

Molecular and cytogenetic identification of new wheat-*Dasypyrum breviaristatum* additions conferring resistance to stem rust and powdery mildew

Cheng Liu¹), Guangrong Li¹), Hongfei Yan²), Jianping Zhou¹), Lijun Hu¹), Mengping Lei¹), Ling Ran¹) and Zujun Yang*¹)

¹) School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan, China

²) College of Plant Protection, Agricultural University of Hebei, Baoding 071000, He'bei, China

Two cytologically stable wheat-*Dasypyrum breviaristatum* addition lines, Y93-1-6-6 and Y93-1-A6-4, were identified by integrated molecular and cytogenetic techniques. C-banding and genomic *in situ* hybridization (GISH) showed that Y93-1-6-6 and Y93-1-A6-4 were different wheat-*D. breviaristatum* additions. A total of 51 markers (primer/enzyme combinations), including 6 PCR-based Landmark Unique Gene (PLUG) markers and 45 Sequence-Tagged-Site (STS) markers, were selected from 3,774 primer/enzyme combinations to further characterize these two additions. Marker haplotypes suggested that both *D. breviaristatum* chromosomes in Y93-1-6-6 and Y93-1-A6-4 were rearranged. Stem rust resistance screening indicated that both additions were highly resistant to race RKQQC, whereas only Y93-1-6-6 was resistant to race TTKSK (Ug99). Powdery mildew resistance screening showed that only Y93-1-6-6 was resistant. Pedigree analysis suggested that the stem rust and powdery mildew resistance of Y93-1-6-6 was derived from *D. breviaristatum*, indicating that the *D. breviaristatum* chromosomes in Y93-1-6-6 possess a new powdery mildew resistance gene(s), and new stem rust resistance gene(s). These two additions could be used as stem rust or powdery mildew resistance sources in wheat breeding programs.

Key Words: *D. breviaristatum*, addition line, *Puccinia graminis tritici*, *Blumeria graminis tritici*, GISH.

Introduction

The genus *Dasypyrum* consists of two species, namely *D. villosum* (L.) Candargy and *D. breviaristatum* (Lindb. F.) Frederiksen. The annual diploid *D. villosum* (V genome), is distributed mainly from the Mediterranean region to the Caspian Sea, and in south-western Asia, Russia and the Caucasus areas (Sears 1953), whereas the perennial tetraploid *D. breviaristatum* is mainly distributed in North Africa (Algeria, Morocco), with isolated populations in Greece (Frederiksen 1991, Ohta and Morishita 2001). Recently, a diploid cytotype in *D. breviaristatum* was rediscovered in Morocco, enabling assignment of one genome of *D. breviaristatum* as V^b (Ohta *et al.* 2002). These species of *Dasypyrum* possess many useful characteristics such as disease resistance, high protein quality and tolerance to drought (Qi *et al.* 2011, Yildirim *et al.* 1998). *D. villosum* has been extensively hybridized with wheat, and the disease resistance genes *Pm21*, *PchDv* and *Wss1* were transferred to wheat (Chen *et al.* 1995, Yildirim *et al.* 1998, Zhang *et al.* 2005). However, in comparison with *D. villosum*, research on *D. breviaristatum* is very limited.

Since *Triticum-D. breviaristatum* partial amphiploids were developed (Jiang *et al.* 1992), we initiated the introduction of the rust and powdery mildew resistance genes from *D. breviaristatum* to common wheat by crossing the amphiploids with cultivated wheat (Yang *et al.* 2005, 2006). We produced a large quantity of wheat-*D. breviaristatum* introgression lines, but the cytogenetic structures of the *D. breviaristatum* chromatin in these introgression lines are still unknown and need further identification.

Wheat powdery mildew, caused by *Blumeria graminis* (DC.) E. Speer f. sp. *tritici* Em. Marchal, is a yield-limiting disease of wheat in environments conducive for disease development. Wheat stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. (*Pgt*), is historically a devastating disease with yield losses exceeding 50% in North Dakota and Minnesota, USA, during epidemic years (Leonard 2001). Resistance in wheat has prevented serious epidemics in the USA since 1956 and worldwide over the last several decades (Leonard 2001), but a new race of *Pgt* known as Ug99 and characterized as race TTKSK (Jin *et al.* 2007), is virulent on the majority of the wheat cultivars worldwide and is spreading throughout Africa and the Middle East. Variants of race TTKSK further broaden the virulence spectrum of race TTKSK and derivatives (Jin *et al.* 2009). New sources of resistance are needed for protection against both the powdery mildew and stem rust pathogens.

