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ORIGINAL PAPER

The genetic characterisation of stripe rust resistance in the German wheat cultivar Alcedo

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Abstract Stripe rust resistance in the German winter wheat cv. Alcedo has been described as durable, the resistance having remained effective when grown extensively in Germany and Eastern Europe between 1975 and 1989. Genetic characterisation of field resistance in a cross between Alcedo and the stripe rust susceptible UK winter wheat cv. Brigadier identified two major QTL in Alcedo located on the long arms of chromosomes 2D (*QPst.jic-2D*) and 4B (*QPst.jic-4B*). Stripe rust resistance was evaluated by measuring the extent of fungal growth, percentage infection (Pi) and the necrotic/chlorotic response of the plant to infection, infection type (IT). Both *QPst.jic-2D* and *QPst.jic-4B* contributed significantly to the reduction in stripe rust infection (Pi), with *QPst.jic-2D* explaining up to 36.20% and *QPst.jic-4B* 28.90% of the phenotypic variation measured for Pi. Both QTL were identified by the IT phenotypic scores, with *QPst.jic-2D* in particular being associated with a strong necrotic phenotype (low IT), *QPst.jic-2D* explaining up to 53.10% of IT phenotypic variation and *QPst.jic-4B* 22.30%. In addition, two small effect QTL for field stripe rust resistance were identified in Brigadier, *QPst.jic-1B* on the long arm of chromosome 1B and *QPst.jic-5A* on the short arm of chromosome 5A. The influence of *QPst.jic-1B* was primarily seen with the Pi phenotype, contributing up to 13.10% of the explained phenotypic variation. *QPst.jic-5A* was only detected using an approximate multiple-QTL model and selecting markers linked to the major effect QTL, *QPst.jic-2D* and *QPst.jic-4B* as co-factors. Seedling stripe rust resistance was also mapped in the cross, which confirmed the location of *Yr17* from Brigadier to the short arm of chromosome 2A. A seedling expressed QTL was also located in Alcedo that mapped to the same location as the field stripe rust resistance *QPst.jic-2D*.

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Introduction

In a world economy faced with global food insecurity the demand for increased agricultural production has never been greater. This demand is set against a background of declining natural resources, increasing land marginalisation and the uncertainties of climate change. The development of low-chemical input, sustainable agricultural systems; is therefore critical (The Royal Society report 2009). Along with rice and maize wheat provides a substantial proportion of the calorific intake of the human population, either directly or through livestock feed (http://faostat.fao.org). Biotic stresses present a major constraint to crop production, with the fungal rusts of wheat being a significant, global problem (http://www.globalrust.org).

Puccinia striiformis f. sp. *tritici*, the causal agent of stripe rust in wheat, is an economically important foliar pathogen particularly prevalent in temperate and maritime wheat growing regions (Boyd 2005). Currently the disease is controlled through the genetic deployment of resistance genes and the application of fungicides. The first genetic

characterisation of disease resistance in wheat was to stripe rust in the cv. Rivet (Biffin 1905). Since then many genes effective against stripe rust have been identified (Boyd 2005; Catalogue of gene symbols for wheat, 2008). However, many major stripe rust resistance genes deployed in wheat cultivars have proven to be race-specific, having a relatively limited effective life (Bayles et al. 2000). In contrast, some wheat cultivars, grown extensively for many years retain a good level of resistance, including Cappelle Desprez (Johnson 1983), Camp Remy (Boukhatem et al. 2002; Mallard et al. 2005) and Alcedo (Meinel 1997). These sources of stripe rust resistance are often referred to as durable. The term 'Durable Resistance' was coined by Dr. Roy Johnson and describes resistance which has 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 1975). Many forms of resistance considered as durable often express a partial, growth stage specific phenotype referred to as adult plant resistance (APR; Boyd 2006).

Race-specific resistance genes generally manifest a hypersensitive response which is thought to be involved in the inhibition of pathogen growth (Bozkurt et al. 2010). The durable APR genes *Yr18* and *Yr29*, used extensively in spring wheat cultivars bred by CIMMYT (Singh et al. 2000), however exhibit a slow-rusting phenotype which is not associated with significant levels of necrosis in the host (Rosewarne et al. 2006). Such durable sources of cereal rust resistance affect fungal growth by increasing latent period and reducing the number and size of uredinia (Kolmer 1996; Pretorius et al. 1988).

Stripe rust resistance in the winter wheat cv. Alcedo represents an uncharacterised source of resistance. Alcedo was grown extensively in Germany and Eastern Europe between 1975 and 1989, and at its peak in 1981 had 47% of the wheat acreage in Germany. Alcedo expresses complete field stripe rust resistance, which is still effective in Europe (Paul Fenwick, personnel communication). For this reason the resistance in Alcedo has been described as durable (Meinel 1997). The resistance in Alcedo either represents the accumulation of a number of partial APR genes with an additive phenotype and/or the presence of one or more major resistance genes. To identify the genetic loci responsible for the stripe rust resistance in Alcedo a cross was made to the UK winter wheat cv. Brigadier. Brigadier was a high yielding feed wheat which when recommended for release in 1993 expressed complete stripe rust resistance due to the seedling resistance genes *Yr9*, derived from *Secale cereale* and *Yr17*. *Yr17* was transferred into hexaploid wheat from *Triticum ventricosum* as part of the *Yr17-Lr37-Sr38* complex and has been mapped to the short arm of chromosome 2A (Bariana and McIntosh 1993; Robert et al. 1999). In 1996, the stripe rust resistance in Brigadier was overcome by an isolate of *P. striiformis* f. sp. *tritici* which was virulent for both *Yr9* and *Yr17* (Bayles 2001), leading to the rapid commercial demise of this cultivar in the UK.

