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Identification and physical mapping of three *Haynaldia villosa* chromosome-6V deletion lines

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Abstract Three deletion lines (del6V#2S-1, del6V#2L-1, and del6V#2L-2) of *Haynaldia villosa* chromosome 6V added to wheat were identified by C-banding and characterized by RFLP analyses. The breakpoints were located at fraction lengths (FL) 0.58 in del6V#2S-1 in the short arm, and FL 0.66 in del6V#2L-1 and FL 0.64 in del6V#2L-2 in the long arm. Thirty-one *Triticeae* homoeologous group-6 DNA probes were used to map RFLP loci in the deletion lines and the wheat-*H. villosa* disomic substitution (DS) line 6V#2(6A). Nine probes failed to detect polymorphism between Chinese Spring and DS6V#2(6A). Ten of sixteen polymorphic short-arm loci were not detected in del6V#2S-1. Thus, the loci are located in the deleted distal chromosome region. Six RFLP markers were mapped in the proximal 58% of 6VS. Of 20 DNA markers specific for 6VL, six mapped in the distal 36% of the long arm, and nine mapped in the proximal 64% of 6VL. The breakpoint of the short arm of 6V#2 occurs between *Xpsr106* and *Xcdo270*, and that of the long arm between *Xpsr915* and *Xmwg934*. The powdery mildew resistance gene *Pm21* is located on the short arm of chromosome 6V#2. *Pm21* is present in del6V#2S-1, and can be further mapped in the proximal 58% of 6V#2S.

Key words *T. aestivum* · *H. villosa* · C-banding · RFLP · Deletion mapping · *Pm21*

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Introduction

Sears (1953) was the first to demonstrate the feasibility of gene transfer from *Haynaldia villosa* (L.) Schur [syn. *Dasypyrum villosa* (L.) Candargy] to common wheat. Over the last 15 years, scientist at the Cytogenetic Institute, Nanjing Agricultural University (Cl, NAU hereafter) have been working in this area. Wheat-*H. villosa* addition, substitution, and translocation lines involving chromosome 6V#2 exhibit high levels of resistance to powdery mildew (*Erysiphe graminis* D.C. ex Merat F. sp. *tritici*) (Pei et al. 1986; Liu et al. 1988; Qi et al. 1995 a). This powdery mildew resistance gene (*Pm21*) is located in the short arm of chromosome 6V#2 (Chen et al. 1995; Qi et al. 1995 b). The expression and stability of gene *Pm21* in different wheat backgrounds has been studied (Liu et al. 1996). The resistant disomic addition (DA) 6V#2 was crossed to the susceptible DA6V#1 (Hyde 1953; Sears 1953) to generate a suitable mapping population and to identify RFLP markers closely linked to *Pm21*. However, observations of C-banded pollen mother cells at metaphase-I of meiosis indicated that the *H. villosa* chromosome failed to pair in most cells. Also, one of the 6V chromosomes was deficient for a telomeric C-band in the short arm. Because of the low pairing it was not possible to use conventional mapping procedures to locate the *Pm21* gene.

In common wheat, deletion lines involving 21 pairs of chromosomes have been developed using a gametocidal chromosome system (Endo and Gill 1995, 1996). These deletion lines have been used to develop cytogenetically based physical maps of the seven wheat homoeologous groups (Werner et al. 1992; Gill et al. 1993, 1996; Kota et al. 1993; Hohmann et al. 1994; Delaney et al. 1995 a, b; Mickelson-Young et al. 1995), and these maps are important for map-based positional cloning.

The present paper reports the identification of three deletion lines of *H. villosa* chromosome 6V#2 and their characterization through deletion mapping.

Materials and methods

Plant material

T. aestivum-H. villosa disomic addition 6V produced by Sears (1953) was designated DA6V#1 according to the nomenclature proposed by Raupp et al. (1995) and is maintained at the Wheat Genetic Resource Center, Kansas State University, Manhattan, USA. The wheat-*H. villosa* disomic substitution 6V(6A), designated DS6V#2(6A), the T6AL·6V#2S translocation line, and a *T. durum-H. villosa* amphiploid were developed at the Cytogenetic Institute, Nanjing Agricultural University. A spontaneous deletion of the short arm of chromosome 6V#2 was found in wheat-*H. villosa* DA6V#2. Two lines with deletions in the long arm of chromosome 6V#2 were selected in derivatives of an F_3 plant irradiated from a cross between the wheat-*H. villosa* DS6V#2(6A) and the wheat cultivar Yangmai 5. The *H. villosa* accession that served as the genome donor for producing wheat-*H. villosa* alien chromosome lines in Cl, NAU was kindly provided by the Cambridge Botanical Garden, UK. The wheat cultivar Yangmai 5 was kindly provided by the Agricultural Institute of Yangzhou, Jiangsu, China.

