

Mapping resistance genes for *Oculimacula acuformis* in *Aegilops longissima*

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Abstract

Key message This study identified three QTL conferring resistance to *Oculimacula acuformis* in *Aegilops longissima* and their associated markers, which can be useful in marker-assisted selection breeding for eyespot resistance.

Abstract *Oculimacula acuformis* is one of two species of soilborne fungi that cause eyespot of wheat, the other being *Oculimacula yallundae*. Both pathogens can coexist in the same field and produce elliptical lesions on stem bases of wheat that are indistinguishable. *Pch1* and *Pch2* are the only two eyespot resistance genes readily available to wheat breeders, but neither provides complete control. A new source of eyespot resistance was identified from *Aegilops longissima* ($2n = 14, 2S^{1S^1}$), a wild relative of wheat. Three QTL for resistance to *O. acuformis* were mapped in chromosomes 1S¹, 3S¹, and 5S¹ using a recombinant inbred line population developed from the cross *Ae. longissima* accessions PI 542196 (R) × PI 330486 (S). The three QTL explained 66 % of phenotypic variation by β -glucuronidase score (GUS) and 84 % by visual rating. These QTL had LOD values of 10.6, 8.8, and 6.0 for GUS score, and 16.0, 10.0, and 13.0 for visual rating. QTL associated with resistance to *O. acuformis* have similar

chromosomal locations as some for resistance to *O. yallundae*, except that a QTL for resistance to *O. yallundae* was found in chromosome 7S¹ but not for *O. acuformis*. Thus, it appears that some genes at the same locus in *Ae. longissima* may control resistance to both eyespot pathogens. QTL effective against both pathogens will be most useful for breeding programs and have potential to improve the effectiveness and genetic diversity of eyespot resistance.

Introduction

Eyespot, a stem base disease of wheat, is caused by *Oculimacula acuformis* Crous & W. Gams (syn: *Tapesia acuformis*, Wallwork & Spooner) and *O. yallundae* Crous & W. Gams (syn: *T. yallundae*) (Crous et al. 2003). These fungi often coexist in the same field and cause eye-shaped elliptical lesions at the stem bases that are indistinguishable. Eyespot occurs in many cool and wet wheat growing areas in the world. In the USA, eyespot is a chronic and economically important disease of winter wheat in the Pacific Northwest (PNW) region including Idaho, Oregon and Washington. Severe eyespot results in stem breakage and multi-directional lodging in the field (Maloy and Inglis 1993). Up to 50 % yield reduction has been documented in severely affected fields (Murray 2010).

Although eyespot was controlled with fungicides for many years, limiting input costs, environmental concerns, and fungicide resistance resulted in the need for planting resistant cultivars. Resistance to eyespot has been investigated since the pathogen was first described (Sprague and Fellowes 1934). Discovery of the wheat relative *Aegilops ventricosa*. Tausch ($2n = 28, DDM^VM^V$) (syn. *Triticum ventricosum* (Tausch) Ces.) by Sprague (1936) led to the introgression of eyespot resistance gene *Pch1* into common

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wheat (Kimber 1967; Doussinault et al. 1983). *Pch1* is the most effective resistance gene to eyespot to date. It is a single dominant gene mapped to the distal end of the long arm of chromosome 7D (Worland et al. 1988). Through breeding line VPM-1 (Ventricosa × Persicum × Marne), *Pch1* has been incorporated into several commercial wheat cultivars for use in the US PNW. Madsen, the first eyespot resistant line in the US PNW, was released in 1988 by the USDA-ARS and Washington State University winter wheat breeding program at Pullman, WA (Allan et al. 1989). It has been widely grown in the PNW for more than two decades (Murray 2010). Lind (1999) found that *Pch1* resistance was more effective in early plant growth stages than at the adult stage. Burt et al. (2010) found that *Pch1* conferred resistance to both *O. acufomis* and *O. yallundae* at the seedling stage.

French variety Cappelle Desprez containing *Pch2* is the only known source of eyespot resistance from hexaploid wheat (Hollins et al. 1988). Law et al. (1976) found evidence that eyespot resistance in Cappelle Desprez is located on chromosomes 1A, 2B, 5D, and 7A, with a major component on 7A. *Pch2* was later mapped to the distal portion of 7AL with RFLP markers (de la Peña et al. 1997). Recently, a major QTL associated with eyespot resistance was mapped on chromosome 5AL of Cappelle Desprez (Burt et al. 2011). Although *Pch2* is less effective than *Pch1* (Johnson 1992), Cappelle Desprez has been grown extensively and its resistance has been transferred to many other wheat varieties in Europe since the 1950s (Hollins et al. 1988). Burt et al. (2010) found that *Pch2* was significantly more effective against *O. acufomis* than *O. yallundae* at the seedling stage and that the QTL on chromosome 5A conferred resistance to both pathogens at the seedling and adult stages (Burt et al. 2011).

Although *Pch1* and *Pch2*, along with the QTL on 5AL of Cappelle Desprez, have been used to effectively protect wheat from eyespot disease, none of them provides complete resistance to eyespot when used alone. Additional resistance genes are desired for incorporation into adapted wheat cultivars to improve the effectiveness and diversity of resistance genes. Wild species of wheat have received considerable attention as sources of eyespot resistance (Jones et al. 1995). A single dominant gene, *Pch3* or *PchD^v*, was mapped to chromosome 4VL of *Dasyphyrum villosum* because of its eyespot resistance (Yildirim et al. 1998), but the gene has not been used commercially. Uslu et al. (1998) found multiple resistance genes in *D. villosum* chromosomes 4V and 5V to *O. yallundae* and *O. acufomis*, respectively; the first report showing differential resistance to these two pathogens. Burt et al. (2010) also demonstrated that *Triticum monococcum* accessions had significantly different resistance reactions to *O. yallundae* and *O. acufomis*.

