Theor Appl Genet (1998) 97 : 1–8 © Springer-Verlag 1998

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Molecular characterization of the genome composition of partial amphiploids derived from *Triticum aestivum* × *Thinopyrum ponticum* and *T. aestivum* × *Th. intermedium* as sources of resistance to wheat streak mosaic virus and its vector, *Aceria tosichella*

Received: 27 January 1998 / Accepted: 10 February 1998

Abstract Wheat streak mosaic virus (WSMV), vectored by the wheat curl mite (WCM), is one of the most important viral diseases of wheat (*Triticum aestivum*) in the world. Genetic resistance to WSMV and the WCM does not exist in wheat. Resistance to WSMV and the WCM was evaluated in five different partial amphiploids namely Agrotana, OK7211542, ORRPX, Zhong 5 and TAF 46, which were derived from hybrids of wheat with decaploid *Thinopyrum ponticum* or with hexaploid *Th. intermedium*. Agrotana was shown to be immune to WSMV and the WCM; the other four partial amphiploids were susceptible to the WCM. Genomic in situ hybridization (GISH) using genomic DNA probes from *Th. elongatum* (EE, $2n = 14$), *Th. bessarabicum* (JJ, $2n = 14$), *Pseudoroegneria strigosa* (SS, $2n = 14$) and *T. aestivum* (AABBDD, $2n = 42$) demonstrated that three of the partial amphiploids, Agrotana, OK7211542 and ORRPX, have almost identical alien genome constitutions: all have 16 alien chromosomes, with 8 chromosomes being closely related to the J^s genome and 8 chromosomes belonging to the E or J genomes and no evidence of any S-genome chromosomes. GISH confirmed that the alien genomes of Agrotana and OK7211542, like ORRPX, were all derived from *Th. ponticum*, and not from *Th. intermedium*. However, in contrast to Agrotana, ORRPX and OK7211542 were susceptible to the WCM and WSMV. The partial amphiploid Zhong 5 had a reconstituted alien genome composed of 4 S-and 4 J^s-genome chromosomes of *Th. intermedium* with 6 translocated chromosomes between the S and J^s genomes. This line was highly resistant to WSMV, but was susceptible to the WCM. TAF 46, which contained a synthetic genome consisting of 3 pairs of S-genome chromosomes and 4 pairs of E- or J-genome chromosomes in addition to the 21 pairs of wheat chromosomes, was susceptible to the WCM, but moderately resistant to WSMV. Agrotana offers great potential for transferring WSMV and WCM resistance into wheat.

Key words Wheat streak mosaic virus · Wheat curl mite · Triticeae · Partial amphiploids · Wheat · Resistance analysis · GISH · Genome

Communicated by G. Wenzel

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Introduction

It is not uncommon in present day, multidisciplinary plant breeding efforts to see increasing emphasis being placed on wild and related species for the genetic improvement of our cultivated crop plants. Species belonging to the "secondary" and "tertiary" gene pools (Jiang et al. 1994) continue to provide much needed genetic variability for various biotic and abiotic resistance breeding. Chromosome manipulation methodologies, as proposed by Feldman and Sears (1981), enable the transfer of novel genes and chromosome segments derived from the species in the Triticeae with homoeologous genomes.

Wheat streak mosaic (WSM), caused by wheat streak mosaic virus (WSMV) and vectored by the wheat curl mite (WCM), *Aceria tosichella* Keifer (syn. *Aceria tulipae* Keifer), is one of the most important viral diseases

of wheat (*Triticum aestivum* L. em Thell). It continues to be a serious threat to wheat production in the Great Plains of the United States, Canada as well as in many other wheat-producing countries of the world. To date, no useful resistance to this disease has been reported in commercial wheat cultivars. However, useful resistance is present in related Triticeae genera such as *Secale* (Martin et al. 1976), *Aegilops* (Thomas and Conner 1986), *Thinopyrum* (Cauderon et al. 1973; Larson and Atkinson 1973) and *Haynaldia* (Chen et al. 1996).

Several $2n = 56$ wheat-*Thinopyrum* partial amphiploids derived from either wheat × *Th. ponticum* (Podp.) Barkworth & D. R. Dewey [syn. *Agropyron elongatum* (Host) P. Beauv. and *Lophopyrum ponticum* (Podp.) Love, $2n = 10x = 70$] or wheat × *Th. intermedium* (Host) Barkworth & D. R. Dewey [syn. *Agropyron intermedium* (Host) Beauv., $2n = 6x = 42$], such as TAF 46, Zhong 5, OK7211542, ORRPX and Agrotana, have been identified as promising sources of multiple disease resistance. Their ability to fully express the resistance derived from wild species and readily cross with wheat has facilitated their extensive use for wheat improvement (Cauderon et al. 1973; Larson and Atkinson 1973; Dvorak 1985; Xin et al. 1988; Friebe et al. 1991; Bai and Knott 1993; Banks et al. 1993; Jiang et al. 1993). These wheat-*Thinopyrum* partial amphiploids also provide useful intermediate material for studying genomic affinities and homoeologous relationships among alien chromosomes. They are also useful for producing wheat-alien addition, substitution and translocation lines which confer resistance to WSMV or WCM (Lay et al. 1971; Liang et al. 1979; Wells et al. 1982; Whelan et al. 1983; Whelan and Hart 1988).

