
Homoeologous relationships of *Haynaldia villosa* chromosomes with those of *Triticum aestivum* as revealed by RFLP analysis

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Homoeologous relationships between *Haynaldia villosa* chromosomes and bread wheat (*Triticum aestivum*) were studied in two sets of wheat-*H. villosa* addition lines and five substitution lines by restriction fragment length polymorphism (RFLP) analysis. *H. villosa* chromosomes 1V to 7V are homoeologous with group 1 to 7 chromosomes of wheat. In wheat-*H. villosa* substitution lines, the wheat chromosomes 3D, 4D, 5D, and 6A were replaced by homoeologous *H. villosa* chromosomes. The addition lines for chromosome 4V and 5V from three different accessions were analyzed by C-banding, and characterized by RFLP markers. Group 4 and 5 translocations were detected on chromosomes 4V#1 and 5V#1 (produced by Sears, unpublished), and 4V#3 and 5V#3 (produced by Lukaszewski, personal communication). The translocation breakpoints were located between *Xpsr1051* and *Xpsr115* on 4VL and between *Xpsr370* and *Xcdo1312* on 5VL. The similarities of the breakpoints within the Triticeae indicates that the specific chromosome regions of the homoeologous groups 4 and 5 are “hot spots” for chromosome breaks. The group-5 homoeologous loci, *Xpsr115*, *Xpsr580*, and *Xcdo484*, also were detected on the chromosome 4V#2 (produced by Liu et al., 1988) with a similar breakpoint between *Xpsr1051* and *Xpsr115*. However, no reciprocal translocation was detected on the chromosome 5V#2. Possible reasons for difference of 4/5 translocation in this species are discussed.

Wild relatives of wheat (*Triticum aestivum* L. em. Thell) are an important source of agronomic traits for wheat improvement. The successful exploitation of alien chromosome in wheat breeding greatly depends on the compensating ability of substituting alien chromosome for the replaced wheat chromosome. Thus, knowledge of the homoeologous relationships between chromosomes of the donor and recipient species is a prerequisite for the development of compensating wheat-alien transfers via homoeologous recombination. In recent years, a large number of RFLP probes identifying each homoeologous chromosome arm in wheat and in Triticeae has been developed (Sharp et al., 1989; Anderson et al., 1992). The presence of translocations involving homoeologous groups 4, 5, and 7 in the Triticeae were also confirmed by RFLP markers (Liu et al., 1992; Devos et al., 1993, 1995; King et al., 1994; Mickelson-Young et al., 1995; Nelson et al., 1995; Kojima and Oghiara, 1998).

*Haynaldia villosa* (L.) Schur (syn. *Dasypyrum villosa* (L.) Candargy) (2n =14, VV), a wild relative of bread wheat, is considered to be an important source of genes for powdery mildew resistance (Liu et al., 1988; Qi et al., 1995), eyespot resistance (Murray et al., 1994; Yildirim et al., 1998), and seed storage protein content and quality (Blanco and Simeone, 1988). The homoeologous relationship, between chromosomes of *H. villosa* and those of wheat were determined primarily by morphology, isozyme markers, and C-banding analysis (Sears, 1953; Hyde, 1953; Liu et al., 1995). The present study was undertaken to further investigate the homoeology of *H. villosa* chromosomes with those of wheat by a molecular analysis.

