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ORIGINAL PAPER

The development of PCR-based markers for the selection of eyespot resistance genes *Pch1* and *Pch2*

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Abstract Two eyespot resistance genes (*Pch1* and *Pch2*) have been characterised in wheat. The potent resistance gene *Pch1*, transferred from *Aegilops ventricosa*, is located on the distal end of the long arm of chromosome 7D (7DL). *Pch2* derives from the variety Cappelle Desprez and is located at the distal end of chromosome 7AL. The RFLP marker *Xpsr121* and the endopeptidase isozyme allele *Ep-D1b*, are very closely linked to *Pch1,* probably due to reduced recombination in the region of the introgressed *A. ventricosa* segment. *Pch2* is less closely linked to these markers but is thought to be closer to *Xpsr121* than to *Ep-A1b*. In the present study simple sequence repeat (SSR) markers were integrated into the genetic map of a single chromosome (7D) recombinant (RVPM) population segregating for *Pch1*. Sequence-tagged-site (STS)-based assays were developed for *Xpsp121* and a 7DL wheat EST containing a SSR. SSR markers *Xwmc14* and *Xbarc97* and the *Xpsr121*-derived marker co-segregated with *Pch1* in the RVPM population. A single chromosome (7A) recombinant population segregating for *Pch2* was screened for eyespot resistance and mapped using SSRs. QTL interval mapping closely associated *Pch2* with the SSR marker *Xwmc525*.

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Introduction

Eyespot, caused by *Oculimacula acuformis* (formerly *Tapesia acuformis*) and *O. yallundae* (*T. yallundae*) (Crous et al. 2003) is one of the most important diseases of the stem base of cereals in temperate regions (Muranty et al. 2002). A wide range of cereals including wheat, barley, oats and rye are susceptible. Symptoms include leaf sheath browning followed by the development of stem base lesions (Fitt 1992). Severe infection results in lodging and premature ripening of grain, reducing crop yield.

Two main sources of resistance effective at the seedling stage have been incorporated into cultivated wheat varieties. The most potent resistance derives from the wild grass species *Aegilops ventricosa*. The single dominant gene *Pch1* was introduced into the wheat line VPM1 by Maia (1967), and is located on the distal end of the long arm of chromosome 7D (7DL) (Doussinault et al. 1983; Worland et al. 1988).

The second resistance originates from the variety Cappelle Desprez which dominated European wheat markets for two decades from 1953 (Hollins et al. 1988). Despite being widely used across Europe (Scott et al. 1989), the Cappelle Desprez resistance has proved highly durable (Johnson 1984; Scott et al. 1989). Resistance is transferable (Scott et al. 1989) and several new varieties have been found to

contain levels of resistance similar to Cappelle Desprez (Johnson 1992). Most of the resistance of Cappelle Desprez was found to be conferred by resistance gene(s) *Pch2*, located on chromosome 7A with additional effects associated with chromosomes 2B and 5D suggesting the involvement of genes of minor effect (Law et al. 1976). De la Peña et al. (1996), however, observed a 1:1 segregation of recombinant substitution lines for eyespot resistance, suggesting that a single gene controls *Pch2* resistance in Cappelle-Desprez.

De la Peña et al. (1997) used RFLP markers to map *Pch2*. A partial map of the long arm of chromosome 7A was constructed but no RFLP markers showed close linkage to resistance gene *Pch2*. However, two markers *Xcdo347* and *Xwg380* could be used to select for *Pch2* as double recombination occurred only in 3% of the recombinant population. *Pch2* mapped to approximately 11 cM proximal to RFLP marker *Xcdo347* and 18.8 cM distal to *Xwg380* on the distal end of the long arm of chromosome 7A (de la Peña et al. 1997).

