Theor Appl Genet (1996) 93: 1033–1039

X. Y. Zhang · A. Koul · R. Petroski · T. Ouellet
G. Fedak · Y. S. Dong · R. R.-C. Wang

Molecular verification and characterization of BYDV-resistant germ plasms derived from hybrids of wheat with *Thinopyrum ponticum* and *Th. intermedium*

Received: 20 April 1996 / Accepted: 24 May 1996

Abstract Twenty-five partial amphiploids (2n=8x=56), which were derived from hybrids of wheat (*Triticum aestivum* L.) with either *Thinopyrum ponticum* (Podpera) Liu & Wang, *Th. intermedium* (Host) Barkworth & D. Dewey, or *Th. junceum* (L.) A. Löve, were assayed for resistance to BYDV serotype PAV by slot-blot hybridization with viral cDNA of a partial coat protein gene. Three immune lines were found among seven partial amphiploids involving *Th. ponticum*. Seven highly resistant lines were found in ten partial amphiploids involving *Th. intermedium*. None of eight partial amphiploids or 13 addition lines of Chinese Spring – *Th. junceum* were resistant to BYDV. Genomic in situ hybridization demonstrated that all of the resistant partial amphiploids, except TAF46, carried an alien genome most closely related to **St**, whether it was derived from *Th. ponticum* or *Th. intermedium*. The two partial amphiploids carrying an intact **E** genome of *Th. ponticum* are very susceptible to BYDV-PAV. In TAF46, which contains three pairs of **St**- and four pairs of **E**-genome chromosomes, the gene for BYDV resistance has been located to a modified 7**St** chromosome in the addition line L1. This indicates that BYDV resistance in perennial polyploid parents, i.e., *Th. ponticum* and *Th. intermedium*, of these partial amphiploids is probably controlled by a gene(s) located on the **St**-genome chromosome(s).

Communicated by J. MacKey

X. Y. Zhang[1] (✉) · Y. S. Dong
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, 30 Bai Shi Qiao Road, Beijing, 100081, P. R. China

A. Koul · R. Petroski · T. Ouellet · G. Fedak
Plant Research Center, Agriculture and Agri-Food Canada, Ottawa, Ontario, K1A, 0C6, Canada

R. R.-C. Wang
United States Department of Agriculture, Agricultural Research Services, Forage and Range Research Laboratory, Utah State University, Logan, UT84322-6300, USA

Present address:
[1] USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, UT84322-6300, USA

Key words *Thinopyrum ponticum* · *Th. intermedium* · *Th. junceum* · Wheat · BYDV · Genome

Introduction

Barley yellow dwarf virus (BYDV) is widely recognized as one of the most damaging viruses in world wheat production (Burnett 1990). It is an aphid-transmitted virus, with many pathotypes such as PAV, MAV, RPV, RMV, SGV, GPV, and GAV, etc. (Burnett 1990; Comeau et al. 1993). Resistance to this virus is not found in the genus *Triticum*, but it is common in perennial *Triticeae* (Comeau et al. 1993; Xu et al. 1994). However, the finding of a species either immune or highly resistant to BYDV does not assure that this species will be a practical source of genes for introgression (Plourde et al. 1992; Comeau et al. 1993).

The genus *Thinopyrum* is a practical source for the introgression of BYDV resistance into wheat (Xin et al. 1988; Banks et al. 1993, 1995; Comeau et al. 1993; Banks and Larkin 1995; Zhong et al. 1994). It is relatively easy to hybridize species of this genus with wheat and to obtain backcross progenies (Cauderon 1973; Sun 1981; Li et al. 1985). More importantly, the expression of the resistance gene(s) is excellent in a wheat background (Xin et al. 1991; Banks and Larkin 1995; Banks et al. 1995; Sharma et al. 1995). Xin et al. (1988) and Banks et al. (1993) identified some highly resistant lines using an enzyme-linked immunosorbent assay (ELISA) in partial amphiploids derived from *Triticum aestivum*×*Thinopryun intermedium* hybrids. Several BYDV-resistant translocation lines have been successfully produced (Xin et al. 1991; Banks and Larkin 1995; Banks et al. 1995), and at least one was proven to originate from centromeric fusion (Mujeeb-Kazi 1994; Fedak et al. unpublished). Zhong et al. (1994) also reported an aneu-partial amphiploid resistant to BYDV, which was derived from *T. aestivum*×*Th. ponticum*.

In this paper we report the screening for BYDV resistance in partial amphiploids derived from crosses of wheat and *Th. ponticum*, *Th. intermedium* or *Th. junceum*, and

addition lines of CS – *Th. junceum*. We also report the molecular characterization of the resistant lines.

