Theor Appl Genet (2005) 111: 932–940
DOI 10.1007/s00122-005-0025-0

ORIGINAL PAPER

H. J. Li · M. Arterburn · S. S. Jones · T. D. Murray

Resistance to eyespot of wheat, caused by *Tapesia yallundae*, derived from *Thinopyrum intermedium* homoeologous group 4 chromosome

Received: 5 February 2005 / Accepted: 14 June 2005 / Published online: 2 August 2005

Abstract *Thinopyrum intermedium* was identified previously as resistant to *Tapesia yallundae*, cause of eyespot of wheat. Using GUS-transformed isolates of *T. yallundae* as inoculum, we determined that wheat lines carrying *Th. intermedium* chromosome 4Ai#2 or the short arm of chromosome 4Ai#2 were as resistant to the pathogen as the eyespot-resistant wheat- *Th. ponticum* chromosome substitution line SS767 (PI 611939) and winter wheat cultivar Madsen, which carries gene *Pch1* for eyespot resistance. Chromosome 4E from *Th. elongatum* and chromosome 4J from *Th. bessarabicum* did not confer resistance to *T. yallundae*. Genome-specific PCR primers confirmed the presence of *Thinopyrum* chromatin in these wheat- *Thinopyrum* lines. Genomic in situ hybridization using an St genomic probe from *Pseudoroegneria strigosa* demonstrated that chromosome 4Ai#2 belongs to the J^s genome of *Thinopyrum*. The eyespot resistance in the wheat- *Th. intermedium* lines is thus controlled by the short arm of this J^s chromosome. This is the first report of resistance to *T. yallundae* controlled by a J^s genome chromosome of *Th. intermedium*.

Communicated by B. Keller

H. J. Li
Institute of Crop Sciences,
Chinese Academy of Agricultural Sciences,
Beijing, 100081, P. R. China

M. Arterburn · S. S. Jones
Department of Crop and Soil Sciences,
Washington State University,
Pullman, WA, 99164-6420, USA

T. D. Murray (✉)
Department of Plant Pathology,
Washington State University,
Pullman, WA, 99164-6430, U.S.A
E-mail: tim_murray@wsu.edu
Tel.: +509-335-9541
Fax: +509-335-9581

Keywords *Thinopyrum intermedium* · Genomic in situ hybridization · PCR · *Pseudocercosporella*

Introduction

Eyespot is a serious disease of winter wheat (*Triticum aestivum* L.) grown in areas where winters are cool and wet, including the U.S. Pacific Northwest (PNW). Susceptible wheat cultivars develop lesions on stem bases as a result of colonization by the pathogen. Significant yield losses can occur in eyespot-susceptible genotypes because of a reduced number of tillers, premature/death of stems, and lodging caused by the pathogen, which results in smaller kernels. The fungus that causes eyespot in the PNW is mainly *Tapesia yallundae* Wallwork & Spooner, which was referred to previously as the W-type strain of *Pseudocercosporella herpotrichoides* (Fron) Deighton. Among the possible approaches available for controlling eyespot of winter wheat, growing resistant cultivars is an effective and environmentally friendly method compared with application of fungicides (Murray 1996).

Three genes, *Pch1*,*Pch2*, and *Pch3*, are known for eyespot resistance (Doussinault et al. 1983; de la Peña et al. 1996; Yildirim et al. 1998). *Pch1*, which was derived from *Triticum ventricosum* Ces., is very effective and the development of eyespot-resistant cultivars adapted to the PNW has relied on this gene (Jones et al. 1995; Murray 1996). *Pch1* is also used in other parts of the world (Johnson 1992; Lucas et al. 2000). Eyespot resistance conferred by *Pch2*, which is present in wheat cultivar Cappelle-Desprez, does not effectively protect wheat from yield losses when disease is severe (Murray and Bruehl 1983). Located on chromosome 4V of *Dasypyrum villosum* L. Candargy (syn. *Haynaldia villosa* L.), *Pch3* appears to be highly effective against *T. yallundae* (Murray et al. 1994). Neither *Pch2* nor *Pch3* are used extensively in wheat breeding programs in the PNW because of their limited effectiveness (*Pch2*) against

eyespot or the difficulty of incorporating them (*Pch3*) into adapted wheat cultivars. Repeated use of the single gene *Pch1*, and limited choices of other resistance genes available to breeders have resulted in genetic vulnerability in host resistance to eyespot. Furthermore, although very effective in limiting disease development, *Pch1*-containing varieties can sustain significant yield loss when eyespot is severe (Allan et al. 1989) and a shift in species composition from predominately *T. yallundae* to equal or greater proportions of *T. acuformis* in the US Pacific Northwest has been documented and may be due to the use of *Pch1*-containing varieties (Douhan et. al 2002) on which *T. acuformis* may be more virulent than *T. yallundae* (Murray, unpublished). Therefore, new genes from different sources are needed to diversify the genetic resources of resistance to eyespot and potentially improve effectiveness of resistance to eyespot.

