Theor Appl Genet (2011) 122:119–128
DOI 10.1007/s00122-010-1427-1

ORIGINAL PAPER

Identification of a QTL conferring seedling and adult plant resistance to eyespot on chromosome 5A of Cappelle Desprez

C. Burt · T. W. Hollins · P. Nicholson

Received: 13 May 2010 / Accepted: 24 July 2010 / Published online: 12 August 2010

Abstract Eyespot is an economically important fungal disease of wheat and other cereals caused by two fungal species: *Oculimacula yallundae* and *Oculimacula acuformis*. However, only two eyespot resistance genes have been characterised and molecular markers made available to plant breeders. These resistances are *Pch1*, introduced into wheat from the relative *Aegilops ventricosa*, and *Pch2*, originally identified in the cultivar Cappelle Desprez (CD). There are drawbacks associated with both resistances; *Pch1* is linked to deleterious traits carried on the *Ae. ventricosa* introgression and *Pch2* has been shown to have limited effectiveness. An additional resistance has been reported on chromosome 5A of CD that confers resistance to eyespot in adult plants. In the present study, we demonstrate that resistance on this chromosome is effective against both *O. yallundae* and *O. acuformis* eyespot pathogens and confers resistance at both seedling and adult plant stages. This resistance was mapped in both seedling bioassays and field trials in a 5A recombinant population derived from a cross between CD and a CD single chromosome substitution line carrying 5A from the susceptible line Bezostaya. The resistance was also mapped using seedling bioassays in a 5A recombinant population derived from a cross between the susceptible line Chinese Spring (CS) and a single chromosome substitution line carrying 5A from CD. A single major QTL on the long arm of chromosome 5A was detected in all experiments. Furthermore, the SSR marker *Xgwm639* was found to be closely associated with the resistance and could be used for marker-assisted selection of the eyespot resistance by plant breeders.

Communicated by J. Dubcovsky.

C. Burt · P. Nicholson (✉)
John Innes Centre, Norwich Research Park, Colney,
Norwich NR4 7UH, UK
e-mail: paul.nicholson@bbsrc.ac.uk

T. W. Hollins
RAGT Seeds Ltd., Grange Road, Ickleton, Essex CB10 1TA, UK

Introduction

Eyespot is an economically important fungal disease of cereal crops such as wheat, barley and rye. It is caused by two species of fungi, *Oculimacula yallundae* (formally *Tapesia yallundae*) and *O. acuformis* (*T. acuformis*). It is widespread in areas where mild and damp autumns promote the growth and spread of the pathogen, particularly northwest Europe and northwest USA. High levels of the disease can lead to significant economic losses (Hardwick et al. 2001; Murray 1996). Unfortunately, control with fungicides may not be cost effective (Nicholson and Turner 2000) and resistance has arisen in numerous eyespot pathogen populations to a number of different chemicals (Parnell et al. 2008). Therefore, the use of disease-resistant cultivars is thought to be the most effective strategy to control the disease.

There are only two sources of resistance that are known to be widely used in commercial wheat cultivars. The more potent of these is the dominant resistance gene *Pch1*, which was derived from the wheat relative *Aegilops ventricosa.* This gene was introduced into hexaploid wheat (Maia 1967) and has been located to the long arm of chromosome 7D (Worland et al. 1988), where it is associated with the SSR markers *Wmc14* and *Barc97* (Chapman et al. 2008) and the STS markers *Orw1, Orw5* and *Orw6* (Leonard et al. 2008). Although *Pch1* significantly reduces the rate of penetration from the outer leaf sheaths into the stem

(Mauler and Fehrmann 1987), there is evidence that the gene is most effective at the seedling stage and that additional quantitative resistances are required to confer a high level of resistance at the adult plant stage (Lind 1999). A further limitation is that it appears to be difficult to break a linkage between *Pch1* and yield-limiting traits also introgressed from *Ae. ventricosa* on the same segment (Koen et al. 2002). Consequently, there is interest in alternative sources of resistance to use either in combination with *Pch1* to increase the level of resistance, or in place of *Pch1* to avoid the problem of linkage drag with deleterious traits.

The second source of resistance is from the cultivar Cappelle Desprez (CD), which was widely grown in Europe for over 20 years from 1953 (Silvey 1978). The resistance observed in CD has been shown to be partly due to a seedling resistance termed *Pch2* on chromosome 7A (Law et al. 1976). This gene has been mapped to the long arm of chromosome 7AL (de la Peña et al. 1996) and is delimited by the SSR markers *Xgwm346*, *Xwmc525* and *Xcfa2040* (Chapman et al. 2008). Additional quantitative resistances are believed to be present in CD, notably an adult plant resistance identified on chromosome 5A (Muranty et al. 2002). Chromosome 5A appears to be an important component of the eyespot resistance observed in CD at the adult plant stage, particularly as *Pch2* appears to confer little adult plant resistance as assessed by field trials (Muranty et al. 2002). Previous studies (Hollins et al. 1988; Lind 1999) have demonstrated that cultivars carrying *Pch1* and with CD in their pedigree, such as Rendezvous, have enhanced adult plant resistance. However, it has not been determined whether this is due to the effect of *Pch2*, as known to be carried by Rendezvous (Burt et al. 2010), or other CD quantitative resistances that are effective at the adult plant stage such as the 5A resistance.

