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Molecular characterization of *Haynaldia villosa* chromatin in wheat lines carrying resistance to wheat curl mite colonization

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Abstract Wheat-*Haynaldia villosa* (L.) Schur. hybrid lines were tested as potential sources of resistance to colonization by the wheat curl mite, the vector of wheat streak mosaic virus. Two lines, Add 6V-1 and Sub 6V-1, were found to be mite-resistant. Fluorescence in situ hybridization using total genomic DNA, from *H. villosa* in the presence of unlabelled wheat DNA, confirmed that Add 6V-1 is a disomic wheat-*H. villosa* chromosome addition line. Sub 6V-1 turned out to be a homoeologous wheat-*H. villosa* chromosome translocation line rather than a substitution. The translocation in Sub 6V-1 occurred between a wheat chromosome and a chromosome from *H. villosa* through Robertsonian fusion of misdivided centromeres. Only the short arm of the group 6 chromosome of *H. villosa* was involved in the genetic control of mite resistance, a conclusion based on the genomic in situ hybridization signal and specific DNA fragments obtained by polymerase chain reaction.

Key words Wheat streak mosaic virus · *Eriophyes tulipae* · Resistance · Fluorescence in situ hybridization · Wheat-*Haynaldia villosa* hybrids

Introduction

The wheat curl mite [*Eriophyes (Aceria) tulipae* Keifer] is the vector of wheat streak mosaic virus (WSMV), which causes wheat streak mosaic, a serious viral disease of wheat (*Triticum aestivum* L.) (Slykhuis 1955; Nault and Styer 1970; Harvey et al. 1994). The development of resistant wheat cultivars would be an efficient way to control this disease. Although a few wheat genotypes have been reported to show moderate tolerance (Martin et al. 1976), there are no effective sources of resistance in wheat. However, high levels of resistance to the wheat curl mite are present in several genera of Triticeae, including *Secale* (Harvey and Livers 1975), *Thinopyrum* (Whelan 1988) and *Aegilops* (Thomas and Conner 1986). Incorporation of resistant gene(s) from these genera into wheat to provide resistance to mite colonization has been realized through the creation of alien chromosome addition or translocation lines by wide hybridization (Friebe et al. 1991). Although the usefulness of the current sources of resistance in wheat has not yet been fully established (Thomas and Conner 1986; Harvey et al. 1994), it is believed that identification of the new resistance sources could be helpful for the further breeding of wheat.

With multiple disease resistance and other desirable agronomic chracters, *Haynaldia villosa* (L.) Schur, an allogamous annual grass (2n = 14, VV) of the Tribe Triticeae, has been an important donor of useful genes for wheat improvement (Qualset et al. 1981). Some alien chromosome addition lines (2n = 44), substitution lines (2n = 42) and even translocation lines derived from wheat × *H. villosa* have been developed (Hyde 1953; Blanco et al. 1987; Liu et al. 1988; Qi et al 1993) and found to possess desirable genes controlling traits such as powdery mildew resistance and high protein content (Liu et al. 1988; Blanco and Simeone 1989; Qi et al. 1993). Further evaluation of such lines and determination of the chromosomal location of resistant genes will provide more useful information for their utilization in wheat breeding.

We report here the results of screening a nearly complete set of addition lines and substitution lines derived from a wheat × *H. villosa* hybrid for resistance to mite colonization and the identification of the alien *H. villosa* chromatin in a wheat background using genomic in situ hybridization (GISH) with *H. villosa* genomic DNA as a probe. DNA fragments amplified by the sequence-tagged-site (STS) Polymerase chain reaction (PCR) primer set G8 located on wheat homoeologous group 6 chromosomes (Talbert et al. 1994) were further

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used for detection of the homoeologous relationships of the added *H. villosa* chromosomes or chromosome arms in the resistant lines.

