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ORIGINAL PAPER

Mapping QTL for resistance to eyespot of wheat in *Aegilops longissima*

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Abstract Eyespot is an economically important disease of wheat caused by the soilborne fungi *Oculimacula yallundae* and *O. acuformis*. These pathogens infect and colonize the stem base, which results in lodging of diseased plants and reduced grain yield. Disease resistant cultivars are the most desirable control method, but resistance genes are limited in the wheat gene pool. Some accessions of the wheat wild relative *Aegilops longissima* are resistant to eyespot, but nothing is known about the genetic control of resistance. A recombinant inbred line population was developed from the cross PI 542196 (R) × PI 330486 (S) to map the resistance genes and better understand resistance in *Ae. longissima*. A genetic linkage map of the S^l genome was constructed with 169 wheat microsatellite markers covering 1261.3 cM in 7 groups. F_5 lines (189) were tested for reaction to *O. yallundae* and four QTL were detected in chromosomes $1S^l$, $3S^l$, $5S^l$, and $7S^l$. These QTL explained 44 % of the total phenotypic variation in reaction to eyespot based on GUS scores and 63 % for visual disease ratings. These results demonstrate that genetic control of *O. yallundae* resistance in *Ae. longissima* is polygenic. This is the first report of multiple QTL conferring resistance to eyespot in *Ae. longissima*. Markers *cfd6*, *wmc597*, *wmc415*, and *cfd2* are tightly linked to *Q.Pch.wsu-1S*^{l}, *Q.Pch.wsu-3S*^{l}, *Q.Pch.wsu-5S*^{l}, and *Q.Pch.wsu-7S*^{l}, respectively. These markers may be useful in marker-assisted selection for transferring resistance genes to wheat to increase the effectiveness of resistance and broaden the genetic diversity of eyespot resistance.

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Introduction

Eyespot is caused by the soilborne fungi *Oculimacula yallundae* (syn: *Tapesia yallundae*, Wallwork & Spooner) Crous & W. Gams and *O. acuformis* Crous & W. Gams (syn: *T. acuformis*) (Crous et al. 2003). These two pathogens were formerly known as the W- and R- pathotypes of *Pseudocercosporella herpotrichoides* (Fron.) Deighton, respectively, before the teleomorph was discovered (Lucas et al. 2000). These fungi infect the stem base of wheat and other cereals and grasses, causing eye-shaped elliptical lesions that result in lodging of infected plants and yield loss (Murray 2010). When eyespot is severe, yield loss of up to 50 % can occur in susceptible cultivars (Murray and Bruehl 1986).

Eyespot has been reported in several wheat-growing areas of the world with cool, wet autumn and winter weather including North and South America, Australia, New Zealand, Europe, and Africa (Lucas et al. 2000). In the US, eyespot is a yield-limiting disease mainly in the Pacific Northwest (PNW) even though the pathogens are widespread.

The most economical and environmentally friendly control method for eyespot is growing resistant wheat cultivars. Cappelle Desprez was the first source of eyespot resistance reported from hexaploid wheat (Law et al.

1976). Resistance from Cappelle Desprez has been used extensively since the 1950s and transferred to many cultivars in Europe (Hollins et al. 1988). The genetic control of eyespot resistance in Cappelle Desprez was first studied by Law et al. (1976) using chromosome substitution lines and monosomic analysis. They found that chromosome 7A was critical for eyespot resistance and chromosomes 1A, 2B, and 5D also contributed to resistance. Law et al. (1976) suggested that the inheritance of eyespot resistance in Cappelle Desprez was complex. Doussinault and Dosba (1977) concluded that resistance to eyespot in Cappelle Desprez was quantitative. This conclusion was supported by Jahier et al. (1979), who found multiple resistance factors in wheat cultivar Roazon, which has Cappelle Desprez in its pedigree. The eyespot resistance gene on chromosome 7A of Cappelle Desprez was designated *Pch2* and mapped to the distal portion of the long arm using RFLP (de la Peña et al. 1996, 1997).

Muranty et al. (2002) found that chromosome 5A of Cappelle Desprez carried a gene for eyespot resistance. Later, Burt et al. (2011) mapped a major QTL on chromosome 5AL and associated it with simple sequence repeat (SSR) marker *gwm639*. Three SSR markers (*wmc346*, *wmc525*, and *cfa2040*) were closely linked to *Pch2* on chromosome 7A (Chapman et al. 2008). The eyespot resistance in Cappelle Desprez has been durable; however, its effectiveness is not sufficient under severe eyespot conditions and fungicide application is required to prevent yield loss (Macer 1966; Hollins et al. 1988).

Johnson (1992) reported that eyespot resistance was difficult to find and exploit because it is not readily available in cultivated wheat. Consequently, wild wheat species have been evaluated as sources of resistance (Jones et al. 1995). Wild species, especially *Aegilops* spp., can broaden the genetic diversity of cultivated wheat (Schneider et al. 2008). *Aegilops ventricosa* was reported to be highly resistant to eyespot (Sprague 1936). The introgression of eyespot resistance gene *Pch1* from tetraploid *Ae. ventricosa* Tausch ($2n = 28$, $DDM^{v}M^{v}$) to breeding line VPM-1 is the most successful example of using eyespot resistance genes from a wild relative of wheat (Maia 1967). Several wheat cultivars with *Pch1* derived from VPM-1 have been developed in the US PNW. One of them, soft winter wheat cultivar Madsen, has been widely grown in the PNW since it was released in 1988 (Murray 2010).

The genetic control of eyespot resistance in VPM-1 was reported to be a single dominant gene and mapped to the distal portion of chromosome 7DL using the endopeptidase marker *EP-D1b* (Worland et al. 1988). Chao et al. (1989) mapped RFLP marker, *psr121*, at the same location as *EP-D1b*. Three sequence-tagged-site (STS) markers (*orw1*, *orw5*, and *orw6*) and three microsatellite markers (*wmc14*, *barc97*, and *cfd175*) were tightly linked to *Pch1* (Leonard et al. 2008). Chapman et al. (2008) also found that markers *wmc14*, *barc97*, and *psr121* were closely linked with *Pch1*. Meyer et al. (2011) reported that *orw1*, *orw6*, and *cfd175* were the most suitable markers for marker assisted selection (MAS).

Aegilops longissima Schweinf. & Muschl. ($2n = 2x = 14$, $S^{l}S^{l}$) is a diploid species in the section Sitopsis of *Aegilops* L. (Van Slageren 1994). Species of section Sitopsis have been valuable sources of genes for wheat improvement and disease resistance (Millet 2007). *Ae. longissima* has provided exploitable traits in grain quality, grain weight, and drought tolerance (Levy et al. 1985; Millet et al. 1988). Resistance in *Ae. longissima* to Septoria glume blotch, powdery mildew, and rusts of wheat has been reported (Ecker et al. 1990; Cenci et al. 2003; Anikster et al. 2005). *Ae. longissima* was recently identified as a new source of resistance to eyespot (Sheng and Murray 2009).

Genetic mapping is needed to discover the eyespot resistance genes and associated molecule markers in *Ae. longissima*. To date, there have been no molecular markers developed directly from the *Ae. longissima* genome. Zhang et al. (2001) used 59 wheat RFLP probes to construct a genetic map of *Ae. longissima* with 7 linkage groups, but it only comprises 67 loci. They found that 62 % of the markers were polymorphic between the parents, and provided evidence that wheat RFLP markers can be used in *Ae. longissima*.