Cytogenetic analysis

The C-banding technique followed that described by Gill et al. (1991). The breakpoint for each deletion chromosome was calculated as a fraction length (FL) of the whole chromosome arm using the method of Endo and Gill (1996). Measurements were made on at least five C-banded chromosomes.

RFLP analysis

Forty homoeologous group-6 probes selected from *Triticeae* species were used for the deletion mapping. These clones included, barley cDNA (BCD), oat cDNA (CDO), and wheat genomic DNA (WG) (provided by Dr. M.E. Sorrells, Ithaca, USA); wheat cDNA or gDNA (PSR) (Dr. M.D. Gale, Norwich, UK); *Ae. tauschi* gDNA (KSU) (Dr. B.S. Gill, Manhattan, USA); and barley gDNA (mWG) and barley cDNA (cmWG) (Dr. A. Graner, Grunbach, Germany).

The DNA extraction, restriction digestion, Southern blotting, probe labeling and hybridization procedures are used as described by Qi et al. (1997).

Powdery mildew test

Three deletion lines were tested for their reaction to powdery mildew in the greenhouse at both NAU and KSU with Yangmai 5 and line DS6V#2 (6A) as the susceptible and resistant controls, respectively. Both lines were tested for disease reaction at the Plant Protection Institute, Chinese Academy of Agricultural Science. The wheat Yangmai 5 is susceptible (rating = 8), and line DS6V#2(6A) highly resistant (rating = 0), to powdery mildew according to a scale of 0–9 (Qi et al. 1995 b). All of the materials were inoculated with natural pathogen populations of *E. graminis* in the greenhouse. Disease reaction was scored 14 days after inoculation at both the seedling and adult stages.

Results

Identification of *H. villosa* chromosome 6V#2 deletion lines

Chromosome 6V#2 is submetacentric with diagnostic centromeric and telomeric bands in both arms. In addition, there are proximal centromere and subtelomeric bands in the long arm (Fig. 1). A spontaneous deletion of part of the short arm of chromosome 6V#2 (del6V#2S-1) was identified in the progeny of wheat-*H. villosa* line DA6V#2 (Fig. 1) with the breakpoint at FL 0.58. Line del6V#2S-1 has 2n = 44 chromosomes and was designated DAdel6V#2S-1.

The cross between DS6V#2 (6A) and Yangmai 5 was made. A line C215 with high resistance to powdery mildew was selected from the F_3 population. A portion of the C215 seeds were treated by Co^{60} gamma rays at a dosage of 20 000 rads. Sixty nine resistant plants were selected from the M_3 population and analyzed by C-banding and in situ hybridization. Ten M_3 plants were wheat-*H. villosa* translocations (T6AL·6V#2S) (Chen et al. 1995; Qi et al. 1995 a). A pair of chromosomes in two plants, 92R112 and 92R119, had a different C-banded pattern from either the *H. villosa* chromosome 6V#2 or the translocated chromosome. Each line was found to have the terminal part of the long arm of chromosome 6V#2 missing (Fig. 1). The breakpoints of del6V#2L-1 (92R112) and del6V#2L-2 (92R119) are located at FL0.66 and FL0.64, respectively. Both lines have 2n = 42 chromosomes, and possess a deleted 6V#2 chromosome substituting for 6A.

RFLP analysis

Both 6V#1 and 6V#2 are considered homoeologous to group-6 chromosomes of the *Triticeae* based on cytogenetic and molecular evidence (Hyde 1953; Sears 1953; Liu et al. 1995; Qi et al. 1998). Because of their different origin and reaction to powdery mildew, these two chromosomes were named 6V#1 and 6V#2 in the present study.