Materials and methods

Mite resistance assessment

The plant materials tested for mite resistance were 6 wheat-*Haynaldia villosa* chromosome addition lines designated Add 2V to 7V and 5 substitution lines designated Sub 2V to 6V by Liu et al. (1993) Check treatments included the mite-resistant lines Rescue-6Ag(6D), a *Thinopyrum ponticum* (syn *Agropyron elongatum* (Host) Beauv.) translocation line with 'Rescue' (Conner et al. 1991) and 'Agrotana', a wheat-*Th. ponticum* amphiploid, 2n = 56 (Chen et al. 1995) as well as the suseptible wheat cultivars 'Norstar' and 'Rescue'. The original *H. villosa* parent was not included as a check because seed of the stock used to create the original hybrid of wheat-*H. villosa* was not available.

Virus-free mites were increased on seedlings of the WSMV resistant line TA1, a disomic substitution line of wheat carrying a pair of chromosomes derived from *Thinopyrum intermedium* (Host) Bark. and Dewey (Thomas and Conner 1986). The protocol for maintaining virus-free mite colonies was the same as that described by Thomas and Conner (1986). The plants tested for mite resistance were grown in Ferdinand style rootrainer trays (Spencer-Lemaire, Edmonton, AB) containing Cornell mix. Plots consisted of 12 plants of a line or cultivar, and these plots were arranged in a randomized complete block design with three replications. The study was repeated once

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 or trapping of leaves. An analysis of variance was carried out on the incidence of resistant, moderately resistant or suseptible plants, and means and standard errors of the means were calculated for the entries.

Genomic in situ hybridization

Chromosome preparation for GISH, DNA isolation, probe labelling and in situ hybridization using total genomic *H. villosa* DNA as the probe were essentially the same as described by Chen et al. (1994,1995). The only exception being that root tips of mite-resistant lines were harvested and pretreated with a mixture solution of colchicine (0.05%), 8-hydroxyquinoline (0.025%) and dimethyl sulfoxide (40 drops/100 ml) for 4 h to accumulate metaphases, and then fixed in a 3:1 mixture of ethanol-acetic acid for 3 days at room temperature.

STS PCR analysis

The amplified DNA fragments obtained with the STS PCR primer set G8 that map the wheat homoeologous group 6 chromosomes (Talbert et al. 1994) were also used for chromosome analysis in the *H. villosa*-wheat addition and translocation lines carrying resistance to wheat curl mite.

DNA amplification was done in a 25-µl volume containing 50 ng genomic DNA,1 unit of *Taq* DNA polymerase, 1× PCR buffer (50 m*M* KCl, 1.5 m*M* $MgCl_2$, 10 m*M* Tris-HCl pH 8.2, 0.001% gelatin), 200 µ*M* each of dATP, dTTP, dGTP and dCTP as well as 200 n*M* primer. Samples were overlaid with 50 µl mineral oil to prevent evaporation Amplifications were carried out in a thermal cycler (TwinBlock, ERICOMP) programmed for 1 cycle at 94 °C for 5 min followed by 35 cycles at 94 °C for 1 min,45 °C for 1 min, 72°C for 1.2 min with a final extension step at 72 °C for 10 min prior cooling to room temperature. PCR products were subsequently digested directly with about 10 units of *Hin*PI per reaction mixture for 2 h at 37 °C *Hin*PI-digested PCR products (10 µl) with 2 µl of loading buffer were separated by electrophoresis in a composite 1% agarose and 1% MetaPhor agarose gel in 1 × TAE buffer. The gel was stained with ethidium bromide for 20 min and washed 20 min. and DNA fragments were visualized under UV light. The image of the gel was captured with a UVP GDS 7500 gel documentation and analysis system and printed with a high-resolution printer (600 dpi). Molecular sizes of the amplified products were estimated using a 1-kb DNA ladder (BRL, Bethesda, Md.)

