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ORIGINAL PAPER

QTL identification and microphenotype characterisation of the developmentally regulated yellow rust resistance in the UK wheat cultivar Guardian

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Abstract Yellow rust (causal agent: *Puccinia striiformis* f.sp. *tritici*) resistance in the UK wheat cultivar Guardian is developmentally regulated, resistance increasing as the plant matures. Yellow rust resistance was assessed under field conditions on plants after ear emergence to ensure maximum expression of resistance. Three quantitative trait loci (QTL) for yellow rust resistance were identified, being located on chromosomes 1B (*QPst.jic-1B*), 2D (*QPst.jic-2D*) and 4B (*QPst.jic-4B*). The largest resistance effect, *QPst.jic-1B* located to the same position on the long arm of chromosome 1B as the known durable source of yellow rust resistance, *Yr29*. Microscopic studies were carried out to determine what effect the resistance in Guardian had on the development of *P. striiformis* f.sp. *tritici*. While the adult plant resistance in Guardian did not prevent germinated urediniospores from establishing an effective infection site, the growth of hyphae within flag leaf tissue was significantly inhibited, slowing the development of microcolonies. 3,3-diaminabenzadine (DAB) and trypan blue staining indicated that this inhibition of hyphal growth was not associated with hydrogen peroxide accumulation or extensive plant cell death.

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Introduction

Puccinia striiformis f.sp. *tritici*, the causal agent of yellow rust in wheat (*Triticum aestivum* L.), is an economically important foliar disease particularly prevalent in temperate and maritime wheat growing regions (Boyd 2005). The disease is generally controlled through a combination of resistance gene deployment and fungicide application. Many major genes for yellow rust resistance deployed in wheat cultivars have proven to be race-specific, having a relatively limited effective life (Bayles et al. 2000). More durable sources of yellow rust resistance are known and these include the partial, adult plant resistance (APR) genes *Yr18, Yr29* and *Yr30* (Boyd 2005, 2006). These sources of yellow rust resistance have been extensively used in spring wheat cultivars bred by CIMMYT (Singh et al. 2000) and have proven globally effective against yellow rust. Due to the economic impact of yellow rust there is increasing interest among European wheat breeders in the deployment of such sources of resistance in winter wheat cultivars.

Race-specific resistance genes generally manifest a hypersensitive response thought to be involved in the inhibition of pathogen invasion (Lamb and Dixon 1997). The durable, APR genes *Yr18* and *Yr29* exhibit a slow-rusting phenotype which is not associated with significant necrosis in the host (Rosewarne et al. 2006). Many 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). These resistance phenotypes are also very sensitive to environmental factors, in particular temperature (Broers et al. 1996).

The wheat cultivar Guardian expresses a partial, growth-stage-specific resistance to yellow rust (Boyd and Minchin 2001; Boyd et al. 2002). At later plant growth stages the latent period increases and fewer sporulating uredinia are observed. The biggest reduction in disease symptoms are that seen during urediniospore production, with significantly fewer urediniospores being produced on older plants.

Puccinia striiformis is a biotrophic fungal pathogen, having only an asexual reproductive cycle (Murray et al. 1995). Air-borne urediniospores germinate on the plant surface, entering the plant through stomatal openings (Goddard 1976; Mares 1979; Garrood 2001). A sub-stomatal vesicle forms within the stomatal cavity from which infection hyphae grow, differentiating a haustorial mother cell at the point of contact with a host mesophyll cell. Feeding structures, haustoria form inside living mesophyll cells, establishing a successful infection site. From these initial infection sites secondary hyphae grow intercellularly within the leaf, forming a microcolony. Runner hyphae grow away from the initial infection site, establishing further haustoria and infection foci within the leaf (Mares and Cousen 1977; Cartwright and Russell 1981).

The objective of this study was to identify the quantitative trail loci (QTL) responsible for the partial, APR to yellow rust in the UK winter wheat cultivar Guardian. Microscopic studies were carried out to determine what effect the growth-stage-specific resistance in Guardian had on the development of *P. striiformis* f.sp. *tritici* and how pathogen development related to the cellular responses of the host plant.

Materials and methods

Plant material and mapping population

An F_2 mapping population of 178 individuals was derived from the cross Guardian × Avocet S. The F_3 population, consisting of 174 families was used to support the F_2 analysis. Guardian (Pedigree: Israel-M-46/Maris-Ranger//Siete-Cerros-66/Maris-Ranger) is a UK winter wheat cultivar, released by Advanta Seeds Ltd in 1982 that expresses partial APR to yellow rust. Avocet S is a yellow rust susceptible selection derived from the Australian spring wheat Avocet (McIntosh et al. 1995).