Communicated by T. Komatsuda

Received July 28, 2011. Accepted August 12, 2011.

*Corresponding author (e-mail: yangzujun@uestc.edu.cn)

The purpose of this study was to characterize wheat-*D. breviaristatum* additions carrying powdery mildew and stem rust resistances that could be further used as new resistance sources in wheat breeding. In order to achieve this, we analyzed the progeny of crosses between a *Triticum-D. breviaristatum* partial amphiploid and wheat lines using C-banding, molecular markers and genomic in situ hybridization (GISH).

Materials and Methods

Plant materials

D. breviaristatum (PI 546317) was obtained from Dr. Harold Bockelman, National Plant Germplasm System (NPGS), USDA-ARS, Aberdeen, Idaho, USA. Wheat line 99E-18 was provided by Sichuan Agricultural University, China. Wheat line Longfu 10 was provided by the Crop Breeding Institute, Heilongjiang Academy of Agricultural Sciences, China. ML19 was provided by International Maize and Wheat Improvement Center (CIMMYT). A wheat-*D. villosum* 6AL/6VS translocation line was provided by Prof. PD Chen, Nanjing Agricultural University, China. *Triticum-D. breviaristatum* partial amphiploid TDH-2 was created and provided by Prof. HR Jiang, Sichuan Agricultural University, China. Wheat-*D. breviaristatum* BC₁F₈ derivatives included Y93-1-6 (involving Longfu10 and ML19)-TDH-2 and Y93-1-A6 (involving 99E-18 and ML19)-TDH-2 (Yang *et al.* 2005, 2006).

DNA isolation, STS-, and PLUG-PCR

Total genomic DNA was prepared from young leaves using an SDS protocol (Yang *et al.* 2006). A total of 943 bin-mapped ESTs were selected from the wheat EST mapping project (<http://wheat.pw.usda.gov/NSF/data.html>), of which 223, 96, 100, 100, 96, 100 and 228 bin-mapped ESTs belonged to homologous groups 1 to group 7, respectively. The sequences of these 943 ESTs (<http://www.ncbi.nlm.nih.gov/Genbank/>) were used to design EST-specific primers using the software Primer 3 (<http://frodo.wi.mit.edu>). STS-PCR amplifications were performed as described by Qi *et al.* (2007). To obtain higher levels of polymorphism, the PCR products were digested with the 4-base cutter enzymes *AluI*, *HaeIII*, *MspI* and *RsaI*. The PCR products were separated on a 2% agarose gel. Four PLUG primer pairs were synthesized for each arm of the seven homoeologous group (1–7) chromosomes according to Ishikawa *et al.* (2007), a total of 56 primer pairs. The PLUG-PCR protocol and PCR product digestion followed that of Ishikawa *et al.* (2007).

Chromosome counting, chromosome C-banding and genomic in situ hybridization (GISH)

Chromosome counting, chromosome C-banding and GISH were performed as described by Ren and Zhang (1995). Microphotographs of C-banded chromosomes and GISH chromosomes were taken with an Olympus BX-51 microscope. The digoxigenin labeled genomic DNA signal

was detected with a fluorescein-conjugated antidigoxigenin antibody (Roche Diagnostics). The slide was finally mounted in Vectashield antifade solution (Vector Laboratories, Burlingame, CA, USA) with (0.25 µg/ml) propidium iodide for only FITC detection.

Powdery mildew and stem rust resistance testing

Powdery mildew responses were scored on a 0–9 rating scale according to Leath and Heun (1990) at the seedling and adult plant stages following inoculation with mixed Sichuan (China) and mixed Kansas (USA) powdery mildew races. Stem rust responses were determined on seedlings using isolates of races RKQQC or TTKSK (Ug99) at the USDA-ARS Hard Winter Wheat Genetics Research Unit (Kansas) and USDA-ARS Cereal Disease Laboratory (Minnesota), respectively. Inoculations of *Pgt* isolates were performed as described in Jin *et al.* (2007). The disease responses were recorded on a 0 to 4 rating scale.