Wheat microsatellite markers, also known as SSR markers, are tandem repeats of short (2–6 bp) DNA sequences (Röder et al. 1998). Over 1,500 SSR markers have been developed from the wheat genome and they are more polymorphic than other marker systems in wheat (Adonina et al. 2005). The majority of SSRs are co-dominant and chromosome-specific (Röder et al. 1998). Wheat SSRs were used to amplify DNA from wheat relatives, including *Ae. longissima* (Sourdille et al. 2001). Adonina et al. (2005) tested 253 wheat SSRs for their transferability to diploid *Aegilops* species and found that 68 % of them amplified in *Ae. longissima*. These results demonstrated the possibility of applying wheat SSRs to *Ae. longissima*.

Since differential genetic control of resistance to *O. yallundae* and *O. acuformis* was reported in *Dasypyrum villosum* (Uslu et al. 1998), the objectives of this study were to determine the genetic control of resistance to *O. yallundae* in *Ae. longissima* and to locate the genes in the S^{l} genome by developing a linkage map using wheat SSR markers. This work will contribute to the long-term goal of transferring new eyespot resistance genes to wheat.

Materials and methods

Mapping population

Ae. longissima accessions PI 542196 and PI 330486 (obtained from the USDA National Small Grains Collection) are resistant and susceptible to both *O. yallundae* and *O. acuformis*, respectively (Sheng and Murray 2009). PI 542196 was originally collected from Izmir, Turkey, and PI 330486 is from an unknown source; both are winter habits. A recombinant inbred line (RIL) population with 189 lines was developed through single-seed descent from the cross PI 542196 (R) × PI 330486 (S).

Phenotypic evaluation

RILs and the parents were evaluated for eyespot resistance in growth chamber experiments using a modified GUS assay (de la Peña and Murray 1994). Winter wheat cultivars Madsen and Hill 81 were the resistant and susceptible controls, respectively. One hundred eighty-nine F_5 RILs were tested twice in a randomized complete block (RCB) design experiment with three blocks; a total of 12 plants per line were tested.

Two seeds were planted into a 6.4 cm square plastic pot (McConkey Co., Sumner, WA) with commercial Sunshine Potting Mix#1/LC1 (SunGro Horticulture, Bellevue, WA) and fertilized with Osmocote (14-14-14, w/v) (The Scotts Company LLC, Marysville, OH). Plastic flats without drain holes (54 × 27 × 6 cm) were used to hold 50 pots; four flats together acted as one block and were always placed in the same growth chamber maintained at 15/13 °C with a 12-h photoperiod and rotated every 2–3 days. Relative humidity was maintained between 98 and 100 %.

Plants were inoculated with a mixture of conidia from β-glucuronidase (GUS) transformed *O. yallundae* isolates tph8934-5-61, tph8934-5-62, tph8934-5-68, and tph8934-5-70 when at the two-leaf stage (de la Peña and Murray 1994). A slurry was made by blending conidia, 1.5 % fresh WA, and water together for a final concentration of 2.1×10^5 conidia per ml. During inoculation, 250 µl of the slurry was pipetted into a 3.3-cm long split drinking straw collar around each plant stem base. The same amount of inoculum was added again 1 or 2 days later (de la Peña and Murray 1994).

Eight weeks after inoculation, at approximately growth stage 23–25 (Zadoks et al. 1974) a 3-cm section of the whole stem was removed from around the inoculation site and briefly washed with tap water to remove soil. Visual disease ratings were performed on a 0–4 scale (Yildirim et al. 2000), where 0 = no symptoms (healthy), 1 = a lesion only on the first leaf sheath, 2 = a lesion on the first leaf sheath and a small lesion on the second leaf sheath, 3 = a lesion covering the first leaf sheath and up to half of the second sheath, and 4 = a lesion covering the first and second sheaths (nearly dead). All tillers (2–4) of each plant were evaluated as a whole. The stems were then wrapped with paper towels and frozen at −20 °C until the GUS assay was performed. GUS activity in stems was used as a surrogate measurement of the amount of fungal colonization.

Frozen stems were ground in a leaf squeezer (Ravenel Specialties Company, Seneca, SC) with 2.5 ml GUS extraction buffer added per sample. GUS extraction buffer includes 0.05 M $NaHPO_4$ (pH 7.0), 0.01 M Na_2EDTA (pH 8.0), 1 g *n*-laurylsarcosine, 1 ml Triton X-100, and 0.8 g DL-dithiothreitol per liter. GUS activity was determined by adding 50 µl extract with 40 µl 10 mM fluorescent substrate 4-methylumbelliferyl β-D-glucoside (MUG) (Sigma Life Science, St. Louis, MO) in a 1.2-ml testing tube, and then incubating at 37 °C for 1 h to produce fluorescent methylumbelliferone (MU). The fluorescence intensity of MU was measured in a Molecular Devices SpectraMax M2 microplate reader (Molecular Devices Co., Sunnyvale, CA). GUS scores were expressed as the $\log_{10}$ transformed ratio [$\log_{10}$(*x*/resistant control) +1] of GUS activity of an individual accession (*x*) compared with the activity of the resistant control (Madsen); therefore, the GUS score of Madsen was 1.0.

Statistical analysis

Homogeneity of variance of the two F_5 experiments was tested with the *F*-ratio of the larger error variance to the smaller error variance (Gomez and Gomez 1984). When the variances are homogeneous, data from the two experiments were combined. Statistical analysis was conducted with SAS Version 9.2 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) for GUS score and visual rating in individual or combined experiments and standard deviation were carried out by PROC GLM. Variance components were based on ANOVA for a random model generated from PROC GLM with variances of lines and experiments considered random effects. The hypothesis of normality for the frequency distribution of GUS scores or visual ratings was tested by Kolmogorov–Smirnov test with PROC UNIVARIATE. Both Tukey's *t* test and Dunnett's *t* test were used for multiple comparisons for the least squares mean (lsmean) of RILs and the genotypes based on presence of specific QTL. Pearson correlation coefficients between visual ratings and GUS scores of combined experiments were calculated by PROC CORR.

Broad-sense heritability (H^2) was calculated as H^2 = Var(G)/Var(P), where Var(G) is the genetic variance and Var(P) is the phenotypic variance, which is the

combination of genetic variance and environmental variance [Var(E)]. The equation for broad-sense heritability based on entry mean is $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{gxe}^2/r + \sigma_e^2/rn)$, where r is the number of experiments and n is the number of plants per line (Shen et al. 2003).

DNA isolation and marker analysis

Genomic DNA was extracted from fresh leaves (growth stage 18–20) of the parents and F_5 RILs as described in the protocol for monocot DNA isolation (Wheat Genetic and Genomic Resources Center at Kansas State University, http://www.k-state.edu/wgrc). DNA extraction buffer includes 0.5 M NaCl, 0.1 M Tris–HCl (pH 8.0), 50 mM EDTA (pH 8.0), 0.84 % (w/v) SDS, 0.38 g sodium bisulfate per 100 ml, and 5 N NaOH to adjust pH to 8.0. DNA was dissolved in sterile distilled H_2O and quantified using a Bio-Rad Fluorescent DNA Quantitation Kit (Bio-Rad laboratories, Hercules, CA) on a Molecular Devices SpectraMax M2 microplate reader (Molecular Devices Co., Sunnyvale, CA).

Marker analyses were performed using tailed-PCR in which the forward primer had a 19-bp M13 sequence (5′-CACGACGTTGTAAAACGAC-3′) at the 5′ end and a M13 fluorescently labeled primer added as the third universal primer. Six hundred fifteen wheat microsatellite (SSR) primer sets covering the A, B, and D genomes were screened for polymorphism between the parents.