Materials and methods

Materials used in screening for BYDV resistance were (1) partial amphiploids derived from *T. aestivum*×*Th. ponticum*: 784, 693, 7631, 68, 7430, 40767-1, 40767-2 and OK7211542; (2) partial amphiploids derived from *T. aestivum*×*Th. intermedium*: Zhong-1, -2, -3, -4, -5, -6, -7, Xiaoyan 78829, TAF46; (3) partial amphiploids derived from *T. aestivum*×*Th. junceum*: AJAP-1, -2, -3, -4, -5, -7, -8, and -9 (provided by Anne Charpentier, INRA, France); (4) disomic addition lines of Chinese Spring wheat – *Th. junceum*: AJDAj-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, HD3505, and HD3508 (provided by Anne Charpentier, INRA, France); and (5) the disomic addition line L1, derived from TAF46, which was well known to be resistant to BYDV.

Thinopyrum elongatum (Host) D. Dewey (**E** genome, 2n=14); *Pseudoroegneria stipifolia* (Czern. ex Nevski) A. Löve (**St** genome, 2n=14), and *P. strigosa* (M. Bieb.) A. Löve (**St** genome, 2n=14) were used in the in situ hybridization study.

Slot-blot hybridization analysis of BYDV resistance

BYDV (strain PAV) transmission through aphids

Two healthy seedlings (with 3–4 leaves) from each line were caged individually along with 7–10 viruliferous aphids carrying PAV. At the same time, another seedling was caged with non-viruliferous aphids as a control. The caged pots were placed in a growth cabinet at 20°C with 14-h light per day for 1 week and then sprayed with insecticide to kill the aphids. The seedlings were kept under the same growing conditions as above for about 2 weeks before they were harvested, then quickly frozen in liquid nitrogen, and stored at –70°C.

Extraction of viral RNA

Each seedling was ground to a fine powder in a mortar with liquid nitrogen. The viral RNA was obtained by the preparation of crude nucleic acid extracts with extraction buffer (0.1 M glycine, 0.1 M NaCl, 10 mM EDTA, pH 9.5), phenol, and chloroform, following modifications of an extraction procedure described by Robertson et al. (1991). The nucleic acid pellet was re-suspended in TE buffer (RNase-free, pH 7.2) and stored at –70°C.

Hybridization analysis

Three levels of concentration (1000, 100, 10 µg of starting plant tissue/100 µl) of the healthy and infected samples were prepared in 5×SSC. A 100-µl sample solution was slot blotted onto a piece of positively charged nylon membrane (Biotran, Inc.). A dilution series (0.1, 0.01, 0.001 ng) of in vitro synthesized RNA, corresponding to the PAV coat protein cDNA clone used as probe, was included as a positive control on the slot blots.

A partial cDNA clone of the PAV coat protein gene was radioactively labelled using the Prime-A Gene kit (Promega). Hybridization of the cDNA probe with the target RNA was performed according to Lister et al. (1990). The membrane was wrapped in cellophane wrap and exposed to X-film at –70°C.

Molecular detection of the genome composition of the resistant lines by genomic in situ hybridization

The slides were treated with 100 µl RNase A (1 mg/ml in 2×SSC) for 1 h at 37°C, denatured in 70% formamide and 2×SSC for 2 min at 70°C, and dehydrated in a 70% / 80% / 95% / 100% ethanol series. The genomic DNA of *P. stipifolia* (**St**) or *P. strigosa* (also **St**) was labelled with biotin by nick translation. The **St** genomic probe was mixed with 35 × wheat (Chinese Spring) DNA, 35–50 × *Th. elongatum* DNA and 35 × salmon-sperm DNA. The mixture was precipitated and re-suspended in 10 µl of 2 × hybridization buffer and 10 µl 20% dextran sulphate to make the final probe concentration of 2–5 ng/µl. The probe solution was denatured at 100°C for 5 min, chilled on ice for 3–4 min; and pre-annealed at 37°C for 30–60 min. The probe solution was applied to the denatured slide, which was then covered with a coverslip and sealed with rubber cement, and incubated at 37°C overnight. The hybridization signal was detected by FITC-Avidin D and biotinylated goat anti-avidin D following the methods of Chen and Armstrong (1994).

Results

Reaction of *T. aestivum*×*Thinopyrum* partial amphiploid and disomic addition lines to barley yellow dwarf virus (BYDV) serotype PAV

Partial amphiploids 693, 7631, 7430, 68, and 784 were produced from *T. aestivum*×*Thinopyrum ponticum* (Li et al. 1985). They were derived from different wheat parents crossed with the same accession of *Th. ponticum*. There was no prior report on their reactions to BYDV. Slot-blot hybridization showed that 784 was immune, while the other four lines were sensitive, to PAV of BYDV (Fig. 1 a, Table 1). The partial amphiploids 40767-1 and 40767-2 were obtained from one accession of *Th. ponticum* that was resistant to BYDV (Zhang and Dong, unpublished). However, 40767-1 was uniformly susceptible and 40767-2 was still segregating with both immune and highly resistant individuals in the same population (Fig. 1 a, Table 1).

OK7211542, bred in Oklahoma, USA, proved to be one of the most resistant partial amphiploids derived from *T. aestivum*×*Th. ponticum*, which was consistent with an earlier test result (Cisar et al. 1982).