Results

Mite resistance

Resistance assessment based on leaf symptoms following exposure to wheat curl mites clearly differentiated resistant and susceptible lines. The analysis of variance indicated that there was not a significant line × test interaction; therefore, the data from the two tests were combined (Table 1). The wheat-*H. villosa* lines carrying *H. villosa* chromosome 6V either as an addition or substitution were as resistant to mite colonization as the highly resistant wheat-*Th. ponticum* amphiploid, 'Agrotana'. They were also more resistant to mite colonization than the *Th. ponticum* translocation line 'Rescue'-6Ag(6D). Extensive rolling or trapping of leaves was evident in all the other wheat-*H. villosa* chromosome substitution and addition lines and the check wheat cultivars, indicating susceptibility to the wheat curl mite.

Alien chromosome detection

Wheat and *H. villosa* chromosomes are similar in size and morphology and could not be distinguished using carmine staining. However, genomic in situ hybridization with biotinylated total genomic DNA from *H. villosa* clearly distinguished between *H. villosa* and wheat chromosomes. The probe hybridization sites were

Fig. 1 a–g Detection of *H. villosa* chromosomes in wheat-*H. villosa* lines by genomic in situ hybridization using total genomic DNA from *H villosa* as a probe: mitotic (**a, c, f, g**) and meiotic (**b,d,e**) chromosome preparations from the wheat-*H villosa* addition line Add 6V-1 and translocation line Sub 6V-1. Sites of probe hybridization (i.e. *H. villosa* chromatin) fluoresce yellow, while unprobed sites (i.e. wheat chromatin) fluoresce red. A 1:40 ratio of labelled *H villosa* DNA to non-labelled wheat DNA was used **a** A root-tip metaphase cell of Add 6V-1 showing 2 yellow *H. villosa* chromosomes and 42 red wheat chromosomes, **b** a meiotic metaphase I (MI) cell of Add 6V-1 showing a yellow ring bivalent between 2 homologous *H. villosa chromosomes*, **c** a root-tip metaphase cell of Sub 6V-1 showing the 2 yellow *H villosa* chromosome arms translocated onto 2 red wheat chromosome arms. The translocation breakpoint was at or very close to the centromere, **d** a MI cell of Sub 6V-1 show the 2 wheat-*H. villosa* translocated chromosomes as a rod bivalent. **e** a MI cell of Sub 6V-1 showing the two wheat-*H. villosa* translocated chromosomes as a ring bivalent, **f** a mitotic prophase cell of Add 6V-1 showing that both arms of the two *H. villosa* chromosomes gave a hybridization signal. **g** a mitotic prophase cell of Sub 6V-1 showing that only the translocated *H. villosa* arms gave a hybridization signal