Polymorphic markers were used to genotype individual 178 F_5 RILs along with parental DNA and water as controls. DNA of 178 F_5 RILs was chosen randomly among the 189 F_5 RILs used for phenotype evaluation because of the limited number of wells in the PCR plate. The 12-µl PCR reaction mix contained 40 ng DNA, 1.2 µl of 10× PCR buffer with 15 mM $MgCl_2$ (New England Biolabs, Ipswich, MA), 0.48 µl of 25 mM $MgCl_2$ (Fermentas, Glen Burnie, MD), 0.24 µl of 250 µM each of dCTP, dGTP, dTTP, and dATP (GenScript USA Inc. Piscataway, NJ), 0.06 µl of 10 µM M13-tailed forward primer, 0.3 µl of 10 µM reverse primer, 0.24 µl of 10 µM M13 primer fluorescently labeled with 6-FAM, VIC, NED, or PET (Applied Biosystems, Foster City, CA), and 0.6 U Taq DNA polymerase (New England Biolabs, Ipswich, MA). Thermocycler conditions consisted of 5 min initial denaturation at 94 °C, 42 cycles of 1 min denaturing at 94 °C, 1 min annealing at primer-specific temperature, 1 min extension at 72 °C, and final extension at 72 °C for 10 min. PCR was conducted in a Bio-Rad iCycler with 384 well Reaction Module (Bio-Rad laboratories, Hercules, CA). PCR products with different fluorophores were mixed and detected on an ABI 3730 Gene Analyzer (Applied Biosystems, Foster City, CA). Fragment analysis was performed using GeneMarker V1.50 software (SoftGenetics, State College, PA).

Linkage map construction and QTL analysis

Marker segregation for resistant to susceptible lines in F_5 was tested by Chi-square (χ^2) analysis for goodness-of-fit to the expected ratio of 17:15. Segregating markers were used to construct linkage maps with Mapmaker V3.0 (Lander et al. 1987). A minimum logarithm of odds (LOD) score of 4.0 was used as the threshold value for grouping markers into linkage groups. Three-point linkage analyses were carried out to order the linked markers with maximum recombination value of 0.5 for calculating the distance between markers. Genetic distance (cM) among markers was computed by the Kosambi map function (Kosambi 1944). Linkage groups were assigned to the putative homoeologous chromosomes of the S^l genome according to the wheat chromosome information provided in GrainGenes (http://wheat.pw.usda.gov/cgi-bin/graingenes). Marker order on the S^l genome was compared with previously published wheat genome maps (Röder et al. 1998; Somers et al. 2004).

QTL analysis was performed using data of F_5 RILs with WinQTLCart V2.5 (Wang et al. 2010). The least squares means for GUS score and visual rating of two experiments for each line were used in the QTL analysis. Single Marker Analysis (Wang et al. 2010) was performed to identify markers with significant effects ($P < 0.05$) for GUS score and visual rating and the chromosome locations of the major QTL for eyespot resistance. Composite Interval Mapping (Wang et al. 2010) was conducted to detect QTL associated with resistance to *O. yallundae*. The LOD threshold value for detecting significant QTL was 2.5 ($P < 0.01$) based on a 1,000-permutation test with 1.0 cM walk speed. The phenotypic variation (R^2) that was explained by significant QTL and additive effects were also carried out with Composite Interval Mapping.

Results

Phenotypic evaluation

Error variance of the two F_5 phenotyping experiments was not significantly different for GUS scores and visual ratings at 95 % significance level; therefore, homogeneity of variance was established and data were combined for analysis. Mean GUS scores of 189 F_5 lines ranged from 1.0 to 1.9, and mean visual ratings ranged from 0.6 to 4.0. Distribution of GUS scores and visual ratings in the F_5 population were continuous and normal based on the Kolmogorov–Smirnov test of normality ($P > 0.15$) (Fig. 1).

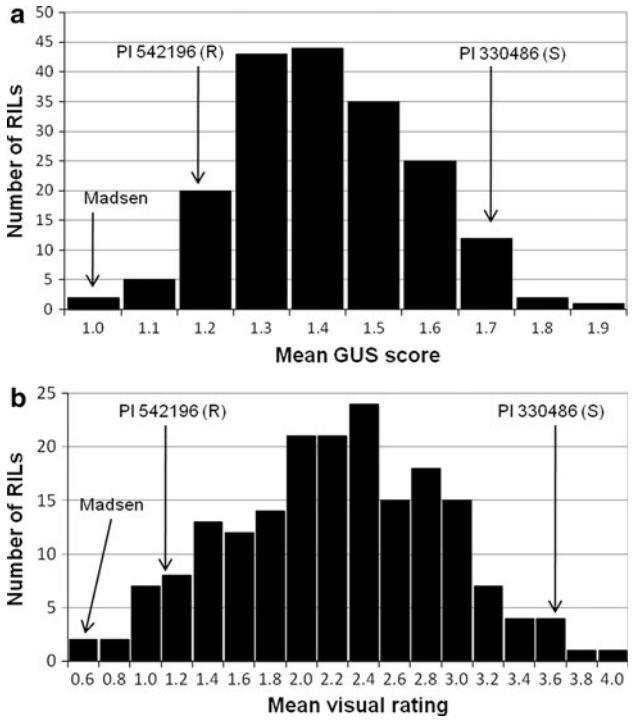

Fig. 1 Distribution of GUS scores (**a**) and visual ratings (**b**) in F_5 population (189 lines, 12 plants/line) of the cross PI 542196 (R) × PI 330486 (S) inoculated with *Oculimacula yallundae*. The null hypothesis of normality was not rejected by the Kolmogorov–Smirnov test with values of 0.0233 and 0.0505 ($P > 0.15$)

GUS scores and visual ratings were significantly correlated ($r = 0.678$, $P < 0.0001$) with combined data in the F_5 population tests. The parental lines, PI 542196 (R) and PI 330486 (S) had significantly ($P < 0.0078$) different GUS scores and visual ratings. The GUS score (1.2) of PI 542196 was not significantly ($P > 0.90$) different from 1.0, the value of Madsen (resistant control), whereas the GUS scores (1.6 or 1.7) of PI 330486 were significantly ($P < 0.0001$) greater than 1.0 in each experiment.

Based on analysis of variance (ANOVA), there was significant variation among RILs for both GUS score and visual rating. Genotypes (RILs) had significantly ($P < 0.0001$) different GUS scores and visual ratings in the F_5 populations (Table 1). The effects of environment (block within experiment) and genotype by environment interaction (Expt. × RILs) were significant ($P < 0.05$). However, neither GUS scores ($P = 0.23$) nor the visual ratings ($P = 0.83$) were significantly different between the two experiments in the F_5 population. Broad-sense heritability (H^2) based on line means in the F_5 population was 82 and 81 % for GUS score and visual rating, respectively (Table 1).

Genetic linkage map

Among 615 wheat SSR markers, 332 (54 %) were polymorphic between the parental lines (PI 542196 and PI 330486) and 189 (57 %) of those were co-dominant. The polymorphic markers were used to genotype F_5 RILs and 215 segregating markers were used for mapping. A linkage map of *Ae. longissima* was constructed with 169 linked markers covering 1261.3 cM in 7 groups. The average distance was 7.46 cM between markers. Putative homoeologous chromosomes were assigned as $1S^l$, $2S^l$, $3S^l$, $4S^l$, $5S^l$, $6S^l$, and $7S^l$ (Fig. 2a, b). Individual chromosomes had 15–39 markers and length ranged from 53.4 to 287.6 cM (Table 2). Sixty percent (101 markers) of the 169 markers were mapped on homoeologous chromosomes (1A, 1B, or 1D) in the S^l genome. Seventy-seven of the 101 markers (76 %) have the co-linearity in wheat homoeologous chromosomes (Table 2).