There is evidence from a number of sources that the genetic basis of resistance to *O. yallundae* and *O. acuformis* can differ. *Pch2* has recently been shown to confer a lower level of resistance to *O. yallundae* than to *O. acuformis* (Burt et al. 2010). Furthermore, $PchD^{v}$ (*Pch3*), a third eyespot resistance mapped to chromosome 4 V of the wheat relative *Dasypyrum villosum* (Yildirim et al. 1998), but not used in wheat cultivars, appears to be less effective against *O. acuformis* than *O. yallundae* (Uslu et al. 1998). There is also evidence that potentially novel resistances identified in the wheat relative *Triticum monococcum* (Burt et al. 2010) may confer differential resistance to the two pathogen species. The species coexist in field populations, and control of one species tends to increase the proportion of the other and fails to prevent disease (Parnell et al. 2008). To provide effective disease control in commercial wheat cultivars any novel sources of resistance that are used, such as the CD chromosome 5A resistance, should be effective against both forms of the pathogen.

There are other genes of agronomic importance on chromosome 5A, such as QTL controlling time to ear emergence (Griffiths et al. 2009) and resistance to Fusarium head blight (Buerstmayr et al. 2003), and therefore it is highly desirable to identify molecular markers linked to the eyespot resistance in order to introduce this alongside genes for other beneficial traits carried on this chromosome in other cultivars. To our knowledge, no previous studies have determined the genetic basis of this resistance or sought to identify molecular markers for its selection by plant breeders.

The aim of the present study was to characterise the adult plant eyespot resistance previously identified on chromosome 5A of CD and determine whether its presence could be detected at the seedling stage and whether it confers resistance towards both *O. yallundae* and *O. acuformis*. We also sought to identify the genetic location of the resistance at both the seedling and adult plant stages and, furthermore, to identify SSR markers suitable for marker-assisted selection of the resistance.

Materials and methods

Plant and fungal material

Cappelle Desprez carries both *Pch2* and 5A resistances, while Chinese Spring (CS) and Bezostaya (Bez) contain no known eyespot resistance genes. The inter-varietal single chromosome substitution lines Chinese Spring-Cappelle Desprez 7A (CS/CD7A) containing *Pch2,* Chinese Spring-Cappelle Desprez 5A (CS/CD5A) containing the chromosome 5A resistance, Cappelle Desprez-Bezostaya 7A (CD/Bez7A) lacking *Pch2* but containing the 5A resistance, and Cappelle Desprez-Bezostaya 5A (CD/Bez5A) lacking the 5A resistance but carrying *Pch2*, were all obtained from the John Innes Centre (JIC) wheat collection. The parental lines CD, CS and Bez were used as controls.

Two chromosome 5A recombinant populations were used to determine the genetic location of the eyespot resistance on chromosome 5A of CD. These were a population of 88 recombinant inbred lines (RILs) previously generated from the cross CD × CD/Bez5A by Tony Worland at the Plant Breeding Institute, Cambridge, and a population of 147 RILs generated from the cross CS × CS/CD5A.

Eyespot isolates were grown on V8 agar (9 g of bacto agar, 50 ml of V8 vegetable juice in 450 ml of de-ionised water) at 15°C for 21 days. In the seedling bioassays conducted at the JIC mixtures of six isolates were used for both the *O. yallundae* and *O. acuformis* inoculations. Inoculum for the field trials was prepared using colonised oat grain as described by Bruehl and Nelson (1964). All 12

isolates were used in equal proportions for the field trials at JIC to provide a 1:1 inoculum mix of the two pathogen species. In the field trials conducted at RAGT Seeds, three *O. yallundae* and two *O. acuformis* isolates were selected from the company's collection and used in a 3:2 inoculum mix. A mixture of different isolates was used to ensure that a successful infection was achieved in case of lack of virulence of one or more of the isolates.

Inter-varietal single chromosome substitution lines experiments

Seedling bioassays were conducted to determine the relative effectiveness of *Pch2* and 5A resistances at the seedling stage using wheat lines CS, CS/CD7A, CS/CD5A, CD, CD/Bez5A, CD/Bez7A, and Bez. In all seedling bioassays, plants were grown in 7 × 7 cm pots in peat and sand compost, with five plants per pot. All plants were grown under 12 h day length in controlled environment rooms (CERs). Twenty pots per line were arranged in a complete randomised block design consisting of ten blocks at 5°C and ten at 10°C, with one pot of each line per block. At each temperature five blocks were inoculated with *O. yallundae* and five blocks were inoculated with *O. acuformis.* Plants were inoculated at growth stage (GS) 12 (Zadoks et al. 1974) using a PVC cylinder and inoculum slurry method as described by Chapman et al. (2008), harvested 6–8 weeks after inoculation, and scored for disease on the basis of leaf sheath penetration (Scott 1971). This experiment was subsequently repeated using identical methods to confirm the findings.

Phenotyping populations

To identify the genetic location of the resistance at the seedling stage, 88 RILs from the CD × CD/Bez5A population were phenotyped for resistance to *O. yallundae* and 147 RILs from the CS × CS/CD5A population were phenotyped for seedling resistance to *O. acuformis* in seedling bioassays. These were conducted as independent randomised complete block experiments, each consisting of six blocks, and each block containing one pot (five plants) per line and three pots (15 plants) per parent line. Seedlings were inoculated as described above. The seedling bioassays of single chromosome substitution lines suggested that the differential in disease between lines with or without the 5A resistance was greater between CD and CD/Bez5A at 5 than at 10°C, whereas the reverse was the case for the differential between CS and CS/CD5A. For this reason the CD × CD/Bez5A experiment was conducted in a CER at 5°C, and the CS × CS/CD5A experiment was conducted at 10°C in an effort to ensure maximal discrimination between lines with and without the chromosome 5A resistance.