Isozyme loci for endopeptidase are located on chromosomes 7AL, 7BL and 7DL (Hart and Langston 1977) and it was shown that *Pch1* co-segregated with the *Ep-D1b* allele (McMillin et al. 1986) with no recombination observed between *Ep-D1* and *Pch1* (Worland et al. 1988). Koebner et al. (1988) refined the isozyme assay for the group 7 endopeptidase-1 (*Ep-1*) homoeoallelic series and this is used by several plant breeders for selection of *Pch1* in breeding material. Recently, a STS marker was developed to an oligopeptidase B gene suggested to encode *Ep-D1* (Leonard et al. 2007). Leonard and colleagues found this marker to be completely linked to *Ep-D1* and *Pch1*. Although *Pch1* and *Ep-D1* are genetically linked these loci may not be in close physical proximity because the presence of the translocated *Ae. ventricosa* chromosome segment may prevent recombination between *Ep-D1* and *Pch1*. This is supported by the report of Chao and colleagues who found *Pch1* to co-localise with both the *Ep-D1* and *Xpsr121-7D* loci in a VPM1 7D single chromosome recombinant population of Hobbit 'S' Chao et al. (1989). In contrast, the *Ep-D1* and *Xpsr121* homoeo-loci were determined to be 9 and 10 cM apart on chromosomes 7A and 7B respectively, with the latter marker being distal. A later study to map *Pch2* found *Ep-A1* to be 3.9 cM proximal to *Xpsp121* (de la Peña et al. 1996). They also reported that *Pch2* was probably closer to *Xpsr121* than to *Ep-A1*, mapping 27.9 cM proximal to *Xpsr121* on the long arm of 7A. The relative positions of *Pch1* and *Pch2* on the long arm of chromosomes 7D and 7A respectively, strongly suggest that they are homoeo-loci (de la Peña et al. 1996).

A sequence tagged site (STS) marker has been developed that is closely linked to *Pch1* (Groenewald et al. 2003) but a significant amount of recombination between *Pch1* and this marker has been found (Santra et al. 2006). To date no PCR-based marker has been reported for the selection of *Pch2*. We sought to identify SSR markers closely linked to *Pch1* and *Pch2* and to use genomics resources to develop additional PCR-based assays to facilitate selection of these two eyespot resistance genes.

Materials and methods

Plant material

The inter-varietal chromosome 7D substitution line, Hobbit 'sib'-VPM7D (HS/VPM7D) carries *Pch1* (Worland et al. 1988). A BC_5 recombinant population (RVPM7D) of 90 lines, between HS/VPM7D and the eyespot susceptible line Hobbit 'sib' (HS), (Worland et al. 1988) was used to map *Pch1*. Similarly the inter-varietal chromosome substitution line Chinese Spring-Cappelle Desprez 7A (CS/CD7A) carries *Pch2*. CS/CD7A was crossed to the eyespot susceptible variety Chinese spring (CS). F_1 plants were selfed to generate an F_2 population of 192 plants and leaf material was removed for genotyping. F_2 plants were grown on and selfed to produce F_3 families that were phenotyped for eyespot resistance in two trials.

SSR analysis

The parental lines in the two populations were screened with SSRs reported to be located on chromosomes 7A and 7D (Table 1). Six sources of SSR markers were used: John Innes Centre hexaploid wheat SSRs (Bryan et al. 1997), Gatersleben D genome bread wheat microsatellite markers (Pestsova et al. 2000), Gatersleben wheat microsatellites (Röder et al. 1998), wheat microsatellite consortium (http://wheat.pw.usda.gov), US Wheat and Barley Scab Initiative (BARC) primers (http://www.scabusa.org/pdfs/BARC_maps_011106.pdf) and the *XustSSR2001-7DL* marker (Groenewald et al. 2003).

Leaf tissue (50 mg) from 5-week-old plants of the recombinant population RVPM and the F_2 CS:CS/CD7A population was harvested into 96-well plates on dry ice. Samples were stored at −70°C prior to DNA extraction using the DNeasy 96 Plant Kit (Qiagen Ltd.) according to manufacturer's instructions.