Field phenotypic assessment of yellow rust resistance

The F_2 (2003–2004) and F_3 (2004–2005) generations were evaluated in field trials for yellow rust resistance at sites near Norwich, UK. The F_2 population was sown in rows of ten plants. The parental cultivars, Guardian and Avocet S were included every tenth row. Every third row was planted with the yellow rust susceptible wheat cultivar Kharchia Local to aid spread of the yellow rust infection within the trial.

In 2004, twelve seeds of each F_3 family were sown as family rows, following a complete randomisation design. Two replicates were sown. Rows of the parental cultivars, Guardian and Avocet S and the susceptible spreader cultivar, Kharchia Local were included as for the F_2 trial. The *P. striiformis* f.sp. *tritici* isolate, WYR68-1 was introduced into the trial site on spreader plants (cultivar Kharchia Local) in March of each season. Previous work had shown isolate WYR68-1 to be fully virulent on seedlings of Guardian and on Avocet S at all plant growth stages (Boyd and Minchin 2001).

Levels of yellow rust infection were scored twice during the growing season (early and late scores), approximately 14 days apart, between June and July. Individual plants were scored for (1) percentage infection (Pi), taken as a percentage of the green leaf area covered in sporulating uredinia, using the modified Cobb scale (Stubbs et al. 1986; Ma and Singh 1996) and (2) infection type (IT; Table 1). The first score was taken when the susceptible parent, Avocet S was exhibiting a Pi value of approximately 70%. The IT scores were converted to numerical values to give an infection type nominal (IT nominal; adapted from McNeal et al. 1971; Table 1).

SSR screening and linkage map construction

Guardian and Avocet S were screened with 279 SSR markers, including GWM (Röder et al. 1998), WMC (Somers et al. 2004), BARC (http://www.scabusa.org) and PSP

Table 1 Description of yellow rust infection type (IT) scores

Infection type	Description	Infection type nominal
Fleck (;)	Necrotic flecks, no uredinia	0.1
Resistant (R)	Larger necrotic regions, no uredinia	0.2
Moderately resistant (MR)	Uredinia surrounded by necrotic tissue	0.4
Moderately resistant/susceptible (MR/MS)	Uredinia surrounded by both necrotic and chlorotic tissue	0.6
Moderately susceptible (MS)	Uredinia surrounded by chlorotic tissue	0.8
Susceptible (S)	Uredinia surrounded by green tissue	1.0
0	No visible symptoms	0

(Bryan et al. 1997) markers. Linkage groups were constructed using JoinMap version 3.0 (van Ooijen and Voorips 2001), a minimum LOD value of 4.0 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 (Röder et al. 1998, Somers et al. 2004) including the Graingenes (http://wheat.pw.usda.gov) and Komugi integrated wheat consensus maps (http://www.shigen.nig.ac.jp/wheat).

QTL analysis of field yellow rust resistance

QTL analysis was performed using MapQTL version 5.0 (van Ooijen 2004). The early and late score Pi and IT nominal data sets from the F_2 and F_3 generations were used for QTL analysis, testing both the untransformed and the logit transformation of the data sets. The predicted means of the F_3 generation Pi and IT nominal data sets were also analysed. Initial analysis was performed using a single marker regression method, the Kruskal-Wallis test. Interval mapping and multiple-QTL-model mapping (MQM) were also carried out. Significant LOD thresholds were determined for each data set by conducting a permutation test with 10,000 permutations. Significant markers identified during interval mapping were selected as co-factors for MQM analysis.

Microscopic analysis of *P. striiformis* development

Plants of cultivar Guardian were grown to growth stages (GS) GS12-13, GS26-27 and GS49-52 (Zadoks et al. 1974) in a spore-free containment greenhouse before simultaneously inoculating with the *P. striiformis* f.sp. *tritici* isolate WYR68-1 (Boyd and Minchin 2001). Plants were grown under a 16/8 h photoperiod cycle, supplemented with sodium lighting (240 µmol/m^2/s) at day/night temperatures of 20 C/15 C and a relative humidity of 60%, both before and after inoculation.

For each time point segments of inoculated leaf (2 cm) were sampled from three plants for analysis of *P. striiformis* development (trypan blue staining) and from two plants for double staining with trypan blue and 3,3-diaminobenzadine (DAB) to detect hydrogen peroxide. For GS12–13 the samples were taken from the second leaf, for GS26–27 the primary leaf of the 6/7th tiller was sampled, while for GS49–52 samples were taken from the flag leaves. The following time points after inoculation are reported. Experiment 1: 0, 12, 24, 48, 72, 96, 120, 168 and 216 h post inoculation (hpi). For Experiment 2 only GS12–13 and GS49–52 were examined, samples being taken at 0, 48, 96 hpi, and 8, 10, 12, 14 and 16 days post inoculation (dpi).