Aegilops longissima Schweinf. & Muschl. ($2n = 2x = 14$, S¹S¹), a diploid species in the section Sitopsis of *Aegilops* L. (Van Slageren 1994), is a distant relative of wheat and potential donor of genes for wheat cultivar improvement, including disease resistance (Friebe et al. 1993). In previous studies, *Ae. longissima* was identified as containing a new source of eyespot resistance (Sheng and Murray 2013). Multiple genes in the S¹ genome were found to be responsible for genetic control of eyespot resistance and some of them reacted differently from *O. yallundae* and *O. acufomis* (Sheng and Murray 2013). A genetic linkage map composed of 169 wheat microsatellite markers (SSR markers) was constructed with the S¹ genome with a recombinant inbred line population of *Ae. longissima* (Sheng et al. 2012). Furthermore, four QTL conferring resistance to *O. yallundae* were mapped in chromosome 1S¹, 3S¹, 5S¹, and 7S¹ (Sheng et al. 2012). The objective of this study was to determine the genetic control of resistance to *O. acufomis* in the S¹ genome of *Ae. longissima* by QTL mapping 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

Aegilops longissima accessions PI 542196 and PI 330486 provided by the USDA National Small Grains Collection (NSGC) were identified as resistant and susceptible to both *O. yallundae* and *O. acufomis*, respectively (Sheng and Murray 2013), and were selected as the resistant and susceptible parents for development of a recombinant inbred line (RIL) population (Sheng et al. 2012). The population was developed to F₈ through single-seed descent.

Phenotypic evaluation

One-hundred and seventy-eight F₈ RIL of PI 542196 (R) × PI 330486 (S) were tested for resistance to *O. acufomis* in two growth chamber (Controlled Environments Limited, Winnipeg, Manitoba, Canada) experiments. The parent lines and the winter wheat cultivar Madsen were included in the experiments as controls. Each experiment was a randomized complete block (RCB) design with two subsamples and three blocks. Thus, a total of 12 plants per line were evaluated for phenotypic variation. The planting method and growth chamber conditions were the same as for the phenotypic evaluation of F₅ RIL to *O. yallundae* (Sheng et al. 2012).

Disease evaluation was conducted after inoculating seedlings with β-glucuronidase (GUS) transformed *O.*

acuformis isolates tph98-2-34A, tph98-2-34D, tph98-2-34E, and tph98-1-54aa (de la Peña and Murray 1994). Each two-leaf stage seedling was inoculated twice with 250 μ l slurry of conidia containing 1.2×10^5 conidia per ml (Sheng et al. 2012).

Visual disease ratings were performed at growth stage 23–25 (Zadoks et al. 1974), about 8 weeks after inoculation. All tillers (2–4) of each plant were cut into a 3 cm-long section above the soil line. After washing in water to remove soil, stems were evaluated visually for disease severity on a 0–4 scale, where 0 = healthy to 4 = nearly dead (Sheng et al. 2012). GUS activity in stems, a surrogate measurement of fungal growth, was determined with a fluorescent GUS assay using a methylumbelliferone (MU) substrate. Fluorescence was measured in a SpectraMax M2 microplate reader (Molecular Devices Co., Sunnyvale, CA). Each sample was ground with 2.5 ml GUS extraction buffer (Sheng et al. 2012). Fluorescence intensity was expressed as the \log_{10} transformed ratio [$\log_{10}(x/\text{resistant control}) + 1$] (Sheng et al. 2012) of an individual accession (x) compared to the resistant control (Madsen).

Statistical analysis

SAS Version 9.2 (SAS Institute Inc., Cary, NC) was used for statistical analysis. Homogeneity of variance of the two experiments was tested and if the hypothesis of homogeneity was accepted, data from the two experiments were combined for further analysis (Gomez and Gomez 1984). PROC GLM was used for analysis of variance (ANOVA) and standard deviation of two dependent variables (GUS score and visual rating). Variance components were generated by a random model with lines and experiments as random effects in ANOVA. The Kolmogorov-Smirnov (KS) test was used to test normality of the phenotype distribution. Dunnett's t test was used for multiple comparisons of the least squares mean (lsmean) of each RIL compared with the resistant parent (PI 542196). The genotypes based on presence of specific QTL were compared by Tukey's t test with least squares means (lsmean). The correlation between visual ratings and GUS scores was evaluated by Pearson correlation coefficients. Broad-sense heritability (H^2) was calculated as the ratio of the genetic variance [$\text{Var}(G)$] to the phenotypic variance [$\text{Var}(P)$] (Sheng et al. 2012).

Linkage mapping and QTL analysis

In the previous study (Sheng et al. 2012), a genetic linkage map of *Ae. longissima* was established based on the genotypic data of 178 F_5 RIL of (PI 542196 \times PI 330486) with 169 wheat microsatellite (SSR) markers. Based on seven linkage groups of that map and phenotypic data of same RIL advanced to the F_8 , QTL analysis was performed with

WinQTLCart V2.5 (Wang et al. 2010). Composite Interval Mapping was conducted to identify the chromosome locations of major QTL associated with resistance to *O. acuformis* (Sheng et al. 2012).

Results

Phenotypic evaluation

Based on the F ratio test, the error variances of GUS scores and visual ratings were not significantly different at 95 % significance level between the two experiments. Therefore, homogeneity of variance was accepted for both GUS scores and visual ratings, and data from the two experiments were combined for further analysis. The mean GUS scores were normally distributed (KS test, $P > 0.15$) and ranged from 0.8 to 1.8 (Fig. 1). Mean visual ratings were also normally distributed and ranged from 0.8 to 3.7 (Fig. 1).