Development of SSR marker to7D using wheat EST deletion bins

Wheat EST sequences mapping to deletion bin 7DL3-0.821-1.00, located at the distal end of chromosome 7D were obtained from the *GrainGenes* database (http://wheat.pw.usda.gov). ESTs were analysed for SSR sequences

Table 1 SSR markers used to map *Pch1* and *Pch2* on chromosomes 7D and 7A respectively showing allele sizes for the parental lines used to generate the mapping populations in each case

7D marker	Allele size (bp)		7A marker	Allele size (bp)	
	HS	HS/VPM7D		CS	CS/CD7A
gdm86	154	150	barc108	159	155
wmc221	298	260	barc29	199	191
gdm150	104	–	gwm130	128	126
gdm46	150	140	gwm276	93	130
gdm67	140	134	gwm332	230	211
psp3045	115	–	gwm60	213	217
gwm428	152	–	psp3050	159	125
wmc273	260	175	wmc168	328	312
gwm37	201	240	wmc346	210	204
ustSSR2001-7DL	220	240	wmc83	163	155
wmc14	252	–	wmc525	210	208
barc97	257	–	cfa2019	–	221
W7DESTSSR	350	–	cfa2040	297	317

using Tandom Repeats Finder version 3.21 (Benson 1999). EST BE446395 was the only sequence found to contain SSR elements. A primer set (W7Dest-F: CACTAATCTTC TTGCTCTCTCT and W7Dest-R: CAATCTCTTTGTCAG TATCT) was designed for BE446395 using Primer3 (http://fokker.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and tested for secondary structures, hairpins, primer dimers and annealing temperatures using the Genosys website (http://www.sigma-genosys.co.uk/oligos/frameset.html). W7Dest primers were tested on the parental varieties HS and HS/VPM7D and were mapped using the *Pch1* RVPM7D mapping population. PCR conditions for SSRs were as described by Bryan et al. (1997) with W7DestF/R having an annealing temperature of 50°C. PCR products were separated on 6% polyacrylamide gel and visualised by silver staining (Bassam et al. 1991).

Marker development from *Xpsr121*

The RFLP probe *Xpsr121* encodes a partial sequence for a beta-glucanase. Wheat EST BG604756 is annotated as encoding for beta-glucanase and maps to the distal 7DL wheat deletion bin (7DL3-0.821-1.00), located at the end of chromosome 7D and was used to develop a 7D specific marker. PCR primers to the BG604756 sequence (B-glu7D-F: TGGAGGTTCTGTACCCGTTC and B-glu7D-R: GAAGTCCGGTGGGTTGTCTA) were designed and mapped as above using a PCR programme with 60°C annealing temperature. PCR products were examined by single strand conformational polymorphism (SSCP) assay using Sequa Gel® MD (National Diagnostics, U.K Ltd) and visualised by silver staining (Bassam et al. 1991).

Phenotyping F_3 population CS:CS/CD7A families for *Pch2* resistance

Trial 1 (2005)

Nine plants from each of 162 F_3 families were grown in 5 × 5 cm pots (Plantpak™ Cookson Plantpak Ltd), containing peat and sand compost. Three replicates of each F_3 family were arranged in a randomised complete block design in propagation trays (20 pots/tray). Parental lines, CS and CS/CD7A, were included as references in each tray. Plants were grown in a 10°C growth chamber with 12 h day length and 3 cm long transparent PVC cylinders (5 mm I.D), were placed over emerging shoot tips. After 21 days plants were inoculated by pipetting agar slurry (400 µl) into each cylinder. Isolates of *O. acuformis* (P37, P38, AG98/167, AG98/174, AG98/119, C93/786) from the JIC culture collection were grown on V8 agar (9 g of bacto agar, 50 ml of V8 in 450 ml of de-ionized water) at 15°C for 21 days. Inoculum was prepared by homogenising the agar and associated fungal colonies with water (2:1). Trays were well watered and propagator lids were used to increase humidity to aid fungal infection. Plants were returned to incubate at 10°C for 8 weeks when plants were harvested and disease was scored according to Scott (1971).

Trial 2 (2006/7)

Eyespot resistance was assessed for 183 F_3 families by a seedling bioassay in an unheated unlit glasshouse at RAGT Seeds Ltd., Cambridge, between December 2006 and February 2007. This experiment was conducted in complete randomised blocks with six replicates. In each replicate, six seeds of

each F_3 family and twelve seeds of both parental lines were sown onto peat and sand compost in 35 cm × 20 cm seed trays, with 20 lines per tray. Seeds were inoculated by applying a mixture of oat grains previously colonised by *O. acuformis* using the method of Bruehl and Nelson (1964), and peat and sand compost, through which the seedlings emerged. *O. acuformis* isolates were kindly provided by Bill Hollins from the collection of RAGT Seeds Ltd. Plants were harvested 10 weeks after sowing and scored as above.