To map the 5A resistance at the adult plant stage, 88 RILs from the population CD × CD/Bez5A were grown in two independent field trials at RAGT Seeds, Cambridge, UK, and at JIC, Norwich, UK. Both trials were drilled in autumn 2007 and plants were harvested and scored in summer 2008. Each trial was arranged in a randomised block design, each block containing two plots of each line and the two parents. Each plot consisted of a 1-m strip with three rows of plants. Using inoculum mixes as described above, both trials were inoculated when seedlings had reached GS 12 at an application rate of 20 g/m^2. To prevent unwanted foliar fungal pathogens, plants in the JIC trial were sprayed once at approximately GS 45 with 'Amistar Opti' (Azoxystrobin and Chlorothalonil) at a rate of 1.5 l ha^{-1}. Plants in the RAGT trial were untreated with fungicides. Plants were assessed for penetration of eyespot into the main stem at GS 70, using the method of Scott and Hollins (1974). It was not possible to conduct a field trial to map adult plant resistance in the CS × CS/CD5A population because of the very poor agronomic performance and growth habit of CS-based materials in the field.

Statistical analysis

For the chromosome substitution line seedling bioassays, *O. yallundae* and *O. acuformis* inoculations were analysed as separate experiments. General linear modelling (GLM) was used to calculate predicted mean disease scores for each substitution line and control line across the two replicated seedling bioassay experiments and temperatures within each experiment, also accounting for the environmental effects of blocks. Mean disease scores for lines were compared using *t* probabilities calculated within the GLM.

Data from the population phenotyping experiments were also analysed using GLM to assess variability due to block and genotype. Interactions between block and genotype were also assessed as each field trial block contained two plots per genotype and each seedling bioassay block contained five plants per genotype. GLMs were used to predict mean disease scores for RILs from the CD × CD/Bez5A population seedling bioassays and field trials, and for RILs from the CS × CS/CD5A population seedling bioassay. The predicted means were subsequently used for the QTL analysis detailed below. All analyses were conducted using Genstat v.12 (Copyright 2009 Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

SSR analysis

The parent lines of the two populations, CS, CS/CD5A, CD and CD/Bez5A, were screened with 47 publicly available SSR markers, reported to be located on chromosome 5A, to identify markers which were polymorphic in either or both

populations. Primer sets used were from IPK Gatersleben (*Gwm*), Wheat Microsatellite Consortium (*Wmc*), Beltsville Agricultural Research Station (*Barc*) and INRA (*Cfa/Cfd/Gpw*), and are described on the GrainGenes website (http://wheat.pw.usda.gov/cgi-bin/graingenes/). Markers were identified to provide an even coverage of chromosome 5A on the basis of deletion bin locations (Goyal et al. 2005) and published consensus maps (Somers et al. 2004). Wherever possible, markers polymorphic in both populations were used for mapping, in order to make direct comparisons.

Fresh leaf tissue (50 mg) from 5-week-old plants of the two populations was harvested into 96-well plates on dry ice. DNA was extracted from samples, quantified using a PicoDrop spectrophotometer (Picodrop Ltd.), and diluted to 6 ng/μl in sterile distilled water for use in PCRs. A 6.25 μl reaction volume consisted of 2.5 μl of DNA, 3.125 μl of Taq mastermix (Qiagen) and 0.625 μl of the relevant primer pair (2 μM). The forward primer for each marker was labelled with 6-FAM, NED, PET or VIC fluorescent dyes (Applied Biosystems). PCR conditions were as described by Bryan et al. (1997) with annealing temperatures as indicated by the GrainGenes website. Samples were prepared by adding 1 μl of a 1:40 dilution of the PCR product to 10 μl formamide and 0.2 μl of LIZ 500 size standard (Applied Biosystems). Samples were run on an ABI 3700 capillary sequencer (Applied Biosystems), and the output data were analysed using Peak Scanner v1.0 (Applied Biosystems) to determine the product size of the amplicons.

Map construction and QTL analysis

Linkage maps were generated for both populations in JoinMap® (version 3.0) using 0.4 as the maximum recombination fraction and 3.0 as the logarithm of the odds ratio (LOD). Linkage map data were combined with phenotypic data from the seedling bioassays and two field trials of the populations independently for a QTL analysis using Map-QTL® version 4.0 (van Ooijen and Maliepaard 1996). QTLs were initially identified using the Kruskal–Wallis test. Second, approximate locations of the QTLs were determined using interval mapping for each experiment independently. Finally, multiple QTL mapping (MQM) was carried out to finalise the locations, using the QTLs detected as co-factors. The minimum significant logarithm of the odds (LOD) scores was calculated by permutation tests (1,000 permutations) to identify the appropriate significance thresholds ($P < 0.05$) to declare the presence of a QTL for eyespot resistance.

Verification experiments

To confirm the QTL location of resistance to both pathogen species at the seedling stage, seedling bioassays were conducted on sub-sets of 13 lines from the CD × CD/Bez5A population and 28 lines from the CS × CS/CD5A population. These lines were selected on the basis of recombination around the detected QTL. This experiment consisted of five blocks, each block consisting of two pots per line, one of which was inoculated with *O. yallundae* and the other was inoculated with *O. acuformis*. CS, CS/CD5A, CD and CD/Bez5A were included as controls in each block. Mean disease scores from the seedling bioassays of recombinant lines were used alongside marker data in a single marker regression analysis to confirm QTL location for *O. yallundae* and *O. acuformis* resistance separately.

Results

Seedling bioassays of inter-varietal single chromosome substitution lines

The *Pch2* and the 5A resistances were similarly expressed in the experiments conducted at 5 and 10°C and therefore combined data across temperatures are presented in Fig. 1. However, the differential between CD and CD/Bez5A was greater, but not significantly so, at 5°C than at 10°C whereas the reverse was the case between CS and CS/CD5A. We have no clear explanation for this, although it could partly be influenced by differences in growth habit between CD and CS.

The substitution line CS/CD5A exhibited a significantly lower mean disease score than CS ($P < 0.001$) when inoculated either with *O. yallundae* or *O. acuformis* (Fig. 1a), demonstrating that chromosome 5A of CD confers resistance at the seedling stage in a susceptible CS background. The level of resistance observed in CS/CD5A was similar to that observed in CS/CD7A, which contains *Pch2* in the same susceptible background (Fig. 1a).