β -Glucuronidase score (GUS) scores were significantly correlated ($r = 0.7025$, $P < 0.0001$) with visual ratings in the F_8 population. The resistant parent (PI 542196) had significantly ($P < 0.0001$) lower GUS scores and visual ratings

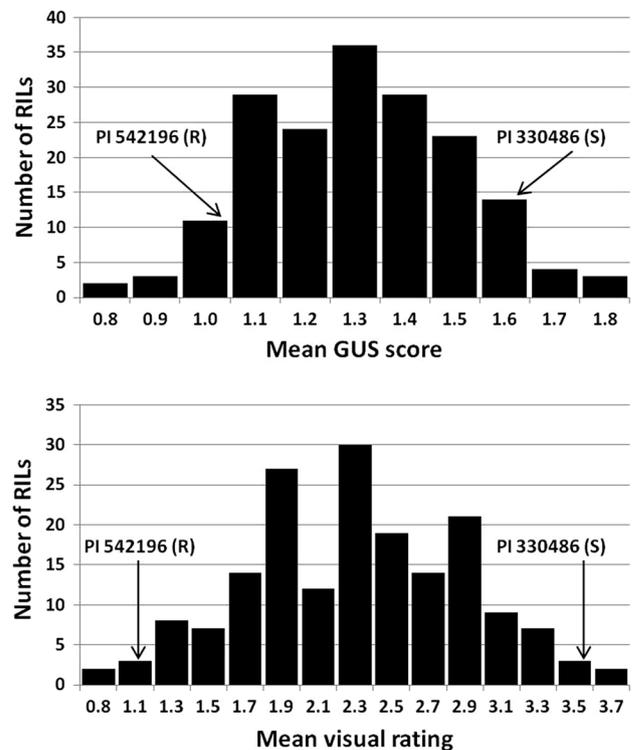


Fig. 1 Distribution of GUS scores and visual ratings in F_8 population (178 lines, 12 plants/line) of the cross PI 542196 (R) \times PI 330486 (S) inoculated with *Oculimacula acuformis*. The null hypothesis of normality was accepted based on the Kolmogorov–Smirnov test with values of 0.0475 and 0.0559 ($P > 0.15$)

Table 1 Variance components of GUS scores and visual ratings and broad-sense heritability (H^2) for F_8 recombinant inbred lines (RIL) derived from the cross PI 542196 (R) \times PI 330486 (S) and inoculated with *Oculimacula acufiformis*

Source of variation	df	GUS score		Visual rating	
		Mean square	F value	Mean square	F value
RIL ^a	177	0.49	4.75**	4.19	3.66**
Block (expt.)	4	7.3	70.8**	27.32	23.85**
Experiment	1	57.66	7.9	35.89	1.31
Expt. \times RIL	177	0.198	1.92**	2.57	2.24**
Error	1,730	0.103		1.146	
H^2 (based on line means)		69.4 %		56.6 %	

** $P < 0.0001$ ^a 178 lines; 12 plants/line were included in two experiments

than the susceptible parent (PI 330486). PI 542196 had mean GUS score (1.1) and mean visual rating (1.1), which were not significantly ($P > 0.05$) different from those of Madsen (1.0 and 1.2, respectively). The mean GUS score (1.6) and visual rating (3.5) of PI 330486 were both significantly ($P < 0.0001$) greater than the mean values for Madsen.

The variance components of GUS scores and visual ratings of F_8 RIL were determined by analysis of variance (ANOVA). There were significantly ($P < 0.0001$) different reactions to *O. acufiformis* among the 178 F_8 RIL for both GUS score and visual rating (Table 1). The environment effect (block within experiment) and genotype by environment interaction (Expt. \times RIL) were also significant ($P < 0.0001$) for both. However, experiments were not significantly different for either GUS score ($P = 0.1597$) or visual rating ($P = 0.3156$). The F_8 population demonstrated 69.4 % broad-sense heritability (H^2) based on line means for the reaction to *O. acufiformis* by GUS score and 56.6 % by visual rating (Table 1).

QTL analysis

Three QTL conferring resistance to *O. acufiformis* were identified on chromosomes 1S¹, 3S¹, and 5S¹ (Fig. 2a–c). All of them were detected using both GUS scores and visual ratings and were contributed by the resistant parent, PI 542196. These QTL were designated as *Q.Pch-oa.wsu-1S¹*, *Q.Pch-oa.wsu-3S¹*, and *Q.Pch-oa.wsu-5S¹*.

The QTL detected on chromosome 1S¹, *Q.Pch-oa.wsu-1S¹*, had LOD values of 10.6 and 16.0 for GUS score and visual rating and explained 27 and 31 % of the phenotypic variation of GUS score and visual rating, respectively (Fig. 2a). SSR markers *cfid6* and *gdm132* flanked this QTL, spanning six other markers (*gdm67*,

gwm642, *cfid48*, *barc128*, *gwm32*, and *cfid83*) covering 30.3 cm. All eight markers were significantly associated with *Q.Pch-oa.wsu-1S¹* ($P < 0.0001$). Markers *cfid6*, *gdm67*, *gwm642*, *cfid48*, *barc128*, and *gwm32*, which were clustered in an 8.7 cm interval on chromosome 1S¹, were most closely linked to *Q.Pch-oa.wsu-1S¹*. An additive effect (0.13 for GUS score and 0.43 for visual rating) was contributed from the resistant parent PI 542196. Markers *gwm642* and *cfid48* are located on the long arms of wheat chromosome 1D and 1B (GrainGenes, <http://wheat.pw.usda.gov/cgi-bin/graingenes>), respectively, indicating that *Q.Pch-oa.wsu-1S¹* is on the long arm of chromosome 1S¹. At markers *gdm67*, *gwm642* and *cfid48*, the mean GUS score and visual rating of lines with the resistant allele (PI 542196) were significantly ($P < 0.0001$) less than lines with the susceptible allele (PI 330486) (Fig. 3).