Materials and methods

Plant material and mapping population

A doubled haploid (DH) population of 192 lines was developed from the cross Alcedo × Brigadier by Limagrain UK Ltd, Station Road, Docking, UK. Alcedo [Pedigree: (Record × Poros) × Carstens VIII; Wheat Pedigree On Line http://genbank.vurv.cz/wheat/pedigree] is a German cultivar released in 1975 which expresses complete stripe rust resistance under field conditions. Brigadier [Pedigree: Squadron × Rendezvous] is a UK winter wheat released by ICI Ltd in 1993. The Avocet stripe rust near-isogenic lines (NILs) carrying stripe rust resistance genes *Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr24*, *Yr26*, *Yr27* and *YrA* were obtained from Colin Wellings, Plant Breeding Institute, Cobbitty, Sydney, Australia (Wellings et al. 2004). The World and European wheat stripe rust differential sets were as described by Boshoff et al. (2002). Line VPM1 is derived from the introgression of *Yr17* from *T. ventricosum* into hexaploid wheat (McIntosh et al. 1995).

Puccinia striiformis f. sp. *tritici* isolates

The *P. striiformis* f. sp. *tritici* isolates used in this study are part of a Rust Collection held by L. A. Boyd at the JIC. The avirulence/virulence profiles of each *P. striiformis* f. sp. *tritici* isolate used were confirmed by screening on the World and European stripe rust wheat differential sets (Boshoff et al. 2002) and the stripe rust Avocet NILs (Wellings et al. 2004). The isolates used in this study were virulent for the following stripe rust resistance genes;

IPO82069	virulent for *Yr2, Yr3, Yr4, Yr6, Yr9, Yr25, Yr26, YrSd, YrSu, YrHVII* and *YrA*
WYR 1996-502	virulent for *Yr1, Yr2, Yr3, Yr4, Yr6, Yr9, Yr17, Yr25, YrSd, YrHVII* and *YrA*
WYR 1993-54	virulent for *Yr1, Yr2, Yr3, Yr4, Yr6, Yr9, Yr13, Yr14, Yr17, Yr25, YrSd, YrHVII* and *YrA*.

Seedling phenotypic assessment of stripe rust resistance

Seedlings of Alcedo, Brigadier and each DH line were grown to growth stage 12–13 (Zadoks et al. 1974) in a spore-free containment level 2 greenhouse, under a 16/8 h

Table 1 Description of seedling stripe rust infection type scores

Infection type	Infection type description	Infection type nominal
0	No visible symptoms	0.5
;	Small necrotic flecks	1
n;	Necrotic regions greater than 1 mm in diameter	2
0^{n}	Necrotic regions greater than 2 mm in diameter	3
0^{nn}	Spreading necrotic regions greater than 4 mm in diameter	4
0^{nc}	Regions showing necrosis and chlorosis	5
1	Small, sporulating uredia surrounded by necrotic tissue	6
2^{n}	Moderately sized, sporulating uredia surrounded by necrotic tissue	7
2^{c}	Moderately sized, sporulating uredia surrounded by chlorotic tissue	8
3	Medium sized, sporulating uredia surrounded only by chlorotic tissue	9
4	Large, sporulating uredia surrounded by green tissue	10

photoperiod cycle, supplemented with sodium lighting (240 µmol m^{-2} s^{-1}), at day/night temperatures of 20°C/15°C and a relative humidity of 60%. Stripe rust disease tests were carried out using five seedlings per genotype for each test. Seedlings were inoculated with a single *P. striiformis* f. sp. *tritici* isolate using the inoculation procedure described by Boyd and Minchin (2001). Stripe rust infection was scored 14–16 days after inoculation using the seedling infection type (IT) score shown in Table 1, scoring the IT on the 1st and 2nd seedling leaves independently. These qualitative IT scores were converted to a numerical scale for all statistical and QTL analyses (Table 1).

Field phenotypic assessment of stripe rust resistance

The DH population was evaluated in field trials for stripe rust resistance at independent sites near Norwich, UK in 2005 (year 1) and again in 2006 (year 2). The full DH population was sown in replicate beds, consisting of rows, following a complete randomisation design. Each DH line was represented by ten plants per row. The parents Alcedo and Brigadier were sown every tenth row. Every fourth row was planted with the stripe rust susceptible cv. Avocet S to aid spread of the stripe rust infection within the trial. Two replicate beds were sown in each year. The Avocet stripe rust NILs and World and European differential set lines were included in the field trial to monitor the virulence profile of the *P. striiformis* f. sp. *tritici* population for additional, naturally introduced virulences.

Stripe rust was introduced into the field trials in March of each year by the introduction of spreader plants of Avocet S inoculated with *P. striiformis* f. sp. *tritici* isolates WYR 1996-502 and WYR 1993-54, to which both Alcedo and Brigadier were susceptible at the seedling growth stage 12–13 (Zadoks et al. 1974). Levels of stripe rust infection were scored on three dates in year 1 (Early-Yr1E, Middle-Yr1M and Late-Yr1L) and on two dates in year 2 (Early-Yr2E and Late-Yr2L), approximately 14 days apart in June and July. Scoring began once flag leaf emergence was seen for Alcedo and Brigadier, and Pi scores of greater than 60% were recorded on Brigadier.