Leaf segments were fixed and cleared by submerging in excess chloral hydrate (CH) solution (300 ml 95% ethanol, 125 ml 90% lactic acid, 800 g chloral hydrate, made up to 1L with chloroform; Garrood 2001). The CH solution was changed every 24 h until leaf tissue was translucent. Trypan blue stains both fungal cell walls and plant cells where the plasma membrane has been compromised (Görg et al. 1993). Cleared leaf tissue was stained in 0.1% trypan blue in lactoglycerol (lactic acid:glycerol:H_2O; 1:1:1) for 18 h (Garrood 2001).

The DAB stain was applied using a vascular uptake method (Thordal-Christensen et al. 1997). The freshly cut end of the leaf segment was dipped into 0.5 ml of DAB solution (1 mg/ml DAB dissolved in H_2O and adjusted to pH 3.8 using 3 M HCl) for a minimum of 8 h. The leaf segments were then fixed and cleared as described above and doubled stained with trypan blue to visualise fungal structures. A wheat mutant line derived from Guardian, M66 that was known to exhibit a strong necrotic phenotype (Boyd and Minchin 2001) was used as a positive control to confirm that the DAB stain had been taken up by the leaf material.

Stained leaf samples were mounted in lactoglycerol and observed using brightfield light microscopy or UV light to resolve DAB staining (Nikon Microphot II). The following *P. striiformis* developmental stages were measured: (1) percentage germination—the number of urediniospores that had germinated as a proportion of the total numbers of spores on the leaf surface; (2) percentage infection sites—the number of germinated urediniospores that had successfully entered a stomatal opening, forming an infection site as a proportion of the number of germinated urediniospores, and (3) percentage microcolonies—the number of infection sites that had produced secondary hyphae, forming a microcolony within the leaf as a proportion of the number of infection sites. The number of *P. striiformis* infection sites associated with DAB-stained mesophyll cells was recorded as a percentage of the total number of infection sites.

Statistical analysis

The analyses were done using the statistical package Genstat for Windows, release 8.2 (Genstat 5 Committee 2005). The F_3 generation Pi and IT nominal data sets underwent a logit transformation to achieve independence of means and variances. General linear regression, selecting for accumulated analysis of variance, was performed to access phenotypic variability between F_3 families and replicates, and for the analysis of pathogen development across plant growth stages. Further outputs from the analysis of variance provided the predicted means of the F_3 generation Pi and IT nominal data sets. Urediniospore germination, establishment of infection sites and microcolony development were

analysed for variance across the different plant growth stages at each time point independently. Only if the F-statistic for plant growth stage was significant at the 0.1% level were the effects of growth stage examined further using a *t* test comparison.

Results

Guardian × Avocet S $F_{2:3}$ phenotypic trait analyses

Yellow rust resistance was evaluated by measuring the extent of uredinia formation (percentage infection; Pi) and the necrotic/chlorotic response exhibited by the plant (infection type; IT). There were highly significant differences between F_3 families ($P < 0.001$) for both traits, at both score dates (Table 2). Highly significant differences were also observed between replicates for Pi (Table 2). For both Pi scores transgressive segregation was seen in the F_2 (data not shown) and F_3 generations, indicating that Avocet S was contributing a small effect to the yellow rust resistance segregating in the Guardian × Avocet S cross (Fig. 1).

Construction of a genetic map of the Guardian × Avocet S cross

A total of 136 SSR markers were polymorphic (48% polymorphism) in the Guardian × Avocet S cross, of which 110 (81% mapped) were assigned to 33 linkage groups (LOD threshold of 4.0), representing all 21 wheat chromosomes and covering a total distance of 1238 cM. All SSR markers mapped to their predicted chromosomes as determined by comparison with published wheat consensus maps (Röder et al. 1998; Somers et al. 2004; Graingenes; http://wheat.pw.usda.gov; Komugi; http://www.shigen.nig.ac.jp).

Table 2 Analysis of variance of yellow rust resistance in the F_3 families from the cross Guardian × Avocet S

Trait	Score	Source	*df*	m.s.	*F* value
Pi	Early	Replicate	1	535.6	197.6***
		F_3 family	173	55.1	20.3 ***
		Residual	3,439	2.7	
	Late	Replicate	1	120.8	13.5***
		F_3 family	173	187.4	20.9***
		Residual	3,439	9.0	
IT	Early	Replicate	1	0.9	4.1*
		F_3 family	173	3.3	14.9***
		Residual	3,439	0.2	
	Late	Replicate	1	2.3	9.2**
		F_3 family	173	3.6	14.0***
		Residual	3,439	0.3	

F value levels of significance are shown at 0.1% ($P < 0.001$; ***), 1% ($P < 0.01$; **) and 5% ($P < 0.05$; *) for percentage infection (Pi) and infection type (IT) nominal data sets. *df* degrees of freedom, *m.s.* mean sum of squares