Thinopyrum ponticum (Podp.) Liu and Wang (syn. *Agropyron elongatum* (Host) Beauv., JJJ^sJ^s genome, 2n = 70) and *Th. intermedium* (Host.) Barkworth and D. R. Dewey (syn. *A. intermedium* (Host) Beauv., $StJJ^s$ genome, 2n = 42) are important wheatgrass species that have provided resistance genes for improvement of wheat against diseases such as leaf rust (*Puccinia triticina* Eriks.), stem rust (*P. graminis* Pers. f. sp. *tritici* Eriks. & Henn.), *Wheat streak mosaic virus* (WSMV), and *Barley yellow dwarf virus* (Friebe et al. 1996b). More recently, these wheatgrasses, as well as certain wheat-*Thinopyrum* derivatives, were identified as sources of resistance to eyespot (Cox et al. 2002). A factor(s) located on homoeologous group 4 chromosome that belongs to the J genome is responsible for resistance to *T. yallundae* in the wheat- *Th. ponticum*-derived line SS767 (PI 611939) (Li et al. 2004a). The chromosome in *Th. intermedium* that carries eyespot resistance gene(s) is not known; whether chromosomes that originate from diploid *Thinopyrum* species such as *Th. elongatum* (Host) D. R. Dewey (syn. *Lophopyrum elongatum* (Host) Á. Löve, E genome, 2n = 14) or *Th. bessarabicum* (Savul & Rayass) Á. Löve (J genome, 2n = 14), confer resistance to eyespot remains to be determined. Wheat genotypes that carry homoeologous group 4 chromosomes from different genomes of *Th. intermedium* have been developed (Cauderon et al. 1973; Wells et al. 1973; Friebe et al. 1996a) and resistance of these lines to WSMV was characterized (Pfannenstiel and Niblett 1978; Wells et al.1982; Friebe et al. 1991, 1996a; Seifers et al. 1995; Li et al. 2004b). Group 4 chromosomes were also introduced from *Th. elongatum* and *Th. bessarabicum* into wheat for genetic studies (Dvořák 1980; M. Anatasova, personal communication). Since the wheatgrasses share some related genomes, it is of interest to determine whether these *Thinopyrum* group 4 chromosomes are associated with eyespot resistance.

The present study was initiated to test the reaction of wheat- *Th. intermedium* lines to *T. yallundae* and to determine the chromosome(s) that confer resistance to eyespot. This study was also conducted to determine whether the group 4 chromosomes from diploid *Thinopyrum* species, *Th. elongatum* and *Th. bessarabicum*, carry genes for eyespot resistance.

Materials and methods

Plant materials

The chromosome composition of wheat- *Thinopyrum* hybrids used in this study is summarized in Tables 1 and 2. Six wheat- *Th. intermedium* lines, in which the homoeologous group 4 chromosomes or chromosome arms of *Th. intermedium* were added to wheat genomes or exchanged with the corresponding wheat chromosomes, were tested for reactions to *T. yallundae*. Line L4 (Wheat Genetic Resource Center, Manhattan, KS, accession number TA3650) is a chromosome disomic addition (DA) line in which *Th. intermedium* chromosome 4Ai#1 is added to wheat cultivar Vilmorin (Cauderon et al. 1973). Line CI 15092 (TA3519) is a wheat- *Th. intermedium* disomic substitution (DS) line DS 4Ai#2(4A) (Lay et al. 1971; Wells et al. 1973; Friebe et al. 1991). Lines CI 17881 (TA3513), CI 17885 (TA3517), and CI 17884 (TA3516) are chromosome addition DA 4Ai#2, chromosome substitution DS 4Ai#2(4D), and chromosome translocation T4DL·4Ai#2S lines, respectively, all of which were derived from crosses with CI 15092 as a parent (Wells et al. 1982; Friebe et al. 1991). In addition to alien chromosomes from *Th. intermedium*, lines CI 17884 and CI 17885 also contain a pair of chromosomes 7S from *Aegilops speltoides* Tausch., which replace a pair of chromosomes 7A from wheat (Friebe et al. 1991). Line TA7700 is a ditelosomic chromosome addition line in which the long arm of *Th. intermedium* chromosome 4Ai#3 was added to Chinese Spring (CS) wheat (Friebe et al. 1996a). Line PC261 is a 4J chromosome addition line that was derived from a cross of CS × *Th. bessarabicum* (M. Anatasova, personal communication). Wheat- *Th. elongatum* chromosome substitution line DS 4E(4D) (Dvořák 1980), chromosome addition line CS + 4E (TA3667), and wheat- *Th. ponticum* DS 4J(4D) chromosome substitution line SS767 (PI 611939) were also included in this study (Table 1). The eyespot-resistant controls were wheat cultivars Madsen, carrying *Pch1* (Allan et al. 1989), and Cappelle-Desprez, carrying *Pch2* (de la Peña et al. 1996). Eleven sib lines with or without gene *Wsm1* for WSMV resistance conferred by the short arm of chromosome 4Ai#2 of *Th. intermedium* (Sharp et al. 2002), together with their parents and check cultivars, were screened for reaction to the eyespot pathogens to determine the association of this chromosome arm with eyespot resistance. The chromosome arm of 4Ai#2 in these lines originated from a wheat- *Th. intermedium* T4DL·4Ai#2S translocation line KS93WGRC27, which was derived from CI 17884 (Gill et al. 1995). The spring wheat cultivar Chinese Spring and the winter wheat cultivar Hill 81 were used as susceptible checks. An accession of *Th. ponticum* (PI

Table 1 Reaction of wheat- *Thinopyrum* lines to inoculation with GUS-transformed isolates of *T. yallundae*

Lines (accession)	Chromosome constitution or eyespot-resistance genes[a]	Disease severity[b]	GUS score[c]
CI 15092 (TA3519)[d]	DS 4Ai#2(4A)[e]	1.3	1.1
CI 17881 (TA3513)	DA 4Ai#2[f]	1.2	1.0
CI 17885 (TA3517)	DS4Ai#2(4D), DS 7S(7A)	1.5	1.1
CI 17884 (TA3516)	T4DL·4Ai#2S, DS 7S(7A)	1.6	1.2
L4 (TA3650)	DA 4Ai#1	2.6*	1.5*
TA7700	DA 4Ai#3L	2.9*	1.6*
CS 4E(4D)	DS 4E(4D)	3.3*	2.0*
TA3667	DA 4E	3.1*	1.9*
PC261	DA 4J	3.1*	1.8*
SS767	DS 4J(4D)	1.4	1.0
Madsen	*Pch1*	1.5	1.0
Cappelle-Desprez	*Pch2*	1.6	1.3
Chinese Spring	Susceptible	3.4*	1.8*
Hill 81	Susceptible	2.8*	1.7*
MSD[g] (P = 0.05)		0.73	0.36