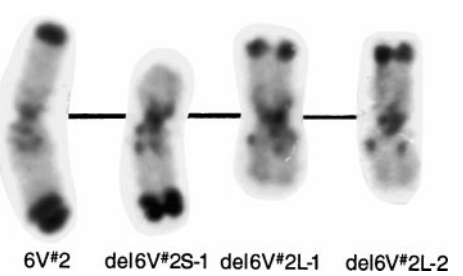

Fig. 1 C-banded chromosome 6V#2, and deletion chromosomes del6V#2S-1, del6V#2L-1, and del6V#2L-2 of *H. villosa*

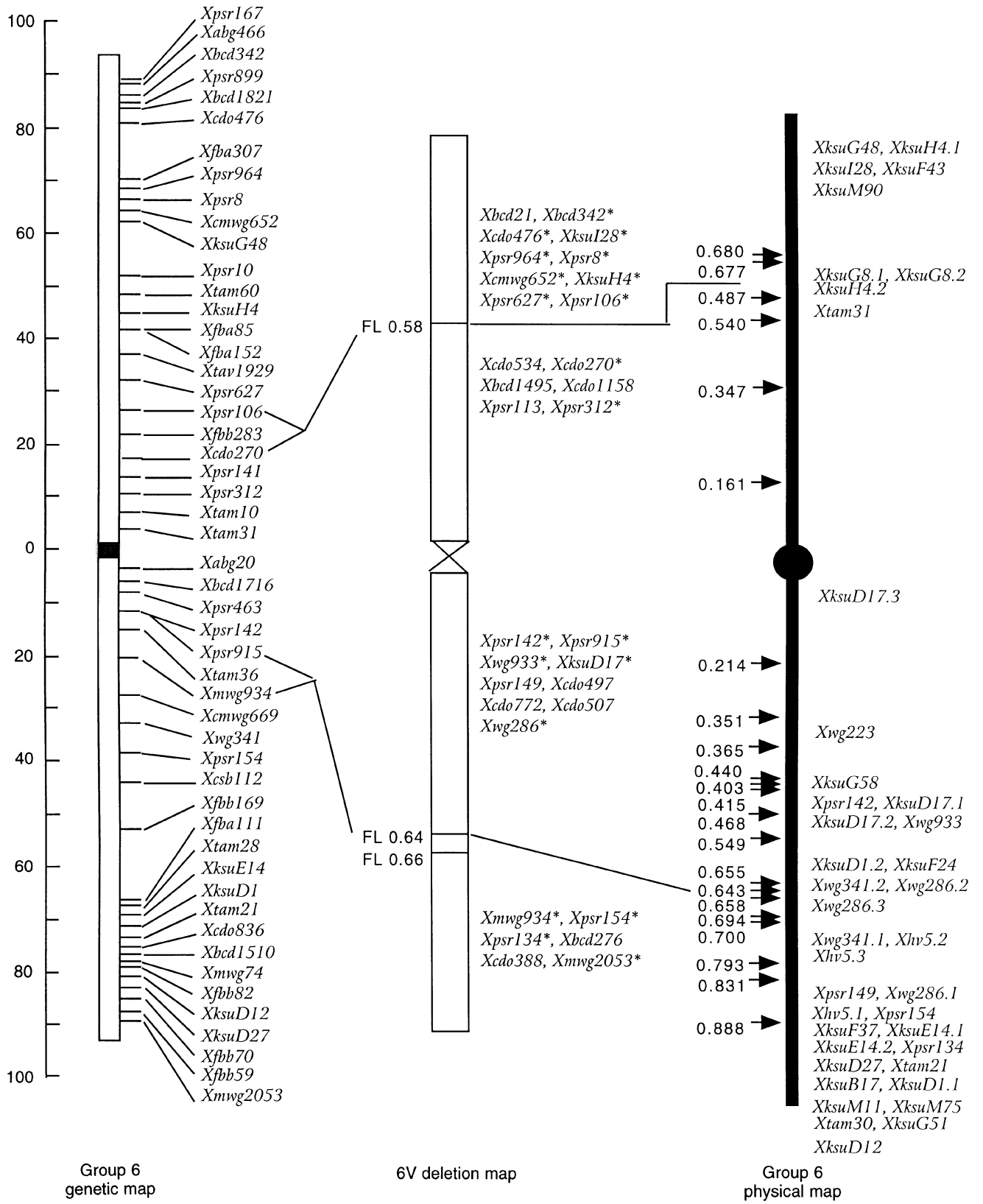

Fig. 2 Comparison of the 6V deletion map with the wheat group-6 genetic linkage and physical maps. Consensus RFLP linkage map of *Triticeae* homoeologous group-6 chromosomes adapted from Marino et al. (1996), and the physical map of homoeologous group 6 from Gill et al. (1993). *: These markers are also found on the group-6 genetic or physical maps

Genomic DNA of each of the wheat-alien lines, deletions, and donors were separately digested with five restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, and *Bam*HI). Forty probes selected from the *Triticeae* homoeologous group 6 were individually hybridized to Southern blots. Of the 40 clones, nine failed to detect any polymorphism between Chinese Spring (CS) and DA6V#1 or DS6V#2 (6A). We concluded that one or two of the RFLP bands in *H. villosa* were similar to those in CS. Six of the 40 probes detected DNA fragments different from CS in chromosomes 6V#1 or 6V#2, but no polymorphism was observed between 6V#1 and 6V#2. The remaining 25 probes (62.5% of DNA clones tested) not only detected RFLP bands in CS and lines containing chromosome 6V, but also were polymorphic for chromosomes 6V#1 and 6V#2.