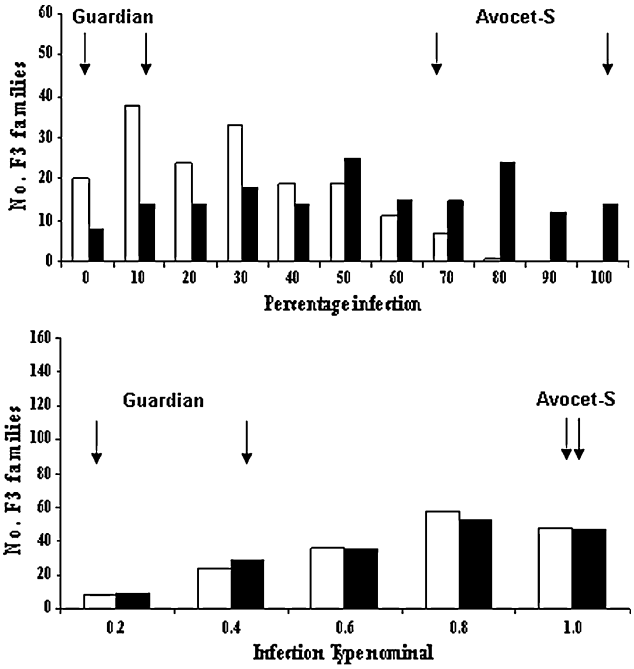

Fig. 1 Segregation of mean percentage infection and infection type nominal phenotypes in the F_3 families from the cross between cultivars Guardian × Avocet S. Infection data is shown for the early (*white bars*) and the late (*black bars*) score dates. The *arrows* represent the phenotypes of the parental cultivars, Guardian and Avocet S at the early (*broken arrow*) and late (*solid arrows*) score dates

QTL analysis of yellow rust resistance in the Guardian × Avocet S cross

QTL analyses were carried out using the early and late scores of Pi and IT trait data from both the F_2 individuals and the F_3 families. Because of the large variation between replicates the predicted Pi and IT F_3 means were also analysed. The results obtained with single marker regression (Kruskal-Wallis; data not shown) analysis were consistent with the outputs from interval mapping and MQM analyses.

Both the Pi and the IT phenotypes located at QTL on the long arm of chromosome 1B (*QPst.jic-1B*; Table 3). *QPst.jic-1B* mapped to the distal end of 1BL, positioned between loci *Xgwm818* and *Xgwm259* (Fig. 2). *QPst.jic-1B* explained up to 45% of the variation for Pi segregating in the cross and 22% of the IT phenotype. In addition to *QPst.jic-1B* two small effect QTL were detected on chromosomes 2D (*QPst.jic-2D*) and 4B (*QPst.jic-4B*; Table 3). *QPst.jic-2D* was only detected by the late disease scores, while *QPst.jic-4B* was only detected by the F_3 data sets.

At all three QTL locations the resistance effect was contributed by the Guardian allele, irrespective of the disease

Table 3 Yellow rust resistance QTL detected in the cross Guardian × Avocet S

Pop[a]	Score	Trait	Chrom	Locus	LOD threshold[b]	Peak LOD[c]	Expl. % variance[d]	Phenotypic means[e]		
								G	(ab)	Av-S
F_2	Early	Pi	1B	*Xgwm259*	2.3	7.6	18	24	39	50
		IT		*Xgwm818*	2.4	5.6	22	0.7	0.9	1.0
F_2	Late	Pi			2.3	12.6	45	50	70	100
		IT			2.3	4.3	15	0.8	1.0	1.0
F_3	Early	Pi			2.3	5.7	15	24	35	45
		IT			2.3	3.8	10	0.6	0.8	0.8
F_3	Late	Pi			2.3	11.7	28	40	60	90
		IT			2.3	6.5	18	0.6	0.8	0.9
F_2	Late	Pi	2D	*Xgwm539*	2.3	2.1	8	60	76	85
F_3	Late	Pi		*Xgwm349*	2.3	4.4	11	50	55	75
		IT			2.3	4.3	14	0.7	0.7	0.9
F_3	Early	Pi	4B	*Xwmc652*	2.0	3.2	8	25	35	40
F_3	Late	Pi		*Xwmc692*	1.9	4.6	12	50	60	75
		IT			1.9	2.5	7	0.7	0.7	0.9

[a] The values shown for the F_3 generation are those obtained using the Pi and IT nominal predicted means

[b] LOD threshold at a P value of 0.05

[c] Peak LOD value at the centre of the QTL

[d] Percentage phenotypic variance explained

[e] Phenotypic means of each allelic class at marker locus closest to QTL peak. *G* cultivar Guardian, *Av-S* cultivar Avocet S, *ab* heterozygote allelic class

phenotype used in the analysis. Each QTL detected using the Pi data sets exhibited partial or incomplete dominance, individuals heterozygous for the marker alleles having an intermediate mean disease phenotype compared to the homozygous individuals (Table 3). For *QPst.jic-2D* and *QPst.jic-4B* the IT data sets suggested that the Guardian allele may be dominant. MQM mapping did not result in the detection of any additional QTL within this cross.