Statistical analysis

Regression analysis and analysis of variance (ANOVA) were calculated in Genstat, ninth edition (Copyright 2006, Lawes Agricultural Trust (Rothamsted Experimental Station)) to assess variability attributed to replicate and genotype. The mean disease score for each line was estimated using generalized linear modelling and was used in the subsequent QTL analysis.

Map construction and QTL analysis

Linkage maps were calculated from recombination frequencies (0.4) and a LOD of 3.0 was used in JoinMap® (version 3.0). QTL analysis was carried out using MapQTL® version 4.0 (van Ooijen and Maliepaard 1996). Interval mapping was carried out on the two linkage groups created following the map construction of chromosome 7A using JoinMap® (version 3.0). A permutation test (1,000 cycles) was used to determine the LOD score at which the QTL was deemed to be present in the given genomic region with a confidence interval of 99%.

Results

SSR and PCR marker analysis and map construction for chromosome 7DL

The HS and HS/VPM7D parents of the 7D recombinant (RVPM) population were screened using 46 SSR markers on the long arm of chromosome 7D. Twelve (26%) of the 46 SSRs and the *XustSSR2001-7DL* marker were polymorphic between HS and HS/VPM7D and co-dominant producing allele sizes shown in Table 1.

Single sequence repeats (SSRs) development using wheat EST deletion bins

Wheat ESTs mapping to the distal portion of the long arm of chromosome 7D were used to develop additional SSR markers for mapping of *Pch1*. Only one EST (BE446395) in the distal deletion bin 7DL3-0.82-1.00 was found to contain SSR elements (CT and CA repeats) (data not shown). The W7Dest primer set amplified a product in HS but not in HS/VPM7D, providing a dominant (presence / absence) marker.

Marker development using 7D Beta-glucanase wheat EST

Initial efforts to produce a PCR primer set based upon the RFLP probe *Xpsr121* that encodes a beta-glucanase were not successful. Following screening of nullisomic lines of Chinese Spring, it was determined that this probe derives from wheat Group 1 chromosomes. The wheat EST BG604756 maps to wheat deletion bin 7DL3-0.821-1.00, located at the distal end of chromosome 7D. BG604756 is annotated as encoding for beta-glucanase and this sequence was used to develop a marker specific for beta-glucanase on chromosome 7D. The B-glu7D primer set amplified a product in HS but not in HS/VPM7D, providing a dominant marker.

The RVPM population was screened with 14 SSR markers (12 conventional SSRs, and the *XustSSR2001-7DL* and *XW7Dest* SSR markers targeted to *Pch1*) and the B-glu7D primer set.

A genetic map of chromosome 7D was constructed by combining the data obtained in the present study with the RFLP and phenotype data, including location of *Pch1* from Chao et al. (1989). The RFLP based map of chromosome 7D produced by Chao et al. (1989) was constructed from 68 RVPM lines and an additional two lines had to be excluded from the analysis because of missing data. JoinMap 3.0 resolved three linkage groups and the map was orientated and the position of the centromere was determined on the basis of the Somers et al. (2004) SSR consensus map for chromosome 7D (Fig. 1). Two SSR markers (*Xwmc14* and *Xbarc97*) mapped to the same position as *Pch1* and the markers *Ep-D1* and *Xpsr121* that were shown to co-segregate with *Pch1* previously (Chao et al. 1989). The B-glu7D marker designed to EST BG604756 (beta-glucanase) also co-segregated with *Pch1* (Fig. 1). The full set of RVPM lines were screened with *Xwmc14*, *Xbarc97* and *XB-glu7D* and all three markers were found to co-segregate with *Pch1* (results not shown). The W7Dest SSR marker derived from the SSR-containing EST (BE446395) within deletion bin 7DL3-0.82-1.00 was found to map to the distal end of chromosome 7D but did not co-segregate with *Pch1,* being separated by 6 cM (Fig. 1).