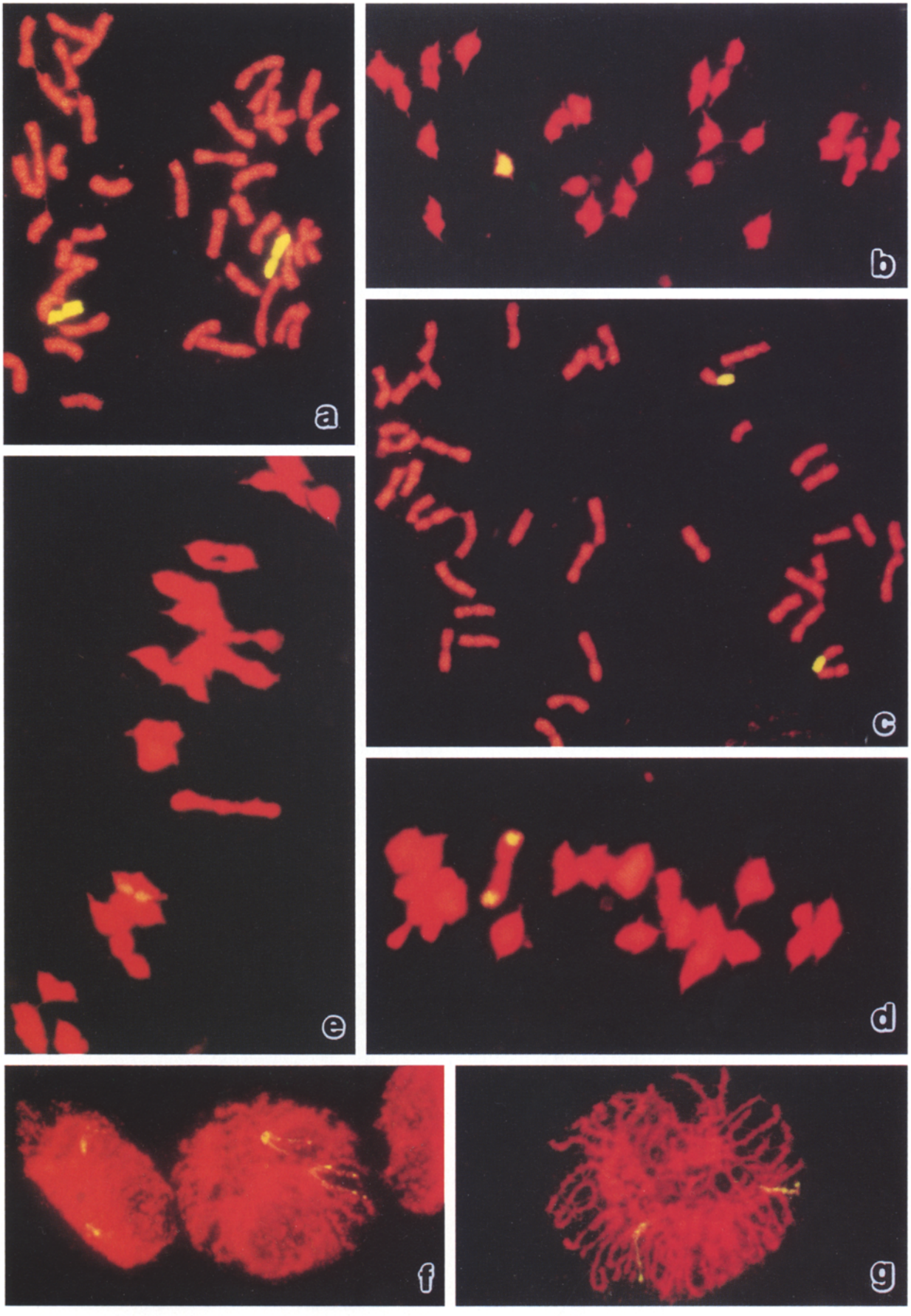
a
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Table 1 Frequency (%) of mite-resistant plants in wheat, wheat-alien hybrids and wheat-*H. villosa* chromosome addition and substitution lines

Lines	2n	Number of plants tested	Resistance	Moderate resistance	Susceptible
Norstar	42	58	100 ± 0[a]	0	0
Rescue	42	66	0	0	100 ± 0
Agrotana	56	42	100 ± 0	0	0
Rescue-6Ag(6D)	42	70	66 ± 3.8	31 ± 4.0	3 ± 1.8
Add 2V-1	44	35	2 ± 2.4	2 ± 2.1	96 ± 2.8
Add 3V-1	44	58	0	0	100 ± 0
Add 4V-1	44	45	0	2 ± 2.4	98 ± 2.4
Add 5V-1	44	51	0	0	100 ± 0
Add 6V-1	44	59	100 ± 0	0	0
Add 7V-1	44	63	0	2 ± 1.7	98 ± 1 7
Sub 2V-1	42	68	0	0	100 ± 0
Sub 3V-1	42	66	0	0	100 ± 0
Sub 4V-1	42	60	0	0	100 ± 0
Sub 5V-1	42	68	0	2 ± 1.5	98 ± 1.5
Sub 6V-1	42	62	98 ± 1 5	2 ± 1.5	0

[a] Mean ± standard error of the mean

detected by the bright yellow color of the fluorescein isothiocyanate-conjugated antibodies under blue light excitation, whereas nonhybridized chromatin fluoresced orange-red from the propidium iodide counterstain.