Each DH line was given a score based on (1) percentage infection (Pi), taken as the percentage of green leaf area covered in 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 (McIntosh et al. 1995). The first score was taken when the susceptible parent, Brigadier was exhibiting a Pi value of approximately 60%. The field IT scores were converted to numerical values to give an infection type nominal (IT nominal, Melichar et al. 2008; Table 2).

Genetic linkage map construction

The parental lines Alcedo and Brigadier were screened with over 1,000 SSR markers by Limagrain UK Ltd, including GWM (Röder et al. 1998), WMC (Somers et al. 2004), BARC (http//www.scabusa.org), GPW (Genoplante) and PSP (Bryan et al. 1997) markers. Polymorphic SSR markers were then mapped against all 192 DH lines from the Alcedo × Brigadier cross on Li-COR 4200 DNA sequencers. Linkage groups were constructed using JoinMap version 3.0 (Van Ooijen and Voorrips 2001), a minimum LOD value of 5.0 and a maximum recombination frequency of 0.45. Recombination values were converted into genetic distances using the Kosambi mapping function (Kosambi 1944). The ordering of markers and the assignment of linkage groups to chromosomes were checked against publicly available wheat consensus maps (http://wheat.pw.usda.gov) and (http://www.shigen.nig.ac.jp/wheat).

Table 2 Description of field stripe rust infection type scores

Infection type	Infection type description	Infection type nominal
0	No visible symptoms	0
Fleck (;)	Necrotic flecks, no uredinia	0.05
Resistant (R)	Larger necrotic regions, no uredinia	0.1
R/MR	Combination of necrotic regions with no uredinia and some uredinia surrounded by necrotic tissue	0.2
MR/R	Combination of uredinia surrounded by necrotic regions and some necrosis without uredinia	0.3
Moderately Resistant (MR)	Uredinia surrounded by necrotic tissue	0.4
MR/MS	Uredinia surrounded by necrotic and/or chlorotic tissue	0.5
MS/MR	Uredinia surrounded by chlorotic and/or necrotic tissue	0.6
Moderately Susceptible (MS)	Uredinia surrounded by chlorotic tissue	0.7
MS/S	Uredinia surrounded by chlorotic tissue and/or green tissue	0.8
S/MS	Uredinia surrounded by green tissue and/or chlorotic tissue	0.9
Susceptible (S)	Uredinia surrounded by green tissue	1.0

AFLP marker identification

Polymorphic AFLP markers were identified by screening resistant (R) and susceptible (S) DNA bulks with 72 AFLP primer combinations (Smith et al. 2002). Bulks were designed that contained both the 2D and 4B QTL (R bulk), neither of the QTL (S bulk) and each QTL individually (2D bulk and 4B bulk). Each bulk was made up of DNA from between six and ten DH lines, lines being selected based on the alleles at the three closest SSR marker loci identifying each QTL and the field phenotype of the individual DH lines. In addition to this, the SSR marker loci across the whole genome were examined to ensure that no other genomic region was fixed in the bulks by chance from either Alcedo or Brigadier.

QTL analysis of stripe rust resistance

QTL analyses were performed using MapQTL version 5.0 (van Ooijen 2004). Interval mapping (Lander and Botstein 1989) was carried out to determine the locations of QTL. 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 significant LOD thresholds were obtained for each data set analysed by carrying out a permutation test with 1,000 permutations.

Pi and IT nominal data sets obtained from the field trials were used to identify QTL for field stripe rust resistance in the Alcedo × Brigadier DH population. 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 that from the predicted means for the data sets Yr1EPi, Yr1EIT, Yr1MPi, Yr1MIT, Yr1LPi, Yr1LIT, Yr2EPi, Yr2EIT, Yr2LPi and Yr2LIT. The seedling IT nominal scores for both the 1st and 2nd seedling leaf were analysed to identify resistance genes expressing stripe rust resistance at the seedling growth stage GS12–13.

Statistical analyses

All analyses were performed using the statistical package Genstat for Windows, release 12 (Genstat 5 Committee 2005). The Pi and IT nominal scores were transformed to achieve near normality and independence of the 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).

$$\text{Logit}^{+} = \log_n[(\text{Pi score} + (\min\% + 0.25))/ \times((\max\% + 0.25) - \text{Pi score})]$$

$\log_n$ = natural logarithm. Pi score = percentage infection

The IT nominal scores were transformed using an adjusted log transformation (Ramburan et al. 2004).

$$\text{Log transformation} = \ln(\text{IT nominal} + 1)$$

An analysis of variance (ANOVA) was carried out on the transformed data 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 and IT nominal data sets. The values of variance obtained from

the ANOVA were used to calculate narrow sense heritability (Lillemo et al. 2008) using the formula;

$h^2 = \sigma_g^2/\sigma_p^2$

where $\sigma_g^2 = \left(\sigma_l^2 - \sigma_e^2\right)/r$ and $\sigma_p^2 = \sigma_g^2 + \sigma_e^2$

σ_g^2 = genetic variance

σ_p^2 = phenotypic variance

σ_l^2 = variance of DH lines

σ_e^2 = error variance

r = number of replications

For each data set, the total phenotypic variation explained by the QTL identified was divided by the h^2 value to provide a measure of the genetic variation detected. For the 16 QTL genotypes, variation in Pi and IT values was analysed using the general linear regression model and *t* test comparisons.