Six disomic addition (DA) lines of *T. aestivum* cv. Chinese Spring (CS) each containing a pair of chromosomes from *H. villosa* were developed by Sears (unpublished), in which addition lines of chromosome 1V, 6V and 7V were made from the accession “Italian” of *H. villosa*, and 2V, 4V and 5V from “Greek” (Lukaszewski, personal communication). The set of additions are designated as DA1V#1 to DA7V#1 except DA3V#1 which is missing,
according to the nomenclature proposed by Raupp et al. (1995). These lines are maintained at the Wheat Genetics Resource Center, Kansas State University, Manhattan, Kansas, USA. The *T. durum-H. villosa* amphiploid, six different chromosome additions (DA2V#2 to DA7V#2) in different wheat backgrounds, five disomic substitution (DS) lines (DS2V#2 to DS6V#2), and T6A-6V#2S translocation line were developed at the Cytogenetic Institute, Nanjing Agricultural University (CI, NAU, hereafter) (Liu et al., 1988, 1995; Qi et al., 1995). Two disomic addition lines 4V#3 and 5V#3 from a Sicilian accession in Chinese Spring background (DA4V#3 and DA5V#3) were kindly provided by Dr. A. J. Lukaszewski, University of California, Riverside, California, USA. The wheat accession line 4V#3 and 5V#3 from a Sicilian accession in Chinese Spring background (DA4V#3 and DA5V#3) were kindly provided by Dr. A. J. Lukaszewski, University of California, Riverside, California, USA. The *H. villosa* accession was introduced from Cambridge Botanical Garden, UK, which served as the genome donor for producing wheat-*H. villosa* alien chromosome lines in CI, NAU. The wheat variety ‘Yangmai 5’ was kindly provided by the Agricultural Institute of Yangzhou, Jiangsu, China, which, as latest recurrent parent, was used to produce DA2V#2, DA4V#2, DA6V#2, and DS6V#2. For chromosome identification of above mentioned, the C-banding technique described by Gill et al. (1991) was used.

Twenty-eight probes (Table 1) from seven homoeologous groups of the Triticeae were selected. These clones included BCD (barley cDNA), CDO (oat cDNA), and WG (wheat genomic DNA) provided by Dr. M. E. Sorrells, Cornell University, Ithaca, NY USA, and PSR (wheat cDNA or gDNA) clones provided by Dr. M. D. Gale, John Innes Centre, Norwich, UK. DNA extraction, restriction digestion, Southern blotting, probe labelling and hybridization are described in Qi et al. (1997).

**Homoeology of *H. villosa* chromosomes.** Most probes detected two or three restriction fragments (range 1–8) on *H. villosa* chromosomes. Of total 28 markers tested, only one marker, BCD433, failed to detect the polymorphism between *H. villosa* and Chinese Spring. Two group-1 probes, which specifically detect the loci on the homoeologous chromosomes of group-1, hybridized to unique fragments in DA1V#1 (1V#2 is not available), that were not present in any other addition lines, indicating that at least the chromosome regions of 1V#1 which have been detected by the two group-1 probes are belonging to homoeologous group-1 and not to other groups. The same observation was made for group-3 probes with DA3V#2 and DS3V#2 (Fig. 1).

<table>
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<th>Probe</th>
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<th>Enzyme</th>
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<th>Group</th>
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* Those probes were specifically used to detect the 4/5 translocation and the translocation breakpoints.

Table 1. The list of the probes and enzymes used for RFLP analysis
Homoeology of *H. villosa* chromosomes

Fig. 1. Southern hybridization of the wheat homoeologous group-3 probe PSR596 to EcoRI-digested genomic DNA. Lanes 1. *H. villosa*, 2. *T. durum-H. villosa* amphiploid, 3. Yanhmai 5, 4. Chinese Spring, 5. DA1V#1, 6. DA2V#1, 7–10. DA4V#1 to DA7V#1, 11–16. DA2V#2 to DA7V#2, 17–21. DS2V#2 to DS6V#2, 22–26. T6AL·6V#2S. The arrowheads show the polymorphic bands. Two 3D specific bands present in DA3V#2 (lane 12) were absent in *T. durum-H. villosa* amphiploid (lane 2) and DS3V#2 (lane 18).

Fig. 2. Southern hybridization of the wheat homoeologous group-4 probe PSR584 to EcoRI-digested genomic DNA. Lanes 1 to 26 same as in Fig. 1. DA4V#1 (lane 7), DA2V#2 (lane 11), DA4V#2 (lane 13), DS4V#2 (lane 19). The arrowheads show polymorphic bands. A 4D specific band was absent in *T. durum-H. villosa* amphiploid (lane 2) and DS4V#2 (lane 19).

RFLP screening using a set of probes not only determined homoeologous relationships between alien chromosomes and those of wheat, but also identified the missing wheat chromosomes in the substitution lines. The 20 probes also were used to study five wheat-*H. villosa* substitution lines 2V#2 to 6V#2. The results from four group-2 probes indicated that the alien chromosome in DS2V#2 was lost, and that this line had only the normal wheat chromosome complement. The DNA fragments mapped on chromosomes 3D, 4D, and 5D of wheat were
missing in DS3V#2, DS4V#2, and DS5V#2 respectively. The missing bands in three lines were also missing in the *T. durum*-*H. villosa* amphiploid (AABBVV) (Figs. 1 and 2). In the substitution DS6V#2, the wheat chromosome 6A was replaced by the *Haynaldia* chromosome 6V#2. The present study provided molecular evidence in agreement with the previous results. *H. villosa* chromosomes 1V to 7V are homoeologous with their corresponding wheat chromosomes. All substitution lines are of compensating types and occurred between homoeologous chromosomes.