[a]The chromosome composition of the wheat- *Thinopyrum* lines were determined previously (Cauderon et al. 1973; Friebe et al. 1991; Li et al. 2004a; Dovřák 1980; and M. Atanasova, personal communications).
[b]Symptom severity of eyespot was rated visually on a 1 to 4 scale, where 1 = no lesion, and 4 = a lesion covering the entire first leaf sheath and two-thirds of the second sheath. Figures are the mean of two experiments with four replicates of two plants each.
[c]The mean GUS-score is presented as the ratio of the individual line to the resistant wheat control Madsen plus one, i.e., log10 (X/Madsen) + 1. Figures are the mean of two experiments with four replicates of two plants each.
[d]Accession number designated by the Wheat Genetic Resource Center, Manhattan, KS.
[e]DS: disomic chromosome substitution.
[f]DA: disomic chromosome addition.
[g]MSD = minimum significant difference at P = 0.05 according to Dunnett's t tests using Madsen as the resistant control. Genotypes significantly different than Madsen are indicated with an asterisk (*).

206624), *Th. intermedium* (PI 264770), *Th. elongatum* (PI 547326), *Th. bessarabicum* (PI 531710), and *Pseudoroegneria strigosa* (M. Bieb) Á. Löve (St genome, 2n = 14) (PI 499493) were used as controls in molecular detection of *Thinopyrum* chromatin.

Preparation of inoculum

Isolates of *T. yallundae* used in this study (tPh8934-5-61, tPh8934-5-62, tPh8934-5-68, and tPh8934-5-70) were isolated originally from infected wheat plants collected from eastern Washington State, U.S.A. They were transformed with a plasmid containing the *Escherichia coli gusA* gene that was attached to the constitutive glyceraldehyde-3-phosphate dehydrogenase (*gpd*) promoter fragment originating from *Aspergillus nidulans*, following a method described by Bunkers (1991). Inoculum was prepared by culturing the β-glucuronidase (GUS)-transformed isolates of *T. yallundae* separately on 1.5% water agar plates at 13°C under ultraviolet light for 3 weeks to induce sporulation and then collecting the conidia. Suspensions of conidia containing equal proportions of each GUS-transformed isolate were mixed in a blender to form a water-agar slurry at a concentration of 1×10^5 conidia/ml and used as inoculum.

Evaluation of reaction to *T. yallundae* and analysis of GUS activity

The wheat- *Thinopyrum* lines, together with controls, were arranged in a complete block design with 4 replicates of 2 plants per plot. The 11 wheat- *Th. intermedium* sib lines that differ with respect to presence or absence of translocated chromosome T4DL·4Ai#2S were tested with the same procedure and design. All experiments were carried out twice. Individual plants at the 2-leaf-stage were inoculated with 250 µl of inoculum of *T. yallundae* as described previously (de la Peña and Murray 1994). The same amount of inoculum was applied again to each plant 2 days after the initial inoculation. Inoculated plants were incubated in a growth chamber at 15/13°C (day/night) and 100% humidity for 8 weeks before assessment of disease severity. Plants were rated visually on a 1 to 4 scale based on development of lesions on the basal part of the main tiller, where 1 = no lesion, 2 = a lesion on the first leaf sheath or tiny lesions on the first or second sheath (hypersensitive reaction), 3 = a lesion on the first and second sheaths, and 4 = a lesion covering the entire first leaf sheath and two-thirds of the second sheath (de la Peña and Murray 1994).

Stem segments 3-cm-long were sampled from the base of each main tiller for determination of GUS activity. The stem segments were ground individually in 2.5 ml of extraction buffer, which was composed of 50 mM $NaHPO_4$, pH 7.0, 5 mM dithiothreitol, 10 mM Na_2EDTA, 0.1% sodium lauryl sarcosine (w/v), and 0.1% Triton-100 (v/v) (Jefferson et al. 1987). GUS activity was determined by conversion of 4-methylumbelliferyl β-D-glucoside (MUG) to 4-methylumbelliferone (MU) by GUS as described by de la Peña and Murray 1994. The fluorescence intensity was determined with a Fluorolite 1000 fluorimeter (Dynatech Laboratories, Inc., Chantilly, VA). Values were log-transformed prior to statistical analysis. Analysis of variance was conducted using SAS PROC GLM (SAS Institute, Raleigh, NC) and means were separated using Dunnett's T-test (MSD, P = 0.05) using Madsen as the resistant control to which other genotypes were compared. A line was considered susceptible to *T. yallundae* when its mean GUS score was significantly larger than that of Madsen.

Polymerase chain reaction (PCR)

PCR amplification was used to detect alien chromatin in wheat- *Thinopyrum* derivatives. The primers 2P1 (5' ACAATCTGAAAATCTGGACA 3') and 2P2 (5' TCATATTGAGACTCCTATAA 3') were derived from

a repetitive DNA sequence from *Th. elongatum*, pLeUCD2, and are specific for *Thinopyrum* genomes (Wang and Wei 1995). PCR was carried out in a 25 µl reaction volume containing 1× buffer, 3.0 µl $MgCl_2$ (50 mM), 2.0 µl dNTP (10 mM each), 100 ng of each primer, 75 ng of sample DNA, and 1 unit of Taq DNA polymerase. Amplification was performed with a GeneAmp9600 thermocycler (Perkin Elmer, Norwalk, CT). The reaction mixture was incubated at 94°C for 3 min. For amplification using the genome-specific primers, 35 cycles of 94°C for 1 min, 47–52°C ramp annealing for 45 s, 72°C for 1 min were performed, followed by an extension at 72°C for 5 min. The amplified products were separated on a 1.5% agarose gel. The presence of chromosome arm 4Ai#2 in wheat- *Th. intermedium* sib lines were previously confirmed by PCR with primers STSJ15 (left primer 5' GTAGCAGGGGAAGCTGAAGA 3' and right primer 5' CCGAGCTCACAC GCTAATTT 3'), which are specific for gene *Wsm1* (Sharp et al. 2002).