The partial amphiploids Zhong-1, -2, -3, -4, -5, -6, and -7 were probably derived from the same perennial parent (Sun 1981). Previous tests on their reactions to BYDV serotype PAV by ELISA indicated that five of them, except Zhong-1 and Zhong-2, were resistant (Banks et al. 1993; Xin et al. 1988). Slot-blot hybridization of viral cDNA showed that Zhong-3, -4, -5, -6, -7, and Xiaoyan 78829 could be infected by the virus, but inhibited viral multiplication (Fig. 1 b, Table 1). They were classified as highly resistant. The Xiaoyan 78829 and Zhong series share a common accession of the perennial parent *Th. intermedium* (S. Y. Chen, personal communication).

TAF46, isolated in the 1960s by Dr. Cauderon from a cross of wheat and *Th. intermedium*, has been widely used as a germplasm resistant to rust fungi and BYDV in wheat breeding (Cauderon 1973; Brettle et al. 1988; Xin et al. 1991). The resistance to BYDV was located on $7Ag^{i}$ (Brettle et al. 1988). By slot-blot hybridization, TAF46 was also demonstrated to be highly resistant.

In the tested eight partial amphiploids (Fig. 1 c, Table 1) and 13 disomic addition lines involving *Th. junceum*, none was resistant to BYDV serotype PAV. Probably, the parental *Th. junceum* was susceptible to BYDV.

Table 1 Reaction to BYDV serotype PAV of wheat×*Thinopyrum* partial amphiploids and disomic addition lines

Lines	Chromosome-number	Derived from	Sources	Reaction to BYDV-PAV
693	56	Wheat×*Th. ponticum*	Li et al. 1985	S
7631	56	Wheat×*Th. ponticum*	Li et al. 1985	S
7430	56	Wheat×*Th. ponticum*	Li et al. 1985	S
68	56	Wheat×*Th. ponticum*	Li et al. 1985	S
784	56	Wheat×*Th. ponticum*	Li et al. 1985	R
40767-1	56	Wheat×*Th. ponticum*	Zhang XY	S
40767-2	49–52	Wheat×*Th. ponticum*	Zhang XY	R
OK7211542	56	Wheat×*Th. ponticum*	Sando 1930s	R
Zhong-1	49–56	Wheat×*Th. intermedium*	Sun 1981	S
Zhong-2	56	Wheat×*Th. intermedium*	Sun 1981	S
Zhong-3	56	Wheat×*Th. intermedium*	Sun 1981	R
Zhong-4	56	Wheat×*Th. intermedium*	Sun 1981	R
Zhong-5	56	Wheat×*Th. intermedium*	Sun 1981	R
Zhong-6	56	Wheat×*Th. intermedium*	Sun 1981	R
Zhong-7	56	Wheat×*Th. intermedium*	Sun 1981	R
78829	56	Wheat×*Th. intermedium*	Chen SY	R
TAF46	56	Wheat×*Th. intermedium*	Cauderon 1973	R
AJAP-1	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-2	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-3	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-4	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-5	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-6	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-7	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-8	56	Wheat×*Th. junceum*	Charpentier	S
AJAP-9	56	Wheat×*Th. junceum*	Charpentier	S
AJDAj-1	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-2	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-3	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-4	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-5	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-6	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-7	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-8	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-9	44	Wheat×*Th. junceum*	Charpentier	S
AJDAj-11	44	Wheat×*Th. junceum*	Charpentier	S
HD3505	44	Wheat×*Th. junceum*	Charpentier	S
HD3508	44	Wheat×*Th. junceum*	Charpentier	S
HD3515	44	Wheat×*Th. junceum*	Charpentier	S

If we take into consideration the previous cytogenetic data (Xin et al. 1988; Banks et al. 1993; Zhang and Dong 1994) and the reaction to BYDV of the partial amphiploids, we can detect a relationship between BYDV resistance and the genomic composition of the partial amphiploids.

Molecular characterization of the genomic composition in the BYDV-resistant lines

The partial amphiploid 784 had 14 chromosomes labelled by FITC after hybridization with the **St** genomic DNA probe, and blocked only by the **ABD** genomic DNA of common wheat. But it actually had six pairs of the **St**-genome chromosomes and one pair of the **E**-genome chromosomes, as shown by adding *Th. elongatum* (**E**-genome) DNA to the **ABD** block (Fig. 2 a, Table 2).

The partial aneu-amphiploid 40767-2 had 14 chromosomes from *Th. ponticum*, including one pair of **E**-genome chromosomes, four pairs of **St**-genome chromosomes, one pair of Robertsonian translocation chromosomes between one **St**-genome chromosome and the 1**B** of common wheat, and one pair of a **St/E** translocation (Fig. 2 b, Table 2).

