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Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew

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Abstract Several *Triticum aestivum* L. -*Haynaldia villosa* disomic 6VS/6AL translocation lines with powdery mildew resistance were developed from the hybridization between common wheat cultivar Yangmai 5 and alien substitution line 6V(6A). Mitotic and meiotic C-banding analysis, aneuploid analysis with double ditelosomic stocks, in situ hybridization, as well as the phenotypic assessment of powdery mildew resistance, were used to characterize these lines. The same translocated chromosome, with breakpoints near the centromere, appears to be present in all the lines, despite variation among the lines in their morphology and agronomic characteristics. The resistance gene, conferred by *H. villosa* and designated as *Pm21*, is a new and promising source of powdery mildew resistance in wheat breeding.

Key words Wheat · *Haynaldia villosa* · Alien translocation · Powdery mildew resistance · In situ hybridization

Introduction

Powdery mildew caused by *Erysiphe graminis* D. C. ex Merat *tritici* E. Marchal has been a severe disease in China and a number of other wheat-growing countries, especially with the increasing utilization of nitrogenous fertilizers and improved irrigation. Globally, powdery mildew resistance genes from related species have been successfully used in wheat breeding and have played an important role in the control of this disease. However, some resistant genes have been rendered ineffective due to variation of the pathogenic virulence. For example, the resistance gene *Pm8* derived from 1RS of rye was incorporated into a number of wheat cultivars such as Kavkaz, Aurora, Bezostaja, Veery and Alondra"S" (McIntosh 1983). But, unfortunately, *Pm8* resistance has been overcome in some wheat growing regions (Xiong et al. 1983; Wu 1990).

In the early 1980s, *Haynaldia villosa* (2n = 14, VV) was identified to be a potential source of powdery mildew resistance and transfer of this resistance into wheat was initiated in our institute in order to enrich the vulnerable gene pool for resistance to powdery mildew (Chen and Liu 1982). A single gene on chromosome arm 6VS of *H. villosa* was shown to be responsible for this resistance and the 6V alien addition line and 6V(6A) alien substitution line of wheat were developed in 1986 and 1988, respectively (Chen and Liu 1986; Pei et al. 1986; Liu et al. 1988). Both showed a high level of resistance to powdery mildew in several countries (Qi et al. 1995). However, the lack of homology between 6V and its wheat homoeologues hindered the direct use of this gene in wheat breeding. This led to attempts to use irradiation and screening among the progeny of hybrids between substitution line 6V(6A) and elite wheat cultivars in order to induce resistant wheat-alien chromosome translocations. The production and molecular cytogenetic analysis of these translocation lines are reported in this paper.

Materials and methods

The powdery mildew resistant substitution line 6V(6A) was developed and identified by N- and C-banding analysis on mitotic metaphase root-tip cells (RTCs) or meiotic metaphase-I (MI) pollen mother cells (PMCs). The homoeologous group was designated based on its compensation ability as well as on isozymatic analysis (Liu et al. 1993).

Seeds of the common wheat cultivar Yangmai 5 were provided by Dr. S. H. Chen, Yangzhou Institute of Agricultural Sciences, Jiangsu, China. It is high yielding and widely cultivated in southeast China but is susceptible to powdery mildew.

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For mitotic preparations, two root tips of germinating seeds were harvested and the remaining one was kept for seedling growth. Collected root tips were pre-treated in icy water for 20–22 h prior to fixation in acetic alcohol (1:3) for 3–7 days.

Anthers were fixed in acetic alcohol (1:3) for 2–7 days and squashed in 45% acetic acid or 1% acetocarmine for C-banding and in situ hybridization, respectively.

For in situ hybridization using genomic DNA as a probe (GISH), total genomic DNA was isolated from young leaves of *H. villosa* following the procedure of Saghai-Maroof et al. (1984) and labelled with biotin-11-dUTP through nick translation (nick translation kit, Enzo Diagnostics). In situ hybridization followed Mukai and Gill (1991). The ratio of probe DNA and blocking DNA was about 200 to 1. Signals were detected with a DETEK-HRP Detection Kit (Enzo Diagnostics) following the suggested protocol.