Results

Assessment of field stripe rust resistance in the Alcedo × Brigadier cross

In order to assess the stripe rust APR in the Alcedo × Brigadier cross *P. striiformis* f. sp. *tritici* isolates virulent on both Brigadier and Alcedo at the seedling growth stage 12–13 were selected. Brigadier is known to carry the seedling resistance genes *Yr9* and *Yr17* and *P. striiformis* f. sp. *tritici* isolates WYR 1996-502 and WYR 1993-54 were confirmed to be virulent for *Yr9* and *Yr17* by screening against the World and European stripe rust differential sets, the Avocet NILs, cv. Kavkaz (*Yr9*) and the *Yr17* containing line, VPM1. Both isolates gave reactions in the susceptible range on seedlings of Alcedo (1st leaf IT $2^c/3^c$; 2nd leaf IT 3/4) and Brigadier (1st leaf IT 4; 2nd leaf IT 4), although Alcedo appeared less susceptible than Brigadier.

Stripe rust resistance was evaluated in the field by measuring the extent of uredinia formation (Pi) and the necrotic/chlorotic response exhibited by the plant (IT). On Alcedo, the Pi score never exceeded 0%, while the maximum IT nominal obtained was 0.1. On Brigadier, the maximum Pi score obtained was 85% and the highest IT nominal 0.8. There were highly significant differences between DH lines in both years, for both phenotypes (Table 3). No significant differences were seen between field replications except in year 1 for the early and middle Pi scores (Yr1EPi and Yr1MPi) and probably reflects uneven disease across the field trial early in the season (Table 3). High Pi and IT heritability values (h^2), that increased as the disease pressure increased during the season, indicated a strong genetic component affecting both stripe rust infection phenotypes (Table 3). For both Pi and IT phenotypes, DH lines exhibiting more stripe rust infection than Brigadier (Brigadier maximum Pi = 85% and IT nominal = 0.8) were observed, indicating transgressive segregation due to resistance derived from Brigadier (data not shown).

QTL analysis of field stripe rust resistance in the Alcedo × Brigadier cross

A genetic map comprising 547 SSR markers, with a genetic length of 3,069 cM was developed that represented all 21 chromosomes as 33 linkage groups. This genetic map was used in a QTL analysis of the stripe rust field data. The Pi and IT field scores were analysed independently to identify QTL that contributed to both or independently to each stripe rust infection phenotype. Interval mapping identified two major QTL from Alcedo located on the long arms of chromosomes 2D (*QPst.jic-2D*) and 4B (*QPst.jic-4B*) (Fig. 1). However, both QTL located at the limits of the linkage groups and, therefore, presented ill-defined locations. To improve the genetic maps of these two major QTL regions, AFLP marker loci linked to each QTL were identified using a bulk-segregant analysis approach. For both *QPst.jic-2D* and *QPst.jic-4B*, an AFLP marker was identified that mapped to the end of the 2D and 4B linkage groups, loci *P25M61_280* and *S24M37_130*, respectively, extending both linkage groups by 4 cM.

The QTL analysis was repeated with the two AFLP loci added to the SSR genetic map. The position of *QPst.jic-2D* did not change, however, the AFLP marker *P25M61_280* clearly improved the definition of the location of *QPst.jic-2D*, the SSR marker locus *Xgwm301* now flanking *QPst.jic-2D* (Fig. 1a). The addition of the AFLP locus *S24M37_130* to the linkage group on chromosome 4B also improved the definition of the location of *QPst.jic-4B*, indicating that both the marker loci *Xcfd039* and *S24M37_130* flank *QPst.jic-4B* (Fig. 1b). The addition of the AFLP marker loci did not significantly alter the LOD values, or the contribution to the stripe rust resistant phenotype explained by each QTL (data not shown).

QPst.jic-2D was detected by all the datasets used, explaining up to 36.20% of the phenotypic variation for Pi and 53.10% of the variation for IT segregating in the cross (Table 4). *QPst.jic-4B* was also identified by all the datasets analysed, but explained less of the phenotypic variation of both infection phenotypes, contributing up to 28.90% to Pi and 22.30% to IT (Table 4). In addition to the

Table 3 Analysis of field stripe rust infection phenotypes in the cross Alcedo × Brigadier

Infection phenotype (data sets)	Source	*df*	*F* value	h^2	% phenotypic variance	% genotypic variance
Yr1EPi	Rep	1	27.75***	0.67	70.70	105.52
	DH line	188	3.08***			
Yr1EIT	Rep	1	0.04	0.75	71.40	95.20
	DH line	188	7.03***			
Yr1MPi	Rep	1	21.83***	0.77	69.50	90.26
	DH line	188	7.58***			
Yr1MIT	Rep	1	2.87	0.75	65.00	86.67
	DH line	188	6.94***			
Yr1LPi	Rep	1	0.96	0.82	75.90	92.56
	DH line	188	10.09***			
Yr1LIT	Rep	1	6.91	0.82	61.20	74.63
	DH line	188	10.22***			
Yr2EPi	Rep	1	0.66	0.73	64.50	88.36
	DH line	188	6.39***			
Yr2EIT	Rep	1	0.08	0.75	68.90	91.87
	DH line	188	6.81***			
Yr2LPi	Rep	1	1.11	0.82	63.30	77.20
	DH line	188	10.22***			
Yr2LIT	Rep	1	5.17	0.79	71.00	89.87
	DH line	188	8.55***			