Results

Primary identification of wheat-*D. breviaristatum* derivatives using C-banding, chromosome counting and GISH

The karyotype of the *D. breviaristatum* chromosomes in TDH-2, temporarily named V^{b1} to V^{b7}, had already been established (Yang *et al.* 2008). Using C-banding and GISH as screening approaches, two wheat-*D. breviaristatum* addition lines, A6-5 and Y88-15, were selected from wheat-*D. breviaristatum* introgression lines, in which the alien *D. breviaristatum* C-banding patterns were V^{b3} and V^{b7}, respectively. In order to further screen new wheat-*D. breviaristatum* addition lines, C-banding was performed on Y93-1-6 and Y93-1-A6 families of wheat-*D. breviaristatum* derivatives. The results indicated that Y93-1-6-6 and Y93-1-A6-4 were different addition lines with *D. breviaristatum* chromosomes V^{b1} and V^{b5}, respectively. Chromosome counts indicated that both lines had 2n = 44. Genomic *in situ* hybridization (GISH) analysis using *D. breviaristatum* genomic DNA as probe indicated that a pair of *D. breviaristatum* chromosomes showed yellow to green signals in metaphases of both Y93-1-6-6 and Y93-1-A6-4 as shown in Fig. 1A, 1B, further suggesting that Y93-1-6-6 and Y93-1-A6-4 were new wheat-*D. breviaristatum* addition lines.

Molecular marker screening

In order to further identify the homoeologous groups of the *D. breviaristatum* chromosomes in Y93-1-6-6 and Y93-1-A6-4, 943 pairs of STS primers were synthesized for screening STS and PLUG marker polymorphism between *D. breviaristatum* and Chinese Spring (CS). A total of 44 markers were polymorphic. After 4-base cutter enzyme digestions with *HaeIII*, *MspI*, *RsaI* and *AluI*, PCR products from 130, 100, 130 and 70 primers were polymorphic between *D. breviaristatum* and CS, respectively. The rate of polymorphism was about 10% ((44 + 130 + 100 + 130 + 70)/