SSR analysis and map construction of chromosome 7A

The two parents CS and CS/CD7A were screened using 16 7A SSR markers. Thirteen (81%) of the 7A SSRs were found to be polymorphic between CS and CS/CD7A (Table 1). The 13 polymorphic SSRs were used screen 192 CS:CS/CD7A F_2 lines and the data used to construct a map of chromosome 7A. The software package JoinMap®

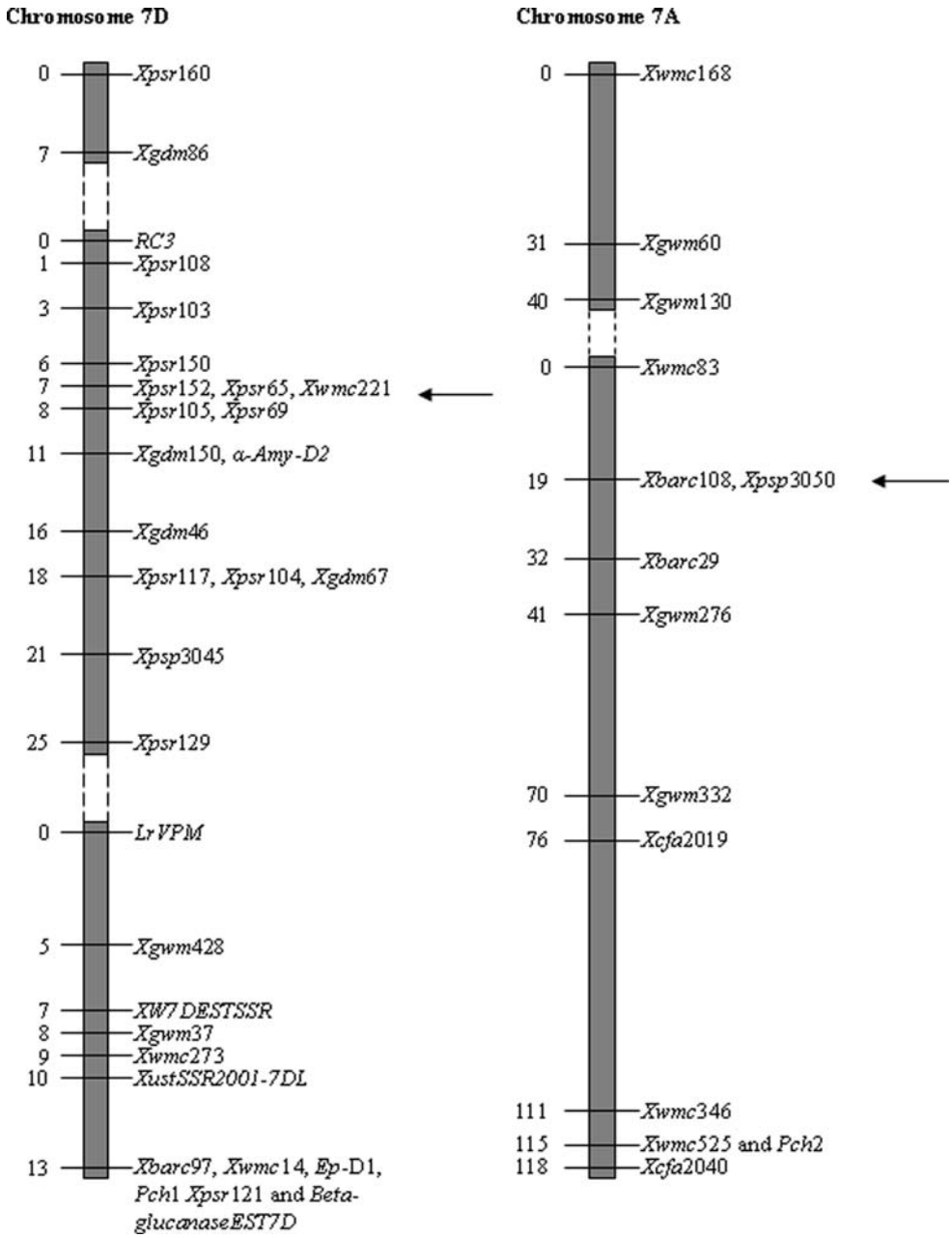

Fig. 1 Genetic map of chromosome 7D in the Hobbit-sib (HS): Hobbit-sib/VPM7D (HS/VPM7D) recombinant population (RVPM lines) and a genetic map of chromosome 7A in Chinese Spring (CS): CS chromosome substitution line Cappelle Desprez (CS/CD7A) recombinant lines. Distances are measured in Kosambi cM units. The *broken vertical line* represents no linkage. The centromere is indicated by the *arrow*