The partial amphiploid OK7211542 had 16 chromosomes from *Th. ponticum*, which were clearly indicated by the result of in situ hybridization with the **St** genomic DNA probe and the wheat genomic DNA block. One unique pair of **E**-genome chromosomes was recognized, because they only hybridized with the **St** probe at the centromere region when *Th. elongatum* genomic DNA was added to the **ABD** blocking solution (Fig. 2 e).

The partial amphiploid Zhong-4 had 14 chromosomes labelled by the **St** probe and 42 chromosomes blocked by wheat genomic DNA. One pair of chromosome was a prominent Robertsonian translocation between **St** and **E**, and another had an **St/E** intercalary translocation within one arm of a **St**-genome chromosome. This line also had one pair of very small **St** chromosomes which was not present in other lines derived from *Th. intermedium* (Fig. 2 c, Table 2).

The partial amphiploids Zhong-3, -5, and -7 had a nearly identical alien genome. The two pairs of translocation

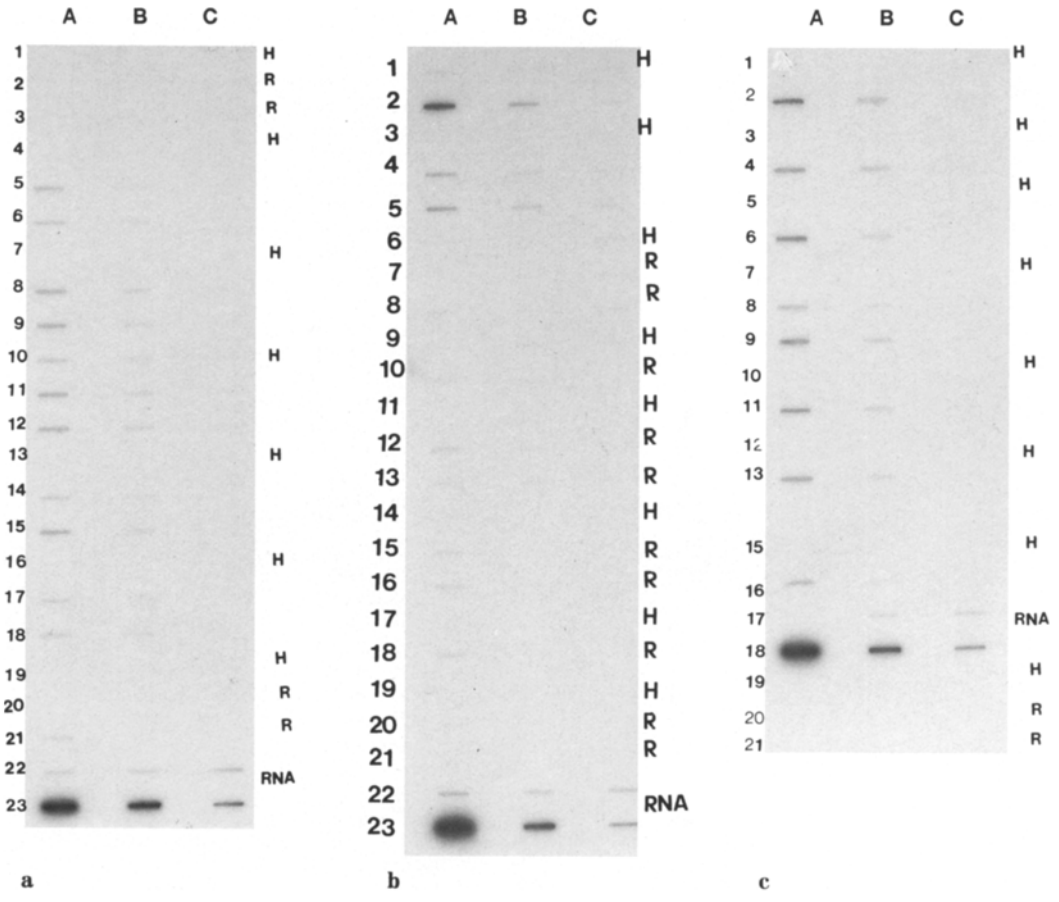

Fig. 1 a–c Detection of BYD virus in partial amphiploids. *A*, *B*, *C*: different levels of concentration of the extracted solution. *A*, 1000 µg (starting tissue)/100 µl; *B*, 100 µg/100 µl; *C*, 10 µg/100 µl. *H*, healthy individuals (affected by the nonviruliferous aphids). *R*, Resistant or highly tolerant. Un-numbered slots, no sample was loaded. Slots not marked by *H* or *R* mean susceptibility. **a** Partial amphiploids derived from *T. aestivum*×*Th. ponticum*. *1*, 784 (H); *2–3*, 784 (PAV); *4*, 7631 (H); *5–6*, 7631 (PAV); *7*, 693 (H); *8–9*, 693 (PAV); *10*, 68 (H); *11–12*, 68 (PAV); *13*, 7430 (H); *14–15*, 7430 (PAV); *16*, 40767–1 (H); *17–18*, 40767–1 (PAV); *19*, 40767–2 (H); *20–21*, 40767–2 (PAV); *22–23*, in vitro synthesized RNA from the PAV coat-protein gene. **b** Partial amphiploids from *T. aestivum*×*Th. intermedium*. *1*, Zhong-1 (H); *2*, Zhong-1 (PAV); *3*, Zhong-2 (H); *4–5*, Zhong-2 (PAV); *6*, Zhong-3 (H); *7–8*, Zhong-3 (PAV); *9*, Zhong-4 (H); *10*, Zhong-4 (PAV); *11*, Zhong-5 (H); *12–13*, Zhong-5 (PAV); *14*, Zhong-6 (H); *15–16*, Zhong-6 (PAV); *17*, Zhong-7 (H); *18*, Zhong-7 (PAV); *19*, Xiaoyan 78829 (H); *20–21*, 78829 (PAV); *22–23*, in vitro synthesized RNA from the PAV coat-protein gene. **c** Partial amphiploids derived from *T. aestivum*×*Th. junceum*. *1*, AJAP1 (H); *2*, AJAP1 (PAV); *3*, AJAP2(H); *4*, AJAP2 (PAV); *5*, AJAP3 (H); *6*, AJAP3 (PAV); *8*, AJAP4 (PAV); *9*, AJAP5 (PAV); *10*, AJAP7 (H); *11*, AJAP7(PAV); *12*, AJAP8 (H); *13*, AJAP8 (PAV); *15*, AJAP9 (H); *16*, AJAP9 (PAV); *17–18*, in vitro synthesized RNA from the PAV coat-protein gene