The evaluation of powdery mildew resistance was conducted both in the greenhouse and in the field under favorable conditions. In 1993, all materials were also sent to the Plant Protection Institute, Chinese Academy of Agricultural Sciences in Beijing, for further assessment (for details see Qi et al. 1995).

Results

Production of translocation lines

The cross of substitution line 6 V(6 A) with Yangmai 5 was conducted in May 1986. Individual plants resistant to powdery mildew were selected in the F_2 generation and grown as lines to produce a F_3 generation. A highly resistant line C215, derived from B33 of the F_2, was selected in 1989. Part of the C215 seeds were irradiated with Co^{60} gamma rays at a dosage of 20 000 rads and then sent to Kuenming in the Yunnan Province to grow the first mutation generation (M1) in the summer. The resulting M2 seeds were planted as spike lines. Meanwhile, the remaining seeds of line C215 were grown as an F_4 in the fall of 1989 at Nanjing. A total of 100 resistant plants were selected from the M3 (69) and F_4(31) populations. Selection and cytological analysis were carried out among their offspring.

From mitotic C-banding patterns, 17 lines selected from the above-mentioned 100 plants (five from F_5, 12 from M4) were all shown to have the same pair of translocated chromosomes with one characteristic dark terminal band, one light centromere band, one interstitial band near the centromere of the short arm, and two interstitial bands on the long arm. Compared with the C-banding patterns of *H. villosa* (Liu et al. 1993) and common wheat (Gill et al. 1991), this pair of chromosomes was revealed to be composed of 6 VS from *H. villosa* and 6 AL from wheat (Fig. 1). The chromosome pairing, 0.00–0.8 I + 20.69–21.00 II per PMC, suggested that all of the 17 lines were homozygous disomic translocation lines, and it seems very possible that this translocation happened spontaneously in line C215.

C-banding analysis on testcross hybrids

Testcrosses of five of the 17 translocation lines (92R089, 92R090, 92R091, 92R141 and 92R149) with Yangmai 5 and substitution line 6 V (6 A) were made in 1992. Hybridization between 92R090, 92R091 and the double ditelosomic 6 A of Chinese Spring was also carried out in this same year. C-banded mitotic and meiotic chromosome pairing analysis was made on these three types of F_1 hybrids. One rod bivalent formed by chromosome 6A and the translocated chromosome 6 VS/6 AL was observed in PMCs of the F_1 plants of the translocation line with Yangmai 5 (Fig. 2). In hybrids of the translocation line × substitution line cross, the short arm of 6 V and the translocated chromosome 6 VS/6 AL paired as one rod bivalent with a dark C-band at the chiasma position. In the F_1 plants obtained from the crosses of double ditelosomic 6 A with translocation lines (92R090, 92R091), telosome 6 AL paired with the translocated chromosome 6 VS/6 AL forming a heteromorphic rod bivalent, while telosome 6 AS remained unpaired as an univalent. These results provided further evidence that the translocated chromosome consisted of 6 AL and 6 VS.

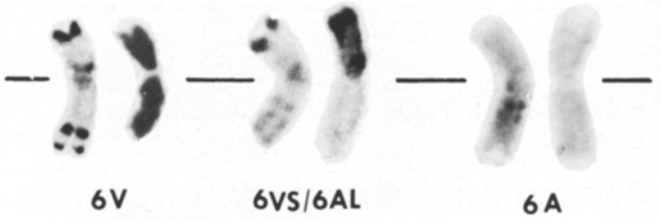


Fig. 1 C-banded (left) and GISH (right) mitotic chromosomes from 6 V, the 6 VS/6 AL translocation and 6 A

In situ hybridization

The C-banding patterns of the translocated chromosomes suggested that the translocation breakpoints were located close to the centromere. To determine the position of the breakpoint more precisely, biotin-labeled genomic DNA form *H. villosa* was hybridized to mitotic metaphase and meiotic MI chromosomes of five translocation lines (92R089, 92R090, 92R137, 92R149 and 92R178). The results indicated that in all cases translocation breakpoints were at or very close to the centromere (Figs. 1, 3a). Moreover, the two translocated chromosomes paired as one bivalent (Figs. 2, 3 b) indica-