Genomic in situ hybridization (GISH)

Using biotin-14-dATP-labeled St genomic DNA from *Ps. strigosa* as a probe and sheared genomic DNA of Chinese Spring (ABD genomes) as a blocker, GISH analysis was conducted to detect alien chromatin in wheat- *Th. intermedium* lines. The genome to which a *Thinopyrum* chromosome belongs was determined based on distribution and intensity of fluorescein isothioncyanate (FITC)-signals on chromosomes; St genome-chromosomes display strong hybridization signals along their entire length, J and E genome-chromosomes are completely labeled but less intensely than St genome-chromosomes, and J^s genome-chromosomes express obvious fluorescent signals around the centromeric regions and faint signals over the rest of the chromosomes (Chen et al. 1999a). The preparation of chromosomes, labeling of probes, and detection of fluorescent hybridization signals were carried out as described previously (Chen et al. 1999a). The yellow-greenish fluorescent signals were viewed with a Zeiss fluorescent microscope (Carl Zeiss, Oberkochen, Germany) and the results of GISH were recorded with a digital camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA).

Results

Reaction to *T. yallundae*

Data from each experiment were subjected to analysis of variance and then combined after determining error variances were not significantly different (Gomez and Gomez 1984). Accessions CI 17881, CI 17884, CI 17885, and CI 15092, which carry chromosome 4Ai#2 or the short arm of chromosome 4Ai#2 from *Th. intermedium*, developed no symptoms or hypersensitive reactions, and were similar to the resistant control Madsen and the wheat- *Th. ponticum* line SS767 following inoculation with *T. yallundae* (Table 1). Line TA3650 (L4), a chromosome 4Ai#1 addition line, and line TA7700, a telocentric chromosome 4Ai#3L addition line, developed severe disease and had visual disease scores that were significantly greater than Madsen. Wheat- *Th. bessarabicum* chromosome 4J addition line PC261, wheat- *Th. elongatum* chromosome substitution line CS 4E(4D), and chromosome 4E addition line TA3667 (CS + 4E), were as susceptible to *T. yallundae* as Chinese Spring and Hill 81, which exhibited lesions that nearly covered the stem bases (Table 1). Cappelle-Desprez developed obvious symptoms of eyespot in some plants, but the mean disease score was significantly less than Chinese Spring and Hill 81.

GUS analysis confirmed the results of visual symptom assessment and were significantly correlated with visual rating (r^2 = 0.71 and 0.76 for test 1 and 2, respectively). GUS scores for Cappelle-Desprez, SS767, CI 17881, CI 17884, CI 17885, and CI 15092 were comparable to Madsen (Table 1). GUS scores for TA3650, TA7700, and PC261 were significantly greater than that of Madsen. Lines CS 4E(4D) and TA3667 had the greatest GUS scores in both experiments.

PCR analysis. All wheat- *Th. intermedium* lines, i.e., CI 15092, CI 17881, CI 17885, CI 17884, TA3650, and TA7700 and the wheat- *Th. ponticum* line SS767, produced the 277 bp diagnostic fragment using primers 2P1 and 2P2 similar to *Th. intermedium* and *Th. ponticum*, which indicated that these lines contained *Thinopyrum* chromatin. The primers amplified a band in CS 4E(4D), TA3667, and PC261 identical in size (277 bp) to their alien parents *Th. elongatum* (E genome) and *Th. bessarabicum* (J genome), and *Ps. strigosa* (St genome). No such product was amplified from Madsen, Cappelle-Desprez, Chinese Spring, or Hill 81, indicating that they did not contain *Thinopyrum* chromatin (Fig. 1, Table 2).

GISH analysis

Two alien chromosomes or chromosome arms belonging to different genomes of *Thinopyrum* were detected by GISH in all wheat- *Thinopyrum* derivatives, either resistant or susceptible to eyespot. Fluorescent hybridization signals in alien chromosomes carried by CI 17881, CI 17885, and CI 15092 were brightest in the centromeric regions, indicating that they are likely J^s genome chromosomes. In addition to the J^s genome chromosomes that were added to CI 17881, a pair of wheat- *Th. intermedium* terminal translocated chromosomes were detected in mitotic cells of this line (Fig. 2a). In CI 17884, the alien chromosome arms that were translocated onto wheat chromosome arms from *Th. intermedium* also displayed GISH patterns typical of J^s genome chromosomes, indicating that CI 17884 is likely a wheat-J^s chromosome translocation line (Fig. 2b). Line TA7700 contained one or two chromosome arms

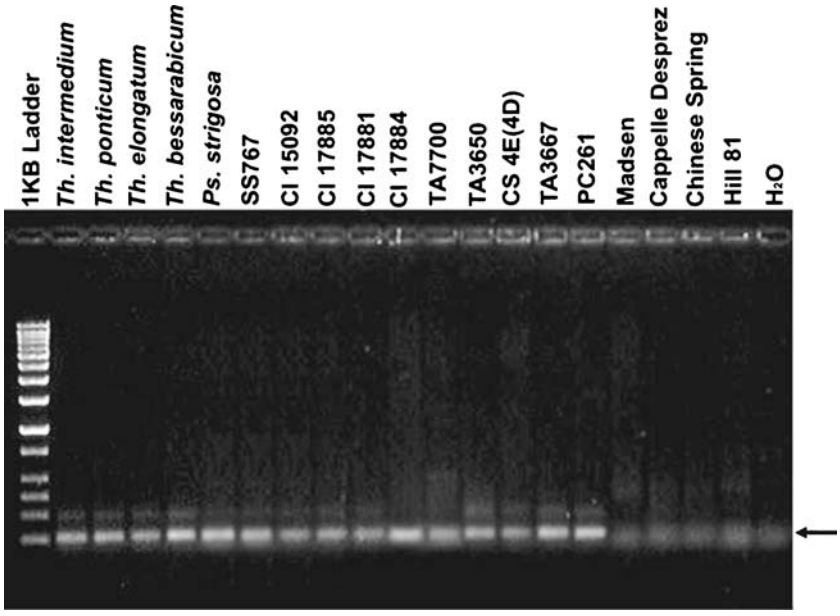

Fig. 1 PCR products amplified with genome-specific primers 2P1 and 2P2 originating from a repetitive DNA sequence, pLeUCD2, from *Th. elongatum*, which amplifies a 277 bp DNA fragment specific to genus *Thinopyrum* (*arrow*)