Fig. 2 a–f Genomic in situ hybridization of BYDV-resistant lines. Probe, **St**; block, **ABD** + **E** + hering-sperm DNA. **a** Partial amphiploid 784, which has 6″ St- + 1″ E-genome chromosomes. The two **E**-genome chromosomes were hybridized by the **St** probe only at highly repeated regions. One **St**-chromosome was washed away. **b** A partial aneu-amphiploid individual (2n=49) of 40767–2 showing 4″ **St**-genome + 1″ **E**-genome + 1″ **St**/1**B** Robertsonian translocation + 1″ **St/E** translocation chromosomes. **c** Zhong-4 showing 5″ **St** + 1″ **St/E** Robertsonian translocation + 1″ **St/E** intercalary translocation chromosomes. *MC*, minute-size chromosomes. **d** 78829 showing 7″ **St**-genome chromosomes without detectable translocation. **e** OK7211542 carrying 7″ **St** + 1″ **E**. **f** In L1 (2n=44), the pair of alien chromosome was identified to be 7**St** with insertion of **E**-genome chromatin in the centromeric region

chromosomes found in Zhong-4 were also present in these three lines (Table 2). The partial amphiploid Zhong-6 showed 14 chromosomes labeled by the **St** genomic probe. Only one translocation between **St**- and **E**-genome chromosomes (the Robertsonian translocation) was evident (Table 2).

The partial amphiploid Xiaoyan 78829 was the only one having a nearly intact **St**-genome from *Th. intermedium*. No obvious translocation was found (Fig. 2 d). Because this line and its sister lines had a similar resistance to BYDV (Fig. 1 b), the BYDV resistance in these lines must be controlled by a gene(s) on the **St**-genome chromosomes.

The partial amphiploid TAF46 carried three pairs of **St**- and four pairs of **E**-genome chromosomes. According to in situ hybridization results with the probe $pSchet_1$, Xin et al. (1991) reported that there are two pairs of **X**- (=**St**-) and five pairs of **E**-genome chromosomes in TAF46. Consequently, the BYDV-resistant gene's location could be determined only via the addition lines derived from TAF46.

The disomic addition line L1, produced from TAF46, was highly resistant to BYDV and the resistance on 7$\mathbf{Ag}^{i}$ had been transferred into wheat by tissue culture (Xin et al. 1991; Banks et al. 1995). Xin et al. (1991) believed that

