Identification of Wheat–*Dasypyrum breviaristatum* addition lines with stripe rust resistance using C-banding and genomic in situ hybridization

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ABSTRACT

The species *Dasypyrum breviaristatum* (genome V^bV^b) has many agronomically important traits, which can be used to wheat improvement. The progenies from the cross and backcross of wheat - *D. breviaristatum* partial amphiploid TDH-2 with common wheat lines were used to produce the wheat-*Dasypyrum* introgression lines. Both stripe rust resistance screening and the diagnostic PCR analysis confirmed that the introduced *D. breviaristatum* chromatin were responsible to the strip rust resistance in the introgression lines. The sequential C-banding and Genomic in situ hybridization (GISH) analysis of TDH-2 indicated that the C-banding karyotype of V^b chromosomes of *D. breviaristatum*, temporarily named V^b1-7, are significantly different from the V genome of *D. villosum*. Two new wheat–*Dasypyrum* addition lines A6-7 and Y88-15 with high resistance to strip rust were developed, and C-banding revealed that they contained the V^b3 and V^b7 chromosomes from *D. breviaristatum*, respectively. The new wheat-*Dasypyrum* addition lines will be a promising donor to produce stripe rust resistance wheat translocation lines for wheat breeding.

INTRODUCTION

The genus *Dasypyrum* (or *Haynaldia*), consisting of two species, *Dasypyrum villosum* and *D. breviaristatum*, has many agronomically important traits including disease resistance, high protein quality, and drought tolerance and, therefore, is a valuable source for wheat improvement[1]. Since the diploid *D. breviaristatum* genotypes were found, the symbol V^b was assigned to genome of *D. breviaristatum*, which is distantly related to the V genome of *D. villosum* [2]. The *D. villosum* has been extensively hybridized to wheat, and several disease resistance genes were successfully transferred to wheat[3]. However, a few studies concerned the introduction of *D. breviaristatum* chromatin to wheat[4].

Stripe rust of wheat caused by *Puccinia striiformis* f. sp. *tritici* has been periodically epidemic and severely damaged wheat production both in China and throughout the world. More and more wheat cultivars are broke down by the incursion of mutative physiological races, it is urgent to develop novel wheat varieties containing new stripe rust resistance genes. In the present study, we produced and characterized wheat- *D. breviaristatum* addition lines with aim to transfer novel rust resistance genes to wheat.

MATERIALS AND METHODS

Plant materials

Wheat lines Chinese Spring (CS), Mianyang11 (MY11), and wheat- *D. breviaristatum* partial amphiploid (TDH-2) were provided by Sichuan Agricultural University, China. *D. breviaristatum* accession was obtained from Dr. Harold Bockelman, National Plant Germplasm System (NPGS), USDA-ARS, Aberdeen, Idaho USA. Wheat- *D. briviaristatum* introgression lines were produced from the F7 population of the crosses between MY11 and TDH-2.

Stripe rust resistance testing and PCR amplification

The powdery mildew resistances were identified at seedlings in the field by inoculating stripe rust mixed isolates of CYR31 and CYR32, and the response observations were according to Yang et al (2005) [4]. The *Dasypyrum* specific PCR primer pairs H12F and H12R, and the PCR amplification programs were executed according to Yang et al (2006)[5].

C-banding and Genomic in situ hybridization (GISH) analysis

Chromosome C-banding of metaphases from root tips was according to references reported (Ren and Zhang, 1995) [6]. Using *D. breviaristatum* genomic DNA as probe and CS as block, GISH was as described in Mukai et al (1993)[7].

RESULTS AND DISCUSSION

Stripe rust testing of the wheat- *D. breviaristatum* introgressions

Wheat lines CS, MY11 and CY12, *D. breviaristatum*, wheat-*D. breviaristatum* partial amphiploid TDH-2, and introgression lines A6-5 and Y88-17 were inoculated with stripe rust races to investigate the disease resistance. The *D. breviaristatum* was immune to these isolates, implying that *D. breviaristatum* genome carried novel stripe rust resistance genes. The introgression lines A6, Y88-17 plants showed the high resistance to stripe rust, while its wheat parents CS and MY11 were highly susceptible. It is suggested that the stripe rust resistance from *D. breviaristatum* expressed in the wheat background.