Q.Pch-oa.wsu-3S¹, located on chromosome 3S¹, had LOD values of 8.8 and 10.0 for GUS score and visual rating and explained 27 and 30 % of the phenotypic variation, respectively (Fig. 2b). *Q.Pch-oa.wsu-3S¹* was associated with both GUS score and visual rating in a 40.6 cm interval between markers *cfid79* and *barc71*. Marker *cfid9* was in an interval about 6 cm from the QTL peak. All three markers (*cfid79*, *cfid9*, and *barc71*) had significant ($P < 0.0001$) effects on both GUS score and visual rating. The additive effects from the resistant parent PI 542196 were 0.11 and 0.34 for GUS score and visual rating, respectively. *Q.Pch-oa.wsu-3S¹* probably is located on the short arm of chromosome 3S¹ since markers *gwm314*, *barc147*, and *cfid79* are either on 3BS or 3DS of wheat chromosomes according to GrainGenes (<http://wheat.pw.usda.gov/cgi-bin/graingenes>). The mean GUS score and visual rating were significantly ($P < 0.0029$ and $P < 0.0044$, respectively) different between lines with opposite alleles of markers *cfid79*, *cfid9*, and *barc71* (Fig. 3).

Q.Pch-oa.wsu-5S¹ had LOD values of 6.0 and 13.0 for GUS score and visual rating, and explained 12 and 23 % of the phenotypic variation, respectively (Fig. 2c). *Q.Pch-oa.wsu-5S¹* was between markers *wmc415* and *barc319* in a 24.9 cm interval. Six markers (*wmc415*, *cfid12*, *gwm408*, *gwm271*, *wmc160*, and *barc319*) fell in this interval and all were significantly ($P < 0.0001$) associated with *Q.Pch-oa.wsu-5S¹* by GUS score and visual rating. Markers *cfid12*, *gwm408* and *gwm271* are in a 6.3-cm interval and the most closely linked. Additive effects were 0.06 for GUS score and 0.29 for visual rating, which came from the resistant parent PI 542196. *Q.Pch-oa.wsu-5S¹* probably is on the long arm of chromosome 5S¹ since the five markers associated with this QTL are located on wheat chromosomes 5AL, 5BL or 5DL (<http://wheat.pw.usda.gov/cgi-bin/graingenes>). At the closest linked markers *cfid12*, *gwm408*, and *gwm271*, the mean GUS score and visual rating were significantly