The study of genomic structure and the chromosome constitution of the partial amphiploids continues to be an interesting area of research, as it provides valuable information for facilitating the efficient transfer of useful traits from these partial amphiploids to wheat (Xin et al. 1988; Banks et al. 1993; Friebe et al. 1992; Chen et al. 1995; Zhang et al. 1996a). From chromosome pairing analysis, Xin et al. (1988) and Banks et al. (1993) reported that Zhong 5 and TAF 46 have very different synthetic genomic constitutions although both of them are derived from *Th. intermedium*. Based on C-banding and fluorescence in situ hybridization analyses, Friebe et al. (1992) indicated that TAF 46 has at least three pairs of alien chromosomes belonging to the X genome (S genome, Liu and Wang 1993). Zhang et al. (1996b) concluded that the wheat × *Th. intermedium* partial amphiploids Zhong 4 and Zhong 5 both have five pairs of S-genome chromosomes and 4 translocated chromosomes involving the S and E genomes. They further concluded that the *T. aestivum* × *Th. ponticum* partial amphiploid OK7211542 has seven pairs of S-genome chromosomes and two E-genome chromosomes (Zhang et al. 1996a). However, using genomic in situ hybridization (GISH) with different genomic DNA probes, Chen et al. (1998b) determined that the ten genomes of *Th. ponticum* belong to either the J or J^s genomes and that there is no S genome in *Th. ponticum*. These two different interpretations of the genome composition of these wheat-*Thinopyrum* partial amphiploids were likely due to the highly polyploid nature of these *Thinopyrum* species and the close relationship among the E, J, J^s and S genomes within the Triticeae (Wang 1992; Zhang et al. 1996a, b; Chen et al. 1998b, 1998c).

In this paper, we report the results of resistance analysis to the WCM and WSMV among the different partial amphiploids derived from crosses of wheat and *Th. ponticum* or *Th. intermedium*. A detailed examination of the genomic composition of these partial amphiploids was conducted using fluorescent in situ hybridization.

Materials and methods

Plant material

Germplasms used in screening for WCM and WSMV resistance included (1) three partial amphiploids derived from *Triticum aestivum* × *Th. ponticum*: Agrotana, OK7211542 and ORRPX; (2) two partial amphiploids derived from *T. aestivum* × *Th. intermedium*: Zhong 5 and TAF 46, which are known to be resistant to BYDV and other diseases (Cauderon et al. 1973, Xin et al. 1988; Banks et al. 1993); (3) two wheat cultivars, 'Chinese Spring' and 'Rescue', were included as the susceptible checks.

Thinopyrum elongatum (Host) D. Dewey (E genome, $2n = 14$), *Th. bessarabicum* (Savul. & Rayss) A. Love (J genome, $2n = 14$), and *Pseudoroegneria strigosa* (M. Bieb) A. Love (S genome, $2n = 14$) and *Triticum aestivum* cv 'Chinese Spring' (CS) (ABD genomes, $2n = 42$) were used as a source of genomic DNA in the GISH study.

Mite resistance assessment

Virus-free mites were increased on seedlings of the WSMV-resistant line TAi, a disomic substitution line of wheat carrying a pair of chromosomes derived from *Th. intermedium* that was isolated from CI 15093 (Larson and Atkinson 1973). The protocol for maintaining virus-free mite colonies was the same as that described by Thomas and Conner (1986). The genetic material to be tested for mite resistance was grown in Ferdinand-style Rootrainer trays (Spencer-Lemaire, Edmonton, AB) containing Cornell mix (Boodley and Sheldrake 1973). Plots consisted of six plants of a line or cultivar, and these were arranged in a randomized complete-block design with eight replications in experiment 1 and four replications in experiment 2.

At emergence, the test seedlings were exposed to mite-infested plants for 4 days. Two weeks after exposure, the seedlings were individually rated for susceptibility to mite colonization based on the degree of rolling and trapping of the leaves (Thomas and Conner 1986). An analysis of variance was carried out on the percentage of resistant and susceptible plants over the two experiments. Means and standard errors were calculated for the entries.

WSMV resistance evaluation

The germplasm was seeded in Ferdinand-style Rootrainers containing Cornell mix. Plots were seeded in single rows of six plants that

were arranged in a randomized complete-block design with eight replications in experiment 1 and four replications in experiment 2.

An isolate of WSMV was maintained under greenhouse conditions on the susceptible wheat cultivar 'Rescue'. Inoculum was prepared by grinding the leaves of 6-week-old WSMV-infected plants with tap water in a mortar and pestle. Sap from the macerated plant material was then filtered through four layers of cheesecloth, and carborundum (320 grit) was added at a rate of 1 g per 50 ml of plant extract. Plants at the two-leaf stage were inoculated with an artist's airbrush model AF-689 (Paasche Airbrush, Harwood Heights, Ill.) set at a pressure of 270 kPa. Plants were examined for symptoms of WSM 7, 9, 16, 23 and 30 days after inoculation. A combined analysis of variance for the two experiments was carried out on the percentage of plants with symptoms of WSM at each date the plants were rated. The means and standard errors were calculated for each entry.