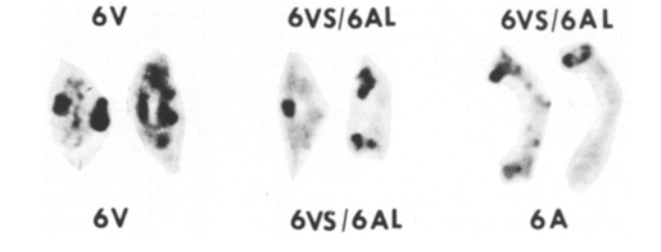
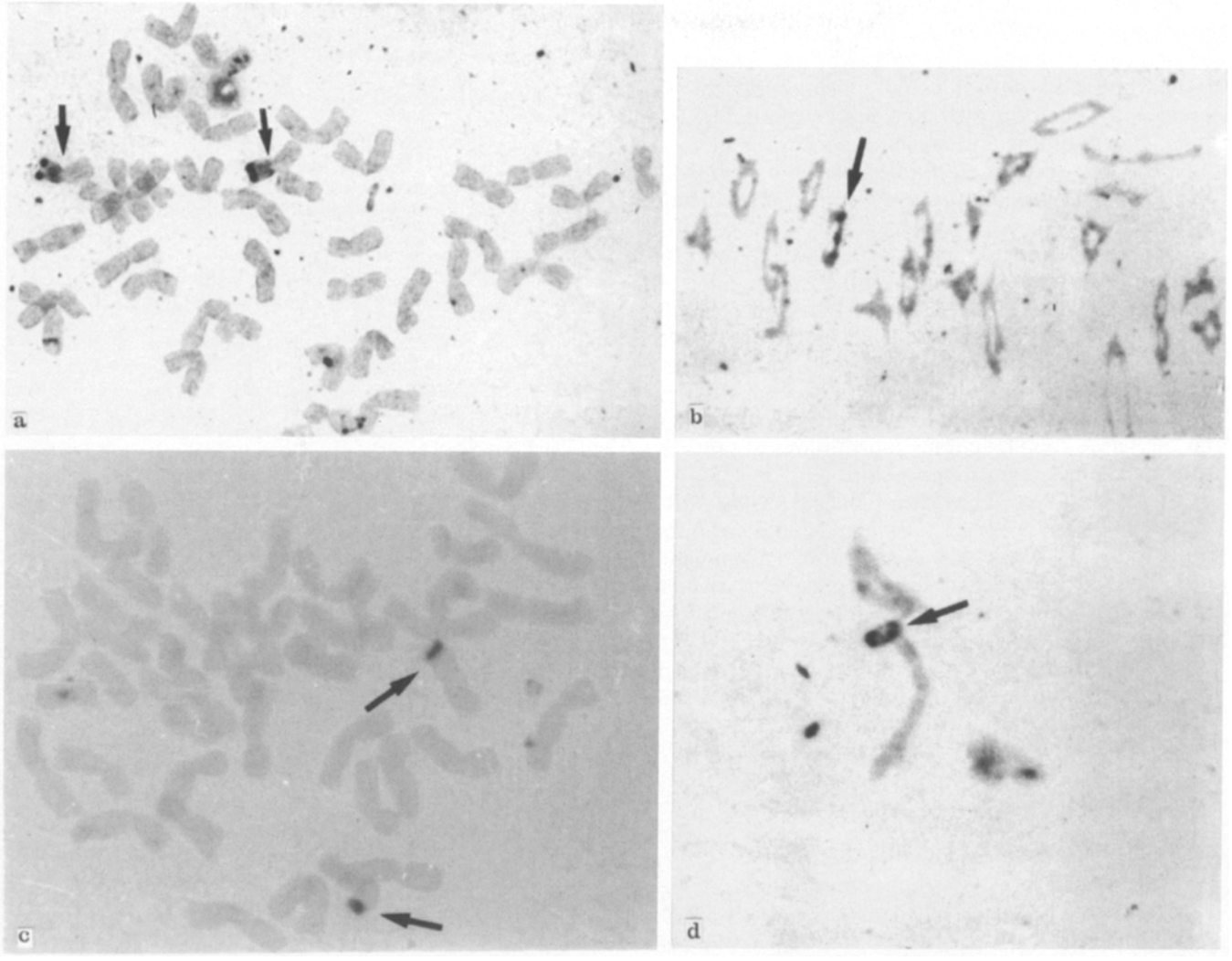