The substitution line CD/Bez5A exhibited a mean disease score that was significantly higher than CD ($P < 0.001$) when inoculated with either *O. yallundae* or *O. acuformis* (Fig. 1b). This demonstrates that chromosome 5A provides an important component of the eyespot seedling resistance observed in CD, because when chromosome 5A from CD was replaced by chromosome 5A from the susceptible line Bezostaya, the level of resistance conferred to both pathogens was significantly reduced. CD/Bez7A, which contains the CD5A resistance but lacks *Pch2*, had significantly higher disease scores than CD ($P < 0.05$) when inoculated with *O. acuformis,* but not when inoculated with *O. yallundae* (Fig. 1b), suggesting that the loss of any effect of *Pch2* in the CD resistance reduces the level of resistance to *O. acuformis,* but not to *O. yallundae*.

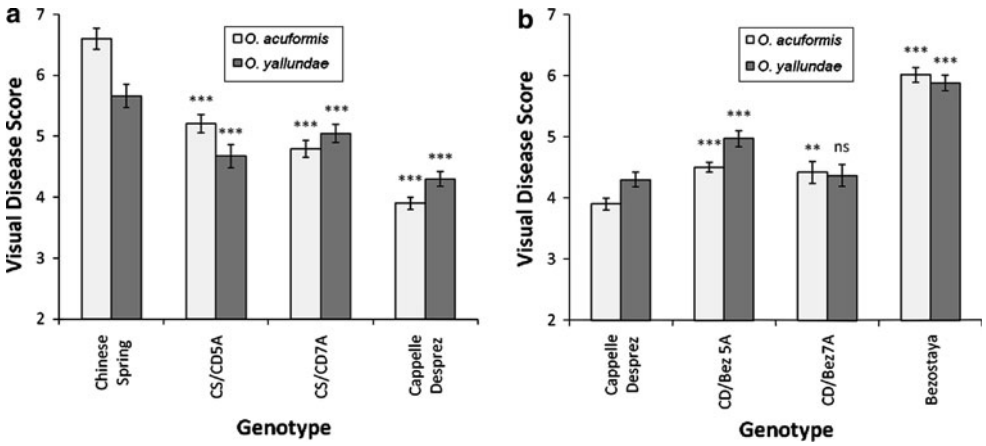

Fig. 1 Predicted mean disease scores for **a** Chinese Spring–Cappelle Desprez substitution lines and **b** Cappelle Desprez–Bezostaya substitution lines when inoculated with *Oculimacula yallundae* and *Oculimacula acuformis* in seedling bioassays. *Error bars* are all ±standard error of the mean. Mean disease scores are compared to Chinese Spring in **a**, and to Cappelle Desprez in **b**, using *t* probabilities calculated within general linear models: *ns* non-significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Seedling and adult plant resistance in CS × CS/CD5A and CD × CD/Bez5A populations

Analysis of variance demonstrated that the effect of genotype was highly significant ($P < 0.001$) in all trials apart from the field trial of CD × CD/Bez5A conducted at RAGT, in which it was also significant, albeit at a lower level ($P < 0.05$). A highly significant block effect ($P < 0.001$) was observed in all experiments. This was higher in the field trials than the CER seedling bioassays and was particularly high in the RAGT field trial. The large block effect in both field trials may in part have been due to fungal development within stems during the harvesting and scoring process. Trials were harvested and scored in blocks, each block taking approximately 2 days to complete, during which time the fungus continued to grow within the stems. This would increase block variation but would limit the residual component of the analysis of variance. No significant block × genotype interaction could be detected in either field trial although a significant interaction was observed in the seedling bioassays of the populations (Table 1).

SSR analysis, map construction and QTL analysis

Of the 47 SSR markers tested 40% (19) were polymorphic between CS and CS/CD5A and 36% (17) were polymorphic between CD and CD/Bez5A. All of the 17 that were polymorphic between CD and CD/Bez5A were also polymorphic between CS and CS/CD5A. These markers were applied to the populations and linkage maps were calculated using JoinMap (version 3.0). The markers were resolved into identical orders in both populations and it was possible to compare QTL locations in the two populations directly (Fig. 2). However, the linkage map of chromosome 5A was shorter in CD × CD/Bez5A (76 cM) than in CS × CS/CD5A (131 cM). This is probably due to the combined effect of a smaller population size and a lower level of recombination in this population.

QTLs were detected in the same location in both populations conferring both seedling and adult plant resistance (Fig. 2). In the CD × CD/Bez5A population, a QTL for eyespot resistance at the adult plant stage was detected on the long arm of chromosome 5A, centred on the SSR marker

Table 1 Variance components of visual disease scores from phenotyping experiments, calculated using general linear modelling

Variance Component	CS × CS/CD5A		CD × CD/Bez5A					
	Seedling OA		Seedling OY		JIC field		RAGT field	
	MS	*F* value	MS	*F* value	MS	*F* value	MS	*F* value
Block	28.6	25.0***	44.3	31.8***	445.4	19.6***	5174.5	111.0***
Genotype	4.9	4.3***	8.5	6.1***	41.2	1.8***	62.7	1.3*
Block × Genotype	2.4	2.1***	3.7	2.7***	28.0	1.2^{ns}	41.9	0.9^{ns}
Residual	1.1		1.4		22.7		46.6	

OA, *Oculimacula acuformis*; OY, *Oculimacula yallundae*; MS, mean squares; ns, not significant