Genomic in situ hybridization

Root tips (2–3 cm) were removed from germinated seeds and placed in ice water for 30 h at 4°C to accumulate metaphase cells and then fixed in a 3 : 1 mixture of ethanol-acetic acid for 2–3 days at −20°C. The fixed root tips were squashed in 45% acetic acid on a 95% ethanol washed slide. The slides were frozen at −80°C for 30 min before removing the coverslip, then the chromosomes on the slide were then treated in 45% acetic acid for 10 min, air dried, and stored at −20°C until they were used.

The slides were treated with 100 µl of RNase A (100 µg/ml in 2 × SSC) for 1 h at 37°C, washed quickly in 2 × SSC, denatured in 70% formamide in 2 × SSC at 70°C for 150 s, dehydrated in a graded ethanol series (70, 80, 95 and 100%) at −20°C and air-dried.

The genomic DNA from *T. aestivum*, *Th. elongatum*, *Th. bessarabicum* and *Ps. strigosa* were used as sources of the ABD, E, J and S genomes, respectively, and were labelled as probes with biotin by nick translation. The genomic DNA probe was mixed in a 1 : 20–40 ratio with non-labelled block genomic DNA to differentiate the E-, J^s- or S-genome chromosomes from the ABD wheat chromosomes in the partial amphiploids. The probe mixture was denatured at 76°C for 15 min, chilled on ice for 5 min and then loaded onto the denatured chromosome preparation on a slide under a 22 × 30-mm plastic cover slip. The slide preparation was redenatured for 8 min at 80°C, and then incubated overnight in a humidity chamber at 37°C. The hybridization signal was detected by FITC-Avidin DN (5 µl/ml) and amplified by biotinylated goat anti-avidin D (20 µg/ml). The slide was counterstained with 100 µl propidium iodide (0.5µg/ml) in 2 × SSC for about 20 min at room temperature. After draining, slides were mounted with one drop of antifade, then examined and photographed (Chen et al. 1995, 1996).

Results

Response to mite colonization

The results from the two WCM tests were pooled in Table 1 since the analysis of variance indicated that there was no significant ($P = 0.05$) line × experiment interaction. Within the study, 100% of the plants of the two wheat cultivars 'Chinese Spring' and 'Rescue' quickly developed typical symptoms of leaf curling and trapping that characterizes WCM-infested plants (Table 1), while the wheat-*Th. ponticum* partial amphiploid Agrotana did not exhibit any symptoms of mite colonization. However, rolling or trapping of leaves on the other four wheat-*Thinopyrum* partial amphiploids was as extensive as that found on the two susceptible wheat lines, indicating no resistance to WCM was present in any of these lines (Table 1).

Response to WSMV inoculation

As shown in Fig. 1, systemic symptoms of WSM appeared in 95% of the 'Chinese Spring' and 'Rescue' plants within 8 days after inoculation, and in all the plants within 10 days, whereas all the plants of Agrotana were free of WSM symptoms at every sampling date (Fig. 1). Agrotana proved to be the most resistant amphiploid to WSMV.

Differences in WSMV resistance were observed between Agrotana and the other two partial amphiploids derived from the same *T. aestivum* × *Th. ponticum* hybrids, OK7211542 and ORRPX. At 7 days after inoculation, no WSM symptoms appeared on any of the plants of OK7211542 or ORRPX. However, after this incubation period, the frequency of plants with systemic symptoms greatly increased as WSM symptoms developed in 40–50% of the plants at 16–20 days after inoculation and increased to 90% of the plants after 23 days, whereas no infected plants were detected in Agrotana during the same period.

Table 1 Reaction to wheat curl mite of wheat and wheat × *Thinopyrum* partial amphiploids

Lines	2n	Derived from	Number of plants Tested	Resistance[a] (%)
Agrotana	56	Wheat × *Th. ponticum*	68	100
Oklahoma	56	Wheat × *Th. ponticum*	68	0
ORRPX	56	Wheat × *Th. ponticum*	68	0
Zhong 5	56	Wheat × *Th. intermedium*	68	0
TAF46	56	Wheat × *Th. intermedium*	24	0
Rescue	42	Wheat cv	60	0
Chinese Spring	42	Wheat cv	60	0

[a] Number of plants showing no symptoms 2 weeks after exposure to mite-infested plants/no. of plants inoculated