PCR analysis of the resistance lines

The reported primer pair H12F and H12R specifying to target Sabrina-like LTR sequence pDb12H for *D. breviarisatatum* was used to amplify the DNA of

the resistant lines A6 and Y88-17(Figure 1). The result showed that all resistant plants give rise to the target bands, while the remaining susceptible lines have no amplification. The fact further supported that the introduced *D. breviaristatum* chromatin was responsible for the stripe rust resistance.

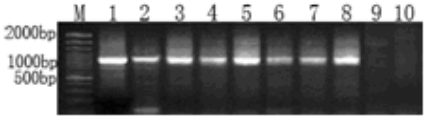

Figure 1. PCR of pDb12H fragments in wheat-*D. breviaristatum* introgressions.

M: DNA Ladder, 1: *D. breviaristatum*, 2: TDH-2, 3-5: A6, 6-8: Y88-17; 9: CS, 10: MY11

Chromosomal identification of the wheat- *D. breviaristatum* addition lines

By using the sequential C-banding and GISH, we can distinguish the *D. breviaristatum* chromosomes in TDH-2, and obtained the C-banding karyotype of seven pairs of *D. breviaristatum* chromosomes, temporally named Vb1-Vb7 (Fig.2). Based on the reported the C-banded pattern of *D. villsoum* chromosomes [8], the *D. breviaristatum* had much lower terminal bands, the fact suggesting that the V^b genome D. breviaristatum differed from the V geneome of *D. villsoum*.

Figure 2. The karyotype of the *D. breviaristatum* chromosomes in TDH-2

Chromosomal counting, C-banding and GISH were performed to the resistant lines A6-5 and Y88-17. The results revealed that their chromosome numbers were 43 to 44. The GISH revealed the introduction of a pair of *D. breviaristatum* chromosomes. C-banding indicated that plant A6-5 introduced the chromosomes were V^b3 with only the strong bands around the centromere (Figure 3), while Y88-17 contained a pair of long chromosomes with faint bands, which thus to be chromosome V^b7, respectively. Therefore, it is concluded that the two *D. breviaristatum* chromosomes carried stripe rust resistance genes.

In the present study, the wheat - *D. breviaristatum* addition lines containing V^b3 and V^b7 were high resistance to stripe rust. The addition lines A6-5 and Y88-17 had been used as a donor to induce the translocation lines to transfer the resistance genes to cultivated wheat by using the chromosomal manipulation strategies, and the *D. breviaristatum* specific marker pDb12H can efficiently assist to target the chromosome introgression.

ACKNOWLEDGEMENTS

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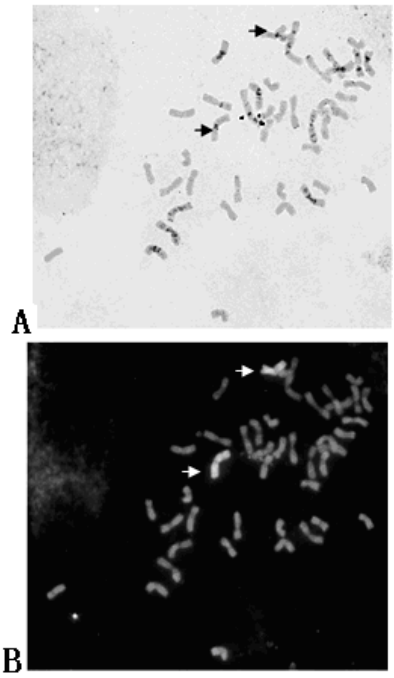

Figure 3. C-banding (A) and GISH (B) patterns of A6-5 plant with V^b3 chromosomes arrowed.

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