$* P < 0.05$, $**P < 0.01$, $***P < 0.001$

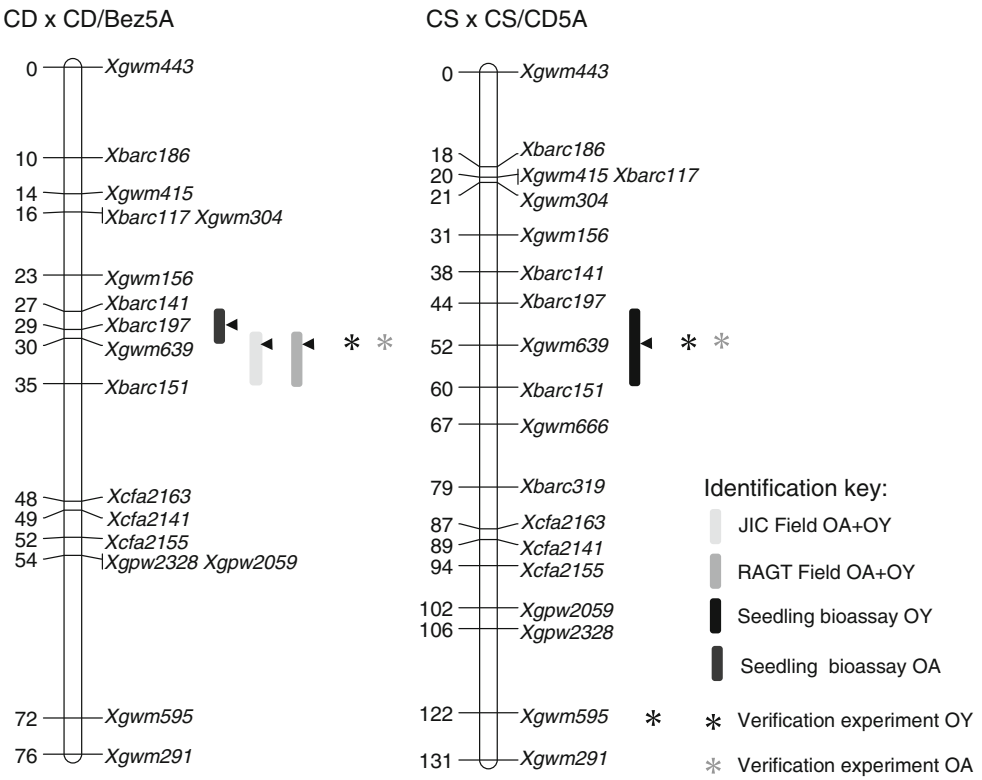

Fig. 2 Genetic maps of chromosome 5A in CD × CD/Bez5A and CS × CS × CS/CD5A populations. Distances are measured in Kosambi cM units. QTL positions for resistance to eyespot are shown to the *right* of the *genetic maps* by *bars* that indicate areas on the map with a LOD score greater than the significance threshold ($P < 0.05$) and *arrowheads* indicate the location of the peak LOD score. *Asterisks* indicate markers with significant associations ($P < 0.05$) with resistance to *Oculimacula yallundae* and *Oculimacula acuformis* in the verification experiments using a single marker regression analysis. *OA* refers to inoculation with *Oculimacula acuformis* and *OY* refers to inoculation with *Oculimacula yallundae*

Table 2 QTL identified in CS × CS/CD5A and CD × CD/Bez5A populations

Population	Test	Pathogen	Closest marker	Map position	LOD threshold	LOD	R^2
CS × CS/CD5A	Seedling	OA	*Xgwm639*	52	2.0	10.62	33.9
CD × CD/Bez5A	Seedling	OY	*Xbarc197*	29	1.9	4.62	23.9
CD × CD/Bez5A	Field JIC	OA + OY	*Xgwm639*	30	1.8	4.72	23.2
CD × CD/Bez5A	Field RAGT	OA + OY	*Xgwm639*	30	1.8	4.83	23.5

OA, *Oculimacula acuformis;* OY, *Oculimacula yallundae*; LOD, Logarithm of the odds ratio; R^2, % phenotypic variance explained

Xgwm639 (Table 2), in both the JIC (LOD 4.7, R^2 24%) and RAGT field trials (LOD 4.8, R^2 23%). The seedling bioassay of CD × CD/Bez5A identified a QTL for seedling resistance to *O. yallundae* (LOD 4.6, R^2 24%), which was most significantly associated with the SSR marker *Xbarc197* (Table 2). Although this QTL is centred on a different SSR marker to that identified in the field trials, *Xbarc197* is only 1 cM proximal to *Xgwm639*, and the QTL regions overlap (Fig. 2). The seedling bioassay of the CS × CS/CD5A population also identified a single major QTL for resistance to *O. acuformis* in the same location (Table 2), again centred on marker *Xgwm639* (LOD 10.6, R^2 34%).

Verification experiment

The single marker regression in the verification experiment confirmed *Xgwm639* as the marker most significantly associated with seedling resistance to *O. yallundae* and *O. acuformis* in both populations (Fig. 2). The presence of a CD allele at the *Xgwm639* locus was associated with a significant reduction in disease scores for both pathogen species in the recombinant lines from CD × CD/Bez5A ($P < 0.05$), explaining 29.1% of phenotypic variance for resistance to *O. acuformis* and 36.2% of phenotypic variance for resistance to *O. yallundae* (Table 3). Similarly, the presence of a CD allele at *Xgwm639* was associated with a highly significant reduction in disease scores for both pathogen species in recombinant lines from CS × CS/CD5A ($P < 0.001$), explaining 43.9% of phenotypic variance for resistance to *O. acuformis* and 43.3% of variance for resistance to *O. yallundae* (Table 3). In addition, it was possible to detect a minor resistance to both pathogens at the *Xgwm595* locus on the distal portion of 5AL from CD in the CS × CS/CD5A recombinant lines, but not in the CD × CD/Bez5A recombinant lines. The presence of a CD