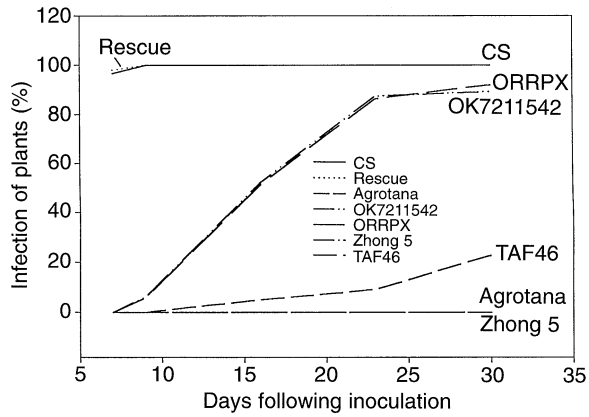


Fig. 1 Percentage of plants infected with symptoms of wheat streak mosaic over time in wheat cultivars and wheat-*Thinopyrum* partial amphiploids

The two *T. aestivum* × *Th. intermedium* partial amphiploids Zhong 5 and TAF 46 showed a higher level of resistance to WSMV than OK7211542, ORRPX or the two susceptible wheat lines. Zhong 5 was uniformly resistant to WSMV, but TAF 46 showed a low percentage of susceptible plants (Fig. 1).

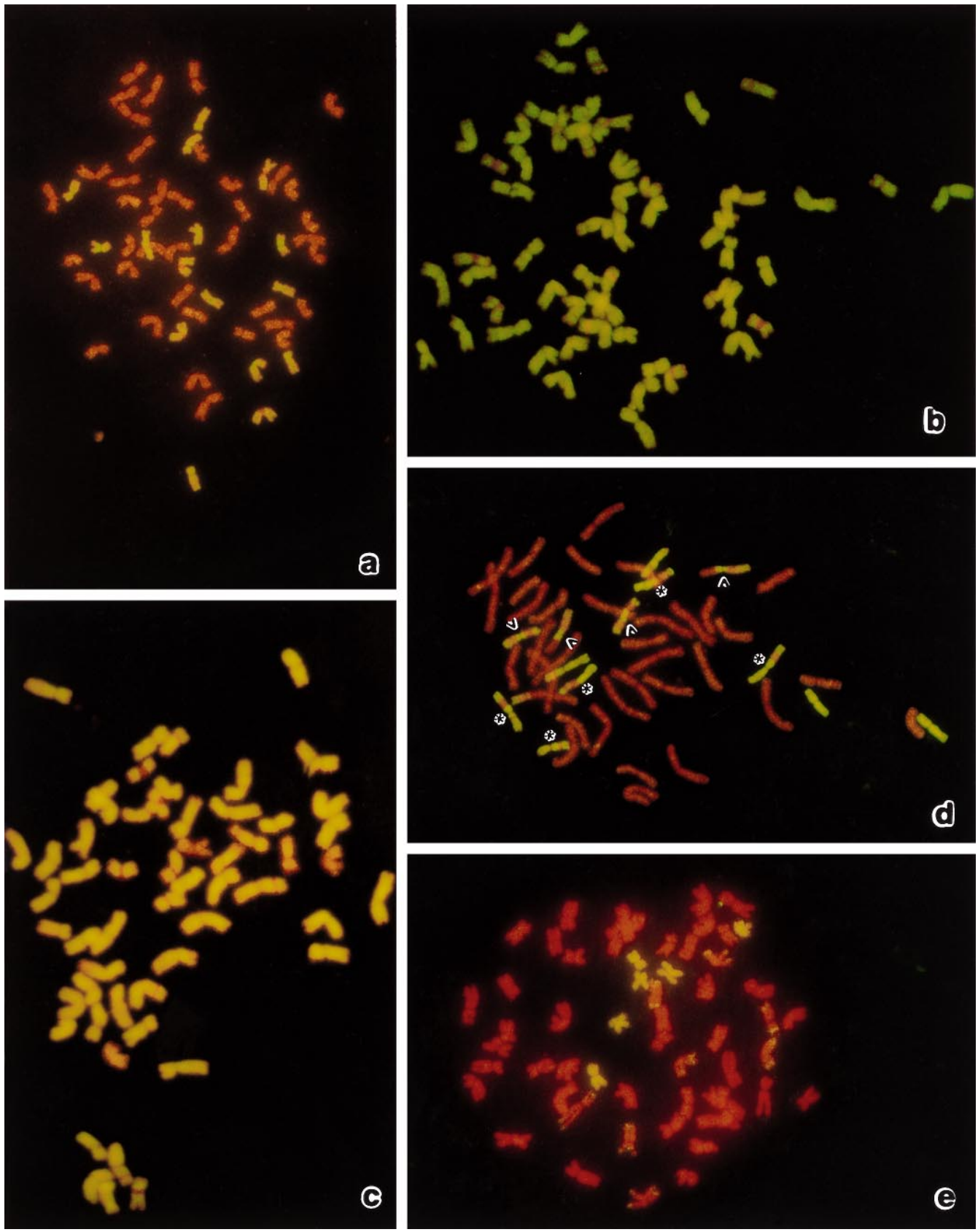
Molecular characterization of the genomic composition in the partial amphiploids