QTL analysis

Four QTL for eyespot resistance were detected on chromosomes $1S^l$, $3S^l$, $5S^l$, and $7S^l$, respectively (Fig. 2a, b). All QTL were significant using both GUS scores and visual ratings, and were contributed by the resistant parent, PI 542196. These QTL were designated as *Q.Pch.wsu-1S*^{l}, *Q.Pch.wsu-3S*^{l}, *Q.Pch.wsu-5S*^{l}, and *Q.Pch.wsu-7S*^{l}.

Q.Pch.wsu-1S^l was detected on chromosome $1S^l$ with LOD values of 5.2 and 7.1 for GUS score and visual rating, respectively (Fig. 2a). *Q.Pch.wsu-1S*^{l} explained 11 and 15 % of the phenotypic variation with GUS score and visual rating, respectively, and is flanked by SSR markers *barc119* and *cfd83*, including 12 markers and covering a 25.7-cM interval. All 12 markers were significantly associated with *Q.Pch.wsu-1S*^{l} ($P < 0.001$) and 9 of them are co-dominant markers. Markers *cfd6*, *gdm67*, *gwm642*, and *cfd48*, which were clustered at a 3.1-cM interval on chromosome $1S^l$, were most closely linked to *Q.Pch.wsu-1S*^{l}*-1* ($P < 0.0001$). Three of them are co-dominant. For both GUS score and visual rating, the mean of lines with the resistant allele (PI 542196) was significantly less (more resistant) than the susceptible allele (PI 330486) ($P < 0.0005$) at markers *cfd6* and *gdm67* (Fig. 4). An additive effect (0.06 for GUS score and 0.19 for visual rating) was contributed from the resistant parent PI 542196.

Q.Pch.wsu-3S^l explained 14 and 9 % of the phenotypic variation with GUS score and visual rating, respectively. Its LOD values were 5.3 and 3.4 for GUS score and visual rating, respectively (Fig. 2a). *Q.Pch.wsu-3S*^{l} was associated with both GUS score and visual rating in a 39.2-cM interval between markers *wmc169* and *wmc231* on chromosome $3S^l$. *Q.Pch.wsu-3S*^{l} may be located on the short arm because the two flanking markers are located on the short arm of wheat chromosome 3A and 3B, respectively. Markers *gdm72* and *wmc597*, in an interval of 6.1 cM, had a significant ($P < 0.0001$) effect on both GUS score and visual rating. Marker *wmc597* was about 7 cM from the

Table 1 Variance components of GUS scores and visual ratings and broad-sense heritability (H^2) for F_5 recombinant inbred lines derived from the cross PI 542196 (R) × PI 330486 (S) and inoculated with *Oculimacula yallundae*

Source of variation	*df*	GUS score		Visual rating	
		Mean square	*F* value	Mean square	*F* value
RILs[a]	188	0.27	4.54**[b]	4.94	5.65**
Block (Expt.)	4	2.69	46.17**	34.76	39.76**
Experiment	1	5.71	2.12	1.84	0.05
Expt. × RILs	188	0.09	1.6**	1.65	1.88**
Error	1,660	0.058		0.87	
H^2 (based on line means)		82 %		81 %	

[a] 189 lines; 12 plants/line in two experiments

[b] ** $P < 0.01$

QTL peak. Both GUS score and visual rating were significantly ($P < 0.0001$ and $P = 0.0005$, respectively) different between resistant and susceptible lines at the closest marker *wmc597* (Fig. 4). The additive effects from the resistant parent were 0.21 and 0.05 for GUS score and visual rating, respectively.

Q.Pch.wsu-5S^{l} had LOD values of 4.3 and 17.1 for GUS score and visual rating, and explained 10 and 28 % of the phenotypic variation, respectively (Fig. 2b, 3). *Q.Pch.wsu-5S*^{l} was flanked between markers *gwm213* and *gwm271* in a 24-cM interval. Six markers fell in that range and all were significantly ($P < 0.0001$) associated with *Q.Pch.wsu-5S*^{l} by GUS score and visual rating. It is possible that *Q.Pch.wsu-5S*^{l} is on the long arm because all these markers are located at long arm of wheat chromosome group 5. Markers *gwm639*, *wmc415*, and *cfd12*, in a 13.1-cM interval, were the most closely linked markers. At these markers, GUS score and visual rating were significantly less for the resistant parental alleles ($P < 0.0085$ and $P < 0.0001$, respectively) (Fig. 4). Additive effects were 0.06 for GUS score and 0.32 for visual rating, which came from the resistant parent.

Q.Pch.wsu-7S^{l} was detected with both visual rating and GUS score near the end of chromosome 7S^{l}L since some homoeologous markers have similar locations on wheat homoeologous group 7. The flanking markers, *gdm132* and *cfd2*, are in a 12.5-cM interval (Fig. 2b). Both markers were significantly ($P < 0.003$) associated with the QTL. *Q.Pch.wsu-7S*^{l} had LOD values of 3.3 and 4.8 and explained 9 and 11 % of the phenotypic variation of GUS score and visual rating, respectively. At marker *cfd2*, both GUS score and visual rating were significantly lower ($P = 0.0024$ and 0.0017, respectively) for the resistant parental types (Fig. 4). The resistant parent had additive effects with values of 0.05 and 0.24 for GUS score and visual rating, respectively.

The QTL in *Ae. longissima* exhibited additive effects ranging from 0.05 (*Q.Pch.wsu-7S*^{l}) to 0.32 (*Q.Pch.wsu-5S*^{l}). In total, the additive effect was 0.38 for GUS scores and 0.80 for visual ratings. Epistatic effects were detected between some QTL, but they were not significant.

Sixteen genotypes with three to eighteen lines each were produced from 178 RILs based on presence of these four QTL. Genotypes were defined by combinations of the resistant parental allele at the closest marker to each QTL. *cfd6*, *wmc597*, *wmc415*, and *cfd2* are the closest markers to *Q.Pch.wsu-1S*^{l}, *Q.Pch.wsu-3S*^{l}, *Q.Pch.wsu-5S*^{l}, and *Q.Pch.wsu-7S*^{l}, respectively. The LSmeans for GUS score and visual rating for each genotype were calculated (Fig. 5). Both GUS score and visual rating were significantly ($P < 0.0001$) different among the 16 genotypes. Lines with no QTL had the greatest GUS score (1.6) and visual rating (3.1), and the lines with all QTL had the lowest GUS score (1.2) and visual rating (1.4). GUS scores and visual ratings for genotypes with one QTL were not significantly different ($P > 0.15$ and $P > 0.14$, respectively) from lines with no QTL or from each other, but were significantly ($P < 0.0001$) greater than the lines with all QTL.

Six genotypes with combinations of two QTL had significantly ($P < 0.05$) lower GUS scores, and four of them had significantly ($P < 0.05$) lower visual ratings than the 'no QTL' genotype. The combination of *Q.Pch.wsu-3S*^{l} and *Q.Pch.wsu-5S*^{l} was as effective as three QTL combinations and was not significantly different than lines with all QTL for either GUS score or visual rating ($P = 0.81$ and $P = 0.49$, respectively).