Table 3 Single marker regression of predicted mean disease scores, calculated in a general linear model, for recombinant lines from CD × CD/Bez5A and CS × CS/CD5A

Marker	CD × CD/Bez5A				CS × CS/CD5A			
	O. acuformis		*O. yallundae*		*O. acuformis*		*O. yallundae*	
	R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value
Xgwm443	0	0.789	0	0.864	1.1	0.269	0	0.940
Xbarc186	2.2	0.283	0	0.41	0	0.479	0.1	0.322
Xbarc117	6.3	0.206	7.9	0.182	2.9	0.123	5.9	0.123
Xgwm415	2.5	0.278	0	0.437	6.1	0.109	7.1	0.092
Xgwm304	8	0.205	8.7	0.179	6.1	0.109	7.1	0.092
Xgwm156	6.6	0.202	8.9	0.168	0.8	0.281	0.8	0.278
Xbarc141	1.2	0.311	6.1	0.21	0	0.635	0	0.846
Xbarc197	9.1	0.167	3	0.267	9.1	0.064	4.8	0.135
Xgwm639	29.1	0.031	36.2	0.017	43.9	<0.001	43.3	<0.001
Xbarc151	0	0.698	1.5	0.296	5.4	0.123	0	0.328
Xcfa2163	0	0.721	0	0.356	0	0.456	0	0.635
Xcfa2141	0	0.721	0	0.356	0	0.958	0	0.699
Xcfa2155	0	0.622	0	0.758	0	0.858	0	0.991
Xgpw2059	0	0.674	0	0.578	0	0.595	0	0.777
Xgpw2328	0	0.674	0	0.578	0	0.641	0	0.915
Xgwm595	0	0.582	0	0.987	8.8	0.077	12.6	0.042
Xgwm291	0	0.977	0	0.53	4.1	0.154	6.8	0.097

R^2, percentage phenotypic variance explained

allele at *Xgwm595* in CS × CS/CD5A explained 8.8% of phenotypic variance for resistance to *O. acuformis* and 12.6% of variance for resistance to *O. yallundae*. Only the *O. yallundae* resistance was below the $P = 0.05$ significance threshold ($P < 0.042$) (Fig. 2). This probably represents a minor resistance, too small to be detected in the original QTL analysis, which can only be detected in a fully susceptible background. Alternatively, it is possible that this represents a minor effect that is carried by both CD and Bezostaya, but is absent from CS.

Discussion

Resistance to eyespot in the cultivar CD has previously been attributed to the seedling resistance gene *Pch2* on chromosome 7A (de la Peña et al. 1996; Law et al. 1976) and to an unmapped adult plant resistance located on chromosome 5A (Muranty et al. 2002). Through seedling bioassays we determined that the CD chromosome 5A resistance is also effective at the seedling stage, and that it is an important component of the resistance observed in CD, conferring a similar level of resistance to *Pch2*. The resistance was demonstrated in the absence of *Pch2* in a fully susceptible background in the substitution line CS/CD5A (Fig. 1), suggesting it could be used alone to provide eyespot resistance in cultivars. Furthermore, the resistance was also observed in the presence of *Pch2* in lines from the CD × CD/Bez5A population that contained both resistances, suggesting that the 5A resistance confers an enhanced effect when combined with *Pch2*, and therefore could be introduced into cultivars alongside *Pch2* to provide a higher level of protection against the disease.

In contrast to our findings, neither Law et al. (1976) nor Muranty et al. (2002) were able to detect any significant resistance conferred by CD chromosome 5A at the seedling stage. The apparent contradiction between these studies and our data may be due to differences between the methods of inoculation, particularly considering the environmental variability often associated with a necrotrophic fungus such as eyespot (Chapman et al. 2008; de la Peña et al. 1996). In our seedling bioassays, inoculum slurry was pipetted into a PVC cylinder around each seedling stem base as described by Chapman et al. (2008). In contrast, both Law et al. (1976) and Muranty et al. (2002) used the Macer technique whereby seedlings are infected from inoculated straw (Macer 1966). The PVC cylinder and slurry method is likely to provide a more uniform infection than the Macer technique, as inoculum is maintained in contact with the entire surface of the stem base, and this may prevent disease escape or delays in infection and so enable the detection of moderate seedling resistances, such as that conferred by CD chromosome 5A. However, it is possible that other differences in experimental conditions such as temperature, humidity and plant growth stage at inoculation or harvest could also contribute to the disparity between our findings and those of previous studies.

Although adult plant resistances have been identified that are only activated once a plant reaches a particular

developmental stage (Hugot et al. 1999), other adult plant resistances have been identified in wheat that can also be detected at the seedling stage, particularly towards rust diseases (Ma and Singh 1996; Singh and Huerta-Espino 2003). For example, the broad-spectrum adult plant resistance gene *Lr34*, which was first determined to confer resistance to leaf rust at the adult plant stage (Dyck 1987), was shown in later studies to be expressed at the seedling stage. Near-isogenic lines of *Lr34* have demonstrated some evidence of resistance as early as the first leaf stage and a highly significant level of resistance by the 4-leaf stage when inoculated with the causal agent of leaf rust, *Puccinia triticina* (Singh and Huerta-Espino 2003). This suggests that resistance genes once thought to be specific to adult growth stages, such as *Lr34* resistance to leaf rust and resistance to eyespot on CD chromosome 5A, may be active at earlier stages and that this can be detected with accurate phenotyping methods. It is also possible that resistances such as these may have a cumulative effect becoming more evident in older plants and although not conferring complete resistance may inhibit pathogen development sufficiently to prevent the disease becoming a significant problem.