(version 3.0) resolved two linkage groups (Fig. 1) and the linkage groups were orientated according to the Somers et al. (2004) SSR consensus map for chromosome 7A.

Pch2 resistance in the CS:CS/CD7A F_3 families

A total of 162 CS:CS/CD7A F_3 families were phenotyped for *Pch2* resistance to *O. acuformis* in the first trial and 183 F_3 families were phenotyped in the second trial. Analysis of variance demonstrated that genotype was highly significant in both trials but that variance due to replicate was also high in Trial 2 carried out in an unheated glasshouse (Table 2).

QTL interval mapping analysis

QTL interval mapping was also used to determine the genomic location of *Pch2* on chromosome 7A and identify SSR markers associated with the resistance. The QTL analysis of results from the individual trials revealed that *Pch2* was associated with three SSRs; (*Xwmc346*, *Xwmc525* and *Xcfa2040*) that mapped together on the distal end of the long arm of chromosome 7A (Fig. 1). The marker most significantly associated with eyespot disease in all instances was SSR *Xwmc525*. This marker explained 35.1% of the phenotypic variance observed in Trial 1 and 40.1% in Trial 2 (Table 3). The combined data from the two trials also

Table 2 Variance components of visual eyespot disease scores using generalised linear modelling for the two disease trials of the CS × CS/CD7A F_3 families

Source of variation	Trial 1		Trial 2	
	MS	*F* value	MS	*F* value
Replicate	8.32	8.76***	164.55	80.66***
Genotype	28.27	29.78***	16.94	8.3***
Replicate x Genotype	2.35	2.48***	4.16	2.04***
Residual	0.95		2.04	

MS mean squares

*** $P < 0.001$

showed that *Xwmc525* was the marker most significantly associated with resistance (*Pch2*) explaining 36.3% of the phenotypic variance observed (Table 3).

Discussion

Pch1 on chromosome 7D.

We have integrated SSR marker data into the RFLP-based map of 7D from RVPM lines generated by Chao et al. (1989) to provide PCR-based markers for *Pch1*. Two SSR markers, *Xbarc97* and *Xwmc14*, co-segregated with the *Pch1* locus that confers eyespot resistance. These two SSR markers were also found to be completely linked to *Pch1* in a large mapping population (Leonard et al. 2007). However, evidence was presented to suggest that linkage between *Pch1* and these SSRs can be broken. Unfortunately, both SSR markers are dominant, failing to amplify the *Ae. ventricosa* allele, making them suitable only for screening homozygous material in breeding programmes. Furthermore, exclusive use of dominant markers makes it impossible to detect failed reactions (Varshney et al. 2005). This problem can be easily overcome by including a second co-dominant marker in the PCR reaction. Overall, six of the thirteen SSRs used herein were found to be dominant markers (absent in VPM), in the RVPM population. Other studies have also found SSR markers to be less transferable between closely related genera than within a genus (Röder et al. 1995; Peakall et al. 1998). For example, Gupta et al. (2003) found 66% of EST-based SSRs to be polymorphic between 18 alien species and *Triticum aestivum* L. due to null alleles in the alien. We found the *XustSSR2001-7DL* marker to be the closest co-dominant SSR marker to *Pch1* mapping 3 cM from the resistance locus. The SSR *XustSSR2001-7DL* was recently reported as a highly polymorphic marker linked to *Pch1* and *Ep-D1*, with a calculated recombination frequency of 2% (Groenewald et al. 2003). However, use of this marker to predict eyespot resistance (*Pch1*) is reported to be only about 90% efficient and it has been concluded that the *Ep-D1* isozyme marker was more efficient in the MAS selection of *Pch1* than *XustSSR2001-7DL* (Santra et al. 2006). A single EST in the 7DL distal deletion bin (7DL3-0.821-1.00) was found to contain SSR motifs. The SSR marker (*XW7Dest*) designed to this EST mapped to the distal end of chromosome 7D but 6 cM away from *Pch1*, and therefore, like *XustSSR2001-7DL*, cannot be used for reliable MAS selection of *Pch1*, as there is a high probability that recombination would occur between the marker and *Pch1*.