Deletion mapping

RFLP loci were allocated within specific chromosome regions by scoring the presence or absence of chromosome arm-specific bands. Sixteen of the twenty short arm probes hybridized to specific DNA fragments in DS6V#2(6A). Ten of these probes failed to hybridize with fragments in del6V#2S-1, indicating that these DNA markers are located in the 6V#2 chromosome region distal to FL0.58. The other six RFLP markers detected fragments in del6V#2S-1 and mapped in the proximal 58% of the short arm. The breakpoint of the short arm appears to be between *Xpsr106* and *Xcdo270* (Fig. 2). Of the 20 DNA markers specific for 6VL, six were mapped in the distal 36% of the long arm, and nine were mapped in the proximal 64% of 6VL (Fig. 2). The breakpoint of del6V#2L-1 was close to that of del6V#2L-2, and none of the RFLP markers tested were able to differentiate between these two lines. The long-arm breakpoint appears to be between *Xpsr915* and *Xmwg934* (Fig. 2).

Powdery mildew resistance

Resistance to powdery mildew in del6V#2S-1 and the two long-arm deletion lines was expressed at a high levels. When inoculated with natural pathogen populations of *E. graminis* in the greenhouse, three deletion lines consistently had no disease symptoms similar to line DS6V#2 (6A). Yangmai 5 was susceptible. The resistance gene *Pm21* is located in the short arm of chromosome 6V#2. The fact that del6V#2S-1 is highly resistant to powdery mildew places *Pm21* in the proximal 58% of 6VS.

Discussion

The RFLP data in this paper indicate that two *H. villosa* 6V chromosomes are highly polymorphic. Because chromosomes 6V#1 and 6V#2 do not pair and recombine, *Pm21* could not be localized by conventional mapping. Alternatively, *Pm21* could be mapped by crossing the resistant *H. villosa* accession with a *Pm*-susceptible accession. We screened 46 different *H. villosa* accessions of different origins. Unfortunately, no susceptible accession was identified.

In the present study, the deletion line del6V#2S-1 occurred spontaneously in DA6V#2. Two long-arm deletion lines were recovered in the derivatives of an irradiated F_3 from the cross of DS6V#2 and Yangmai 5. Whether the deletion lines occurred spontaneously or were induced by irradiation is not known. Spontaneous deletions were also reported in common wheat (Payne et al. 1984; Kota and Drorak 1986). Limited numbers and types of spontaneous deletions make it hard to develop high-density physical maps in the *Triticeae*. Using gametocidal chromosomes to develop a set of deletion lines of chromosomes 6V#2 could be a possible approach for a more detailed physical mapping of *Pm21*.

In the present study, 77.5% of the homoeologous group-6 probes (31 of 40 probes) detected polymorphism between chromosome 6V and the group-6 chromosome of CS. Although a genetic map of chromosome 6V is not available, the location of these segments appears to be conserved in 6V. The linear order of the loci was similar in both chromosome 6V and the wheat chromosome of group 6 (Fig. 2) when compared with the published genetic and physical maps of wheat group 6 (Gill et al. 1993; Marino et al. 1996). The markers located in the distal 42% of the short arm and the distal 36% of the long arm of chromosome 6V span a range of 25 to 90 cM in the short and long arms of the consensus *Triticeae* homoeologous group-6 RFLP linkage map. The physical location of most markers in chromosome 6V is consistent with that of wheat group-6 chromosomes, indicating that gene order is conserved between the *H. villosa* genome and that of wheat. The set of wheat group-6 probes could be used to develop genetic and high-density physical maps of chromosome 6V.

Pm21, located in the proximal portion of the short arm of chromosome 6V, is an important new source of resistance to powdery mildew. Some DNA markers were mapped in the proximal 58% of 6VS, and the breakpoint of del6V#2S-1 appears to be located between *Xpsr106* and *Xcdo270*. On the basis of this evidence a selection strategy using the ph mutant and RFLP markers to introgress this gene into wheat by induced homoeologous chromosome pairing could easily be developed.

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