The results of GISH on metaphase chromosomes of the addition line Add 6V-1 are shown in Fig. 1a. The *H. villosa* chromosomes were unequivocally distinguished by their yellow fluorescence, the remaining 42 wheat chromosomes were not labelled by the probe and appeared red. At meiosis metaphase I, the 44 chromosomes paired to form 22 bivalents in most pollen mother cells, with 1 bivalent fluorescing yellow (Fig. 1b), confirming that this line is a disomic addition. Sometimes, 2–4 univalents were observed, but these fluoresced red, indicating their wheat origin. No bivalent involving pairing between wheat and *H. villosa* chromosomes was observed.

Chromosome constitution in the 42-chromosome mite-resistant line Sub 6V-1 analyzed using GISH showed that the site of probe hybridization was on the short arms of one pair of chromosomes in this 2n = 42 line, whereas the long arms and the other 40 chromosomes stained red (Fig. 1c). It appeared that the arm of a chromosome from *H. villosa* had translocated onto a wheat chromosome. The translocation fusion is located at or very close to the centromere, indicating that this Robertsonian translocation resulted from centric fusion of a wheat and a *H. villosa* chromosome. At meiotic metaphase I, the 2 translocated chromosomes paired together to form a rod (Fig. 1d) or ring bivalent (Fig. 1e), indicating that this line is homozygous for the wheat-*H. villosa* translocated chromosome.

The *H. villosa* chromatin was also distinguishable from wheat chromatin in prophase cells (Fig. 1f and g). In the additon line Add 6V-1 both arms of the *H. villosa* chromosome stained yellow (Fig. 1f), whereas in the translocation line Sub 6V-1 only the translocated arm appeared yellow (Fig. 1g).

Analysis of G8 PCR products

G8 STS primers amplified multiple fragments from total DNA isolated from both 'Chinese Spring' (CS) and *H. villosa* and gave clear polymorphisms between amplifi-

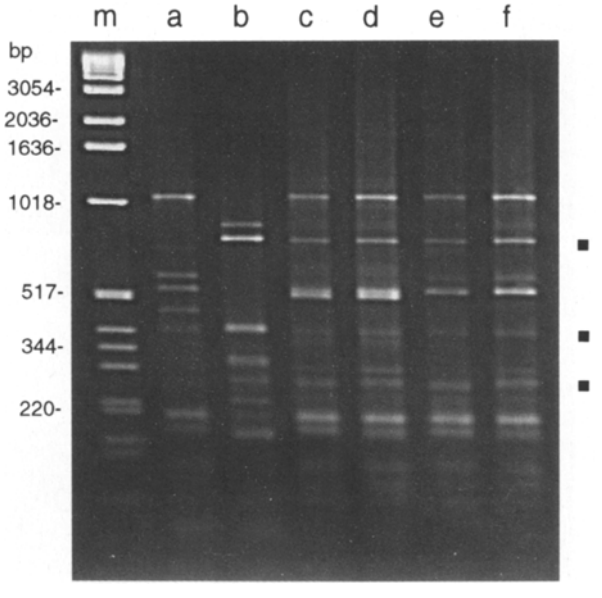

Fig. 2 PCR amplification products using STS PCR primer set G8 followed by digestion with *Hin*PI on a composite 1% agarose and 1% MetaPhor agarose gel. *Lane a* 'Chinese Spring', *b H. villosa*, *c*, *d* 2 plants of addition line Add 6V-1, *e*, *f* 2 plants of translocation line Sub 6V-1, *m* 1-kb DNA ladder. The size of the marker bands is given in base pairs. The *squares* indicate three diagnositc fragments (800 bp, 400 bp and 280 bp) of *H. villosa*