F value levels of significance are shown at 0.1% (***$p < 0.001$). Narrow sense heritability estimates (h^2) and the phenotypic and genotypic variance explained by the QTL identified in Table 4, are shown for Percentage infection (Pi) and Infection Type (IT) nominal data sets in years 1 (Yr1) and 2 (Yr2) for the early (E), middle (M) and late (L) score dates

df degrees of freedom

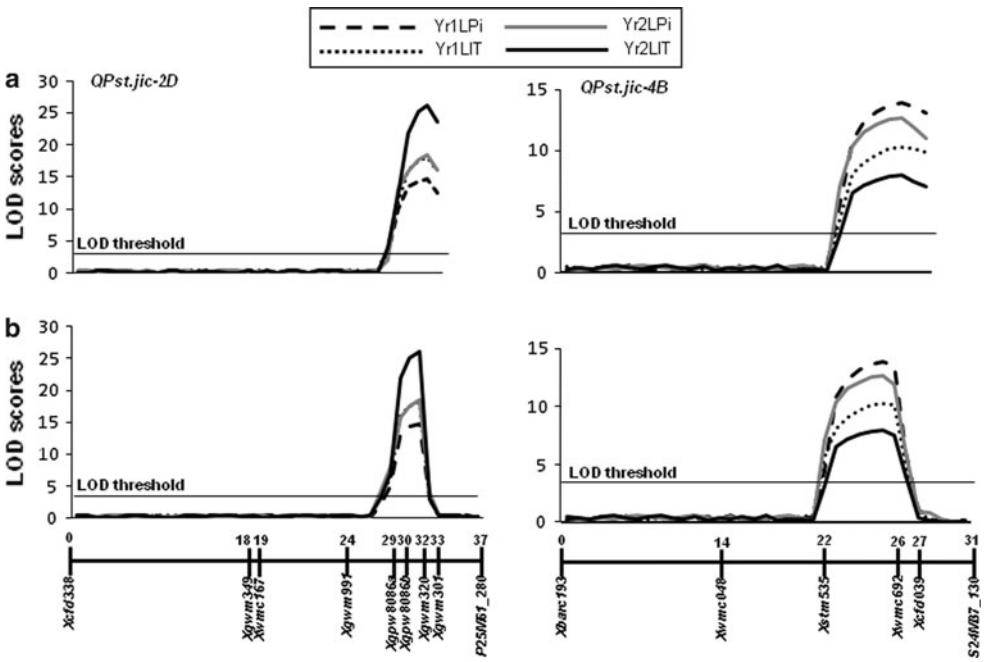

Fig. 1 LOD profile of QTL on the long arms of chromosomes 2D and 4B identified by interval mapping. QTL located using SSR genetic map (**a**) and with AFLP loci added to the genetic map (**b**). The LOD profiles for the data sets Yr1LPi, Yr1LIT, Yr2LPi and Yr2LIT are shown. The marker linkage maps corresponding to the LOD profiles show the order of and distance (cM) between marker loci

two major resistance QTL contributed by Alcedo, a small effect QTL was identified in Brigadier, located on the long arm of chromosome 1B, *QPst.jic-1B* (Table 4). *QPst.jic-1B* was identified primarily by the Pi score data sets and contributed up to 13.10% of the phenotypic variation in Pi (Table 4).

Table 4 Field expressed stripe rust resistance QTL detected in the cross Alcedo × Brigadier by interval mapping

Dataset[a] (LOD threshold)[f]	Chromosome[b]	Parent[c]	Locus[d]	LOD[e]	Expl. % variance[g]	Phenotypic means[h]	
						Al	Br
Yr1EPi	1B	Br	*Xwmc735*	3.34	7.90	15.81	6.95
(3.2)	2D	Al	*Xgwm320*	17.08	34.20	1.22	19.37
	4B	Al	*Xwmc692*	13.76	28.60	2.82	19.42
Yr1MPi	1B	Br	*Xwmc735*	3.88	9.70	21.77	8.78
(3.1)	2D	Al	*Xgwm320*	18.36	36.20	1.33	26.04
	4B	Al	*Xwmc692*	12.04	25.50	4.38	25.17
Yr1LPi	1B	Br	*Xwmc735*	3.87	9.10	28.33	10.71
(3.3)	2D	Al	*Xgpw8086b*	14.36	31.50	1.76	33.97
	4B	Al	*Xwmc692*	13.89	28.90	3.48	34.37
Yr2EPi	1B	Br	*Xwmc735*	4.50	11.10	18.92	6.84
(3.0)	2D	Al	*Xgwm320*	14.71	30.10	1.83	21.56
	4B	Al	*Xwmc692*	11.10	23.80	3.85	21.41
Yr2LPi	1B	Br	*Xwmc735*	4.35	13.10	33.84	12.23
(2.9)	2D	Al	*Xgwm320*	18.43	36.20	3.28	38.96
	4B	Al	*Xwmc692*	12.64	26.60	7.48	38.10
Yr1EIT	2D	Al	*Xgwm320*	21.40	40.90	0.12	0.37
(3.0)	4B	Al	*Xwmc692*	9.20	20.30	0.17	0.35
Yr1MIT	2D	Al	*Xgwm320*	23.56	43.70	0.12	0.39
(3.2)	4B	Al	*Xwmc692*	9.54	20.80	0.17	0.36
Yr1LIT	1B	Br	*Xwmc735*	3.23	9.90	0.31	0.18
(3.1)	2D	Al	*Xgwm320*	18.06	36.70	0.03	0.12
	4B	Al	*Xwmc692*	10.30	22.30	0.03	0.15
Yr2EIT	2D	Al	*Xgwm301*	20.44	41.30	0.09	0.31
(3.2)	4B	Al	*Xwmc692*	9.88	22.00	0.12	0.28
Yr2LIT	2D	Al	*Xgwm301*	27.57	53.10	0.07	0.32
(3.3)	4B	Al	*Xwmc692*	7.98	17.90	0.13	0.27