Fig. 2 C-banded (left) and GISH (right) meiotic chromosome pairing in substitution line 6 V(6A), translocation line 6 VS/6 AL, and the F_1 hybrid of translocation line 6 VS/6 AL with Yangmai 5



ting that these disomic translocation lines were cytologically stable.

In the PMCs of the hybrid of the 6 VS/6 AL translocation line with Yangmai 5, a rod bivalent was observed consisting of a wheat chromosome and the wheat-*H. villosa* translocated chromosome (Figs. 2, 3 d). No pairing between 6 VS from *H. villosa* and 6 AS was found in more than 100 pollen mother cells examined.

The repeated DNA sequence pHv62, specific to genome V of *H. villosa*, could hybridize with 6 of 7 pairs of the V-genome chromosomes (Li et al. 1995). Each arm of 6 V had a characteristic telo-band. After in situ hybridization to mitotic metaphase chromosomes of the translocation lines (92R091, 92R141) with pHv62 as a probe, only one pair of chromosomes with terminal signals on one arm was observed (Fig. 3 c).

Fig. 3a–d Results of GISH. **a** Mitotic metaphase chromosomes of translocation lines. *Arrows* designate one pair of 6 VS/6 AL translocated chromosomes. **b** Meiotic MI chromosomes of translocation lines. The arrow indicates one ring bivalent formed by the two translocated chromosomes. **c** in situ hybridization using biotin-labelled pHv62 as a probe on mitotic metaphase chromosomes of translocation lines. Arrows indicate two translocated chromosomes with terminal signals on their short arms. **d** One rod bivalent formed by translocated chromosome 6 VS/6 AL and 6 A in MI PMCs of the (6 VS/6 AL translocation line × Yangmai 5) F_1

Discussion

The C-banding technique has been successfully used to identify alien chromosomes, or chromosome segments, introduced into wheat (Lapitan et al. 1984; Hueros et al. 1991). However, it is not sensitive enough to resolve transferred chromosome segments which lack characteristic banding patterns. On the other hand, in situ hybridization has shown a great potential for solving this problem and there are a number of cases in which introduced chromosome segments or introgressed chromatins and translocation breakpoints have been detected using various kinds of in situ hybridization methods (Friebe et al. 1991 a, b, 1993; Le et al. 1989; Jiang and Gill 1993; Jiang et al. 1993, 1994). In the present study, results from C-banding and in situ hybridization showed good agreement and supported each other.

Results from in situ hybridization suggested that the breakpoint of the 6 VS/6 AL translocation was located at or near the centromere. All five lines examined by C-banding and chromosome pairing had the same breakpoint position, although there were differences in their morphology and agronomic characteristics. It seems that breakage and reunion occurs more readily in the regions near the centromere which are rich in heterochromatin.

In the translocation line × Yangmai 5 hybrids, 6AL, but not 6AS, always paired with the translocated chromosome 6 VS/6 AL. At anaphase-I, 6 A and 6 VS/6 AL disjoined normally. Presumably all genes on 6 VS would be transmitted as a unit at gamete formation. We observed that black awnness always co-segregated with powdery mildew resistance, suggesting black awns may be used as a supplementary morphological marker for resistance in segregating populations.

Five 6 VS/6 AL translocation lines (92R089, 92R137, 92R141, 92R149 and 92R178) have been distributed to more than 50 wheat research institutions or universities both at home and abroad. In all cases, they showed high resistance to powdery mildew. This resistance gene has been formally designated as *Pm 21* (Qi et al. 1995). Provided no undesirable effects of the alien chromatin on yield and quality exist, these 6 VS/6 AL translocation lines may become useful genetic resources for powdery mildew resistance in wheat improvement.