Table 2 Detection of *Thinopyrum* chromatin using genomic in situ hybridization (GISH) and polymerase chain reaction with genome-specific primers 2P1 and 2P2

Line	Origin of alien chromosome	2n =	GISH or genome composition	PCR
PI 264770	*Thinopyrum intermedium*	42	JJ^s St[a]	+
PI 206624	*Th. ponticum*	70	$JJJJ^s$ J^s	+
PI 531710	*Th. bessarabicum*	14	J	+
PI 547326	*Th. elongatum*	14	E	+
PI 499493	*Pseudoroegneria strigosa*	14	St	+
CI 15092	*Th. intermedium*	42	2 J^s + 40 W	+
CI 17881	*Th. intermedium*	44	2 J^s + 40 W + 2WL-WS-T[c]	+
CI 17885	*Th. intermedium*	42	2 J^s + 40 W[d]	+
CI 17884	*Th. intermedium*	42	2 WL-J^s S + 40 W[e]	+
TA3650	*Th. intermedium*	44	2 St + 42 W	+
TA7700	*Th. intermedium*	42 + 1t[b] 42 + 2t	1 or 2 JL + 42 W	+
CS 4E(4D)	*Th. elongatum*	42	2 E + 40 W	+
TA3667	*Th. elongatum*	44	2 E + 42 W	+
PC261	*Th. bessarabicum*	44	2 J + 42 W	+
SS767	*Th. ponticum*	42	2 J + 40 W	+
Madsen	None	42	42W	-
Cappelle-Desprez	None	42	42W	-
Chinese Spring	None	42	42W	-
Hill 81	None	42	42W	-

[a] J, J^s, and St: J, J^s, and St genome chromosomes from *Thinopyrum*, respectively. W: wheat chromosomes. T: chromosome segment from *Thinopyrum*. L and S: the long arm or the short arm of a chromosome.
[b] t: telocentric chromosome.
[c] WL-WS-T: a *Thinopyrum* chromosome segment is translocated onto the terminal part of the short chromosome arm from wheat.
[d] and [e] Lines CI 19884 and CI 17885 also contained a pair of chromosome 7S from *Aegilops speltoides* (Friebe et al. 1991).

from the J genome of *Th. intermedium*, as indicated by fluorescent hybridization patterns. The even distribution of fluorescent signals along the chromosome indicated that two *Thinopyrum* chromosomes were present in lines CS 4E(4D) and TA3667, which carry E genome chromosomes, and PC261, which carries the J genome chromosomes. Alien chromosomes in line TA3650 had evenly distributed FITC-signals that were brighter than E or J genome chromosomes, indicating that they belong to the St genome (Table 2).

Association of eyespot resistance with chromosome arm 4Ai#2S of *Th. intermedium*

Symptom assessment and GUS analysis of sib lines derived from wheat- *Th. intermedium* hybrids indicated that lines containing the short arm of chromosome 4Ai#2 were resistant to *T. yallundae*, having disease severity and GUS scores that were comparable to Madsen and the resistant parent KS93WGRC27 (Table 3). In contrast, lines that did not contain the