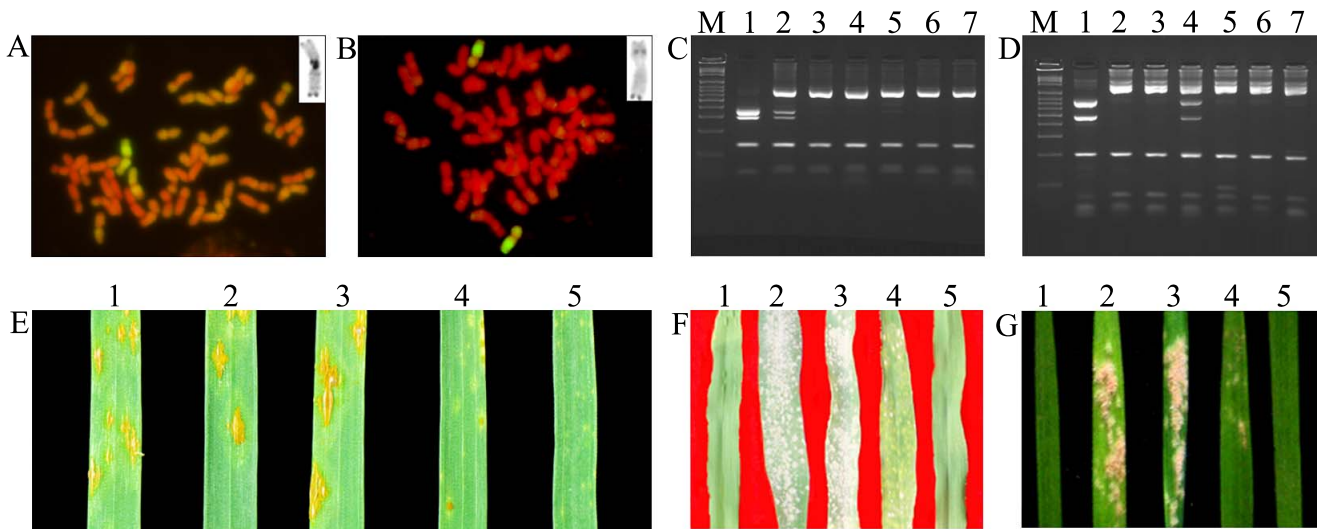


Fig. 1. Cytological, molecular and disease resistance test results from wheat-*D. breviaristatum* addition lines: A and B: GISH of wheat-*D. breviaristatum* addition lines Y93-1-6-6 (A) and Y93-1-A6-4 (B). The top right corners of the figures are the target *D. breviaristatum* chromosome C-banding patterns, which were the same as V^{b1} and V^{b5}, respectively, from Fig. 2 in Yang *et al.* (2008). The scale bar is 20 μ m. C and D: STS-PCR patterns for *D. breviaristatum* (1), Y93-1-6-6 (2), Longfu 10 (3), Y93-1-A6-4 (4), 99E-18 (5), ML19 (6) and CS (7), respectively using BE404728-F, BE404728R (C) and BE517627-F, BE517627 (D) as primer pairs. PCR products were cut by *Rsa*I (C) and *Hae*III (D) separately. M, Marker (DL2000). E: Infection types produced by wheat-*D. breviaristatum* addition lines Y93-1-A6-4 (1), Y93-1-6-6 (4) and their parents Longfu 10 (2), ML19 (3) and TDH-2 (5) when inoculated with *Pgt* race Ug99. F and G: Infection types produced by TDH-2 (1), ML19 (2), Longfu10 (3), Y93-1-6-6 (4) and wheat-*D. villosum* 6AL/6VS translocation line (5) when inoculated with mixed Sichuan (F) and mixed Kansas (USA) *Bgt* races (G) on adult plants.

(943 \times 5)), a value higher than that obtained for rye (5.7%) and *H. villosa* (5.4%) (Cao *et al.* 2009; Wang *et al.* 2009), indicating this set of STS primers can effectively be used for detecting *D. breviaristatum* chromatin in hexaploid wheat genomic background.

We also screened 56 pairs of PLUG primers. One PLUG primer was polymorphic between *D. breviaristatum* and CS. After *Hae*III or *Taq*I digestion, the PCR products from 6 primer pairs were polymorphic between *D. breviaristatum* and CS. The probability of obtaining polymorphism was about 5% (6/(56 \times 2)), lower than using STS. These 6 PLUG markers could thus be used for effective detection of *D. breviaristatum* chromatin in wheat (Table 1).

Identification of wheat-*D. breviaristatum* additions using STS and PLUG markers

PCR was performed on TDB-2, Longfu10, ML19, 99E-18, Chinese Spring (CS), Y93-1-6-6 and Y93-1-A6-4 using the 474 STS marker and 6 PLUG marker candidates obtained above. A total of 45 STS markers and all 6 PLUG markers detected *D. breviaristatum* chromatin in Y93-1-6-6 and Y93-1-A6-4 (Table 1). The PCR patterns for *D. breviaristatum*, Y93-1-6-6, Longfu 10, Y93-1-A6-4, 99E-18, ML19 and CS using BE404728 and BE517627 as primer pairs are displayed in Fig. 1C, 1D. Of these 51 molecular markers, 19 STS markers and 1 PLUG marker identified Y93-1-6-6, whereas 26 STS markers and 5 PLUG markers could be used to identify Y93-1-A6-4.

STS collinear relationships among Triticeae species and

sequence polymorphism among different species (Li *et al.* 2009), suggested that STS markers could be used to identify wheat alien chromosome homoeologous group relationships. In this research, markers from homoeologous groups 1, 2, 6 and 7 identified Y93-1-6-6 (Table 1), suggesting that the *D. breviaristatum* chromosome pair in Y93-1-6-6 was a rearranged chromosome with blocks of chromatin from homoeologous group 1, 2, 6 and 7. In addition, markers from homologous group 6 and 7 identified Y93-A6-4, indicating that the *D. breviaristatum* chromosome pair in Y93-1-A6-4 comprised blocks of homoeologous groups 6 and 7.