Importantly, we determined that the CD 5A resistance functioning at both the seedling and adult plant stages against *O. yallundae* and *O. acuformis* is conferred by the same genetic location. A major QTL, closely associated with SSR marker *Xgwm639*, was identified for adult plant resistance in field trials of the 5A recombinant population CD × CD/Bez5A at both RAGT and JIC sites. As discussed above, we were also able to detect a significant effect of CD5A on eyespot resistance at the seedling stage and therefore we conducted seedling bioassays of both 5A recombinant populations to map the resistance at the seedling stage. A major QTL was detected in CS × CS/CD5A when inoculated with *O. acuformis*, again centred on *Xgwm639*, in the same position as detected in the field trials for adult plant resistance. Although the QTL identified from the seedling test on CD × CD/Bez5A using *O. yallundae* was centred on a different SSR marker, *Xbarc197*, it is probable that they represent the same genetic location as the QTL areas ($P < 0.05$) overlapped, and *Xbarc197* was only 1 cM proximal from the QTL peak position detected in the other three experiments.

A verification experiment was conducted to confirm that resistance to both pathogen species is conferred by the same genetic location. This consisted of seedling bioassays on a sub-set of recombinant lines from CD × CD/Bez5A and CS × CS/CD5A with separate inoculations with *O. yallundae* and *O. acuformis*. A single marker regression analysis on the mean disease scores from this experiment demonstrated that *Xwmc639* was the marker most closely associated with both *O. yallundae* and *O. acuformis* resistance in both populations. Alongside data from the field trials and seedling bioassays of the complete populations, this provides supporting evidence that a single major QTL on 5A, associated with *Xgwm639,* confers resistance to both *O. yallundae* and *O. acuformis*. Although the eyespot resistances; *Pch1* (Chapman et al. 2008), *Pch2* (de la Peña et al. 1996) and *Pch3* (Yildirim et al. 1998), have previously been mapped and annotated as single genes, they provide quantitative phenotypes. Due to the variability associated with screening populations for resistance to eyespot, particularly in field trials, we characterised the CD 5A resistance as a quantitative trait. To our knowledge, this is the first eyespot resistance QTL to be characterised and we propose that is designated as *QPch.jic-5A*.

The QTL detected in the CS × CS/CD5A seedling trial accounted for a higher percentage of phenotypic variation than those detected in field trials. This may be due to the lower level of environmental variation observed in CER experiments compared to field trials (Table 1). However, the QTL detected in the seedling bioassay of CD × CD/Bez5A was also of lower significance and accounted for less phenotypic variation than the QTL detected in the seedling test of CS × CS/CD5A. This difference was also observed in the seedling bioassays for the verification experiment. This may be partly because the CD × CD/Bez5A population has *QPch.jic-5A* segregating always in the presence of *Pch2*. This could reduce the differential between lines with and without the QTL and hence reduce its significance. In comparison, *QPch.jic-5A* in CS × CS/CD5A is segregating in a susceptible CS background and provides more accurate phenotypic data for locating the QTL, and this is reflected in the greater effect of the detected QTL. Any further work to refine the QTL position should focus on the CS × CS/CD5A population as this provides a clearer phenotypic difference. In addition, CS × CS/CD5A is a larger population and was found to have a higher recombination rate than CD × CD/Bez5A, providing a more appropriate resource for mapping *QPch.jic-5A* at a higher resolution.

Although we have identified an SSR marker that appears to be closely linked to *QPch.jic-5A*, it should be possible to improve the predicted genetic location of the gene and to develop more tightly linked PCR based markers. The linked SSR marker, *Xgwm639*, has been physically mapped to the deletion bin location 5AL-6 0.68–5AL-17 0.78 (Goyal et al. 2005). Further PCR markers could be developed in this region using wESTs that have been positioned in this deletion bin. In addition, synteny between wheat and the sequenced genomes of *Brachypodium distachyon* (International Brachypodium Initiative 2010) and *Oryza sativa* (International Rice Genome Sequencing Project 2005) could be used to target the region of interest on chromosome 5A more accurately.

Q.Pch.jic-5A could be of greater use than *Pch2* as it appears to provide resistance to both eyespot species at all growth stages, whilst *Pch2* provides a lower level of resistance to *O. yallundae* (Burt et al. 2010) and confers little effect at adult plant stages (Muranty et al. 2002). Although not in the scope of this study it would be beneficial to validate the 5A resistance QTL to determine whether it is sufficiently potent for use in a range of genetic backgrounds in commercial cultivars. Cultivars could be screened for the presence and absence of CD haplotypes at the SSR loci associated with the resistance, and then phenotyped through seedling tests or field trials, to determine whether the markers identified herein provide a suitable predictor of eyespot resistance.

In conclusion, we identified a single major QTL, *QPch.jic-5A*, on the long arm of chromosome 5A conferring resistance to both *O. yallundae* and *O. acuformis* at both the seedling and adult plant stages. We have also identified *Xgwm639* as a closely linked SSR marker that can be used for the marker-assisted selection of the resistance. This could provide breeders with the ability to select for a previously uncharacterised source of resistance that is effective against both forms of the pathogen and is effective at both seedling and adult plant stages.

Acknowledgments We wish to thank Simone Payne, Joe Nicholson and Ben Annis at the JIC for technical assistance with seedling bioassays and field trials. At RAGT Seeds, we would like to thank Bob Evans and Hilary Butler for assistance with field trials, Mike Boddy for producing and bulking RILs of the CS × CS/CD5A population, and Peter Jack for conducting DNA extracts on both the 5A recombinant populations. This work was jointly funded by Biotechnology and Biological Sciences Research Council (BBSRC) and the Home Grown Cereal Authority (HGCA) through a BBSRC-CASE award to C Burt.