Al cultivar Alcedo, *Br* cultivar Brigadier

[a] The early (E), middle (M) and late (L) Percentage infection (Pi) and Infection type (IT) datasets in year 1 (Yr1) and year 2 (Yr2) analysed by interval mapping using the software MapQTL v. 5.0

[b] Chromosomal location

[c] Parent contributing QTL

[d] Marker locus associated with QTL

[e] Maximum LOD score associated with closest QTL

[f] LOD threshold based on a p value of 0.05

[g] Percentage phenotype explained

[h] Phenotypic means of allelic classes at QTL

MQM mapping was carried out in order to identify additional QTL of small effect. Loci close to the major QTL *QPst.jic-2D* and *QPst.jic-4B* were used as cofactors. The choice of loci as cofactors was confirmed using the backward elimination program of the automatic cofactor selector in MapQTL. MQM mapping supported a QTL on chromosome 5A that was below the LOD threshold in interval mapping, *QPst.jic-5A*. This QTL lay between marker loci *Xwmc752* and *Xgwm786*, placing it near the centromere on the short arm of 5A. *QPst.jic-5A* only reached a significant LOD value using the datasets Yr1MIT (LOD 3.83) and Yr2EIT (LOD 3.71). This minor QTL was contributed by cv. Brigadier and explained 4.0 and 4.6% of the phenotypic variance of each data set, respectively.

The DH lines were divided into 16 genotypes depending on the presence of the four QTL. The parental alleles present at the closest marker loci to each QTL were used to define the resistant QTL. The mean Pi and IT scores of each genotype, for each of the ten data sets, were calculated using the predicted means (Fig. 2). Both *QPst.jic-2D* and *QPst.jic-4B*

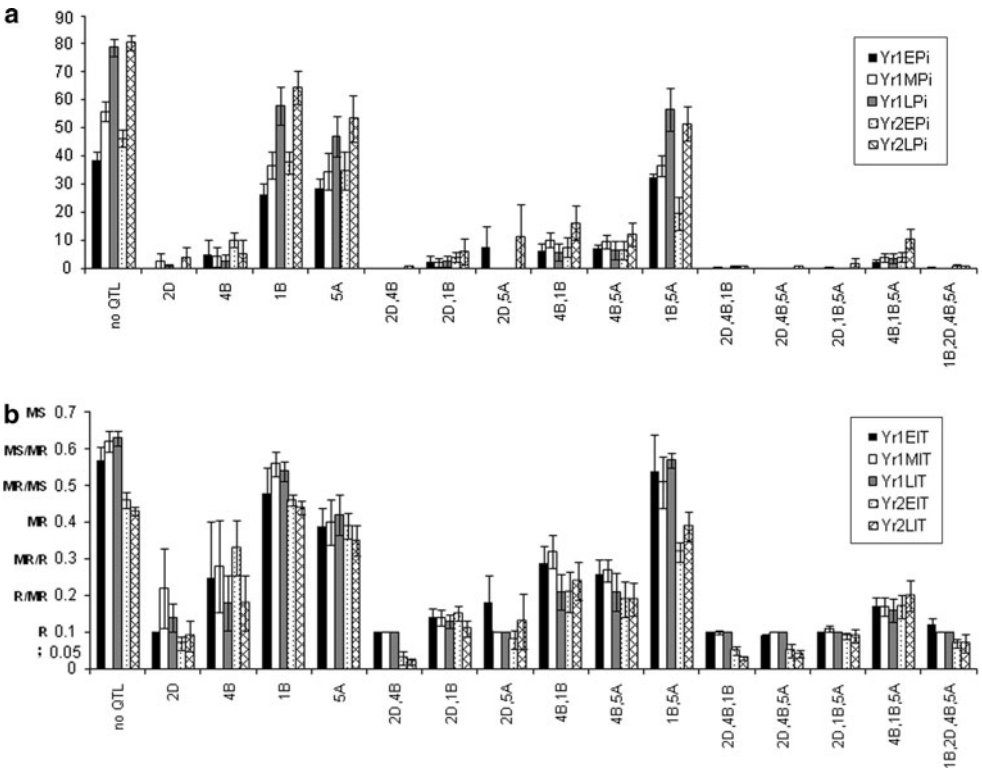

Fig. 2 Mean Percentage infection and Infection Type scores for the DH lines of the Alcedo × Brigadier population divided by the four stripe rust resistance QTL identified in this cross. **a** Percentage infection (Pi) and **b** Infection Type (IT) where the *y*-axis shows both the IT scores; fleck, *R* resistant, *MR* moderately resistant and *MS* moderately susceptible and the IT nominal scale 0–1.0. The *x*-axis identifies the QTL present; no *QTL, 2D QPst.jic-2D*, *4B QPst.jic-4B*, *1B QPst.jic-1B* and *5A QPst.jic-5A*. Error bars show standard errors

significantly reduced fungal growth (*t* test values <0.001), with *QPst.jic-2D* being slightly more effective than *QPst.jic-4B*. *QPst.jic-2D* exhibited an R type IT response (necrosis without sporulation) compared to the R/MR (necrosis with occasional sporulating uredinia) phenotype conferred by *QPst.jic-4B*. Together *QPst.jic-2D* and *QPst.jic-4B* conferred complete immunity, with no sporulation on DH lines carrying both QTL and showing a; to R IT (small to medium necrotic flecks). In year 1, both *QPst.jic-1B* and *QPst.jic-5A* significantly reduced Pi (*t* test values <0.001), however, in year 2 only DH lines with both *QPst.jic-1B* and *QPst.jic-5A* were significantly different from lines with no QTL (*t* test values <0.005; Fig. 2). Only *QPst.jic-5A* in year 1 had a significant effect on the IT phenotype compared to the no QTL DH lines (*t* test value <0.01; Fig. 2).