References

Chen PD, Liu DJ (1982) Cytogenetic studies of hybrid progenies between *Triticum aestivum* and *Haynaldia villosa*. J Nanjing Agric Univ 4:1–16

Chen PD, Liu DJ (1986) Identification of *Haynaldia villosa* chromosomes in alien wheat additions. In: Li ZS (ed) Proc 1st Int Symp on Chromosome Engineering in Plants, Xian, China, pp 31–33

Friebe B, Hatchett JH, Gill BS, Mukai Y, Sebeta EF (1991a) Transfer of Hessian fly resistance from rye to wheat via radiation-induced terminal and intercalary chromosome translocations. Theor Appl Genet 83:33–40

Friebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS (1991b) 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, Jiang J, Gill BS, Dyck PL (1993) Radiation-reduced non-homoeologous wheat-*Agropyron intermedium* chromosomal translocations conferring resistance to leaf rust. Theor Appl Genet 86:141–149

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum* L.). Genome 34:830–839

Hueros G, Gonzales JM. Sanz JC, Ferrer E (1991) Gliadin gene location and C-banding identification of *Aegilops longissima* chromosomes added to wheat. Genome 34:236–240

Jiang JM, Gill BS (1993) A "zebra" chromosome arising from multiple translocations involving non-homoeologous chromosomes. Chromosoma 102:612–617

Jiang JM, Chen PD, Friebe B, Raupp WJ, Gill BS (1993) Alloplasmic wheat-*Elymus ciliaris* chromosome addition lines. Genome 36:327–333

Jiang JM, Morris KLD, Gill BS (1994) Introgression of *Elymus trachycaulus* chromatin into common wheat. Chromosome Res, 2:3–13

Lapitan NLV, Sears RG, Gill BS (1984) Translocation and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. Theor Appl Genet 68:547–554

Le HT, Armstrong KC, Miki B (1989) Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. Plant Mol Biol Rep 7:150–158

Liu DJ, Chen PD, Pei GZ, Wang YN, Qiu BX, Wang SL (1988) Transfer of *Haynaldia villosa* chromosomes into *Triticum aestivum*. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge, UK, pp 355–361

Liu DJ, Chen PD, Raupp JW (1993) Determination of homoeologous groups of *Haynaldia villosa* chromosomes. In: Proc 8th Int Wheat Genet Symp, Beijing, China, pp 181–185

Li WL, Chen PD, Qi LL, Liu DJ (1995) Cloning of repeated DNA sequences specific to *Haynaldia villosa* for detecting its chromatin in wheat. Theor Appl Genet 90:526–533

McIntosh RA (1983) A catalogue of gene symbols for wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp 1218–1225

Mukai Y, Gill BS (1991) Detection of barley chromatin added to wheat by genomic in situ hybridization. Genome 34:448–452

Pei GZ, Chen PD, Liu DJ (1986) A cytogenetic analysis of some powdery mildew-resistant strains of hybrid progeny between wheat and *Haynaldia villosa*. J Nanjing Agric Univ 1:1–9

Qi LL, Chen PD, Liu DJ (1995) The gene *Pm21*–a new source of resistance to wheat powdery mildew. Acta Agron Sin 21(3): 257–260

Saghai-Maroof MA, Soliman KA, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Schafer JF (1987) Wheat disease – rusts, smuts and powdery mildew. In: Heyne EG (ed) Wheat and wheat improvement (2nd edn.). Amer Soc Agron, Madison, Wisconsin, pp 542–584

Wu ZS (1990) Breeding for disease resistance. In Wu ZS (ed) Wheat breeding. Agric Pub Press of China, Beijing, pp 267–270

Xiong EW, Cao Y, Bo YJ, Xia QP, Yang XY, Wu ZF (1983) Identification and analysis of powdery mildew resistance in common wheat cultivars. Plant Protection of China 3:5–7