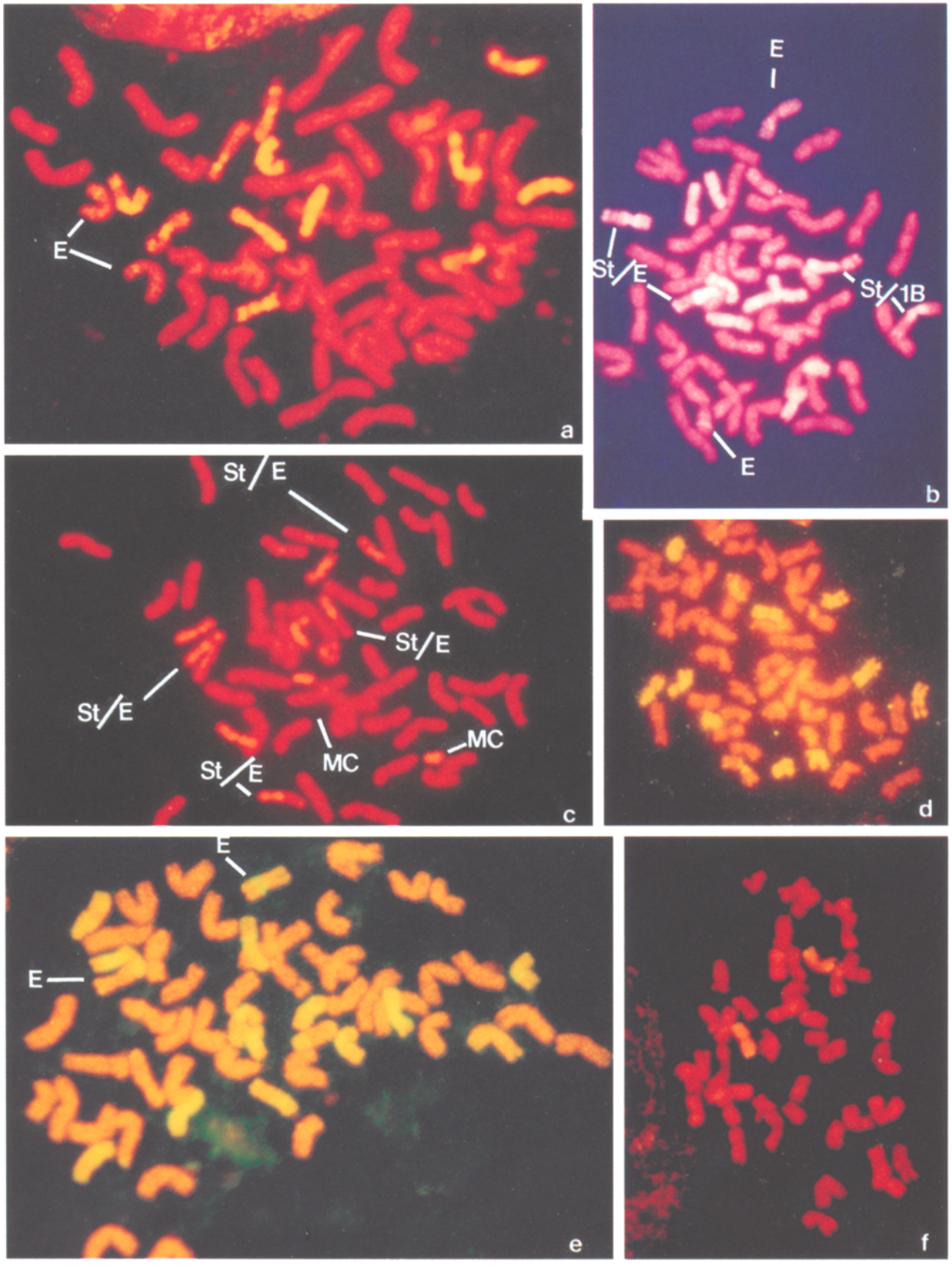
E
a
E
St/E
St/1B
E
b
St/E
St/E
St/E
MC
MC
St/E
c
d
E
E
e
f

Table 2 Genomic in situ hybridization (GISH) analysis of the lines resistant to BYDV

Resistant lines	Chromosome no.	Karyotypes revealed by GISH
784	56	7″ of *Th. ponticum*, 6″ St + 1″ E
40767-2	49	7″ of *Th. ponticum*, 4″ St + 1″ St/1B Robertsonian translocation + 1″ St/E translocation + 1″ E
OK7211542	56	8″ of *Th. ponticum*, 7″ St + 1″ E
Zhong-3	56	7″ of *Th. intermedium*, 5″ St + 1″ St/E Robertsonian translocation + 1″ St/E intercalary translocation
Zhong-4	56	7″ of *Th. intermedium*, 5″ St + 1″ St/E Robertsonian translocation + 1″ St/E intercalary translocation
Zhong-5	56	7″ of *Th. intermedium*, 5″ St + 1″ St/E Robertsonian translocation + 1″ St/E intercalary translocation
Zhong-6	56	7″ of *Th. intermedium*, 6″ St + 1″ St/E Robertsonian translocation
Zhong-7	56	7″ of *Th. intermedium*, 5″ St + 1″ St/E Robertsonian translocation + 1″ St/E intercalary translocation
78829	56	7″ of *Th. intermedium* 7″ St, no translocation found
TAF	56	7″ of *Th. intermedium*, 4″ E + 3″ St
Line-1	44	AABBDD + 1″ 7St-E-St

7**Ag**^{i} should be 7**X** (=7**St**) and Brettle et al. (1988) located the resistance on the long arm of this chromosome. We used in situ hybridization with the **St** genomic probe and the **ABD**+**E** genomic block confirming that the pair of *Th. intermedium* chromosomes in L1 were from the **St** genome but with an insertion of **E**-genome chromatin (Fig. 2 f). Using the translocation lines (Banks and Larkin 1995) we located the BYDV resistance on the terminal region of the critical arm, which was derived from the **St** genome (Wang and Zhang, unpublished).