Genotypes with combinations of three QTL had significantly lower GUS scores and visual ratings ($P < 0.0006$ and $P < 0.0002$, respectively) than 'no QTL'. With the exception of the combination of *Q.Pch.wsu-1S*^{l}, *Q.Pch.wsu-3S*^{l}, and *Q.Pch.wsu-7S*^{l} for visual rating, all were not significantly greater than the 'all QTL' for both GUS score and visual rating ($P > 0.21$ and $P > 0.12$, respectively). When combined with other QTL, *Q.Pch.wsu-5S*^{l} played a critical role in eyespot resistance. Lines containing all QTL had the lowest GUS scores and visual ratings and were significantly lower than most other genotypes.

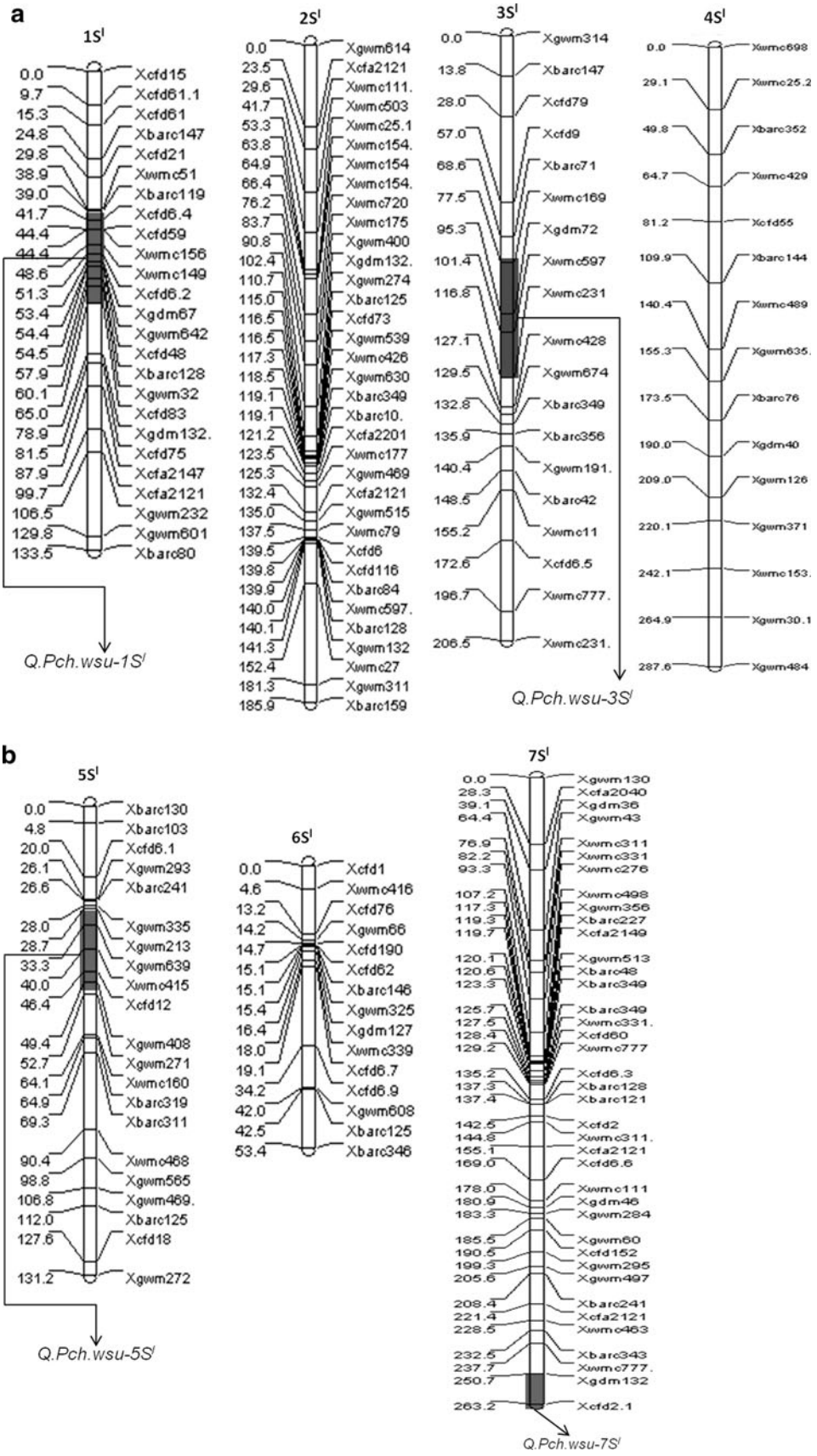

Fig. 2 Linkage map of *Aegilops longissima* chromosomes. **a** $1S^l$, $2S^l$, $3S^l$, and $4S^l$. Chromosomes $1S^l$ and $3S^l$ carry QTL *Q.Pch.wsu-1S*^{l} and *Q.Pch.wsu-3S*^{l} for eyespot resistance, respectively. **b** $5S^l$, $6S^l$, and $7S^l$. Chromosome $5S^l$ and $7S^l$ carry *Q.Pch.wsu-5S*^{l} and *Q.Pch.wsu-7S*^{l} for eyespot resistance, respectively. QTL are indicated on chromosomes as *black rectangles*

Table 2 Genetic linkage groups of *Aegilops longissima* based on the cross PI 542196 (R) × PI 330486 (S) constructed with wheat microsatellite markers

Chromosome	Length (cM[a])	Marker #	Co-dominant markers	Homoeologous markers[b]	Co-linear markers with wheat[c]
$1S^l$	133.5	25	14	15	12
$2S^l$	185.9	35	19	25	19
$3S^l$	206.5	19	5	18	12
$4S^l$	287.6	15	1	3	2
$5S^l$	131.2	21	14	16	14
$6S^l$	53.4	15	10	8	6
$7S^l$	263.2	39	11	16	12

[a] Genetic distance (cM) was computed by the Kosambi map function

[b] Markers homoeologous with chromosomes of A, B, or D genomes

[c] Order of markers on the S^l genome was compared with previously published wheat genome maps

