sets collected in 2006/07 (year 2). *QYr.niab-2D.2*, explaining between 13.7 and 31.8 % of the phenotypic variation, was located in the same region of the wheat genome as a QTL for stripe rust resistance in the cv. Alcedo, *QPst.jic-2D* (Jagger et al. 2011a). Alcedo was introduced into UK winter wheat crossing programs during the 1970s, with the first cultivar derived from Alcedo, Apostle, being released in 1980 (Wheat Pedigree On Line http://genbank.vurv.cz/wheat/pedigree). The QTL on chromosome 2DL in Alcedo was identified in a cross to cv. Brigadier and, in this

background, expressed complete stripe rust resistance in all lines carrying the QTL. As with *QYr.niab-2D.2*, the Alcedo *QPst.jic-2D* QTL expressed at early plant growth stages, with the strength of the gene increasing as the plant developed (Jagger et al. 2011b).

Although stripe rust resistance genes have been found on most chromosomes of wheat (Wellings et al. 2012), the B-genome, and in particular chromosome 2B, is a rich source of resistance (Luo et al. 2008). *QYr.niab-2B* mapped to a region on 2BL where a stripe rust resistance QTL was reported in the cv. Deben (Christiansen et al. 2006). Deben (Pedigree: (Hunter × Buster) × Wasp) was released by Limagrain Ltd in 2002 (formally Nickerson (UK) Ltd), the same breeding company that released Claire; Claire and Deben have a pedigree link through cv. Wasp. *QYr.niab-2B* expressed partial resistance, with the glasshouse adult plant trial indicating a growth stage-dependant expression. *QYr.niab-2B* resistance was strongly expressed in the flag leaf of the 1st tiller, but was less effective in flag leaves of the 2nd and 3rd tillers, whereas resistance on the flag leaf of the 4th tiller was not significant. This may also explain why *QYr.niab-2B* was not detected using glasshouse whole plant scores, where an average score for the whole plant was taken.

A fourth, small effect QTL was detected on chromosome 7BL explaining 10.8 − 13.0 % of the phenotypic variation in stripe rust APR. *QYr.niab-7B* was defined only by DArT markers, which in the integrated CIMMYT map were assigned to chromosome 7BL, with the DArT marker wPt-4057 mapping close to the SSR locus *Xgwm344* (http://wheat.pw.usda.gov/cgi-bin/cmap). Stripe rust APR QTL were reported on the long arm of chromosome 7B in cv. Alpowa (Lin and Chen 2007) and Pastor (Rosewarne et al. 2012). The QTL from Alpowa, between SSR marker loci *Xgwm43* and *Xgwm131*, is at some distance from *Xgwm344* (Wheat Consensus Map 2004; http://wheat.pw.usda.gov/cgi-bin/cmap), but the stripe rust resistance QTL reported in Pastor is in the same region as *QYr.niab-7B*.

The high heritability values and the positively skewed distribution of field Pi scores indicated the presence of a major stripe rust resistance gene in the Claire × Lemhi cross. However, no single QTL explained more than 31.8 % of the phenotypic variation in stripe rust APR. Examination of individual DH lines indicated that *QYr.niab-2D.2* could confer high levels of stripe rust resistance. The variable phenotype seen in some lines containing *QYr.niab-2D.2* may, therefore, be caused by inhibitor gene/s present in Lemhi. This may also explain why *QYr.niab-2D.2* was not consistently detected in year 2 field trials, where uneven establishment of disease resulted in lower phenotypic and genotypic variance associated with all four QTL.

Suppression of rust resistance in incompatible genetic backgrounds is a well-documented phenomenon, and may account for the variable stripe rust resistance phenotypes seen with *QYr.niab-2D.2*. Suppression of stripe rust resistance has been observed in crosses between *Triticum turgidum* × *Ae. tauschii* (Kema et al., 1995; Ma et al. 1995) and suppressors of stem rust resistance were identified in the wheat cultivars Medea (Knott 2000), Canthatch (Kerber and Green 1980; Kerber 1991; Kerber and Aung 1995, 1999) and Chinese Spring (Bai and Knott 1992). The leaf rust resistance gene *Lr23*, located on chromosome 2BS, was suppressed by a gene/s located on chromosome 2DS (Nelson et al. 1997).

In addition to the QTL for stripe rust APR a seedling expressed resistance, no longer effective against UK field races of *P. s.* f. sp. *tritici*, was detected in Claire on chromosome 3BL. The only R-gene for stripe rust resistance reported on chromosome 3B is *Yr4* (Wellings et al. 2012), but this gene was assigned to the short arm of 3B in the Australian cv. Rubric (Bansal et al. 2010). A study of seedling stripe rust resistance in European wheat cultivars also failed to identify any known stripe rust R-genes in Claire (Hovmøller 2007). The stripe rust seedling resistance gene on chromosome 3BL in Claire, therefore, appears to be an uncharacterised R-gene.

Stripe rust was first found on cv. Claire in New Zealand in 2006, with infection levels ranging from 25 to 40 %, depending on location (Chng et al. 2011). In 2011 stripe rust was reported on Claire in the UK (Abel 2012), with inoculated field trails in 2012 showing 30–40 % infection at the end of the season (Rosemary Bayles, personnel communication). Claire has been used extensively in the development of new winter wheat cultivars, so the four stripe rust APR QTL identified in Claire are likely to be present in many modern elite cultivars. Identification of the QTL conferring stripe rust resistance in Claire will enable us to determine which are still effective in the UK and New Zealand, conferring the partial resistance now seen in this cultivar. The DNA markers defining each QTL will provide tools by which breeders can assess the presence of the Claire stripe rust resistance in newer cultivars and to what extent current wheat breeding programs are dependent on the Claire stripe rust resistance.

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