Discussion

Resistance to BYDV has not been found in any true wheats. Partial amphiploids TAF46, Zhong-3, -4, -5, -6, -7, and OK7211542 were previously shown to be resistant to BYDV by ELISA (Cisar et al. 1982; Xin et al. 1988; Banks et al. 1993). Using the more sensitive detection method of slot-blot hybridization, we confirmed the resistance in all these lines. All of them except OK7211542 had the perennial species *Th. intermedium* as a parent. We also found three additional partial amphiploids resistant to BYDV (784, 40767-2, and Xiaoyan 78829). The former two lines were derived from the cross *T. aestivum*×*Th. ponticum*, whereas the latter involved the same accession of *Th. intermedium* as the Zhong series.

Th. ponticum, a decaploid (2n=70) species, has been successfully used in wheat breeding because of its resistance to many wheat diseases (Li et al. 1985; Knott 1989; Kim et al. 1993). Zhong et al. (1994) reported an aneuploid line (2n=47), resistant to BYDV, which carried three pairs and one monosome of *Th. ponticum* chromosomes belonging to the homeologous groups 3, 5, 6, and one of unknown origin. Our analyses by genomic in situ hybridization and genome-specific DNA markers indicated that **E** [including **E**^{e} from *Th. elongatum* and **E**^{b} from *Th. bessarabicum* (Savul. and Rayss) A. Löve] and **St** (occurring in the genus *Pseudoroegneria*) are the basic genomes of the decaploid tall wheatgrass (Zhang et al. 1996). In the two stable BYDV-immune partial amphiploids, 784 carried six pairs of **St**-genome chromosomes and one pair of **E**-genome chromosomes, while OK7211542 carried seven pairs of **St**- and one pair of **E**-genome chromosomes (Fig. 2 a, e, Table 2). We concluded that the BYDV resistance was presumably controlled by the **St**-genome chromosomes. Additional proof for this conclusion was the high susceptibility of 7631 and 693 to BYDV (Fig. 1 a), which had the same wheatgrass parent as 784 but carried an intact **E**^{e} or **E**^{b} genome of *Th. ponticum*.

Th. intermedium is a hexaploid species, with the genome formula $\mathbf{E_1E_2St}$ (Liu and Wang 1993), where $\mathbf{E_1}$ and $\mathbf{E_2}$ originated from diploids *Th. elongatum* and *Th. bessarabicum*, and the **St** from a *Pseudoroegneria* species. Xin et al. (1988, 1991) and Banks et al. (1993) reported BYDV resistance in partial amphiploids derived from a cross of wheat with this species. The screening by slot-blot hybridization in the present study indicated that all the resistant lines could be infected by the virus but that viral amplification in these hosts was inhibited. All these resistant lines, except TAF46, contain an extra genome similar to **St** (Fig. 2, Table 2). Although recombination between **E** and **St** in the Zhong series is obvious, the molecular karyotype of Xiaoyan 78829 suggests that BYDV resistance is located in the **St** genome. This is consistent with results reported by Xin et al. (1988).

Lister et al. (1990) and Xin et al. (1991) located the BYDV-resistant gene (or genes) on 7**X** (=7**St**), which was confimed in the present study by GISH (Fig. 2 f). This conclusion is also consistent with C-banding results (Friebe et al. 1992). However, the **St**-genome chromosomes are shorter than the **E**-genome chromosomes (Hsiao et al. 1986). The alien chromosome in L1 was longer than expected due to the insertion of **E**-genome chromatin in the centromeric region as shown by the GISH results in the present study (Fig. 2 f). Therefore, the alien chromosome in L1 is a modified 7**St** chromosome.

It has been proven that of the three genomes of wheat, **A** and **D** are more closely related to each other than either are to the **B** genome (Joppa 1987). The two basic alien genomes concerned in the present paper, **E** (including **E**^{e} and **E**^{b}) and **St**, are very closely related (Wang 1992). We found

that it is not easy to discriminate between them in both *Th. ponticum* and the offspring of wheat×*Th. ponticum* hybrids by genomic in situ hybridization (Zhang et al. 1996). But with regard to relationships to the wheat genomes, **E** is more closely related to **A** and **D** than it is to **B** (Dvorak 1971, 1980; Hsiao et al. 1994). We found that it was difficult to block cross-hybridization of wheat chromosomes with the $\mathbf{E}^e$ (or $\mathbf{E}^b$) genomic probe, whereas it was easy to prevent the cross hybridization of wheat chromosomes with the **St** genomic probe. Therefore, the **St** genome is more remotely related to the **ABD** of wheat than is the **E** genome. Therefore, transferring the BYDV-resistant gene (s) from these lines to the **D** and **A** genomes is more logical than to the **B** genome. This has been confirmed by breeding results (Banks et al. 1995; Sharma et al. 1995).