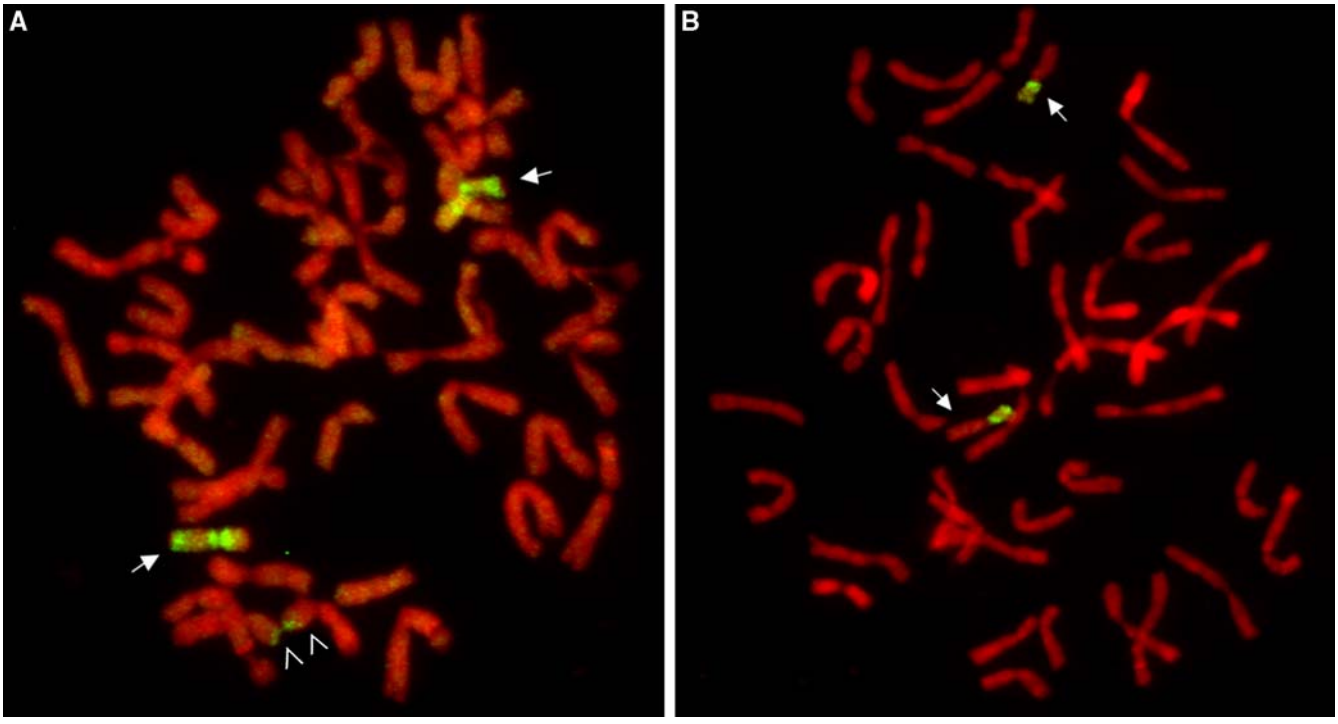

Fig. 2 Genomic in situ hybridization analysis of wheat lines carrying alien chromosomes from *Thinopyrum* using a St genomic probe from *Pseudoroegneria strigosa* and blocking with Chinese Spring genomic DNA. A. CI 17881. A pair of J^s genome chromosomes (*arrows*) and a pair of wheat- *Th. intermedium* terminal translocated chromosomes (open arrows) were detected by GISH. **B**. CI 17884. The *Th. intermedium* chromosome arms that were translocated onto wheat chromosomes belong to the J^s genome, as indicated by their stronger hybridization signals in the centromeric region than in the rest of the chromosome (*arrows*)

short arm of chromosome 4Ai#2 were susceptible to *T. yallundae* as indicated by disease severity and GUS scores that were as high as the wheat parental cultivars McNeal, MT9328, and Amidon, and the check cultivar Chinese Spring; these scores were significantly greater than lines containing chromosome 4Ai#2 (Table 3). The presence or absence of chromosome arm 4Ai#2S was confirmed by amplification of the diagnostic fragment using primer set (2P1 and 2P2) specific for genomes of *Thinopyrum* (Table 3) and gene *Wsm1*, conferring resistance to WSMV, located on the short chromosome arm 4Ai#2 (Sharp et al. 2002).

Discussion

Intermediate wheatgrass, *Th. intermedium*, and some wheat- *Th. intermedium* hybrids were reported to be resistant to *T. yallundae* (Cox et al. 2002). Wheat- *Th. intermedium* lines that carry chromosome 4Ai#2 and the short arm of this chromosome, 4Ai#2S, were resistant to *T. yallundae*. Reaction of sib lines with or without the short arm of chromosome 4Ai#2 further confirmed the association of this chromosome with eyespot resistance. GUS scores of lines containing 4Ai#2S were comparable to Madsen (carrying *Pch1*) and the wheat- *Th. ponticum* chromosome substitution line SS767. However, sib lines without 4Ai#2S were susceptible (Table 3). Eyespot resistance identified in wheat- *Th. intermedium* derivatives in the present study provides new opportunities to improve resistance of wheat against eyespot. This is the first report on genomic control of resistance to *T.yallundae* in intermediate wheatgrass.

Chromosome 4Ai#2 was transferred from *Th. intermedium* into wheat as a source of WSMV resistance over 30 years ago (Lay et al. 1971; Wells et al. 1973). Resistance to WSMV conferred by *Wsm1* on chromosome 4Ai#2 is effective against virus isolates from various wheat producing regions (Stoddard et al. 1987; Baley et al. 2001; Li et al. 2004b), although it is sensitive to temperature (Seifers et al. 1995), and advanced wheat lines containing chromosome arm 4Ai#2S have been developed for use in breeding programs (Sharp et al. 2002). Wheat lines carrying translocated chromosome arm 4Ai#2S had a small yield penalty, which may be overcome by selection for yield. In addition, chromosome arm 4Ai#2S had no deleterious effects on end-use quality parameters (Baley et al. 2001). Together, these make wheat lines with translocated chromosome arm 4Ai#2S useful for simultaneous improvement of resistance to eyespot and WSMV.

Using an St genomic probe prepared from *Ps. strigosa* DNA, GISH analysis demonstrated that chromosome 4Ai#2 is most likely a J^s genome chromosome (Fig. 2a and b; Chen et al. 1999a). Eyespot resistance in these wheat- *Th. intermedium* lines is thus controlled by gene(s) located on chromosome $4J^s$. Previously, group 4 chromosomes from *D. villosum* (Murray et al. 1994) and

Table 3 PCR amplification of *Thinopyrum* chromatin using primers 2P1 and 2P2 specific for a repetitive DNA fragment, pLeUCD2, originating from *Thinopyrum elongatum* and reaction to *Tapesia yallundae* wheat- *Th. intermedium* lines with or without chromosome arm 4Ai#2

Line	Pedigree	PCR using primers specific for		Reaction to T. yallundae	
		Genome	*Wsm1*[a]	Disease severity[b]	GUS score[c]
4161R	McNeal/KS27//MT9328	+	+	1.9	1.2
4165R	McNeal/KS27//MT9328	+	+	1.8	1.2
4266R	Amidon/KS27//McNeal	+	+	1.8	1.3
4274R	Amidon/KS27//McNeal	+	+	1.9	1.2
4292R	Amidon/KS27//MT9328	+	+	1.8	1.2
4168S	McNeal/KS27//MT9328	-	-	2.9*	2.0*
4199S	McNeal/KS27//MT9328	-	-	2.8*	2.0*
4238S	Amidon/KS27//McNeal	-	-	2.9*	2.1*
4241S	Amidon/KS27//McNeal	-	-	2.8*	2.1*
4252S	Amidon/KS27//McNeal	-	-	2.9*	1.8*
4316S	Amidon/KS27//MT9328	-	-	3.1*	1.9*
KS93WGRC27	Resistant parent	+	+	2.1*	1.1
McNeal	Susceptible parent	-	-	3.1*	1.8*
MT9328	Susceptible parent	-	-	3.0*	1.9*
Amidon	Susceptible parent	-	-	2.9*	1.7*
Madsen	*Pch1*	-	-	1.9	1.0
Cappelle-Desprez	*Pch2*			2.0	1.4
Chinese Spring	Susceptible check	-	-	3.0*	1.9*
MSD [d] (P = 0.05)				0.42	0.32