Effects of plant growth-stage on *P. striiformis* development

P. striiformis developed far more rapidly on seedlings of cultivar Guardian (GS12-13) than on either tillering (GS26-27) or heading (GS49-52) plants (Fig. 3). At 12 hpi germinated urediniospores were present on seedlings, but were not observed on the later growth stages until 24 hpi. Successful invasion of stomatal openings was observed on all growth stages by 24 hpi, with no significant differences in the ability to form infection sites being apparent between the growth stages (Fig. 4a, b).

In experiment 1, microcolonies were observed on GS12–13 and GS26–27 at 72 and 120 hpi, respectively (Fig. 4c). On seedling, by 168 hpi the hyphae from independent infection sites had begun to grow together, so that individual microcolonies could no longer be scored (Fig. 3). On GS49–52 microcolonies were not observed at 168 hpi, however at the next sample time (216 hpi) microcolonies had formed and had already started to grow together. Therefore, a second microscopy experiment was undertaken, sampling at time points between 168 and 216 hpi.

Again, in experiment 2, *P. striiformis* developed at a faster rate on seedlings (GS12–13) than on flag leaves (GS49–52). On seedlings microcolonies were seen at 4 dpi (96 hpi) and by 8 dpi (192 hpi) had begun to grow together. On flag leaves microcolonies were not observed until 8 dpi (192 hpi) and had begun to grow together by 10 dpi (240 hpi; data not shown). On all growth stages, all successful infection sites went on to produce secondary hyphae and develop a microcolony (Fig. 3). No infection sites were retarded at earlier stages of pathogen development.

No DAB-staining was found associated with microcolonies, at any growth stage in Guardian (Fig. 4c, e). DAB stain was successfully taken-up, as shown by a positive control line, M66 that exhibits spontaneous necrotic flecking (Fig. 4f). In susceptible seedlings DAB stain was very occasionally found associated with early infection sites at 48 and 72 hpi. However, no DAB stain was found associated with infection sites at later time points in seedlings and never in older plant tissues (Fig. 4d).

Discussion

The UK winter wheat cultivar Guardian, while seedling susceptible to most UK isolates of *P. striiformis* f.sp. *tritici,* expresses a good level of partial resistance to yellow rust in

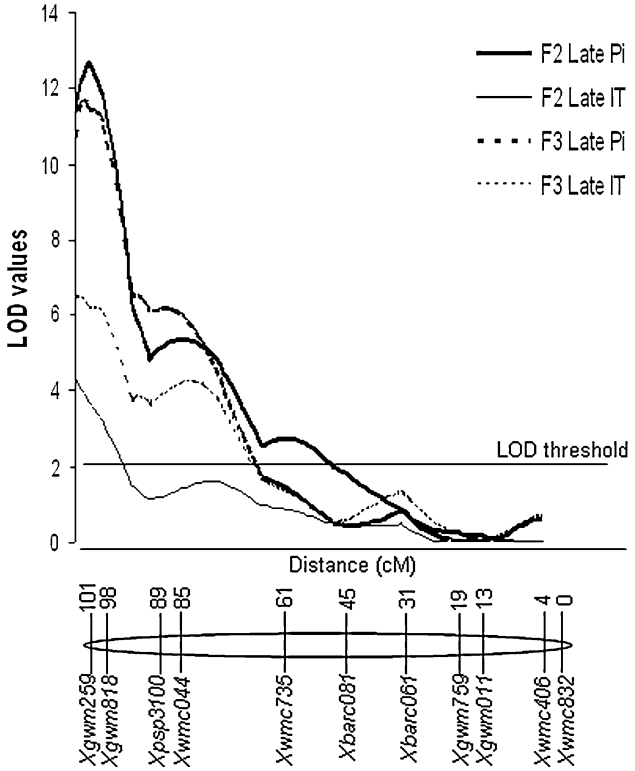

Fig. 2 LOD profiles of QTL on the long arm of chromosome 1B derived by interval mapping (MapQTL, v.5.0). The LOD profiles for the F_2 and F_3 late percentage infection (Pi) and infection type (IT) nominal data sets are shown. The marker linkage map corresponding to the LOD profiles shows the order and distance (cM) between markers. The LOD threshold is that obtained from MapQTL following 10,000 permutations