Stem rust and powdery mildew resistance responses

Tests with *Pgt* race RKQQC showed that additions Y93-1-6-6, Y93-1-A6-4 and all cross parents were resistant (Table 2). For *Pgt* race TTKSK (Ug99), only wheat-*D. breviaristatum* amphiploid TDH-2 and Y93-1-6-6 were resistant; all the other cross parents and Y93-1-A6-4 were susceptible (Table 2 and Fig. 1E). Therefore, the Ug99 resistance of Y93-1-6-6 came from TDH-2.

Mixed Sichuan (China) powdery mildew isolates and mixed Kansas (USA) powdery mildew isolates were used to identify both seeding and adult plant responses. Responses at both growth stages were consistent. Susceptible control CS and resistant control 6AL/6VS translocation were highly susceptible and immune, respectively. Y93-1-A6-4 was highly susceptible, whereas Y93-1-6-6 was resistant. Y93-1-6-6 parents, ML19, Longfu10 and TDH-2, were moderately susceptible, highly susceptible, and immune, respectively

Table 1. Marker haplotypes of the wheat-*D. breviaristatum* additions^a

| Deletion bin ^b | Markers | Y93-1-6-6 | Y93-1-A6-4 | Homologous relationship | Enzyme |
|--|--------------|----------------|------------|-------------------------|---|
| C-2BL2-0.36 | BE517627-STS | - ^c | + | 2L | <i>HaeIII</i> |
| C-2BL2-0.36 | BE443711-STS | - | + | 2L | <i>HaeIII</i> |
| C-2BL2-0.36 | BE591670-STS | - | + | 2L | <i>RsaI</i> |
| 2BL6-0.89-1.00 | BE444894-STS | - | + | 2L | <i>AluI</i> |
| 2AL3-0.77-1.00; 2BL6-0.89-1.00; 2DL6-0.94-1.00 | TNAC1142-STS | - | + | 2L | ^d |
| C-7DL5-0.30 | BE498418-STS | - | + | 7L | <i>AluI</i> |
| C-7DL5-0.30 | BE498849-STS | - | + | 7L | <i>RsaI</i> |
| C-7DL5-0.30; 7AL1-0.39-0.63; 7BL2-0.38-0.63; 7DL5-0.30-0.61 | BF483007-STS | - | + | 7L | ^d |
| 7AL16-0.86-0.90; 7BL6-0.84-0.86; 7DL3-0.82-1.00 | TNAC1815-STS | - | + | 7L | <i>TaqI</i> |
| 7AL21-0.74-0.86 | BE442572-STS | - | + | 7L | <i>RsaI</i> |
| 7DL3-0.82-1.00 | BF294002-STS | - | + | 7L | <i>HaeIII/RsaI</i> |
| 7AL16-0.86-0.90 | BE518357-STS | - | + | 7L | ^b |
| 7DL3-0.82-1.00 | BE637838-STS | - | + | 7L | <i>MspI</i> |
| 7DL3-0.82-1.00 | BE404744-STS | - | + | 7L | <i>MspI/RsaI</i> |
| 7DL3-0.82-1.00 | BG314261-STS | - | + | 7L | <i>HaeIII/AluI</i> |
| 7DL3-0.82-1.00 | BG262960-STS | - | + | 7L | <i>AluI</i> |
| 1BL3-0.85-1.00 | BE446672-STS | - | + | 1L | <i>HaeIII/MspI/RsaI</i> |
| 1BL3-0.85-1.00 | BE591325-STS | - | + | 1L | <i>RsaI</i> |
| C-1BL6-0.32; 7AS2-0.73-0.83; 7BS2-0.27-1.00; 7DS4-0.73-1.00 | BE405613-STS | - | + | 1L | <i>RsaI</i> |
| 7AS1-0.89-1.00 | TNAC1782-STS | - | + | 7S | <i>TaqI</i> |
| 6AS1-0.35-0.65; 6BS4-0.46-0.76; 6DS1-0.54-0.79 | BF473884-STS | - | + | 7S | <i>RsaI</i> |
| C-6AS1-0.35 | TNAC1683-STS | - | + | 6S | <i>TaqI</i> |
| 6DS6-0.99-1.00 | BE445239-STS | - | + | 6S | <i>MspI/RsaI</i> |
| C-6AL4-0.55; C-6BL3-0.36; 6DL6-0.29-0.47 | BG262210-STS | - | + | 6S | <i>MspI</i> |
| C-6BL3-0.36 | TNAC1702-STS | + | - | 6L | <i>TaqI</i> |
| C-6BL3-0.36 | BE498480-STS | + | - | 6L | <i>RsaI</i> |
| C-6BL3-0.36 | BE443951-STS | + | - | 6L | <i>MspI/RsaI</i> |
| 6AL8-0.90-1.00 | BE637963-STS | + | - | 6L | <i>HaeIII</i> |
| C-6BL3-0.36 | BE404912-STS | + | - | 6L | <i>MspI</i> |
| 6DS4-0.79-0.99 | BE591939-STS | + | - | 6S | <i>MspI</i> |
| C-7BS1-0.27 | BE404728-STS | + | - | 7S | <i>RsaI</i> |
| C-7DS5-0.36 | BE585744-STS | + | - | 7S | <i>HaeIII/MspI/RsaI/AluI</i> ^e |
| 7DS4-0.61-1.00 | BE606678-STS | + | - | 7S | <i>HaeIII/RsaI</i> |
| 7DS5-0.36-0.61 | BE489493-STS | + | - | 7S | <i>HaeIII</i> |
| 7DS5-0.36-0.61 | BG262748-STS | + | - | 7S | <i>HaeIII/MspI/RsaI/AluI</i> |
| 7DS4-0.61-1.00 | BE586111-STS | + | - | 7S | <i>RsaI</i> |