cation products after digestion with the restriction enzyme *Hin*PI, an isoschizomer of *Hha*I. 'Chinese Spring' exhibited at least seven fragments and *H. villosa* showed eight fragments (Fig. 2, lanes a and b). Most DNA fragments were polymorphic between CS and *H. villosa*. Among the eight amplifed products of *H. villosa*, three fragments (800 bp, 400 bp and 280 bp) were present in the resistant addition line Add 6V-1 (lanes c and d) and also in DNA from the translocation line Sub 6V-1 (lanes e and f). Since the 800-bp fragment of *H. villosa* (Fig. 2, lane b) was intense and easily discernible, it could be a good marker to indicate resistance to mite colonization in wheat-*H. villosa* lines. There was no missing wheat band in the translocation line Sub 6V-1 relative to the addition line Add 6V-1, but there were some polymorphisms among the wheat bands, suggesting genomic differences between CS and the wheat lines carrying the *H. villosa* chromosomes.

Discussion

This is the first report of a means by which to identify genetic resistance to mite colonization in wheat-*H. villosa* derivatives. The present study showed that resistant line Add 6V-1 carried a homologous pair of chromosomes from *H. villosa* in addition to the full complement of wheat chromosomes. However, GISH revealed that the putative substitution line Sub 6V-1 was in fact a translocation with the breakpoint at or closed to the centromere. Meiotic configurations show that the 2 translocated chromosomes are homologous and that their cytological behavior is therefore likely to be stable.

A prior study with the STS-G8 primer set indicated that fragments amplified by this primer belong to the homoeologous group 6 chromosome (Talbert et al. 1994). In common wheat the 1018-bp fragment and fragments ranging from 600 bp to 100 bp are specific to short arms of chromosome 6D and 6B, respectively (L. Talbert, personal communicatrion). Chromosome 6A is not tagged by this primer. Three of the eight fragments amplifed in *H. villosa* were present in both the addition line Add 6V-1 and the translocation line Sub 6V-1 (Fig. 2 lanes e and f). This supports the finding of Liu et al. (1993) that the V6 chromosome belongs to the sixth homoeologous group and strongly indicates that the arm carrying mite resistance on the *H. villosa* chromosome is homoeologous to the short arm of 6V chromosomes.

Apart from rye, *Secale cereale* L., most wheat curl mite resistant genes in wheat alien hybrids and in the grasses *Aegilops squarrosa* and *Thinopyrum ponticum* have also been mapped to the short arm of group 6 chromosomes (Harvey and Livers 1975;Whelan 1988; Whelan and Thomas 1989). This may indicate a common origin of the resistant gene(s) in *H. villosa* and the other grass species. Since the translocation stock appears well compensated and could be derived from a 6V/6A substitution the presence of the translocation in a putative substitution line is puzzling. The stock was perhaps misidentified. Alternatively, the translocation could have arisen spontaneously during the process of the attempted substitution with 6A. This possibility is supported by the findings of Liu's groups that have also obtained translocation lines from selfed F_3 lines (Qi et al. 1993). The present study confirms that GISH is a very useful technique for the unequivocal identification of alien chromosomes or chromosome segments in wheat-alien lines and for determination of the size of the translocation and location of its breakpoint.

The incorporation of alien chromatin, via chromosome translocation, has proven useful in wheat improvement. The genes for resistance to WSMV and mite colonization have been transferred by translocation into wheat from several grass species (Harvey and Livers 1975; Wells et al. 1982; Martin et al. 1984; Whelan and Hart 1988). The translocation line Sub 6V-1 is, therefore, likely to be more useful as a source of genetic resistance to the wheat curl mite than the addition line Add 6V-1 because of its relatively normal meiotic behavior and fewer potentially deleterious alien genes. In this study, GISH using *H. villosa* genomic DNA as a probe has been a powerful diagnostic tool for detecting small alien *H. villosa* segments introgressed into wheat. The 800-bp fragment specific to *H. villosa*, after amplification with G8 primers in the mite-resistant lines Add 6V-1 and Sub 6V-1, could become useful for marker-based selection to increase the efficiency in using Sub 6V as a new source of mite resistance in wheat breeding programs.

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