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RESEARCH ARTICLE

Genetic behavior of *Triticum aestivum–Dasypyrum villosum* translocation chromosomes T6V#4S·6DL and T6V#2S·6AL carrying powdery mildew resistance

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Abstract

T6V#2S·6AL and T6V#4S·6DL translocation chromosomes developed from the cross of wheat and different *Dasypyrum villosum* accessions have good powdery mildew (PM) resistance, but their pairing and pyramiding behavior remains unclear. Results in this study indicated that the pairing frequency rate of the two differently originated 6VS chromosomes in their F_1 hybrid was 18.9% according to genomic *in situ* hybridization (GISH); the PM resistance plants in the F_2 generation from the cross between T6V#4S·6DL translocation line Pm97033 and its PM susceptible wheat variety Wan7107 was fewer than expected. However, the ratio of the resistant *vs*. the susceptible plants of 15:1 in the F_2 generation derived from the cross between the two translocation lines of T6V#2S·6AL and T6V#4S·6DL fitted well. Plants segregation ratio (homozygous:heterozygous:lacking) revealed by molecular marker for T6V#4S·6DL or T6V#2S·6AL in their F_2 populations fitted the expected values of 1:2:1 well, inferring that the pairing of the two alien chromosome arms facilitates the transmission of T6V#4S·6DL from the F_1 to the F_2 generation. A quadrivalent was also observed in 21% of pollen mother cells (PMCs) of homozygote plants containing the two pairs of translocated chromosomes. The chromosome pairing between 6V#2S and 6V#4S indicates that it will be possible to obtain recombinants and clarify if the PM resistance determinant on one alien chromosome arm is different from that on the other.

Keywords: *Triticum aestivum, Dasypyrum villosum*, translocation, genetic behavior, powdery mildew resistance, GISH, molecular marker

1. Introduction

Wheat is one of the most economically important crops globally. *Dasypyrum villosum* is an important wild relative of wheat that contains many useful genes potentially contributing to plant resistance traits to both biotic and abiotic stresses (Gradzielewska 2006). To improve wheat using *D. villosumas* as a genetic resource donor, it is necessary to develop translocation lines between the two species. The T6V#2S·6AL translocation line was developed from

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a cross between the 6V#2 (6A) disomic substitution line and the wheat variety Yangmai 5 (Chen *et al.* 1995). The 6V#2S alien chromosome arm of T6V#2S·6AL carries the powdery mildew (PM) resistance gene *Pm21* (Qi *et al.* 1995) introgressed from a *D. villosum* accession originating from the Cambridge Botanical Garden in England (Liu *et al.* 1983). Many specific molecular markers have been developed to track the 6V#2S chromosome in a wheat genetic background (Qi *et al.* 1996; Liu *et al.* 1999; Cao *et al.* 2006; Chen *et al.* 2006; Wang *et al.* 2007). Several structural variants with different 6V#2S deletions were obtained *via* spontaneous deletion identification and by using pollen radiation (Qi *et al.* 1998; Chen *et al.* 2008). The use of various 6V#2S deletion lines combined with PM resistance tests enabled the *Pm21* gene to be further physically located on the region between 6V#2S bins 0.45–0.58 (Qi *et al.* 1998; Chen *et al.* 2008). Further, a putative serine/threonine protein kinase gene, *Stpk-V*, was characterized to be located at the *Pm21* locus by applying molecular and cytogenetic techniques, and a major gene for PM resistance at the *Pm21* locus (Cao *et al.* 2010).

Different *D. villosum* germplasm resources likely contain different PM resistance genes. The following lines exhibiting PM resistance were created using *D. villosum* accession No. 1026, which was introduced to Chinese germplasm collections from the former Union of Soviet Socialist Republics (USSR), including durum wheat — *D. villosum* amphidiploids — TH1, TH1W, TH2, TH3, and TH3W; chromosome substitution lines 94G22-1, 94G25-1, 94G32-1, and 94G33-1 (Chen *et al.* 1997; Shang *et al.* 1997). Meanwhile, by crossing and back-crossing TH3 with the PM susceptible wheat variety Wan7107, combining immature embryo/anther culture in the breeding course, three T6V#4S·6DL chromosome translocation lines Pm97033, Pm97034, and Pm97035 resistant to PM were developed (Li *et al.* 2005b).