Ep-D1 is widely used for selecting *Pch1* resistance (Koebner and Summers 2002). It was originally suggested that the eyespot resistance conferred by *Pch1* might be a product of *Ep-D1b* (Worland et al. 1988). Later study showed this not to be the case because wheat line H-93-51 was found to carry the *Ep-D1b* allele but is susceptible to eyespot (Mena et al. 1992). The authors concluded that *Ep-D1b* and *Pch1* can be separated and eyespot resistance is not a product of the *Ep-D1* locus (Mena et al. 1992). However, a recent study reported that a STS marker to *Ep-D1* showed complete linkage to *Pch1* in a cross between the

Table 3 Summary of the QTL interval mapping analysis of *Pch2* on chromosome 7AL

Marker	Map distance (cM)	Trial 1		Trial 2		Combined	
		LOD[a] score	% variance explained	LOD score	% variance explained	LOD score	% variance explained
wmc83	0	0.4	1.3	0.7	2.1	0.7	1.8
barc108	19	0.1	0.2	0.1	0.3	0.3	0.6
psp3050	19	0.1	0.2	0.1	0.3	0.3	0.6
barc29	32	0.3	0.8	0.3	0.7	0.3	0.7
gwm276	41	0.4	1.1	0.1	0.3	0.1	0.3
gwm332	70	0.2	0.7	3.1	8.3	1.9	5.0
cfa2019	76	0.8	3.4	4.5	15.1	3.3	10.2
wmc346	111	10.9	26.7	13.2	31.2	13.4	29.2
wmc525	115	15.1	35.1	18.2	40.1	17.6	36.3
cfa2040	119	11.0	27.1	18.1	40.0	14.1	30.7

[a] Logarithm of the odds score

eyespot resistant cultivar Coda and the susceptible cultivar Brundage (Leonard et al. 2007).

The RFLP marker *Xpsr121* co-segregates with *Pch1* in the RVPM population (Chao et al. 1989). This probe includes sequence for a beta-glucanase gene. Initial attempts to design a PCR-based marker to this probe failed to produce a marker to 7D, amplifying instead products from Group 1 chromosomes (results not shown). The beta-glucanase sequence hybridising to *Xpsr121* on 7D appears to be a paralogue to the original probe. Fortunately, the EST BG604756 encodes beta-glucanase and has been mapped to the distal deletion bin on chromosome 7DL. PCR primers designed to the 7D beta-glucanase EST produced a dominant marker, amplifying a product from HS but not HS/VPM7D. The *XB-glu7D* marker co-segregated with the RFLP marker *Xpsr121* and, hence *Pch1*.

On the basis of RFLP data, recombination within chromosome 7D in RVPM lines carrying the introgressed segment from *Ae. ventricosa* was shown to be significantly reduced relative to that in chromosomes 7A and 7B. Chao et al. (1989) reported that the *Ep-B1* and *Xpsr121* loci on 7B were 9.9 cM apart and, similarly, the *Ep-A1* and *Xpsr121* loci on 7A are separated by 3.9 cM (de la Pena et al. 1997). In contrast, these markers were found to be completely linked for the 7D chromosome of RVPM lines (Chao et al. 1989). This finding is confirmed by the SSR markers added in the present study. For example, loci *Xwmc273* and *Xwmc14* were separated by 4 cM on the 7D chromosome carrying *Pch1* from *Ae. ventricosa* whereas these loci are reported to be separated by 10 cM on the native 7D chromosome of wheat (Somers et al. 2004). This difference in recombination frequency may result from the alien 7D chromosome *Ae. ventricosa*. The alien segment may prevent chiasmas from occurring between the 7D chromosome of wheat and the 7D chromosome of *Ae. ventricosa* reducing recombination (de la Peña et al. 1996). It has also been suggested that an inversion of the VPM segment involving the region between *Ep-D1* and *Xpsr121* would also give an appearance of complete linkage (Chao et al. 1989).