The chromosome composition of the partial amphiploid Agrotana was first determined by using the S-genomic DNA probe in the presence of the wheat genomic DNA as a block. In this case, 16 of the 56 chromosomes were labelled by FITC and fluoresced yellow, while the remaining 40 chromosomes blocked by wheat DNA showed a red fluorescence (Fig. 2a). This confirmed that Agrotana had eight pairs of chromosomes derived from an alien genome (Chen et al. 1995). However, when wheat genomic DNA (ABD genomes) together with *Th. elongatum* genomic DNA (E genome) were used as the probe and the S-genomic DNA was used as the block, all of the 56 chromosomes produced yellow signals and only 8 chromosomes were blocked by S-genomic DNA in the centromeric region (Fig. 2b). This indicated that 8 alien chromosomes carried chromatin from the E or J genome and that S-genome chromatin was present only in the centromeric regions of the other 8 alien chromosomes. Consequently, they belonged to the J^s genome (Chen et al. 1998b) and not the S genome. A weak signal at the NOR region of one pair of chromosomes was also observed (Fig. 2b).

In a simplistic GISH analysis, the partial amphiploids OK7211542 and ORRPX had the same GISH pattern as that observed in Agrotana (i.e. 16 chromosomes labelled by the S-genomic DNA probe and 40 chromosomes blocked by wheat DNA). However, more refined GISH analysis revealed that among the 16 alien chromosomes, only 8 chromosomes could belong to the J^s genome because they hybridized with the S-genomic DNA probe only in the centromeric region when *Th. elongatum* genomic DNA and wheat DNA were used together as the blocking solution. It was observed that 2–4 chromosomes in OK7211542 weakly hybridized with the wheat genomic DNA and *Th. elongatum* genomic DNA probes in the presence of a S-genomic DNA block, resulting in a dispersed type of red fluorescence on these chromosomes (Fig. 2c).

In this study, the partial amphiploid Zhong 5 was not cytologically stable. Its chromosome number varied from 49 to 56 among individual plants, while the number of alien chromosomes present varied from 13 to 14. When mitotic chromosomes of a heptaploid individual of Zhong 5 were probed with S-genomic DNA and blocked with wheat DNA, 4 chromosomes were completely labelled and fluoresced a bright yellow along the entire chromosome, indicating they belonged to the S genome (Fig. 2d). Four other alien chromosomes hybridized with the S-genomic DNA probe only in the centromeric and occasionally in the terminal regions of these chromosomes, indicating that they belonged to the J^s genome. For the other 5 alien chromosomes, one arm was uniformly labelled by S-genomic DNA probe, but the other arm was partial and predominantly labelled near the centromeric and terminal regions. Therefore, these chromosomes were translocations between S and J^s genomes (Fig. 2d). These results were further confirmed by GISH using the S-genomic DNA probe in the presence of ABD- and

Fig. 2a–e Genomic in situ hybridization of wheat-*Th. ponticum* and wheat-*Th. intermedium* partial amphiploids. **a** Agrotana (2n = 56). Probe: S-genomic DNA of *Ps. strigosa*; Block: ABD-genomic DNA of wheat. Of the 56 chromosomes 16 were labelled and *fluoresced yellow*. **b** Agrotana (2n = 56). Probe: ABD of wheat + E-genomic DNA of *Th. elongatum*. Block: S-genomic DNA. All of the 56 chromosomes were labelled and produced yellow signals, only eight chromosomes were blocked by S-genomic DNA in the centromeric region. **c** OK7211542 (2n = 56). Probe: ABD of wheat + E of *Th. elongatum*. Block: S-genomic DNA. Most of chromosomes were labelled and produced *yellow* signals. A few chromosomes hybridized weakly with *Th. elongatum* genomic DNA probe, resulting in a dispersed type of red fluorescence. **d** A heptaploid plant of Zhong 5 (2n = 49) probed with S genomic DNA and blocked with wheat DNA showing 4 S-genome chromosomes which were completely labelled along the entire chromosome, 4 of the J^s genome chromosomes (marked with *arrowheads*) hybridized with S-genomic DNA probe only in the centromeric and occasionally in the terminal regions, and 5 S-J^s translocated chromosomes (marked with *asterisks*) in which one arm was uniformly labelled by S-genomic DNA probe, but the other arm was partial and predominantly labelled near the centromeric and terminal regions **e** TAF 46 (2n = 56) blocked with wheat genomic DNA together with *Th. elongatum* genomic DNA, and probed with the S-genomic DNA of *Ps. strigosa*. Only 6 of the 14 alien chromosomes were completely labelled and showed a *yellow fluorescence*


a
b
c
d
e

E-genomic DNA block in a $2n = 52$ plant of Zhong 5. In this plant, two pairs of S-genome chromosomes, two pairs of J^s chromosomes and three pairs of S-J^s translocation chromosomes were observed. One pair of chromosomes likely consisted of an intercalary translocation within one arm of a wheat chromosome with a portion of a S-genome chromosome (data not shown).