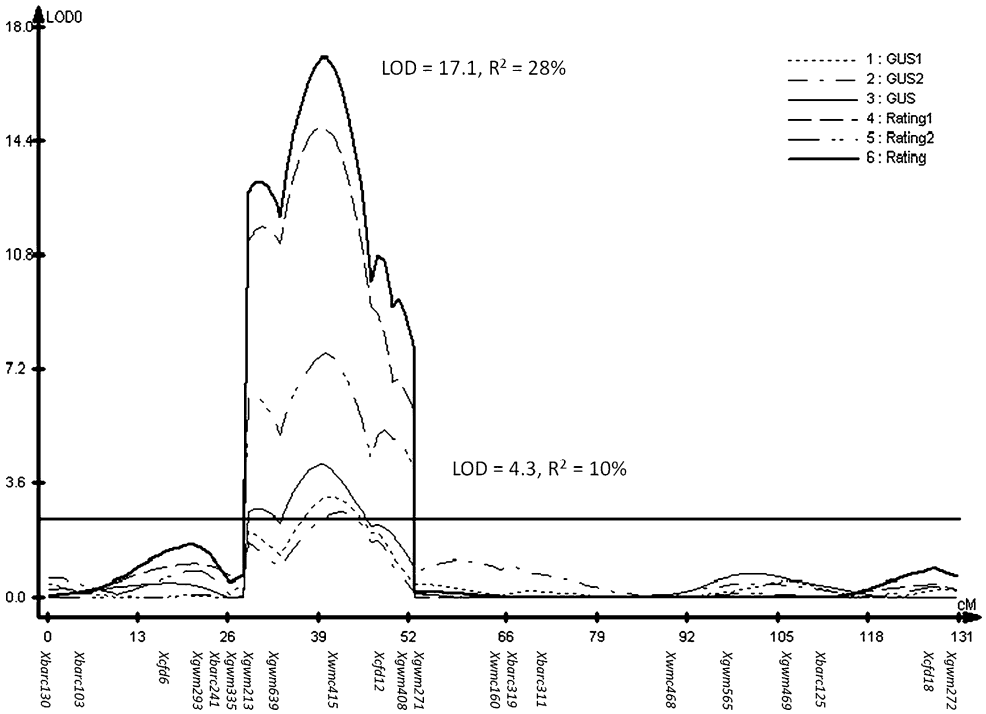

Fig. 3 Major QTL *Q.Pch.wsu-5S*^{l} for eyespot resistance identified on *Aegilops longissima* chromosomes $5S^l$ by GUS score and visual rating with composite interval mapping. The QTL were plotted using data from each individual experiment and combined experiments

Discussion

Four QTL contributing resistance to *O. yallundae* were detected on *Ae. longissima* chromosomes $1S^l$, $3S^l$, $5S^l$, and $7S^l$. These four QTL explained 44 % of the phenotypic variation in GUS scores and 63 % of the variation in visual ratings. These results demonstrate that genetic control of eyespot resistance in *Ae. longissima* RILs of PI 542196 × PI 330486 is polygenic and controlled by QTL. This is the first time that multiple QTL conferring resistance to eyespot in *Ae. longissima* have been reported. To date, only a single QTL for eyespot resistance has been characterized on chromosome 5AL of Cappelle Desprez, which explains 34 % of the phenotypic variation (Burt et al. 2011).

The two commercially available eyespot resistance genes, *Pch1* and *Pch2*, both have been characterized as single genes (Worland et al. 1988; de la Peña et al. 1996). In this study, the distribution of GUS scores and visual ratings in the F_5 population were continuous; therefore, a QTL mapping approach was conducted to determine the inheritance of eyespot resistance in *Ae. longissima*. The identification of multiple QTL confirmed that eyespot resistance in *Ae. longissima* behaved as a quantitatively inherited trait rather than a single gene. Although the resistant wheat control, Madsen, had the lowest GUS score

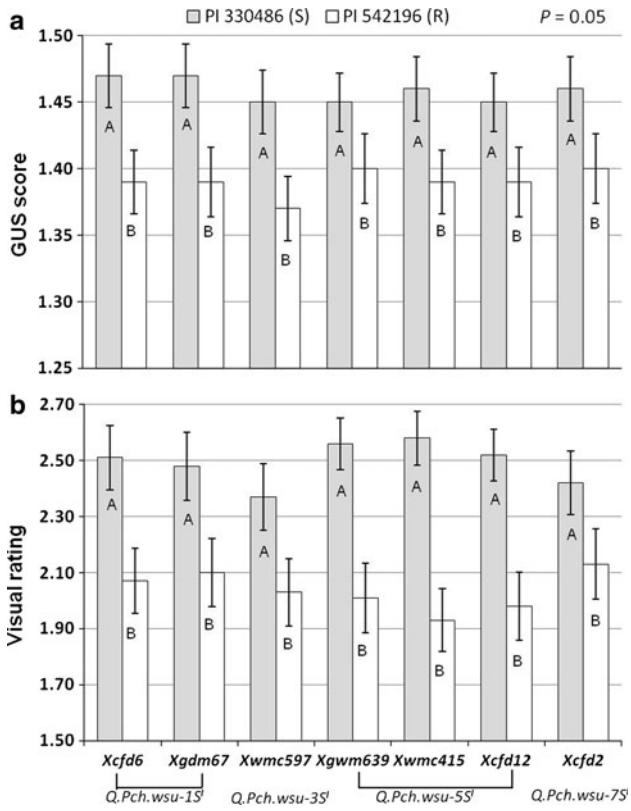

Fig. 4 GUS scores (**a**) and visual ratings (**b**) for RILs of PI 542196 (R) × PI 330486 (S) with different parental alleles at the markers close to each QTL. *cfd6* and *gdm67* are close to *Q.Pch.wsu-1S*^{l}; *wmc597* is close to *Q.Pch.wsu-3S*^{l}; *gwm639*, *wmc415*, and *cfd12* are close to *Q.Pch.wsu-5S*^{l}; and *cfd2* is close to *Q.Pch.wsu-7S*^{l}. The *dark bars* and *light bars* represent the mean GUS scores or visual ratings of RILs with susceptible allele and resistant allele at the marker closet to each QTL, respectively. *Different letters on the bars* indicate the significance ($P < 0.05$). *Error bars* show standard errors

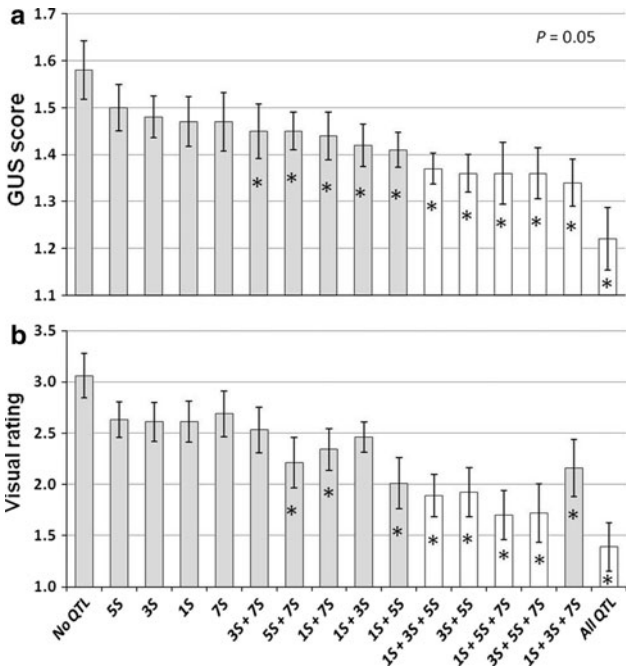

Fig. 5 Resistance of 16 genotypes based on four QTL detected in F_5 RIL populations of PI 542196 (R) × PI 330486 (S) to *Oculimacula yallundae*. Each genotype includes 3–18 lines and each line includes 12 plants. *Bars* represent mean GUS scores or visual ratings of RILs with the same genotype. **a** Mean GUS scores of RILs within each genotype; **b** Mean visual rating of RILs within each genotype. *Bars with an asterisk* are genotypes with significantly ($P < 0.05$) lower GUS scores or visual ratings than 'no QTL'. 1S, 3S, 5S, and 7S represent *Q.Pch.wsu-1S*^{l}, *Q.Pch.wsu-3S*^{l}, *Q.Pch.wsu-5S*^{l}, and *Q.Pch.wsu-7S*^{l}, respectively. *Light bars* are genotypes that were not significantly ($P > 0.05$) greater than 'all QTL' for either GUS score or visual rating. *Error bars* show standard errors

and visual rating, it does not completely prevent yield loss under field conditions. The newly identified QTL in *Ae. longissima* are potentially valuable to increase the effectiveness and genetic diversity of eyespot resistance available to breeding programs.