References

Banks PM, Larkin PJ (1995) Registration of three BYDV-resistant wheat germ plasms: TC5, TC6, and TC9. Crop Sci 35:599–600

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

Banks PM, Larkin PJ, Bariana HS, Lagudah ES, Appels R, Waterhouse PM, Brettell RIS, Chen X, Xu HJ, Xin ZY, Qian YT, Zhou XM, Cheng ZM, Zhou GH (1995) The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. Genome 38:395–405

Brettle RIS, Banks PM, Cauderon Y, Chen X, Cheng ZM, Larkin PJ, Waterhouse PM (1988) A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann Appl Biol 113:599–603

Burnett PA (1990) World perspectives on barley yellow dwarf. CIMMYT, Mexico, D.F. Mexico

Cauderon Y, Saigne B, Dauge M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. Proc 4th Int Wheat Genet Symp, pp 401–407

Chen CF, Amstrong K (1994) Genomic in situ hybridization in *Avena sativa*. Genome 37:607–612

Cisar G, Brown CM, Jedlinski H (1982) Effect of fall or spring infection and sources of tolerance of barley yellow dwarf of winter wheat. Crop Sci 22:474–478

Comeau A, St-Pierre CA, Collin J (1993) Interspecific hybrids of wheat as sources of barley yellow dwarf virus resistance. In: Damania A (ed) Biodiversity and wheat improvement. John Wiley and Sons, Chichester, UK, pp 83–94

Dvorak J (1971) Hybrids between diploid *Agropyron elongatum* and *Aegilops squarrosa*. Can J Genet Cytol 13:90–94

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

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 83:589–596

Hsiao C, Wang RRC, Dewey DR (1986) Karyotype analysis and genome relationships of 22 diploid species in the tribe *Triticeae*. Can J Genet Cytol 28:109–120

Hsiao C, Chatterton NJ, Asay KH, Jensen KB (1994) Phylogenetic relationships of the monogenomic species of the wheat tribe, *Triticeae* (Poaceae), inferred from nuclear rDNA (ITS) sequences. Genome 38:211–223

Joppa LR (1987) Aneuploid analysis in tetraploid wheat. In: Heyne EG (ed) Wheat and wheat improvement (2nd edn.), pp 255-267

Kim N-S, Armstrong KC, Fedak G, Fominaya A, Whelan EWP (1993) Cytogenetical and molecular characterization of a chromosome interchange and addition lines in Cadet involving chromosome 5B of wheat and 6Ag of *Lophopyrum ponticum*. Theor Appl Genet 86:827–832

Knott DR (1989) The wheat rust-breeding for resistance. Springer, Berlin Heidelberg New York

Li ZS, Rong S, Chen S, Zhong GC, Mu SM (1985) Wheat wide hybridization. Published by Scientific Publishing Company, Beijing

Lister RM, Barbara DJ, Kawata EE, Ueng PP, Fattouh F, Larkins BA (1990) Development and use of cDNA probes in studies of barley yellow dwarf virus. In: Burnett PA (ed) World perspectives on barley yellow dwarf. CIMMYT, Mexico, D.F. Mexico, pp 186–193

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

Mujeeb-Kazi (1994) Use of annual and perennial *Triticeae* species for wheat improvement. In: Wang RRC, Jensen KB, Jaussi C (eds) Proc 2nd Int *Triticeae* Symp, Logan, Utah, USA, pp 86–89

Plourde A, Comeau A, St-Pierre CA (1992) Barley yellow dwarf virus resistance in *Triticum aestivum*×*Leymus angustus* hybrids. Plant Breed 108:97–103

Robertson NL, French R, Gray SM (1991) Use of grop-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. J Gen Virol 72:1473–1477

Sharma H, Ohm H, Goulart L, Lister R, Appels R, Benlhabib O (1995) Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome 38:406–413

Sun SC (1981) The approach and methods of breeding new varieties and new species from *Agrotriticum* hybrids. Acta Agron Sinica 7:51–58

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

Xin ZY, Brettell RIS, Cheng ZM, 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

Xin ZY, Xu HJ, Chen X, Lin ZS, Zhou GH, Qian YT, Cheng ZM, Larkin PJ, Banks P, Appels R, Glarke B, Brettell RIS (1991) Development of a common wheat germ plasm-resistant to barley yellow dwarf virus by biotechnology. Sci China (Series B) 34:1055–1062

Xu SJ, Banks PM, Dong YS, Zhou RH, Larkin PJ (1994) Evaluation of Chinese *Triticeae* for resistance to barley yellow dwarf virus. Genet Res Crop Evol 41:35–41

Zhang XY, Dong YS (1994) Cytogenetic research on a hybrid of *Triticum* with *Thinopyrum ponticum* as well as its derivatives. II. Comparative research of eleven partial amphiploids derived from hybrid offspring of *T. aestivum* with both *Th. ponticum* and *Th. intermedium*. Acta Genet Sinica 21:287–296

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

Zhong GY, McGuire PE, Qualset CO, Dvorak J (1994) Cytological and molecular characterization of a *Triticum aestivum*×*Lyphopyrum ponticum* backcross derivative resistance to barley yellow dwarf. Genome 37:876–887