^a Polymorphism was obtained from PCR amplification (and sometimes also with PCR products digested by 4-base cutter enzymes) by all the primers listed in Table 1.

^b Consensus bin location of mapped ESTs taken from http://wheat.pw.usda.gov/cgi4bin/westsql/map_locus.cgi.

^c -, no polymorphism, +, polymorphism detected.

^d Enzyme digestion is not necessary for these primers.

^e Polymorphic PCR products following digestion by *HaeIII*, or *MspI*, or *RsaI*, or *AluI* indicated as Primer/*HaeIII/MspI/RsaI/AluI*.

Table 2. Responses of addition lines and relevant parental materials to stem rust and powdery mildew

| Plant name | <i>Pgt</i> race RKQQC on seedling plants | <i>Pgt</i> race TTKSK (Ug99) on seedling plants | Mixed Kansas (USA) <i>Bgt</i> races on seedling and adult plants | Mixed Sichuan (China) <i>Bgt</i> races on seedling and adult plants |
|------------|--|---|--|---|
| Y93-1-6-6 | ;1 | ;2 = LIF ^a | 3 | 1 |
| Y93-1-A6-4 | ; | 3+ | 6 | 7 |
| TDH-2 | ; | ; | 0 | 0 |
| 99E-18 | ;1 | 4 | 3 | 4 |
| ML19 | 2- | 4 | 6 | 6 |
| Longfu10 | 1- | 3+ | 7 | 7 |
| CS | 2+ | 4 | 6 | 7 |
| 6AL/6VS | NT ^b | NT | 0 | 0 |

^a Low infection frequency.

^b Not tested.

(Table 2 and Fig. 1F, 1G). Presumably, the powdery mildew resistance in Y93-1-6-6 came from TDH-2.

Discussion

New wheat-D. breviaristatum additions identification and chromosome rearrangements

A large number of wheat-*D. breviaristatum* introgression lines were developed by crossing wheat-*D. breviaristatum* amphiploids with cultivated wheat. From the BC₁F₈ introgression lines, Yang *et al.* (2008) selected two stripe rust resistant addition lines. In this study, we further selected and identified two new wheat-*D. breviaristatum* additions, Y93-1-6-6 and Y93-1-A6-4, using molecular markers, C-banding and GISH. The marker data indicated that the *D. breviaristatum* chromosomes in addition lines Y93-1-6-6 and Y93-1-A6-4 were rearrangements of at least two homoeologous groups. Previous FISH data using rye genome repetitive DNA pSc74 as a probe to hybridize *Dasyphyrum* species also showed that *D. breviaristatum* chromosomes were rearranged (Liu *et al.* 2010) as confirmed with marker data herein. This is the first report of rearranged *D. breviaristatum* chromosomes using molecular evidence. In the Triticeae, rearrangements of 3 or more homoeologous groups were reported only in rye (Devos *et al.* 1993). Translocations 6NL-2NS in *Aegilops ventricosa* (Tanguy *et al.* 2005) and 1NS-5NS and 1NL-5NL in *Ae. uniaristata* (Friebe *et al.* 1996) were also reported. Recently, Yang *et al.* (2010) also found rearranged P chromosome in a wheat-*Agropyron cristatum* addition line which suppresses *Ph* gene expression. Though several chromosome rearrangements were reported, we do not know why and how they rearrange.