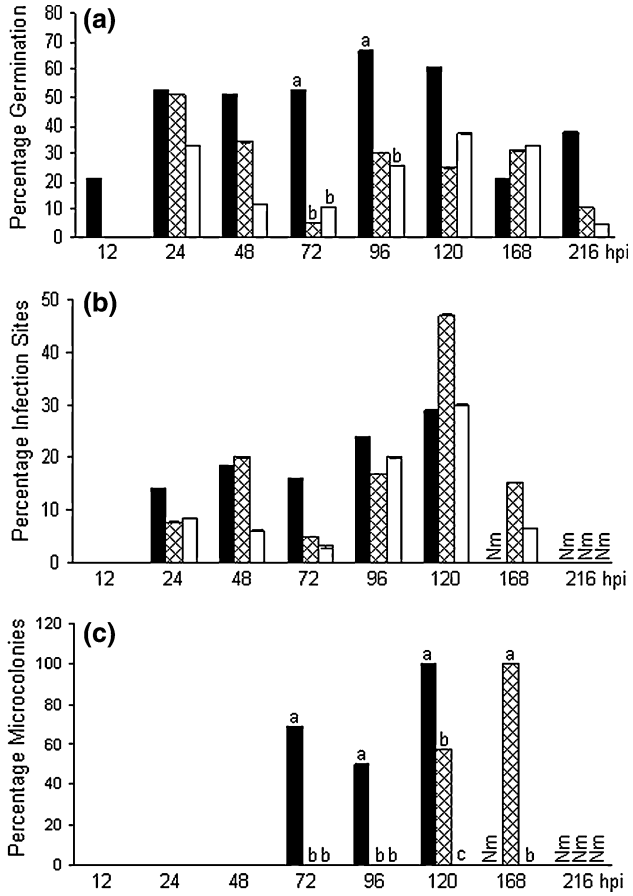

Fig. 3 **a** Percentage of germinated urediniospores, **b** infection sites and **c** microcolonies formed on wheat cultivar Guardian at growth stages GS 12–13, GS 26–27 and GS 49–52 over sampled hours post inoculation (hpi). Those growth stages showing significant differences (*t* values <0.01) at each sample time are indicated by different letters. *Nm* stage of pathogen development not measurable

the field, having been tested against a number of races of *P. striiformis* f.sp. *tritici* (Boyd et al. 2002). The APR in Guardian has previously been shown to express in plants at tillering (GS26–27), with the levels of resistance increasing at later plant growth stages (Boyd and Minchin 2001).

In the cross between Guardian and the yellow rust susceptible cultivar, Avocet S, distribution of both yellow rust Pi and IT phenotypes was continuous, showing quantitative phenotypes. Three QTL for yellow rust resistance were detected, with the resistant allele being derived from Guardian at each QTL. *QPst.jic-1B* was detected using all the phenotypic data sets. However, *QPst.jic-2D* was only detected using the late score data sets and *QPst.jic-4B* exceeded the LOD threshold only when the F_3 data sets were used in the QTL analysis.

A large replicate effect was found for F_3 Pi scores, particularly at the early score date. While it was clear from the parental cultivars used as controls in the field trial that yellow rust established later in one replicate compared to the other, it was also apparent that *QPst.jic-2D* either expressed late in the growing season or is detected only under high disease pressure. It has also been noted that the yellow rust resistance in adult plants of Guardian grown under controlled environment conditions is compromised, indicating the sensitivity of the APR in Guardian to the environmental conditions under which the plants are grown (data not shown).

Not all the phenotypic variance of the Pi and IT scores was explained by the three QTL. Additional QTL for yellow rust resistance may therefore be present in this cross. Regions of the genome for which limited maps were obtained included chromosomes 2B, 4A, 5A and 6B. While additional yellow rust QTL may be present, no unlinked SSR loci exhibited significant associations with either yellow rust phenotype in the single marker regression analysis.

Transgressive segregation was observed with both the early and late Pi scores and previous reports have identified a small resistance effect in Avocet S. A yellow rust resistance effect was detected on chromosome 4A, but only in plants grown in controlled environments (Ramburan et al. 2004), while a QTL for yellow rust resistance was detected