When mitotic chromosomes of TAF 46 were probed with the S-genomic DNA of *Ps. strigosa* and blocked with wheat genomic DNA, 14 chromosomes showed a bright-yellow fluorescence, indicating that TAF 46 had 14 alien chromosomes originating from *Th. intermedium*. However, when *Th. elongatum* genomic DNA was added to ABD as the block, only 6 of the 14 alien chromosomes were completely labelled by the S-genomic DNA probe and showed a yellow fluorescence, while the other 8 alien chromosomes were blocked by the E-genome DNA and fluoresced red with occasional weak signals in the telomeric regions of these chromosomes. Therefore, 6 of the 14 alien chromosomes in TAF 46 belonged to the S genome and the other 8 alien chromosomes belonged to the E or J genome of *Th. intermedium* (Fig. 2e).

Discussion

A comparison of the reactions of wheat and five wheat-*Thinopyrum* partial amphiploids to both the WCM and WSMV showed that different levels of resistance were present in these partial amphiploids, although all of them originate from *Thinopyrum* species. A previous study of the reaction to barley yellow dwarf virus (BYDV) indicated that the partial amphiploids OK7211542 and ORRPX were two of the most resistant *T. aestivum* × *Thinopyrum* partial amphiploids (Comeau et al. 1994; Zhang et al. 1996b; Chen et al. 1997). The present assessment showed that these two partial amphiploids are susceptible to the WCM and WSMV, while another similar partial amphiploid, Agrotana, has complete resistance to WSMV and its vector, the wheat curl mite.

The partial amphiploid ORRPX was selected in 1990 in Quebec from a cross between wheat and *Th. ponticum* cv 'Orbit' by Dr. A. Comeau, while Agrotana and OK7211542 are both wheat-alien partial amphiploids of unknown origins (Xu et al. 1994, Comeau et al. 1994). Based on its morphological resemblance to ORRPX, OK7211542 was assumed to have *Th. ponticum* in its ancestery (Comeau et al. 1994), and chromosome pairing analysis confirmed that OK7211542 carries almost the same alien chromosomes as ORRPX (Chen et al. 1998a). Based on GISH using a *Th. ponticum* genomic DNA probe, Chen et al. (1995) concluded that the origin of the alien chromosomes in Agrotana was *Th. ponticum*, however at that time the exact chromosome constitution of these partial amphiploids was not defined.

The decaploid species *Th. ponticum* and hexaploid species *Th. intermedium* are two highly polyploid perennial grasses, and some of their genomes are closely related. A precise determination of their genomic composition has been difficult. This may explain why various authors have developed different theories on their genomic composition (Dewey 1984; Pienaar 1990; Dvorak 1981; Liu and Wang 1993; Zhang et al. 1996a, b). Recently, based on the distribution of hybridization signals on the chromosomes in GISH using a S-genomic DNA probe from *Ps. strigosa*, Chen et al. (1998b) determined that the hexaploid *Th. intermedium* carried the J, J^s and S genomes. They also demonstrated that decaploid *Th. ponticum* had only the J and J^s genomes. The J^s genome present in these *Th. intermedium* and *Th. ponticum* species was homologous with E or J genomes, but it was quite distinct in the centromeric regions which strongly hybridized with a S-genomic DNA probe. It was also demonstrated that J- or E-genome chromosomes hybridized with a S-genomic DNA probe in the absence of E- or J-genomic DNA blockers but that the S-genomic DNA probe hybridized only with S-genome chromosomes and centromeres of J^s-genome chromosomes when E- or J-genomic DNA was added as block (Chen et al. 1998b, 1998c). Based on these findings, the alien J- or E-, J^s-and S-genome chromosomes in wheat-*Thinopyrum* hybrids can all be identified if S-genome DNA is used as a probe and common wheat DNA (in the absence of J or E genomic DNA) is used as a block (Zhang et al. 1996a, b; Chen et al. 1998b, 1998c). When J- or E-genome DNA together with wheat DNA is used as a block, it is possible to distinquish among J-, J^s-and S-genome chromosomes and identify whole chromosome or arm transfers of particular groups of alien chromosomes (J, J^s or S genomes) (Chen et al. 1998c). Based on this observation, the present study clearly shows that the alien chromosome composition of two wheat × *Th. intermedium* partial amphiploids TAF 46 and Zhong 5 was derived from different combinations of the J-, J^s-and S-genome chromosomes from *Th. intermedium* (Fig. 2d, e).

The genomic composition of *Th. ponticum* consists of five sets of closely related genomes of $J_1J_2J_3J_4J_5$ (Muramatsu 1990; Wang et al. 1991) or $JJJJ^sJ^s$ (Chen et al. 1998b). Consequently, partial amphiploids derived from wheat × *Th. ponticum* would not include any S-genome chromosomes, simply because *Th. ponticum* is devoid of such chromosomes. Our GISH results clearly indicate that the alien genomes in Agrotana, ORRPX and OK7211542 contained only the J and J^s genomes and that there were no S-genome chromosomes present. These results confirm that Agrotana and OK7211942, like ORRPX, all originated from the crosses of wheat × *Th. ponticum*, and not from the crosses of wheat × *Th. intermedium* (Chen et al. 1995; Comeau et al. 1994). These three wheat × *Th. ponticum*

partial amphiploids have a completely different genome constitution from the two wheat × *Th. intermedium* partial amphiploids TAF 46 and Zhong 5 (Cauderon 1966; Xin et al. 1988).