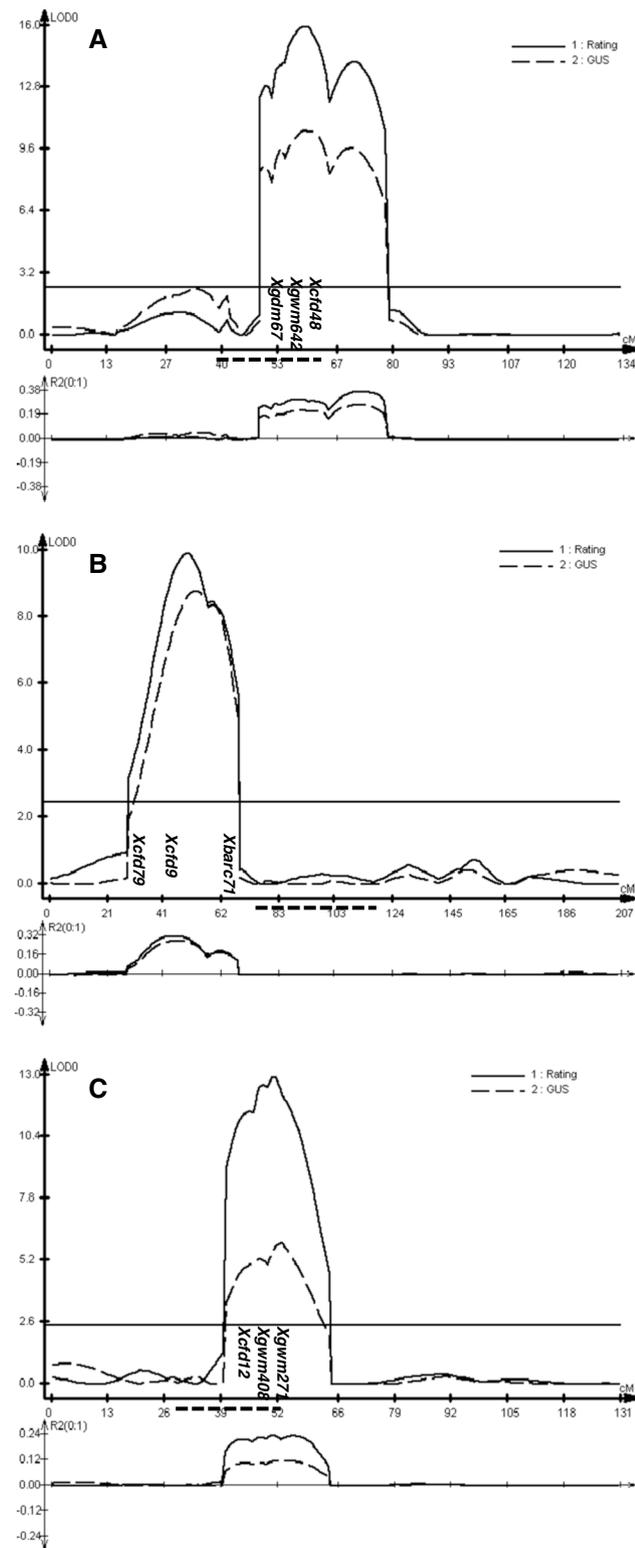


Fig. 2 QTL for resistance to *Oculimacula acufiformis* identified on *Aegilops longissima* chromosomes 1S¹, 3S¹, and 5S¹ by GUS score and visual rating with composite interval mapping. **a** *Q.Pch-oa.wsu-1S¹* on chromosome 1S¹; **b** *Q.Pch-oa.wsu-3S¹* on chromosome 3S¹; and **c** *Q.Pch-oa.wsu-5S¹* on chromosome 5S¹. Dashed lines represent the map locations of *Q.Pch.wsu-1S¹*, *Q.Pch.wsu-3S¹*, and *Q.Pch.wsu-5S¹* to *O. yallundae*, respectively (Sheng et al. 2012)

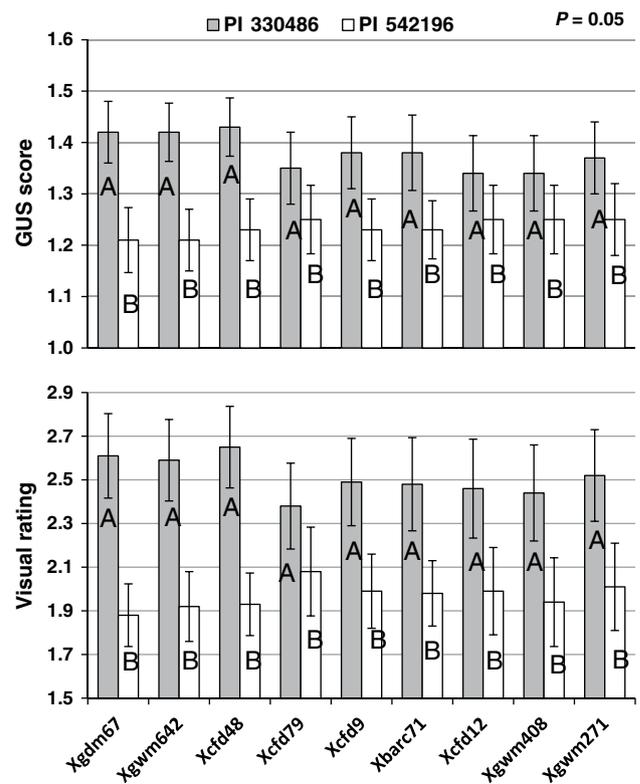


Fig. 3 GUS scores and visual ratings for RIL of PI 542196 (R) × PI 330486 (S) with different parental alleles at the markers close to each QTL. *gdm67*, *gwm642*, and *cf48* are close to *Q.Pch-oa.wsu-1S¹*; *cf479*, *cf49*, and *barc71* are close to *Q.Pch-oa.wsu-3S¹*; and *cf412*, *gwm408*, and *gwm271* are close to *Q.Pch-oa.wsu-5S¹*. The dark and light bars represent the mean GUS scores or visual ratings of RIL with the susceptible and resistant allele at the marker closest to each QTL, respectively. They were all significantly different ($P < 0.05$) between susceptible and resistant allele at the closest markers which were indicated by different letters on the bars. Error bars show standard errors

($P < 0.0045$) less for the resistant parental alleles than the susceptible alleles (Fig. 3).

Eight genotypes, defined by combinations of the resistant parental allele at the closest marker to each QTL, were produced from 178 RIL with 13–33 lines each. Markers *cf448*, *cf49*, and *gwm271* are the closest markers to *Q.Pch-oa.wsu-1S¹*, *Q.Pch-oa.wsu-3S¹*, and *Q.Pch-oa.wsu-5S¹*, respectively. The LSmeans for GUS score and visual rating for each genotype were calculated (Fig. 4) and were significantly ($P < 0.0001$) different among the eight genotypes. Lines with no QTL had the greatest GUS score (1.5) and visual rating (3.0), and the lines with all QTL had the lowest GUS score (1.1) and visual rating (1.6). All the QTL genotypes had significantly ($P < 0.0001$) lower disease severity than the genotype with no QTL. Although the GUS scores and visual ratings for genotypes with one QTL were not significantly different ($P > 0.1975$ and $P > 0.1051$, respectively) from each other, they all had significantly ($P < 0.0266$)