[a]Sharp et al. (2002).
[b]Symptom severity of eyespot was rated visually on a 1 to 4 scale, where 1 = no lesion, and 4 = a lesion covering the entire first leaf sheath and two-thirds of the second sheath. Figures are the mean of two experiments with four replicates of two plants each.
[c]The mean GUS-score is presented as the ratio of the individual line to the resistant wheat control Madsen plus one, i.e., log10 (X/Madsen) + 1. Figures are the mean of two experiments with four replicates of two plants each.
[d]MSD = minimum significant difference at P = 0.05 according to Dunnett's t tests using Madsen as the resistant control. Genotypes significantly different than Madsen are indicated with an asterisk (*).

Th. ponticum (Li et al. 2004a) were associated with resistance to *T. yallundae*. In contrast, eyespot resistance genes *Pch1* and *Pch2* are located on the long arm of group 7 chromosomes (Law et al. 1976; Worland et al. 1988; de la Peña et al. 1996). Furthermore, eyespot resistance gene(s) in *Th. intermedium* is most likely located on the short arm of chromosome $4J^s$ (=4Ai#2) based on the fact that lines containing this chromosome arm were resistant to *T. yallundae* (Tables 1 and 3). Gene *Pch3* for eyespot resistance resides on the long arm of the corresponding chromosome in *D. villosum* (Yildirim et al. 1998).

Wheat lines with *Th. intermedium* chromosome 4Ai#3 or the long arm of chromosome 4Ai#3L are resistant to WSMV (Friebe et al. 1996a). GISH analysis demonstrated that this chromosome arm was derived from the J genome. Nevertheless, chromosome arm 4Ai#3L did not confer resistance to eyespot, since obvious lesions developed on the stem bases and high GUS scores were obtained from TA7700, which carries telocentric chromosome 4Ai#3L (Table 1). Nor did chromosome 4Ai#1, which was characterized as an St genome chromosome (Table 2; Chen et al. 1999b), confer resistance to *T. yallundae*. Resistance to *T. yallundae* was not found in wheat lines carrying chromosome 4E from *Th. elongatum* or chromosome 4J from *Th. bessarabicum*. Although the E and J genomes that are present in the diploid species of *Th. elongatum* and *Th. bessarabicum* are, to some extent, related to the corresponding genomes in polyploid wheatgrasses, it is apparent that the group 4 chromosomes from the diploid *Thinopyrum* spp. tested in this study do not carry genes for eyespot resistance.

The eyespot- and WSMV-resistant lines CI 17884 and CI 17885 are also resistant to greenbug (*Schizaphis graminum* Rond.) due to the presence of chromosome 7S from *Ae. speltoides*, which carries gene *Gb5* for pest resistance (Tyler et al. 1987; Friebe et al. 1991). Although a number of diploid relatives of wheat are resistant to eyespot, resistance has not been reported in *Ae. speltoides* (Sprague 1936; Yildirim et al. 1995; Cadle et al. 1997). Additionally, the chromosome addition line CI 17881, a sib line of CI 17884 and CI 17885, was not resistant to greenbug and does not contain chromosome 7S of *Ae. speltoides* (Tyler et al. 1985; Friebe et al. 1991). However, this line contained chromosome 4Ai#2 (=$4J^s$) and was resistant to eyespot (Table 1) and WSMV (Friebe et al. 1991). Based on these findings, eyespot resistance in these wheat- *Th. intermedium* lines is associated with chromosome 4Ai#2 (=$4J^s$) rather than chromosome 7S of *Ae. speltoides*. Introgression of multiple sources of alien chromosomes can complicate the genomic compositions of wheat making them unsuitable for commercial use. Wheat lines that carry only the short arm 4Ai#2S (=$4J^s$ S) conferring resistance to WSMV have been developed (Gill et al. 1995;

Sharp et al. 2002) and these lines were also resistant to *T.yallundae* (Table 3); they are expected to be more useful in improving wheat against WSMV and eyespot than CI 17884, which carries both *Th. intermedium* and *Ae. speltoides* chromatin.

Acknowledgements Plant Pathology New Series#0385, College of Agriculture and Home Economics Agricultural Research Center Project# 0670. The authors thank Drs. B.S. Gill, L.E. Talbert, W. Jon Raupp, J. Dovřák, and M. Atanasova for providing seeds of wheat- *Thinopyrum* lines that were used in this study. The technical assistance of H.Y. Shen is appreciated. The financial support of the Washington State Wheat Commission is gratefully acknowledged.