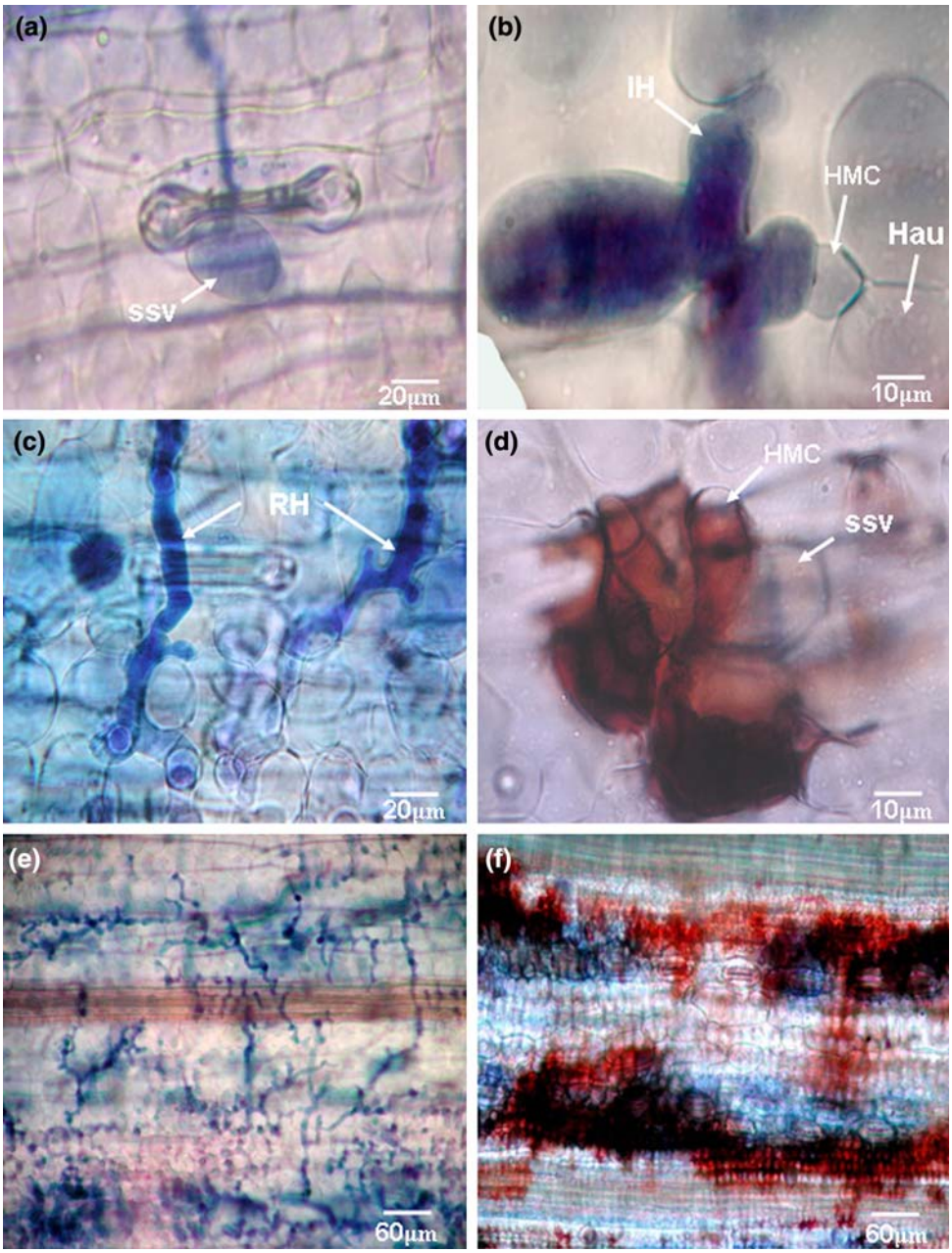

Fig. 4 *Puccinia striiformis* development in the wheat cultivar Guardian. **a** Infection site showing the formation of a substomatal vesicle (SSV) within a stomatal cavity (GS12–13; 48 hpi); **b** development of infection hyphae (IH), haustoria mother cells (HMC) and haustoria (Hau) (GS 12–13; 72 hpi); **c** runner hyphae (RH) extend colonisation away from the initial infection site (GS 26–27; 168 hpi); **d** DAB-stained mesophyll cells associated with an infection site in susceptible seedling leaf tissue (GS12–13; 48 hpi); **e** colony formation in flag leaves of cultivar Guardian stained with DAB (GS 49–52; 14 dpi); **f** colony formation in flag leaves of the positive control line, M66 stained with DAB (GS 49–51; 14 dpi)

on chromosome 6A using field trial data (Singh et al. 2001). However, no Avocet S derived yellow rust resistance effect was detected in this study.

QPst.jic-1B located to the same region on the long arm of chromosome 1B as the known durable source of yellow rust APR, *Yr29* (William et al. 2003). *Yr29* is flanked by the SSR loci *Xwmc044* (10.9 cM), *Xgwm140* (13.3 cM) and *Xgwm259* (19–20 cM; Rosewarne et al. 2006) and *Xbarc80* (10–11 cM: http://maswheat.ucdavis.edu). Unfortunately, neither GWM140 nor BARC80 were polymorphic between Guardian and Avocet S and therefore could not be mapped in this cross. While *Yr29* has been extensively used in spring wheat cultivars bred by CIMMYT (Singh et al. 2001; Rosewarne et al. 2006), there are no reports of its use in European-bred wheat cultivars.