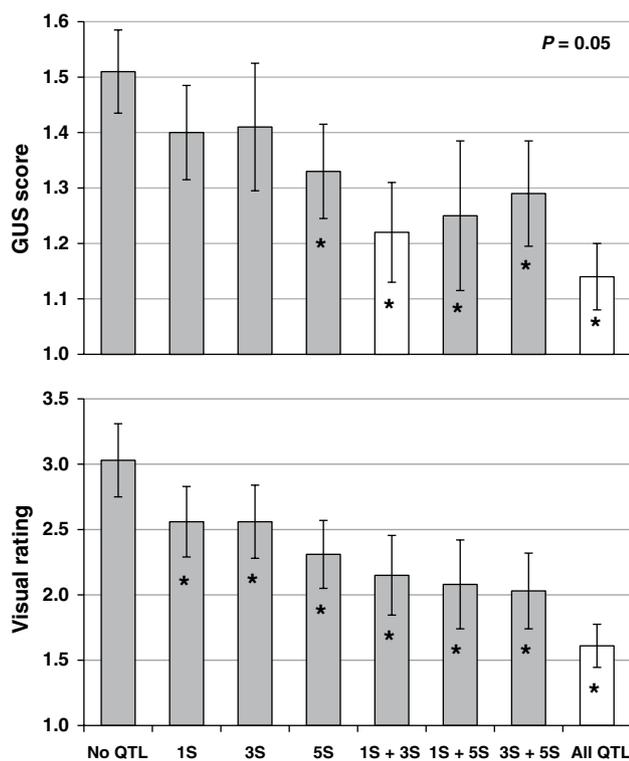


Fig. 4 Mean GUS scores and visual ratings of RIL within each genotype. Resistance of eight genotypes based on three QTL detected in F_8 RIL populations of PI 542196 (R) \times PI 330486 (S) to *Oculimacula acufiformis*. Each genotype includes 13–33 lines and each line includes 12 plants. 1S, 3S, and 5S represent *Q.Pch-oa.wsu-1S^l*, *Q.Pch-oa.wsu-3S^l*, *Q.Pch-oa.wsu-5S^l*, and *Q.Pch.wsu-7S^l*, respectively. Bars with an asterisk are genotypes with significantly ($P < 0.05$) lower GUS scores or visual ratings than ‘no QTL’. 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

lower visual ratings than the genotype with no QTL; however, only *Q.Pch-oa.wsu-5S^l* had significantly ($P = 0.0024$) lower GUS score than the no QTL genotype. All single QTL genotypes had significantly ($P < 0.0003$) greater GUS score and visual rating than the genotype with all QTL.

Three genotypes with combinations of two QTL had significantly ($P < 0.0035$) lower GUS scores and visual ratings than the genotype with no QTL and were not significantly different ($P > 0.3106$ and $P > 0.5762$, respectively) from each other. Only the combination of *Q.Pch-oa.wsu-1S^l* and *Q.Pch-oa.wsu-3S^l* was not significantly ($P = 0.0515$) different from the genotype of all QTL for GUS score (Fig. 4).

Discussion

Three QTL contributing resistance to *O. acufiformis* were detected on *Ae. longissima* chromosomes 1S^l, 3S^l, and

5S^l that explained 66 and 84 % of the phenotypic variation in GUS scores and visual ratings, respectively. This result demonstrates that the resistance to *O. acufiformis* in *Ae. longissima* accession PI 542196 is controlled by multiple QTL. It is also consistent with the finding that multiple QTL confer the resistance to *O. yallundae* in *Ae. longissima* (Sheng et al. 2012). The genetic control of eyespot resistance in *Ae. longissima* is polygenic and the genes are quantitative. Eyespot resistance in Cappelle Desprez is also polygenic (Law et al. 1976; Jahier et al. 1979) but the quantitative character was found only on chromosome 5AL (Burt et al. 2011).

Environment and genotype by environment interactions were significant in the phenotypic tests in this study. A significant block effect occurred because different growth chambers were used. Other factors contributing to the block effect were variation in humidity within chambers and the disease evaluation period, which took about 1 week to complete; experiments were evaluated by block, but continued disease development during this time would result in block variation.

Q.Pch-oa.wsu-1S^l to *O. acufiformis* was detected on chromosome 1S^l and eight SSR markers were significantly associated with it. Five markers (*gdm67*, *gwm642*, *cf448*, *barc128*, and *gwm32*) are co-dominant markers and four of them (*gwm642*, *cf448*, *barc128*, and *cf483*) were also mapped on wheat homoeologous chromosomes 1B or 1D (Somers et al. 2004). We found that the physical arrangements of markers *gwm642*, *cf448*, and *barc128* in *Ae. longissima* and wheat were collinear. *Q.Pch.wsu-1S^l* associated with resistance to *O. yallundae* was mapped in a similar location on chromosome 1S^l as *Q.Pch-oa.wsu-1S^l* with a 13.5 cm overlap (Sheng et al. 2012) and shared seven markers (*cf46*, *gdm67*, *gwm642*, *cf448*, *barc128*, *gwm32*, and *cf483*). Markers *cf46*, *gdm67*, *gwm642*, and *cf448* were most closely linked to both *Q.Pch.wsu-1S^l* and *Q.Pch-oa.wsu-1S^l*. It is possible that *Q.Pch.wsu-1S^l* and *Q.Pch-oa.wsu-1S^l* are linked to the same genes or different genes at the same locus. The LOD values of *Q.Pch-oa.wsu-1S^l* were greater than those of *Q.Pch.wsu-1S^l* for GUS score and visual rating. *Q.Pch-oa.wsu-1S^l* also explained 16 % more phenotypic variation than *Q.Pch.wsu-1S^l* for both GUS score and visual rating. Thus, the gene on chromosome 1S^l may be more effective to *O. acufiformis* than to *O. yallundae*.

The region containing both *Q.Pch.wsu-1S^l* and *Q.Pch-oa.wsu-1S^l* was independently mapped to the long arm of *Ae. longissima* chromosome 1S^l (Sheng et al. 2012). Resistance to both *O. yallundae* and *O. acufiformis* was also found on the long arm of *Ae. longissima* chromosome 1S^l by evaluating *Ae. longissima* ditelosomic addition and substitution lines (Sheng and Murray 2013). The resistant lines will be validated with the markers associated with the QTL identified in PI 542196. Overall, it can be concluded that the long

arm of *Ae. longissima* chromosome 1S¹ contains genes that confer eyespot resistance to both fungal pathogen species and are quantitatively inherited.

Besides *Ae. longissima*, homoeologous chromosome group 1 was found to contain eyespot resistance in other resources. A minor effect to eyespot on chromosome 1A of Cappelle Desprez was reported (Law et al. 1976) but was not specifically for *O. yallundae* or *O. acuformis*. Later, chromosome 1V from *Dasyphyrum villosum*, a wild species of wheat, was found to confer resistance to both *O. yallundae* and *O. acuformis* (Uslu et al. 1998). From our studies, the presence of resistance on homoeologous chromosome group 1 was confirmed.