Assessment of seedling stripe rust resistance in the Alcedo × Brigadier cross

To confirm the presence and location of the stripe rust resistance gene *Yr17* in Brigadier the DH population was screened with *P. striiformis* f. sp. *tritici* isolate IPO82069 at the seedling growth stage 12–13 (Zadoks et al. 1974). At the growth stage 12–13 Alcedo gave an IT $2^c/3^c$ on the 1st seedling leaf and an IT of 4 on the 2nd leaf, while Brigadier gave an IT; on both the 1st and 2nd seedling leaves.

A range of seedling infection phenotypes was observed in the DH population, with some DH lines being more susceptible than Alcedo, suggesting the presence of a seedling resistance in Alcedo effective against IPO82069. This was most evident with the stripe rust IT scores from the 1st leaf. A major QTL, derived from Brigadier was identified on a linkage group assigned to chromosome 2A (Table 5). Interval mapping placed the QTL peak close to the marker locus *Xgwm636,* explaining 39.50 and 48.00% of the phenotypic variation of the 1st and 2nd leaf stripe rust infection scores, respectively. This QTL is considered to represent *Yr17* (Robert et al. 1999).

A second QTL, derived from Alcedo was detected on the same 2D linkage group as the field stripe rust resistance QTL *QPst.jic-2D* (Table 5). The QTL peak was closest to the marker locus *Xgpw8086a,* having LOD scores of 6.34 and 3.22 for the 1st and 2nd leaf datasets, respectively. MQM mapping analysis, however, placed the peak of this QTL closer to marker locus *Xgwm320*. This QTL explained up to 15.90% of the phenotypic variation for the 1st leaf dataset and 8.40% in the 2nd leaf dataset.

Discussion

The German cv. Alcedo has long been considered a source of durable stripe rust resistance (Meinel 1997). Two QTL of large effect were found to be responsible for the field stripe rust resistance seen in Alcedo, *QPst.jic-2D* and *QPst.jic-4B*, each conferring a high level of resistance

Table 5 Seedling expressed stripe rust resistance QTL detected in the cross Alcedo × Brigadier by interval mapping

Dataset[a] (LOD threshold)[f]	Chromosome[b]	Parent[c]	Locus[d]	LOD[e]	Expl. % variance[g]	Phenotypic means[h]	
						Al	Br
1st leaf	2A	Br	*Xgwm636*	17.37	39.5	5.47	2.64
(3.2)	2D	Al	*Xgpw8086a*	6.34	15.9	2.98	4.73
2nd leaf	2A	Br	*Xgwm636*	22.54	48	7.06	3.13
(3.2)	2D	Al	*Xgpw8086a*	3.22	8.4	3.92	5.50

Al cultivar Alcedo, *Br* cultivar Brigadier

[a] The 1st and 2nd seedling leaf stripe rust infection phenotypes analysed by interval mapping using the software MapQTL v. 5.0

[b] Chromosomal location

[c] Parent contributing QTL

[d] Marker locus associated with closest QTL

[e] Maximum LOD score associated with QTL

[f] LOD threshold based on a p value of 0.05

[g] Percentage phenotype explained

[h] Phenotypic means of allelic classes at QTL

throughout the season and across years. Both QTL significantly reduced stripe rust infection (Pi) and conferred a low IT, indicating that arrest of fungal growth was associated with a rapid, confined necrotic response. A QTL for seedling resistance was also detected in the same chromosomal region as *QPst.jic-2D*. This may indicate a linked seedling resistance gene, or that *QPst.jic-2D* begins to express a resistant phenotype as early as growth stage 12–13, as previous studies have shown that stripe rust APR genes that confer a strong field resistance tend to express that resistance at earlier plant growth stages (Ma and Singh 1996; Qayoum and Line 1985).

Alcedo was introduced into UK winter wheat breeding programs during the 1970s, with the first cultivar derived from Alcedo, cv. Apostle being released in 1980 (Wheat Pedigree On Line http://genbank.vurv.cz/wheat/pedigree). However, it is not known to what extent *QPst.jic-2D* and *QPst.jic-4B* are represented in current UK winter wheat cultivars. Field stripe rust resistance on the long arm of chromosome 2D has been reported in the UK cv. Guardian (Melichar et al. 2008) and in the French cv. Cappelle Desprez, where the partial APR *Yr16* has been assigned to 2DL (Worland and Law 1986). However, in both cases these QTL are located some 14 cM from *QPst.jic-2D* in cv. Alcedo, as determined by common markers (Melichar et al. 2008; L. Boyd unpublished data; Renee Prins, personal communication). Therefore, *QPst.jic-2D* in Alcedo appears to represent a previously uncharacterised stripe rust resistance.