Yr29 confers a partial resistance, showing a slow rusting phenotype that is not associated with a strong necrotic response (Singh et al. 2001). In the wheat cultivar Pavon 76 31% of the yellow rust resistance phenotypic variance was attributed to *Yr29* (William et al. 2003), while in this study *QPst.jic-1B* accounted for 45% of the Pi phenotypic variance. The reduction in uredinia production (Pi) seen with *QPst.jic-1B* was associated to some extent with necrotic tissue. However, the Guardian mean IT nominal for this QTL was high (mean IT nominal 0.6–0.8; Table 3), indicating more chlorotic than necrotic tissue.

Yr29 forms an unbroken association with a partial, adult plant leaf rust (*P. triticina*) resistance, *Lr46*. Guardian, however, exhibits high levels of leaf rust infection under high disease pressure. In 1997 and 1998 leaf rust field trials recorded 90 and 60% leaf rust infection, respectively, on Guardian (Boyd et al. 2002). During 2004, a naturally occurring leaf rust infection was observed in the F_2 field trial (data not shown). Although the levels of leaf rust infection were not particularly high, Guardian and Avocet S having between 20–30% and 50–60% infection, respectively, leaf rust Pi was measured in addition to yellow rust on the individual F_2 plants. This data set was used to carry out a preliminary interval mapping analysis against the F_2 genetic map and identified a Guardian-derived QTL for resistance to leaf rust in the same position as the yellow rust resistance QTL, *QPst.jic-1B*. However, this QTL only accounted for 10% of the explained phenotypic variance. An inoculated leaf rust trial was attempted on the F_3 generation, but proved unsuccessful because of unsuitable climatic conditions for leaf rust infection and naturally occurring yellow rust infection within the field trial (data not shown).

To investigate the microphenotype of yellow rust APR in Guardian a detailed microscopic analysis of the pathogen's development, on a progressive series of plant growth stages was carried out (Boyd and Minchin 2001). Three distinct stages in the pathogen's development were analysed; urediniospore germination, infection sites establishment and microcolony formation. Urediniospore germination appeared to be effected by the age of the leaf, germination occurring earlier on seedlings (GS 12–13) than on plants at GS26–27 or GS49–52. Having germinated, germ tubes were equally able to find and enter a stomatal opening, regardless of the age of the leaf. Having formed an infection site the pathogen was able to go on and successfully establish a hyphal colony. However, the development of secondary hyphae was significantly delayed in older leaf tissue, microcolonies not being observed in flag leaves until 8 dpi (192 hpi). This inhibition of hyphal growth was not associated with visible cell death as indicated by trypan blue uptake by mesophyll cells (Görg et al. 1993) or by hydrogen peroxide accumulation (Lamb and Dixon 1997). Although extensive hyphal development was apparent in adult plant tissues of Guardian by 14 dpi, this was not translated into extensive sporulation. Therefore, in addition to retarding hyphal growth the yellow rust APR in Guardian must also inhibit urediniospore development and sporulation (Boyd and Minchin 2001).

Few microscopic studies of yellow rust development have been carried out in adult plant tissues of wheat (Moldenhauer et al. 2006, 2007; Mares 1979; Mares and Cousen 1977). Two major yellow rust APR QTL identified in the South African wheat cultivar Kariega (Ramburan et al. 2004) show a distinct microphenotype associated with each resistance (Moldenhauer et al. 2006, 2007). Little or no plant cell autofluorescence was seen in doubled haploid lines carrying *QYr.sgi-7D*, while extensive autoflurescence was seen associated with fungal hyphae in lines carrying *QYr.sgi-2B*, although hyphal growth was restricted more by *QYr.sgi-7D* than by *QYr.sgi-2B* resistance. When both QTLs occurred together the extent of plant cell autofluroescence was greatly reduced (Moldenhauer et al. 2007). From the chromosomal location of *QYr.sgi-7D* and the pedigree of Kariega this QTL is thought to be the resistance *Yr18*. Like *Yr29, Yr18* has proven to be a durable source of yellow rust APR. The absence of plant cell death associated with *QYr.sgi-7D* in Kariega and the APR in Guardian may be microphenotypically indicative of types of yellow rust APR that have the potential to remain durable.

Three QTL for yellow rust resistance have been identified in Guardian. This APR does not prevent establishment of infection sites, but reduces hyphal growth and slows microcolony formation. It will be interesting to determine whether each QTL influences *P. striiformis* development in the same way. The genetic markers locating each QTL will aid the development of near-isogenic lines containing each QTL independently and in combinations, providing the required genetic material to dissect the microphenotype of each resistance QTL.

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