Q.Pch-oa.wsu-3S¹ was significantly associated with three SSR markers on chromosome 3S¹, which are also located in wheat homoeologous group 3 (Somers et al. 2004). Marker *cf79* along with two other markers at the distal end of the short arm has co-linearity with wheat chromosome 3D. *Q.Pch.wsu-3S¹* was detected 9 cm proximal to *Q.Pch-oa.wsu-3S¹* on chromosome 3S¹ (Sheng et al. 2012). It is possible that these QTL represent two different genes controlling resistance to the two *Oculimacula* species. The LOD values of *Q.Pch-oa.wsu-3S¹* were greater than those of *Q.Pch.wsu-3S¹* for GUS score and visual rating and *Q.Pch-oa.wsu-3S¹* explained 13 and 21 % more phenotypic variation than *Q.Pch.wsu-3S¹* for GUS score and visual rating, respectively. These results suggest that *Q.Pch-oa.wsu-3S¹* may be associated with a more effective gene than the gene conditioning resistance to *O. yallundae*.

Both *Q.Pch-oa.wsu-3S¹* and *Q.Pch.wsu-3S¹* were mapped to the short arm of *Ae. longissima* chromosome 3S¹ (Sheng et al. 2012). Resistance to *O. acuformis* was also identified on the short arm of *Ae. longissima* chromosome 3S¹ when *Ae. longissima* ditelosomic addition and substitution lines were tested (Sheng and Murray 2013). However, those lines did not reveal any evidence of resistance to *O. yallundae* on chromosome 3S¹, further suggesting that different genes on the short arm of chromosome 3S¹ confer resistance to *O. yallundae* and *O. acuformis* independently.

The distal portion of the short arm of *Ae. longissima* chromosome 3S¹ was also reported to carry powdery mildew resistance gene *Pm13* (Cenci et al. 2003), which is in a similar region as the eyespot resistance found in this study. Resistance to both *O. yallundae* and *O. acuformis* also was found on homoeologous chromosome 3V of *Dasyphyrum villosum* (Uslu et al. 1998). Our QTL mapping study provides further evidence that the short arm of *Ae. longissima* chromosome 3S¹ contains genes for disease resistance, especially eyespot.

Q.Pch.wsu-5S¹ associated with resistance to *O. acuformis* was mapped on *Ae. longissima* chromosome 5S¹ with close linkage to six SSR markers. *Q.Pch.wsu-5S¹* associated with resistance to *O. yallundae* was mapped in

a similar location on chromosome 5S¹ as *Q.Pch-oa.wsu-5S¹* with a 9.5 cm overlap (Sheng et al. 2012). These QTL shared three markers (*wmc415*, *cf12*, and *gwm408*), which were the most closely linked markers to both *Q.Pch.wsu-5S¹* and *Q.Pch-oa.wsu-5S¹*. Thus, these two QTL may represent one gene or two different genes at the same locus. Nine markers were associated with the region containing *Q.Pch.wsu-5S¹* and *Q.Pch-oa.wsu-5S¹* on chromosome 5S¹; all are located on wheat homoeologous group 5 chromosomes in collinear order (Somers et al. 2004). Both *Q.Pch.wsu-5S¹* and *Q.Pch-oa.wsu-5S¹* were mapped on the long arm of *Ae. longissima* chromosome 5S¹ in two different studies (Sheng et al. 2012) and to chromosome arm 5S¹L with *Ae. longissima* ditelosomic addition and substitution lines (Sheng and Murray 2013).

Law et al. (1976) reported that chromosome 5D of Cappelle Desprez contributed to eyespot resistance, but it was not characterized since it was less effective than the gene on chromosome 7AL. Muranty et al. (2002) identified quantitative eyespot resistance on chromosome 5A in Cappelle Desprez. Furthermore, Burt et al. (2011) mapped a major QTL conferring resistance to both *O. yallundae* and *O. acuformis* on chromosome 5AL in Cappelle Desprez. They also found that the most closely linked SSR marker was *gwm639*. In our studies, *gwm639* was one of the closest markers associated with *Q.Pch.wsu-5S¹* but not *Q.Pch-oa.wsu-5S¹* on chromosome 5S¹L (Sheng et al. 2012). However, the flanking marker of *Q.Pch-oa.wsu-5S¹*, *wmc415*, was mapped at the distal side of *gwm639* about 6.7 cm away. The other flanking marker of *Q.Pch-oa.wsu-5S¹*, *barc319*, which was 31.6 cm away from *gwm639* on 5S¹L, was 27 cm on the distal side of chromosome 5AL in Cappelle Desprez (Burt et al. 2011). Marker *gwm639* was linked to a QTL for Fusarium head blight on wheat chromosomes 5A and 5B in two different studies, respectively (Gervais et al. 2003; Paillard et al. 2004). Marker *gwm639* on chromosome 5B was also found associated with the QTL for Cephalosporium stripe, another important winter wheat disease in the PNW (Quincke et al. 2011). Overall, the long arms of homoeologous group 5 contain quantitative resistance to several wheat diseases including eyespot, possibly representing homoeoloci.