Lines TAF 46 and Zhong 5 are multi-disease-resistant partial amphiploids which were synthesized independently in France and China, respectively, following several backcrosses of the wheat × *Th. intermedium* hybrid to different wheat cultivars. Our GISH analysis using a S-genomic DNA probe indicate that the alien genome of Zhong 5 contains the 4 S-genome and 4 J^s genome chromosomes and 6 translocated chromosomes involving the S and J^s genomes (Fig. 2d), while TAF 46 carries 6 S-genome chromosomes and 8 J-genome chromosomes (Fig. 2e). This confirms the findings of Friebe et al. (1992), who indicated that three addition lines L1, L4 and L7 originating from TAF 46 were derived from the X genome, which is now designated as the S genome (Liu and Wang 1993).

Resistance derived from *Th. intermedium* is temperature-sensitive and not expressed at high temperatures (Pfannensteil and Niblett 1978; Seifers et al. 1995). However, Pfannensteil and Niblett (1978) observed that resistance originating from *Th. ponticum* was not temperature-sensitive. More desirable WSMV control might therefore be obtained from resistant wheat × *Th. ponticum* partial amphiploids.

Conner et al. (1991) found that a significantly lower percentage of plants developed symptoms of WSM in the WCM-resistant lines than in the mite-susceptible cultivars. They also determined that the WCM resistance derived from *Th. ponticum* was more effective in limiting the spread of WSM than that originating from either rye or *Ae. squarrosa*. These findings strongly suggest that in wheat breeding programs the wheat × *Th. ponticum* partial amphiploid Agrotana which carries WCM and WSMV resistance is likely to be more useful as a source of genetic resistance to control the spread of the WSMV than other wheat × *Thinopyrum* partial amphiploids. Martin et al. (1976) speculated that the genes from *Th. ponticum* controlling resistance to WCM and WSMV are either closely linked or involve the same genes because there was such a close association between these traits. Further analyses are necessary to establish the location of the gene for WCM and WSMV resistance on the chromosome(s) and to determine their genomic affinities in the partial amphiploid. This information will greatly assist the further use of the partial amphiploids in wheat breeding. It is expected that the genes for WCM resistance will be located on one or two of the group 6 J^s chromosomes. Work is in progress to evaluate resistance to WCM and WSMV in addition or substitution lines derived from Agrotana carrying J^s chromosomes.

Acknowledgements We thank A. D. Kuzyk for his technical assistance in this study. The financial support provided by Western Grains Research Foundation Wheat Levy and the Matching Investment Initiative of Agriculture and Agri-Food Canada is gratefully appreciated.

References

Bai D, Knott DR (1993) The effects of level of 2,4-D and time in culture on regeneration rate and chromosome numbers of regenerants from calli of the hybrid *Triticum aestivum* cv 'Chinese Spring' ph 1b × *Thinopyrum ponticum* ($2n = 10x = 70$). Genome 36:166–172

Banks PM, Xu SJ, Wang RRC, Larkin PJ (1993) Varying chromosome composition of 56-chromosome wheat × *Thinopyrum intermedium* partial amphiploids. Genome 36:207–215

Boodley JW, Sheldrake R (1973) Cornell peat-lite mixes for commercial plant growing. Cornell Univ Inf Bull 43

Cauderon Y (1966) Genome analysis in the genus *Agropyron*. Hereditas [Suppl] 2:218–234

Cauderon Y, Saigne B, Dauge M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. University of Missouri, Columbia, Mo., pp 401–407

Chen Q, Conner RL, Laroche A (1995) Identification of the parental chromosomes of the wheat-alien amphiploid Agrotana by genomic in situ hybridization. Genome 38:1163–1169

Chen Q, Conner RL, Laroche A (1996) Molecular characterization of *Haynaldia villosa* chromatin in wheat lines carrying resistance to wheat curl mite colonization. Theor Appl Genet 93:679–684

Chen Q, Collin J, Comeau A, St-Pierre CA, Fedak G (1998b) Comparison of various sources of resistance to barley yellow dwarf virus in wheat-*Thinopyrum* amphiploid lines. Can J Plant Pathol 19:414–417

Chen Q, Ahmad F, Collin J, Comeau A, Fedak G, St-Pierre CA (1998a) Genomic constitution of a partial amphiploid OK7211542 used as a source of immunity to barley yellow dwarf virus for bread wheat. Plant Breed 117:1–6

Chen Q, Conner RL, Laroche A, Thomas JB (1998b) Genome analysis of *Thinopyrum intermedium* and *Th. ponticum* using genomic in situ hybridization. Genome (in press)

Chen Q, Friebe B, Conner RL, Laroche A, Thomas JB, Gill BS (1998c) Molecular cytogenetic characterization of *Thinopyrum intermedium* derived wheat germplasm specifying resistance to wheat streak mosaic virus. Theor Appl Genet 96:1–7