**4/5 translocations in *H. villosa***. The *H. villosa* chromosomes 4V and 5V from three different accessions had polymorphic C-banding patterns (Fig. 3). The three different 4V chromosomes had similar C-banding patterns but differed in C-band size. The size of the C-bands were larger in chromosomes 4V#2 and 4V#3 than in 4V#1. Chromosomes 5V#1 and 5V#3 had a similar interstitial C-band in the distal region of long arm, which was absent in the long arm of the 5V#2.

In the present study, probes PSR115, PSR580, and CDO484 detected 5VL-specific fragments on 4V#1L and 4V#3L, and probes CDO1312, PSR164, and WG114 detected 4VL-specific fragments on 5V#1L and 5V#3L (Fig. 4). These results revealed that a 4/5 translocation is present in *H. villosa* chromosomes 4V#1 and 5V#1, and 4V#3 and 5V#3. The translocation breakpoints appeared to be between Xpsr1051 and Xpsr115 on 4V#1L and 4V#3L, and between Xpsr370 and Xcdo1312 on 5V#1L and 5V#3L (Fig. 5). The translocation breakpoints are similar to those reported in *T. monococcum*, *T. aestivum*, and other related species (King et al., 1994; Devos et al., 1993, 1995; Kojima and Ogihara, 1998). Some regions on chromosomes 4 and 5 are assumed to be more susceptible to breaks, and this property is conserved within the Triticeae.

![Fig. 3. C-banding pattern of the chromosomes 4V and 5V in addition or substitution lines from different accessions of *H. villosa*. The chromosomes 4V#1 and 5V#1 were taken from Friebe et al. (1987). The chromosome 4V#2 were from the DS4V#2.](image-url)
A complex rearrangement was detected in chromosomes 4V#2 and 5V#2. The 4V#2 substitution line carried the group-5 homoeologous loci for \(X_{psr115}\), \(X_{psr580}\), and \(X_{cdo484}\). A similar translocation breakpoint was located between \(X_{psr1051}\) and \(X_{psr115}\) on 4V#2 in DS4V#2 (Figs. 4 and 5). However, no reciprocal translocation was found in chromosome 5V#2. Both addition and substitution lines of chromosome 5V#2 also carried the group-5 homoeologous loci for \(X_{psr115}\), \(X_{psr580}\) and \(X_{cdo484}\) (Figs. 4 and 5). These RFLP markers detected polymorphic bands in DS4V#2 but failed to detect any polymorphic band on DA4V#2. Also, the probes CDO1312, PSR164, and WG114 failed to detect the polymorphic bands in DA4V#2, suggesting that the chromosome segment with the markers may have been deleted in the 4V#2 addition line. This deleted fragment could not be detected under microscope because 4V#2 in DA4V#2 has identical C-banding pattern with that in DS4V#2. Obviously, such a large duplication and deficiency for the segment marked by CDO1312, PSR164, and WG114 would not be tolerated in a diploid strain, and must have occurred during the production of the addition and substi-

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**Fig. 4.** Southern hybridization of the wheat homoeologous group-5 probe PSR115 to EcoRV-digested genomic DNA. Lanes 1. *H. villosa*, 2. Chinese Spring, 3. DA4V#1 4. DA4V#2, 5. DA4V#3, 6. DA5V#1, 7. DA5V#2, 8. DA5V#3, 9. DS4V#2, and 10. DS5V#2. The arrow indicates the polymorphic band. A 5D specific band was missing in DS5V#2 (lane 10).

**Fig. 5.** A drawing of long arms of chromosome 4V and 5V of *H. villosa*. The 4/5 translocations are detected by RFLP markers. The order of the markers is according to Mickelson-Young et al. (1995), Devos et al. (1995), and Gill et al. (1996). TBP: translocation breakpoint. C: centromere.
tution lines.

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REFERENCES