During QTL mapping of resistance to *O. yallundae*, a homoeolocus of *Pch1* and *Pch2*, *Q.Pch.wsu-7S¹*, was identified at the distal end of chromosome 7S¹L (Sheng et al. 2012). Screening *Ae. longissima* ditelosomic addition and substitution lines located the resistance to both *O. yallundae* and *O. acuformis* on the long arms of *Ae. longissima* chromosome 7S¹ (Sheng and Murray 2013). However, there was no evidence of resistance to *O. acuformis* on chromosome 7S¹ in the present study. *Pch1* on chromosome 7DL, a single dominant gene, was effective to both species (Burt et al. 2010). *Pch2* on chromosome 7AL is a single partially

dominant gene (Strausbaugh and Murray 1989) that was more effective to *O. acufiformis* than to *O. yallundae* (Burt et al. 2010). Klos et al. (2014) confirmed that *Pch2* on Cappelle Desprez chromosome 7A conferred some resistance against both species. *Q.Pch.wsu-7S^l* detected on *Ae. longissima* chromosome 7S^l from our previous study (Sheng et al. 2012) was only effective to *O. yallundae*. These results imply that genes from homoeoloci could react differently to the two eyespot pathogens. Thus, it is important to screen lines for resistance to each species separately to provide a gene or genes for both pathogens to the breeding programs.

Each QTL to *O. acufiformis* detected in this study had an additive effect. The genotype containing all QTL had significantly lower disease severity than all other QTL-containing genotypes except one. All two QTL genotypes had more effective eyespot resistance than one QTL genotypes. Although *Q.Pch-oa.wsu-5S^l* had a strong effect, the combination of *Q.Pch-oa.wsu-1S^l* and *Q.Pch-oa.wsu-3S^l* had greater resistance than other combinations containing *Q.Pch-oa.wsu-5S^l* by GUS score. Thus, different QTL combinations demonstrated interactions.

We found three QTL associated with resistance to *O. acufiformis* and four QTL to *O. yallundae* from the same *Ae. longissima* RIL population. These results were not surprising because selection of the donor parent (*Ae. longissima* PI 542196) was based on its resistance to both *O. yallundae* and *O. acufiformis*. PI 542196 is a useful source of eyespot resistance and the markers associated with these seven QTL should prove useful in marker-assisted introgression into adapted wheat lines, addressing the current lack of genetic diversity of eyespot resistance. However, the negative effects of linkage drag must be considered and evaluated during introgression.

Author contributions HS conducted all the experiments of this study, analyzed the data, and wrote the manuscript. DRS provided the guidance for the genotyping work and revised the manuscript. TDM supervised the entire project, and revised the manuscript several times.

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Conflict of interest None of the authors has the conflict of interest.

Ethical standards All experiments and genotyping work were carried out in Pullman, Washington of USA in compliance with the USA laws.

References

- Allan RE, Peterson CJ, Rubenthaler GL, Line RF, Robert DE (1989) Registration of 'Madsen' wheat. *Crop Sci* 29:1575–1576
- Burt C, Hollins TW, Powell N, Nicholson P (2010) Differential seedling resistance to the eyespot pathogens, *Oculimacula yallundae* and *Oculimacula acufiformis*, conferred by *Pch2* in wheat and among accessions of *Triticum monococcum*. *Plant Pathol* 59:819–828
- 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
- 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
- Crous PW, Groenewald 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 *Pseudocercospora herpotrichoides*. *Phytopathology* 84:972–977
- 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, Delibes A, Sanchez-Monge R, Garcia-Olmedo F (1983) Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. *Nature* 303:698–700
- Friebe B, Tuleen N, Jiang J, Gill BS (1993) Standard karyotype of *Triticum longissimum* and its cytogenetic relationship with *T. aestivum*. *Genome* 36:731–742
- Gervais L, Dedryver F, Morlasis JY, Bodusseau V, Negre S, Bilous M, Groos C, Trotet 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 (*Pseudocercospora 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
- Kimber G (1967) The incorporation of the resistance of *Aegilops ventricosa* to *Cercospora herpotrichoides* into *Triticum aestivum*. *J Agric Sci* 68:373–376
- Klos KLE, Wetzel HC, Murray TD (2014) Resistance to *Oculimacula yallundae* and *Oculimacula acufiformis* is conferred by *Pch2* in wheat. *Plant Pathol* 63:400–404
- Law CN, Scott PR, Worland AJ, Hollins TW (1976) The inheritance of resistance to eyespot (*Cercospora herpotrichoides*) in wheat. *Genet Res Camb* 25:73–79
- Lind V (1999) Variation of resistance to *Pseudocercospora herpotrichoides* (Fron) Deighton in wheat genotypes carrying the gene *Pch-1*. *Plant Breed* 118:281–287
- Maloy OC, Inglis DA (1993) Diseases of Washington crops. Wash. St. Univ. Coop. Ext., Pullman, pp 21–46

- 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*, Third Edition. APS Press, Minneapolis, pp 32–34
- 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
- Quincke MC, Peterson CJ, Zemetra RS, Hansen JL, Chen J, Riera-Lizarazu O, Mundt CC (2011) Quantitative trait loci analysis for resistance to Cephalosporium stripe, a vascular wilt disease of wheat. *Theor Appl Genet* 122:1339–1349
- Sheng H, Murray TD (2013) Identifying new sources of resistance to eyespot of wheat in *Aegilops longissima*. *Plant Dis* 97:346–353
- Sheng H, See DR, Murray TD (2012) Mapping QTL for resistance to eyespot of wheat in *Aegilops longissima*. *Theor Appl Genet* 125:355–366
- 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
- Sprague R (1936) Relative susceptibility of certain species of Gramineae to *Cercospora herpotrichoides*. *J Agric Res* 53:659–670
- Sprague R, Fellowes H (1934) *Cercospora* foot rot of winter cereals. *Tech Bull USDA* 428:1–24
- Strausbaugh CA, Murray TD (1989) Inheritance of resistance to *Pseudocercospora 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 (*Pseudocercospora 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 *Pseudocercospora herpotrichoides* on chromosome 4 V 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