A number of stripe rust resistance genes have been identified on the long arm of chromosome 4B. Stripe rust QTL in the region of *QPst.jic.4B* has been found in the cvs Oligoculm (Suenaga et al. 2003), Pavon 76 (William et al. 2006) and Guardian (Melichar et al. 2008). In the cv. Guardian, the QTL was defined by the same marker locus, *Xwmc692* as that linked to the Alcedo QTL *QPst.jic-4B*. However, the Guardian 4B QTL was only detected under low disease pressure, explaining 7.0–12.0% of the stripe rust resistance phenotype seen in Guardian and therefore is unlikely to represent the same allele as that seen in Alcedo at *QPst.jic.4B*. In Pavon 76, the QTL located on 4BL was some 10 cM away from the location of *QPst.jic.4B* and again represented a small effect QTL, explaining between 7.0 and 13.0% of the variation for stripe rust resistance in Pavon 76 (William et al. 2006). Again, in cv. Oligoculm the stripe rust QTL detected on 4BL lay 8 cM distal to *QPst.jic.4B* and explained only 12.0% of the phenotypic variation in this cultivar (Suenaga et al. 2003). The long arm of chromosome 4B would, therefore, appear to represent a region of the wheat genome worthy of further study.

The cv. Brigadier was grown widely after its release in 1994, expressing complete immunity to stripe rust due to the then effective, seedling expressed resistance gene *Yr17*. However, stripe rust resistance in Brigadier was overcome in 1996 when a new *P. striiformis* f. sp. *tritici* race, virulent on *Yr9* and *Yr17* arose in the UK *P. striiformis* f. sp. *tritici* population (Bayles et al. 2000). The presence and map location of *Yr17* was confirmed in Brigadier, mapping to the central region of the short arm of chromosome 2A (Robert et al. 1999).

Although cv. Brigadier was used as the stripe rust susceptible parent in the Alcedo × Brigadier cross, transgressive segregation seen in the field trial data sets indicated that Brigadier still expressed some stripe rust resistance. Two small effect stripe rust resistance QTL were detected in Brigadier, *QPst.jic-1B* and *QPst.jic-5A*. *QPst.jic-1B* located to the long arm of chromosome 1B,

although some distance from the known durable stripe rust resistance gene *Yr29* (William et al. 2003). *QPst.jic-5A* mapped close to the centromere on the short arm of chromosome 5A. A small effect QTL for stripe rust resistance has been reported on chromosome 5A in the ITMI population (Boukhatem et al. 2002), however, this QTL is located at the telomeric end of the long arm of 5A.

Grouping of the DH lines by QTL allowed an assessment of the stripe rust infection phenotypes conferred by each QTL genotype. While alone both *QPst.jic-2D* and *QPst.jic-4B* conferred a high level of stripe rust resistance, when present together an additive effect was observed. The influence of *QPst.jic-1B* and *QPst.jic-5A* on stripe rust resistance was more variable. In year 1, both *QPst.jic-1B* and *QPst.jic-5A* significantly reduced Pi, while in year 2 only an additive effect of *QPst.jic-1B* with *QPst.jic-5A* resulted in significantly less infection than DH lines with no QTL. No additive effect was seen for Pi when *QPst.jic-1B* and *QPst.jic-5A* where present with either *QPst.jic-2D* or *QPst.jic-4B*. *QPst.jic-2D* and *QPst.jic-4B* both had a significant effect on the necrotic response of the plant. However, only *QPst.jic-5A* in year 1 was seen to influence IT compared to the no QTL DH lines, *QPst.jic-1B* having no apparent effect on the necrotic response of the plant. In general, the high levels of genetic variance explained by the QTL detected using each of the data sets would suggest that all significant QTL contributing to stripe rust resistance in the Alcedo × Brigadier cross have been identified.

In order to understand the genetic components underlying the resistance response, a clear, defined method of classifying the resistance phenotypes is required. When evaluating field stripe rust resistance Pi gives a measure of the extent to which the pathogen has infected the plant, while IT classifies the response of the plant to the invading pathogen. Studies have identified QTL that affect both phenotypes (Singh et al. 2000; Suenaga et al. 2003), while other QTL have been found that affect predominantly one phenotype, suggesting that different resistance QTL may confer resistance through differing mechanisms (Singh et al. 2000; Suenaga et al. 2003; Ramburan et al. 2004; Melichar et al. 2008). It has long been known that the durable APR genes *Yr18* and *Yr29* are not consistently associated with a significant necrotic response (Rosewarne et al. 2006; Singh et al. 2005). *Yr18* has now been cloned (Krattinger et al. 2009), encoding for a protein resembling a multi-drug ABC transporter and may indicate that such durable APR genes represent a different mechanism of resistance to that conferred by race-specific stripe rust resistance genes (Bozkurt et al. 2010). As the major stripe rust QTL identified in Alcedo were associated with a significant necrotic response this may indicate that *QPst.jic-2D* and *QPst.jic-4B*, unlike *Yr18*, may represent potential race-specific resistance genes. The resistance phenotypes expressed by *QPst.jic-2D* and *QPst.jic-4B* do not inform as to why the stripe rust resistance in cv. Alcedo remained effective during its many years of cultivation in Germany. The identification of defining molecular markers for *QPst.jic-2D* and *QPst.jic-4B* will now enable wheat breeders to determine to what extent these QTL have already been deployed and to ensure that when exploiting the Alcedo stripe rust resistance that both QTL are deployed together in new wheat cultivars using marker-assisted selection.

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