D. breviaristatum chromosomes in Y93-1-6-6 may possess a new powdery mildew resistance gene(s)

Powdery mildew is one of the most prevalent wheat diseases in China. Wheat cultivars resistant to powdery mildew are the most economical and effective way to control the disease (He *et al.* 2009). Wild relatives of wheat contain resistance genes that can be exploited in wheat. To date, 57 powdery mildew resistance genes at 40 loci have

been formally designated (McIntosh *et al.* 2008). Nevertheless, many wheat cultivars contain common powdery mildew resistance genes such as *Pm2*, *Pm4b*, *Pm5*, *Pm6* and *Pm8* (Zeller and Hsam 1998), whereas widely effective resistance genes, such as *Pm1c*, *Pm12*, *Pm13*, *Pm16* and *Mlxbd*, are not widely used in cultivar improvement because they are either not in well adapted genetic backgrounds or because of linkage drag associated with alien chromosome segments (Qiu and Zhang 2004). Thus, there is a continuous need for identifying new sources of powdery mildew resistance. In the present research, wheat-*D. breviaristatum* addition line Y93-1-6-6 has powdery mildew resistance from *D. breviaristatum*. This gene (temporarily named as *PmDb1*) appears to be a new gene. TDB-2 and addition lines Y93-1-6-6 identified herein could be used as sources of resistance to powdery mildew following further characterization and chromosome engineering.

D. breviaristatum chromosomes in Y93-1-6-6 may possess a Ug99 resistance gene

Stem rust, is a major threat to wheat production in some wheat growing areas. Jan *et al.* (1986) first reported stem rust resistance transferred from *D. villosum* to wheat, but no subsequent studies were reported. Recently, Qi *et al.* (2011) transferred resistance gene, *Sr52*, effective against race Ug99, from *D. villosum* to wheat via a wheat-*D. villosum* 6AS.6VL Robertsonian translocation. There are no reports on transference of stem rust resistance from *D. breviaristatum* to wheat. In the current research, wheat-*D. breviaristatum* amphiploid TDH-2 was nearly immune to *Pgt* races RKQQC and Ug99, whereas the control CS was highly susceptible, indicating the presence of a resistance gene(s) derived from *D. breviaristatum* in TDH-2. Both addition lines identified herein and their cross parents were resistant to race RKQQC, possibly due to *Sr31*, a common gene in Chinese wheats (Yang *et al.* 2005), but only TDH-2 and Y93-1-6-6 were resistant to race Ug99. This latter resistance may be derived from *D. breviaristatum*. The higher resistance in TDH-2 compared with Y93-1-6-6 suggests the presence of a further resistance gene in the partial amphiploid. In conclusion, TDB-2 and addition line Y93-1-6-6 could be used as sources

of resistance to *Pgt* race Ug99 following further characterization and chromosome engineering.

Acknowledgements

We are grateful to Bernd Friebe (Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, USA) for help in chromosome recognition. We are indebted to Robert Bowden (USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, Kansas, USA), and Matthew Rouse and Yue Jin (USDA-ARS Cereal Disease Laboratory, St. Paul, Minnesota, USA) for performing tests with *Pgt* races RKQQC and TTKSK (Ug99), respectively. We also thank Matthew Rouse, Bob McIntosh (the University of Sydney) and three anonymous reviewers for reviewing this manuscript. We are thankful to the National Natural Science Foundation of China (No. 30671288 and 31171542), Fundamental Research Funds for the Central Universities of China (ZYGX2010J099) for their financial support.

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