Compared with the T6V#2S·6AL translocation line, the T6V#4S·6DL translocation line has not been well studied. One of the major reasons for this is that the PM resistance from both materials is from 6VS of *D. villosum*, so the resistance genes in the two translocation lines might be the same.

Li *et al.* (2001) documented the differences among *D. villosum* accessions for disease resistance reactions and found that different derivative lines with 6V or 6VS showed varying resistance levels to both *Wheat streak mosaic virus* (WSMV) and wheat curl mite (WCM), the vector of WSMV. A screened RAPD marker, $OPAL03_{750}$, is specific to T6V#4S·6DL and its amphidiploid and *D. villosum* parent (Li *et al.* 2005b). This was the first molecular marker to distinguish 6V#4 from 6V#2. Additionally, restriction fragment length polymorphism (RFLP) analysis of the two 6VS chromosomes also showed polymorphism (Li *et al.* 2005a). Previously, we isolated a *Stpk-V* homologous gene, *Stpk-V3*, in T6V#4S·6DL translocation line Pm97033, and found polymorphism between the two homologous genes (in both intron and regulatory (5′-UTR) regions). We further confirmed that the transcript levels of the two genes differed following challenge with the PM pathogen (Zhang *et al.* 2012; Lin *et al.* 2013). A recently developed anonymous marker, *MBH1*, can distinguish *Pm21* and *PmV* loci on 6V#2S and 6V#4S unambiguously, respectively (Bie *et al.* 2015). These studies together indicated that the 6V#2S and 6V#4S chromosome arms from different *D. villosum* accessions were different in many DNA sequences. However, it is still unknown if their divergence is enough to affect the pairing and the crossover between the two chromosome arms, which is the base for testing their allelism on PM resistance.

Due to the presence of the pairing homoeologous1 (*Ph1*) gene in allohexaploid wheat, chromosome pairing occurs only between homologous chromosomes. Interestingly, a product encoded by *Ph1* gene can detect some degree of divergence among DNA sequences involved in the recombinational machinery, and exclude them from the crossover pathway (Naranjo and Benavente 2015). It was reported that *D. villosum* chromosomes 6V#1 and 6V#2 do not pair or recombine at meiosis metaphase I in most cells in a wheat background (Qi *et al.* 1998). Thereby, it would be very interesting to see the pairing behavior of the two different chromosome arms of 6V#2S and 6V#4S. In this study, the two different translocated chromosomes T6V#2S·6AL and T6V#4S·6DL were pyramided by crossing the two corresponding translocation lines, and the chromosome pairing behavior of 6V#2S and 6V#4S was investigated in the PMCs of the F_1 hybrid at meiosis. The transmission rate of the T6V#4S·6DL chromosome in the F_2 generations was analyzed, and the homozygous plants with double translocated chromosomes were screened using a specific molecular marker. Our results will provide new information on the structural divergence and inheritance performance for 6V#2S and 6V#4S, and help the development of new genetic materials with ideal PM resistance.

2. Materials and methods

2.1. Plant materials

T6V#2S·6AL translocation line 92R137 was kindly provided by Prof. Chen Peidu of Nanjing Agricultural University, China. A new T6V#2S·6AL translocation line 12X141 was developed by Prof. Chen Xiao in our group using 92R137 as the female parent and wheat variety Wan7107 as a recurrent parent for 7 times backcrossing and then self-crossing for 5 times. *D. villosum* accession No. 1026 was introduced from the former Soviet Union by Prof. Kun Tao at the Institute of