References

Bruehl GW, Nelson WL (1964) Technique for mass inoculations of winter wheat in the field with *Cercosporella herpotrichoides*. Plant Dis Rep 48:863–865

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107:503–508

Burt C, Hollins TW, Powell N, Nicholson P (2010) Differential seedling resistance to the eyespot pathogens, *Oculimacula yallundae* and *Oculimacula acuformis*, conferred by *Pch2* in wheat and among accessions of *Triticum monococcum*. Plant Pathol. doi:10.1111/j.1365-3059.2010.02307.x

Chapman N, Burt C, Dong H, Nicholson P (2008) The development of PCR-based markers for the selection of eyespot resistance genes *Pch1* and *Pch2*. Theor Appl Genet 117:425–433

de la Peña RC, Murray TD, Jones SS (1996) Linkage relations among eyespot resistance gene *Pch2*, endopeptidase Ep-A1b, and RFLP marker Xpsr121 on chromosome 7A of wheat. Plant Breed 115:273–275

Dyck PL (1987) The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467–469

Goyal A, Bandopadhyay R, Sourdille P, Endo T, Balyan H, Gupta P (2005) Physical molecular maps of wheat chromosomes. Funct Integr Genomics 5:260–263

Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, Alibert L, Orford S, Wingen L, Herry L, Faure S, Laurie D, Bilham L, Snape J (2009) Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theor Appl Genet 119:383–395

Hardwick NV, Jones DR, Slough JE (2001) Factors affecting diseases of winter wheat in England and Wales, 1989–98. Plant Pathol 50:453–462

Hollins TW, Lockley KD, Blackman JA, Scott PR, Bingham J (1988) Field performance of Rendezvous, a wheat cultivar with resistance to eyespot (*Pseudocercosporella-herpotrichoides*) derived from *Aegilops ventricosa*. Plant Pathol 37:251–260

Hugot K, Aimé S, Conrod S, Poupet A, Galiana E (1999) Developmental regulated mechanisms affect the ability of a fungal pathogen to infect and colonize tobacco leaves. Plant J 20:163–170

International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763–768

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436:793–800

Koen E, Labuschagne MT, Viljoen CD (2002) The influence of eyespot resistance genes on baking quality and yield in wheat. J Sci Food Agric 82:1537–1540

Law CN, Scott PR, Worland AJ, Hollins TW (1976) The inheritance of resistance to eyespot (*Cercosporella herpotrichiodes*) in wheat. Genet Res Cambridge 25:73–79

Leonard J, Watson C, Carter A, Hansen J, Zemetra R, Santra D, Campbell K, Riera-Lizarazu O (2008) Identification of a candidate gene for the wheat endopeptidase *Ep-D1* locus and two other STS markers linked to the eyespot resistance gene *Pch1*. Theor Appl Genet 116:261–270

Lind V (1999) Variation of resistance to *Pseudocercosporella herpotrichoides* (Fron) Deighton in wheat genotypes carrying the gene *Pch1*. Plant Breeding 118:281–287

Ma H, Singh RP (1996) Expression of adult resistance to stripe rust at different growth stages of wheat. Plant Dis 80:375–379

Macer RCF (1966) Resistance to eyespot disease (*Cercosporella herpotrichoides* Fron) determined by a seedling test in some forms of *Triticum, Aegilops, Secale* and *Hordeum*. J Agric Sci 67:389–396

Maia N (1967) Obtention de blés tendres résistants au piétin-verse (*Cercosporella herpotrichoides*) par crôisements interspécifiques blés × *Aegilops*. Comptes Rendus de l'Académie d'Agriculture de France 53:149–155

Mauler A, Fehrmann H (1987) Evaluation of the susceptibility of wheat to *Pseudocercosporella herpotrichoides*. 2. The development of a resistance test with young plants. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 94:637–648

Muranty H, Jahier J, Tanguy AM, Worland AJ, Law C (2002) Inheritance of resistance of wheat to eyespot at the adult stage. Plant Breed 121:536–538

Murray TD (1996) Resistance to benzimidazole fungicides in the cereal eyespot pathogen, *Pseudocercosporella herpotrichoides*, in the Pacific Northwest 1984 to 1990. Plant Dis 80:19–23

Nicholson P, Turner AS (2000) Cereal stem base disease—a complex issue. In: Nicholson P, Turner AS (eds) Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK, pp 99–106

Parnell S, Gilligan CA, Lucas JA, Bock CH, van den Bosch F (2008) Changes in fungicide sensitivity and relative species abundance in *Oculimacula yallundae* and *O.* acuformis populations (eyespot disease of cereals) in Western Europe. Plant Pathol 57:509–517

Scott PR (1971) The effect of temperature on eyespot *(Cercosporella herpotrichoides*) in wheat seedlings. Ann Appl Biol 68:169–175

Scott PR, Hollins TW (1974) Effects of eyespot on yield of winter-wheat. Ann Appl Biol 78:269–279

Silvey V (1978) The contribution of new varieties to increasing cereal yield in England and Wales UK. J Natl Inst Agric Bot 14:367–384

Singh RP, Huerta-Espino J (2003) Effect of leaf rust resistance gene *Lr34* on components of slow rusting at seven growth stages in wheat. Euphytica 129:371–376

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Uslu E, Miller TE, Rezanoor NH, Nicholson P (1998) Resistance of *Dasypyrum villosum* to the cereal eyespot pathogens, *Tapesia yallundae* and *Tapesia acuformis*. Euphytica 103:203–209

van Ooijen JW, Maliepaard C (1996) MapQTL^{TM} version 4.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen

Worland AJ, Law CN, Hollins TW, Koebner RMD, Giura A (1988) Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. Plant Breed 101:43–51

Yildirim A, Jones SS, Murray TD (1998) Mapping a gene conferring resistance to *Pseudocercosporella herpotrichoides* on chromosome 4V of *Dasypyrum villosum* in a wheat background. Genome 41:1–6

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421