Law et al. (1976) detected minor contributions to eyespot resistance on chromosomes 1A, 2B, and 5D of Cappelle Desprez in addition to the major contribution from 7A in a monosomic analysis. Strausbaugh and Murray (1989) found one semidominant gene for eyespot resistance in Cappelle Desprez and suggested the possibility of more genes involved in resistance since their samples were collected at 4 weeks after inoculation. Burt et al. (2011) identified a QTL (*QPch.jic-5A*) for eyespot resistance in Cappelle Desprez in both seedling and adult plants. However, Law et al. (1976) was not able to detect resistance on chromosome 5A of Cappelle Desprez seedlings. Muranty et al. (2002) evaluated eyespot resistance in Cappelle Desprez at both the seedling and adult stages; they found a major gene on chromosome 7A was only effective at the seedling stage and another gene on 5A was only effective at the adult stage. Polygenic eyespot resistance has been confirmed in Cappelle Desprez (Law et al. 1976; Jahier et al. 1979), but not all genes in Cappelle Desprez have been characterized and mapped. In our study, four QTL were detected with phenotypic data collected 8 weeks after inoculation (10-week-old plants), which was approximately growth stage 23–25 (Zadoks et al. 1974). Eyespot resistance in *Ae. longissima* may be effective in both seedling and adult stages and inherited in a similar manner to that of Cappelle Desprez, rather than the single dominant gene in VPM-1.

In QTL mapping, accurate phenotypic evaluation is critical. In this study, GUS assay was conducted to evaluated eyespot severity in addition to visual ratings. The GUS reporter gene system quantifies pathogen growth and provides an objective and sensitive tool to assess phenotype that complements visual ratings (de la Peña and Murray 1994; de la Peña et al. 1996, 1997; Yildirim et al. 2000). During the GUS assay, inoculation was conducted

similar to Macer's straw-cylinder technique (Macer 1966), in which an inoculum slurry was pipetted into a straw collar around the stem base. This technique provides both uniformity and accuracy because other pathogens rarely attack the stem base (Doussinault and Dosba 1977). de la Peña and Murray (1994) developed the GUS assay to more accurately assess the eyespot resistance phenotype. GUS scores reflect eyespot pathogen growth and were highly correlated with eyespot resistance based on field evaluation of adult plants (de la Peña and Murray 1994). In our study, all QTL were identified with both visual ratings and GUS scores, which confirms that GUS scores are positively correlated with eyespot severity. The high broad-sense heritability in GUS scores (82 %) and visual ratings (81 %) also indicated that phenotypic measurements were reproducible.

Q.Pch.wsu-1S^{l} was detected on chromosome 1S^{l} and 12 wheat SSR markers were significantly associated with it. Resistance to eyespot has been found in homoeologous chromosome Group 1 in other studies. Law et al. (1976) reported that chromosome 1A of Cappelle Desprez was implicated in resistance to eyespot. Uslu et al. (1998) found resistance in *Dasypyrum villosum* chromosome 1V to both *O. yallundae* and *O. acuformis*. Resistance to both pathogens was also identified in *Ae. longissima* chromosome 1S^{l} when *Ae. longissima* addition and substitution lines were tested (Sheng and Murray, unpublished data). However, there were no associated markers reported in those studies.

Q.Pch.wsu-3S^{l} on chromosome 3S^{l} plays a critical role in eyespot resistance since GUS scores and visual ratings were significantly lower when a line had the resistant allele near associated marker *wmc597*. Finding a major QTL conferring resistance to *O. yallundae* on chromosome 3S^{l} is not consistent with the results of *Ae. longissima* addition or substitution lines study, in which only one substitution line containing 3S^{l} was resistant to *O. acuformis* (Sheng and Murray, unpublished data). This may be due to different *Ae. longissima* accessions used in that study. However, *Dasypyrum villosum* chromosome 3V was associated with resistance to both *O. yallundae* and *O. acuformis* (Uslu et al. 1998). The powdery mildew resistance gene *Pm13* was also located on *Ae. longissima* chromosome 3S^{l}S (Cenci et al. 2003). In our study, *Q.Pch.wsu-3S*^{l} may be on the short arm. However, *Pm13* is near the distal portion and *Q.Pch.wsu-3S*^{l} is not.

Q.Pch.wsu-5S^{l} was the most significant QTL identified in this study. Homoeologous chromosome group 5 was reported to contain eyespot resistance in several studies (Law et al. 1976; Uslu et al. 1998; Muranty et al. 2002; Burt et al. 2011). When Burt et al. (2011) detected resistance to eyespot on chromosome 5AL of Cappelle Desprez, SSR marker *gwm639* was the closest marker. A QTL linked to *gwm639* for Fusarium head blight resistance was detected in two different winter wheat populations (Gervais et al. 2003; Paillard et al. 2004). Thus, X*gwm639* may be a critical locus for disease resistance. In our study, the closest marker, *wmc415*, is 6.7 cM away from *gwm639*. SSR marker *cfd12* was closely linked to an adult plant stripe rust resistance QTL from the diploid A genome species *Triticum boeoticum* on chromosome 5A (Chhuneja et al. 2008). Marker *cfd12* is 6.4 cM away from *wmc415* on chromosome 5S^{l}L in our study. An unmapped resistance to *O. yallundae* was also identified in *Ae. longissima* chromosome 5S^{l} when *Ae. longissima* addition and substitution lines were tested (Sheng and Murray, unpublished data).

Q.Pch.wsu-7S^{l} was located near the distal end of the long-arm chromosome 7S^{l}. *Q.Pch.wsu-7S*^{l} may be a homoeolocus of *Pch1* and *Pch2* because they are located in the distal portion of chromosome 7DL of VPM-1 (Worland et al. 1988) and 7AL of Cappelle Desprez (de la Peña et al. 1997), respectively. It has been suggested that *Pch1* and *Pch2* are homoeoloci (de la Peña et al. 1997; Chapman et al. 2008). More markers are needed on the distal side of *Q.Pch.wsu-7S*^{l} to confirm if it is a homoeolocus. Resistance to eyespot was found in *Ae. longissima* addition and substitution lines with *Ae. longissima* chromosome 7S^{l} but a linked marker was not reported (Sheng and Murray, unpublished data).

The additive effects of the QTL in *Ae. longissima* were confirmed when the RILs were grouped by genotype. No single QTL genotypes had significantly greater resistance than other single QTL genotypes. However, all of the two QTL combinations resulted in greater eyespot resistance than single QTL. Most of the three QTL combinations also showed greater resistance than the two QTL combinations. Each QTL contributed to reducing GUS scores and visual ratings, but the combination of all QTL had the greatest effect in reducing disease, although it was not significantly different than some three QTL genotypes.

Although SSR markers are highly polymorphic, they are not always useful in related genera (Röder et al. 1995). Adonina et al. (2005) reported that the transferability of wheat SSR markers to *Ae. longissima* was 68 % and the polymorphism within *Ae. longissima* was 75 %. In our study, similar transferability and polymorphism were observed. Thus, wheat SSR markers were useful for mapping genes in *Ae. longissima* and enabled the first genetic linkage map covering seven linkage groups of the S^{l} genome to be constructed. Furthermore, they will be useful in transferring these QTL to wheat.