References

Allan RE, Peterson CJ Jr, Rubenthaler GL, Line RF, Roberts DE (1989) Registration of 'Madsen' wheat. Crop Sci 29:1575–1576

Baley GJ, Talbert LE, Martin JM, Young MJ, Habernicht DK, Kushnak GD, Berg JE, Lanning SP, Bruchner PL (2001) Agronomic and end-use qualities of *Wheat streak mosaic virus* resistant spring wheat. Crop Sci 41:1779–1784

Bunkers GJ (1991) Expression of the *Escherichia coli* β-glucuronidase gene in *Pseudocercosporella herpotrichoides*. Appl Environ Microbiol 57:2896–2900

Cadle MM, Murray TD, Jones SS (1997) Identification of resistance to *Pseudocercosporella herpotrichoides* in *Triticum monococcum*. Plant Dis 81:1181–1186

Cauderon Y, Saigne B, Dange M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: Proc 4th Intl Wheat Genet Symp August 6–11, 1973, Columbia Agricultural Experiment Station, College of Agriculture, University of Missouri. pp 401–407

Chen Q, Conner RL, Laroche A, Fedak G, Thomas JB (1999a) Genomic origins of *Thinopyrum* chromosomes specifying resistance to wheat streak mosaic virus and its vector, Aceria tosichella. Genome 42:289–295

Chen Q, Conner RL, Laroche A, Ji WQ, Armstrong KC, Fedak G (1999b) Genomic in situ hybridization analysis of *Thinopyrum* chromatin in a wheat- *Th. intermedium* partial amphiploid and six derived chromosome addition lines. Genome 42:1217–1223

Cox CM, Murray TD, Jones SS (2002) Perennial wheat germ plasm lines resistant to eyespot, Cephalosporium stripe, and wheat streak mosaic. Plant Dis 86:1043–1048

Douhan GW, Murray TD, Dyer PS (2002) Species and mating-type distribution of *Tapesia yallundae* and *T. acuformis* and occurrence of apothecia in the U.S. Pacific Northwest. Phytopathology 92:703–709

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

Dvořák J (1980) Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can J Genet Cytol 22:237–259

Friebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS (1991) Identification of alien chromatin specifying resistance to wheat streak mosaic and greenbug in wheat germ plasm by C-banding and in situ hybridization. Theor Appl Genet 81:381–389

Friebe B, Gill KS, Tuleen NA, Gill BS (1996a) Transfer of wheat streak mosaic virus resistance from *Agropyron intermedium* into wheat. Crop Sci 36:857–861

Friebe B, Jiang JM, Raupp WJ, McIntosh RA, Gill BS (1996b) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 191:59–87

Gill BS, Friebe B, Wilson DL, Cox TS (1995) Registration of KS93WGRC27 wheat streak mosaic virus-resistant T4DL·4Ai#2S wheat germplasm. Crop Sci 35:1236

Gomez, KA, Gomez, AA (1984) Statistical Procedures for Agricultural Research. Wiley, New York, p 680

Jefferson RA, Kavanagh AJ, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

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. Annu Rev Phytopathol 33:429–443

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

Lay CL, Wells DG, Gardner WAS (1971) Immunity from wheat streak mosaic virus in irradiated *Agrotricum* progenies. Crop Sci 11:431–432

Li HJ, Arterburn M, Jones SS, Murray TD (2004a) A new source of resistance to *Tapesia yallundae* associated with a homoeologous group 4 chromosome in *Thinopyrum ponticum*. Phytopathology 94:923–937

Li HJ, Conner RL, Chen Q, Graf RJ, Laroche A, Ahmad F, Kuzyk AD (2004b) Promising genetic resources for resistance to *Wheat streak mosaic virus* and the wheat curl mite, in wheat- *Thinopyrum* partial amphiploids and their derivatives. Genet Res Crop Evol 51:827–835

Lucas JA, Dyer PS, Murray TD (2000) Pathogenicity, host-specificity, and population biology of *Tapesia* spp., causal agents of eyespot disease of cereals. Adv Bot Res 33:225–258

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

Murray TD, Bruehl GW (1983) Role of thickening in basal stem internodes in resistance to strawbreaker foot rot in winter wheat. Phytopathology 73:261–268

Murray TD, de la Peña RC, Yildirim A, Jones SS (1994) A new source of resistance to *Pseudocercosporella herpotrichoides*, cause of eyespot disease of wheat, located on chromosome 4V of *Dasypyrum villosum*. Plant Breed 113:281–286

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

Pfannenstiel MA, Niblett CL (1978) The nature of the resistance of Agrotricums to wheat streak mosaic virus. Phytopathology 68:1204–1209

Seifers DL, Martin TJ, Harvey TL, Gill BS (1995) Temperature sensitivity and efficacy of wheat streak mosaic virus resistance derived from *Agropyron intermedium*. Plant Dis 79:1104–1106

Sharp GL, Martin JM, Lanning SP, Blake NK, Brey CW, Sivamani E, Qu R, Talbert LE (2002) Field evaluation of transgenic and classical sources of *Wheat streak mosaic virus* resistance. Crop Sci 42:105–110

Sprague R (1934) Relative susceptibility of certain species of Gramineae to *Cercosporella herpotrichoides*. J Agri Res 53:659–670

Stoddard SL, Gill BS, Lommel SA (1987) Genetic expression of wheat streak mosaic virus resistance in two wheat-wheatgrass hybrids. Crop Sci 27:514–519

Tyler JM, Webster JA, Smith EL (1985) Biotype E greenbug resistance in wheat streak mosaic virus-resistant wheat germ plasm lines. Crop Sci 25:686–688

Tyler JM, Webster JA, Merkle OG (1987) Designation of genes in wheat germplasm conferring greenbug resistance. Crop Sci 27:526–527

Wang RR-C, Wei JZ (1995) Variations of two repetitive DNA sequences in several Triticeae genomes revealed by polymerase chain reaction and sequencing. Genome 38:1221–1229

Wells DG, Wong RSC, Lay CL, Gardner WAS, Buchenau GW (1973) Registration of C.I. 15092 and C.I. 15093 wheat germplasm. Crop Sci 13:776

Wells DG, Kota RS, Sandhu HS, Gardner WAS, Finney KF (1982) Registration of one disomic substitution line and five translocation lines of winter wheat germ plasm resistant to wheat streak mosaic virus. Crop Sci 22:1277–1278

Worland AJ, Law CN, Hollins TW, Kobner RMD, Guira 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, Cox TS, Line RF (1995) Resistance to stripe rust and eyespot diseases of wheat in *Triticum tauschii*. Plant Dis 79:1230–1236

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