Comeau A, Makkouk KM, Ahmad F, St-Pierre CA (1994) Bread wheat × *Agrotricum* crosses as a source of immunity and resistance to the PAV strain of barley yellow dwarf luteovirus. Agronomie 2:153–160

Conner RL, Thomas JB, Whelan EDP (1991) Comparison of mite resistance for control of wheat streak mosaic. Crop Sci 31:315–318

Dewey DR (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed) Gene manipulation in plant improvement, vol 16. Plenum Press, New York, pp 16:209–279

Dvorak J (1981) Genome relationships among *Elytrigia* (= *Agropyron*) *elongata*, *E. stipifolia*, "*E. elongata* 4X" *E. caespitita*, *E. intermedia*, and "*E. elongata* 10X". Can J Genet Cytol 23:481–492

Dvorak J (1985) Transfer of salt tolerance from *Elytrigia pontica* (Podp.) Holub. to wheat by the addition of an incomplete *Elytrigia* genome. Crop Sci 21:306–309

Feldman M, Sears ER (1981) The wild gene resource of wheat. Sci Am 244:98–109

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 germplasm by C-banding and in situ hybridization. Theor Appl Genet 81:381–389

Friebe B, Mukai Y, Gill BS, Cauderon Y (1992) C-banding and in situ hybridization analyses of *Agropyron intermedium*, a partial wheat × *Ag. intermedium* amphiploid, and six derived chromosome addition lines. Theor Appl Genet 84 : 899–905

Jiang J, Friebe B, Dhaliwal HS, Martin TJ, Gill BS (1993) Molecular cytogenetic analysis of *Agropyron elongatum* chromatin in wheat germplasm specifying resistance to wheat streak mosaic virus. Theor Appl Genet 86 : 41–48

Jiang J, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73 : 199–212

Larson RI, Atkinson TG (1973) Wheat-*Agropyron* chromosome substitution lines as sources of resistance to wheat streak mosaic virus and its vector, *Aceria tulipae*. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. Columbia, Mo. University of Missouri, Columbia, Mo., pp 173–177

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

Liang GH, Wang RRC, Niblett CL, Heyne ER (1979) Registration of B-6-37-1 wheat germplasm. Crop Sci 19 : 427

Liu ZW, Wang RRC (1993) Genome analysis of *Elytrigia caespitosa*, *Lophopyrum nodosum*, *Pseudoroegneria geneiculata* ssp. Scythica and *Thinopyrum intermedium* (Triticeae Gramineae). Genome 36 : 102–111

Martin TJ, Harvey TL, Livers RW (1976) Resistance to wheat streak mosaic virus and its vector, *Aceria tulipae*. Phytopathology 66 : 346–349

Muramatsu M (1990) Cytogenetics of decaploid *Agropyron elongatum* (*Elytrigia elongata*) ($2n = 70$). I. Frequency of decavalent formation. Genome 33 : 811–817

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

Pienaar R de V (1990) Wheat × *Thinopyrum* hybrids. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 13: wheat. Springer, Berlin Heidelberg New York, pp 167–217

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

Thomas JB, Conner RL (1986) Resistance to colonization by the wheat curl mite in *Aegilops squarrosa* and its inheritance after transfer to common wheat. Crop Sci 26 : 527–530

Wang RRC (1992) Genome relationships in the perennial Triticeae based on diploid hybrids and beyond. Hereditas 116 : 133–136

Wang RRC, Marburger JE, Hu CJ (1991) Tissue-culture-facilitated production of aneuploid haploid *Thinopyrum ponticum* and amphiploid *Hordeum violaceum* × *H. bogdenii* and their use in phylogenetic studies. Theor Appl Genet 81 : 151–156

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

Whelan EDP, Hart GE (1988) A spontaneous translocation that transfers wheat curl mite resistance from decaploid *Agropyron elongatum* to common wheat. Genome 30 : 289–292

Whelan ED, Atkinson TG, Larson RI (1983) Registration of LRS-IF 193 wheat germplasm. Crop Sci 23 : 194

Xin ZY, Brettell RIS, Cheng EM, Waterhouse PM, Appels R, Banks PM, Zhou GH, Chen X, Larkin PJ (1988) Characterization of a potential source of barley yellow dwarf virus resistance for wheat. Genome 30 : 250–257

Xu J, Conner RL, Laroche A (1994) C-banding and fluorescent in situ hybridization studies of a wheat-alien hybrid Agrotana. Genome 37 : 477–481

Zhang XY, Dong YS, Wang RRC (1996a) Characterization of genomes and chromosomes in partial amphiploids of the hybrids *Triticum aestivum* × *Thinopyrum ponticum* by in situ hybridization, isozyme analysis, and RAPD. Genome 39 : 1062–1071

Zhang XY, Koul A, Petroski R, Ouellet T, Fedak G, Dong YS, Wang RRC (1996b) Molecular verification and characterization of BYDV-resistant germplasms derived from hybrids of wheat with *Thinopyrum ponticum* and *Th. intermedium*. Theor Appl Genet 93 : 1033–1039