QTL mapping with SSR markers has been used for resistance to Fusarium head blight (Gervais et al. 2003; Shen et al. 2003; Paillard et al. 2004), powdery mildew (Lillemo et al. 2008), and stripe rust (Carter et al. 2009; Lin and Chen 2009) of wheat. With the same approach, Leonard et al. (2008) linked markers to eyespot resistance

gene *Pch1* and a major QTL conferring resistance to eyespot on chromosome 5A of Cappelle Desprez was detected (Burt et al. 2011). In our study, four QTL conferring resistance to *O. yallundae* were mapped in the genome of *Ae. longissima*. The markers tightly linked to these QTL can be used in breeding programs for marker-assisted selection. This is the first study to use wheat microsatellite markers for genetic dissection of disease resistance QTL in a wild relative of wheat. In the near future, this RIL population and genetic map of S^l genome will be used to map QTL for resistance to *O. acuformis,* and the genes conferring eyespot resistance will be introgressed into a suitable wheat genetic background for use in breeding programs.

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References

Adonina IG, Salina EA, Pestsova EG, Röder MS (2005) Transferability of wheat microsatellites to diploid *Aegilops* species and determination of chromosomal localizations of microsatellites in the S genome. Genome 48:959–970

Anikster Y, Manisterski J, Long DL, Leonard KJ (2005) Resistance to leaf rust, stripe rust, and stem rust in *Aegilops* spp. in Israel. Plant Dis 89:303–308

Burt C, Hollins TW, Nicholson P (2011) Identification of a QTL conferring seedling and adult plant resistance to eyespot on chromosome 5A of Cappelle Desprez. Theor Appl Genet 122:119–128

Carter AH, Chen XM, Garland-Campbell K, Kidwell KK (2009) Identifying QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. tritici) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. Theor Appl Genet 119:1119–1128

Cenci A, D'Ovidio R, Tanzarella OA, Ceoloni C, Pasquini M, Porceddu E (2003) Genetic analysis of the *Aegilops longissima* 3S chromosome carrying the *Pm13* resistance gene. Euphytica 130:177–183

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Chapman NH, 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

Chhuneja P, Kaur S, Garg T, Ghai M, Kaur S, Prashar M, Bains NS, Goel RK, Keller B, Dhaliwal HS, Singh K (2008) Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. Theor Appl Genet 116:313–324

Crous PW, Groeneward JZ, Gams W (2003) Eyespot of cereals revisited: ITS phylogeny reveals new species relationships. Eur J Plant Pathol 109:841–850

de la Peña RC, Murray TD (1994) Identifying wheat genotypes resistant to eyespot disease with a β-glucuronidase-transformed strain of *Pseudocercosporella herpotrichoides*. Phytopathology 84:972–977

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

de la Peña RC, Murray TD, Jones SS (1997) Identification of an RFLP interval containing *Pch2* on chromosome 7AL of wheat. Genome 40:249–252

Doussinault G, Dosba F (1977) An investigation into increasing the variability for resistance to eyespot in wheat. Eyespot-variability in the subtribe *Triticinae*. Z Pflanzenzücht 79:122–133

Ecker R, Cahaner A, Dinoor A (1990) The inheritance of resistance to Septoria glume blotch III. The wild wheat species *Aegilops longissima*. Plant Breeding 104:224–230

Gervais L, Dedryver F, Morlasis JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003) Mapping of quantitative trait loci for field resistance to *Fusarium* head blight in an European winter wheat. Theor Appl Genet 106:961–970

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley, New York, pp 319–322

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

Jahier J, Doussinault G, Dosba F, Bourgeois F (1979) Monosomic analysis of resistance to eyespot in the variety 'Roazon'. In: Proceedings 5th International Wheat Genetics Symposium, New Delhi, India, pp 437–440

Johnson R (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3–22

Jones SS, Murray TD, Allan RE (1995) Use of alien genes for the development of disease resistance in wheat. Ann Rev Phytopathol 33:429–443

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly JM, Lincoln SE, Newberg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

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

Leonard JM, Watson CJW, Carter AH, Hansen JL, Zemetra RS, Santra DK, Campbell KG, 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

Levy AA, Galili G, Feldman M (1985) The effect of additions of *Aegilops longissima* chromosome on grain protein in common wheat. Theor Appl Genet 69:429–435

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155–1166

Lin F, Chen XM (2009) Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. Theor Appl Genet 118:631–642

Lucas JA, Dyer PS, Murray TD (2000) Pathogenicity, host specificity, and population biology of *Tapesia* spp., causal agents of eyespot disease in cereals. Adv Bot Res 33:226–258

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 Agr Sci 67:389–396

Maia N (1967) Obtention de blés tenders résistants au piétin-verse (*Cercosporella herpotrichoides*) par crôisements interspécifiques blés x *Aegilops*. C R Acad Agric Fr 53:149–155

Meyer N, Lind V, Heindorf M, Korzun V, Friedt W, Ordon F (2011) Diagnostic value of molecular markers linked to the eyespot resistance gene *Pch1* in wheat. Euphytica 177:267–275

Millet E (2007) Exploitation of *Aegilops* species of section Sitopsis for wheat improvement. Israel J Plant Sci 55:277–287

Millet E, Avivi Y, Zaccai M, Feldman M (1988) The effect of substitution of chromosome 5Sl of *Aegilops longissima* for its wheat homoeologues on spike morphology and on several quantitative traits. Genome 30:473–478

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 (2010) Eyespot (strawbreaker foot rot). In: Bockus WW, Bowden RL, Hunger RM, Murray TD, Smiley RW, Morrill W (eds) Compendium of wheat diseases and insects, 3rd edn. APS Press, Minneapolis, pp 32–34

Murray TD, Bruehl GW (1986) Effects of host resistance to *Pseudocercosporella herpotrichoides* and foot rot severity on yield and yield components in winter wheat. Plant Dis 70:851–856

Paillard S, Schnurbusch T, Tiwari R, Messmer M, Winzeler M, Keller B, Schachermayr G (2004) QTL analysis of resistance to *Fusarium* head blight in Swiss winter wheat (*Triticum aestivum* L.). Theor Appl Genet 109:323–332

Röder MS, Planschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wandehake K, Planschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schneider A, Molnár I, Molnár-Láng M (2008) Utilization of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica 163:1–19

Shen X, Zhou M, Lu W, Ohm H (2003) Detection of *Fusarium* head blight resistance QTL in a wheat population using bulked segregant analysis. Theor Appl Genet 106:1041–1047

Sheng H, Murray TD (2009) Identifying resistance genes for eyespot of wheat in *Aegilops longissima*. Phytopathology (abstr) 99:S119

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

Sourdille P, Tavaud M, Charmet G, Bernard M (2001) Transferability of wheat microsatellites to diploid Triticeae species carrying the A. B. and D genomes. Theor Appl Genet 103:346–352

Sprague R (1936) Relative susceptibility of certain species of Gramineae to *Cercosporella herpotrichoides*. J Agr Res 53:659–670

Strausbaugh CA, Murray TD (1989) Inheritance of resistance to *Pseudocercosporella herpotrichoides* in three cultivars of winter wheat. Phytopathology 79:1048–1053

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 Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Wageningen, The Netherlands, pp 1–7

Wang SC, Basten J, Zeng ZB (2010) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Worland AJ, Law CN, Hollins TW, Koebner RD, 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, Line RF (2000) Evaluation of *Dasypyrum villosum* populations for resistance to cereal eyespot and stripe rust pathogens. Plant Dis 84:40–44

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

Zhang H, Reader SM, Liu X, Jia JZ, Gale MD, Devos MK (2001) Comparative genetic analysis of the *Aegilops longissima* and *Ae. sharonensis* genomes with common wheat. Theor Appl Genet 103:518–525