Pch2 on chromosome 7A

Eighty one percent of the SSRs were polymorphic between CS and CS/CD7A. This figure is high compared to the level of polymorphism identified for Group 7 chromosomal RFLP probes in diverse wheat recombinant populations, where polymorphism levels averaged 9% (Chao et al. 1989). Even though non-genic SSRs tend to have higher levels of polymorphism compared to cDNA derived RFLP markers (Chalmers et al. 2001) the level of SSR polymorphism is generally 20–40% in single-wheat mapping populations (Somers et al. 2004), which is lower than that observed here between CS and CS/CD7A. The genetic map developed herein agrees well with that of Somers et al. (2004) with the exception of the relationship between *Xcfa2019* and *Xwmc346*. While the distance between these loci was estimated as 6 cM (Somers et al. 2004) we found the distance to be much greater, being approximately 49 cM. The reasons for this disparity are not known.

Despite the difficulties of carrying out screening for resistance to a necrotrophic pathogen using an F_3 population, the phenotype data from the first trial indicated that *Pch2* functions as a single gene, which corresponds with previous studies (Strausbaugh and Murray 1989; de la Pena et al. 1997). However, *Pch2* was analysed as a quantitative trait due to its lack of potency compared to *Pch1* (Hollins et al. 1988) and because of the variability associated with using an F_3 population, particularly in the uncontrolled conditions of the second trial. QTL interval mapping associated *Pch2* resistance with three SSRs, *Xwmc346*, *Xwmc525* and *Xcfa2040*. The most significant association was with SSR *Xwmc525* which accounted for 35.1 and 40.1% of variance explained in the first and second trial, respectively (Table 2). Thus, results from both trials indicated that *Pch2* is located at the distal end of the long arm of chromosome 7A and is closely linked to SSR *Xwmc525*.

Pch2 has previously been mapped to the long arm of chromosome 7A, being located between RFLP markers *Xcdo*347 and *Xwg380* (de la Peña et al. 1997) which are separated by 29.8 cM (de la Peña et al. 1997). The authors concluded that the RFLP markers *Xcdo*347 and *Xwg380* could be used in combination to select for *Pch2* resistance because double recombination between these markers occurred in only 3% of the population. In the current study we have identified three SSRs (*Xwmc346*, *Xwmc525* and *Xcfa2040*) that are closely linked to *Pch2*. Our results indicate that *Pch2* maps close to *Xwmc525* within the 7 cM interval flanked by *Xwmc346* and *Xcfa2040*. These provide the first reported PCR-based markers to this eyespot resistance gene.

Taken together with the results from previous reports, the results from the present study strongly suggest that *Pch1* and *Pch2* are homeoloci. The 7D locus of *Xcfa2040* is close to that for *Xwmc14* which we have shown to co-localise with *Pch1* in the RVPM population, suggesting that this locus is close to *Pch1,* whilst we have also shown that the 7A locus of *Xcfa2040* is close to *Pch2*. Probably as a result of reduced recombination, the endopeptidase marker *Ep-D1b* and the RFLP marker *Xpsr121* are closely linked to *Pch1* and may be used for the selection of *Pch1* resistance (Chao et al. 1989). However, on chromosome 7A, the homeologous endopeptidase marker *Ep-A1b* is only weakly linked to *Pch2* (Koebner and Martin 1990), and cannot be used to follow this resistance in breeding programmes (de la Peña et al. 1996). Both *Xpsr121* and *Ep-A1b* appear

distal to *Pch2* on chromosome 7A (de la Peña et al. 1997), although the former is closer to *Pch2*. Assuming that *Pch1* and *Pch2* are homeologous it would be anticipated that the *XB-glu7D* marker developed in the present study, would be closer to *Pch1* than the endopeptidase marker *Ep-D1b*. In the current study we have developed a series of PCR-based markers to facilitate selection of both the major eyespot resistance genes, *Pch1* and *Pch2,* in wheat breeding programmes.

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