Molecular cytogenetic identification of a novel dwarf wheat line with introgressed *Thinopyrum ponticum* chromatin

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Novel dwarfing germplasms and dwarfing genes are valuable for the wheat breeding. A novel semi-dwarf line, 31505-1, with reduced height compared with its common wheat parent, was derived from a cross between common wheat and *Thinopyrum ponticum*. Cytological studies demonstrated that 31505-1 contained 42 chromosomes and formed 21 bivalents at meiotic metaphase I. Genomic *in situ* hybridization (GISH) analysis showed that 31505-1 had no large *Th. ponticum* chromosome fragments. Fluorescence *in situ* hybridization (FISH) results revealed the absence of a pAs1 hybridization band on 2DL chromosome of 31505-1. Two SSR markers (*Xwmc41* and *Xcfd168*) and two STS markers (*Xmag4059* and *Xmag3596*), which were located on 2D chromosome, amplified unique bands of *Th. Ponticum* in 31505-1. These revealed presence of an introgressed *Th. ponticum* segment in 2DL chromosome of dwarf line 31505-1, although the alien segment could not be detected by GISH.

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1. Introduction

Semi-dwarfism is an important trait in crop breeding because it increases lodging resistance and possibly the proportions of assimilates delivered into the grains. Short-statured cultivars in wheat and rice were the basis of the 'Green Revolution' in the 1960s (Hedden 2003) and are now used by plant breeders worldwide.

In wheat, 20 semi-dwarfing loci (*Rht*) and 25 alleles are associated with semi-dwarf growth habit (Konzak 1987), including 11 alleles found naturally, viz. *Rht-B1b, Rht-B1c* (formerly *Rht3*)*, Rht-B1d, Rht-B1e, Rht-B1f* located on 4B, *Rht-D1b*, *Rht-D1c* (formerly *Rht10*), *Rht-D1d* located on 4D, *Rht8* located on 2DS, *Rht9* located on 7BS and *Rht6*. A further 14 alleles were obtained by mutagenesis, including *Rht-B1g*, *Rht4* (located on 2BL), *Rht5* (3BS), *Rht7* (2A), *Rht11*, *Rht12* (5AL), *Rht13* (7BS), *Rht14*, *Rht15*, *Rht16*, *Rht17*, *Rht18*, *Rht19* and *Rht20.*

Although many semi-dwarfing genes have been reported, only a few are used in wheat breeding programmes. Alleles *Rht-B1b* and *Rht-D1b*, which were transferred from the Japanese cv. 'Norin 10', are widely used in wheat breeding worldwide. Evans (1998) showed that *Rht-B1b* and *Rht-D1b* were present in over 70% of the registered wheat cultivars globally, and Guedira *et al.* (2010) reported that 90% of recent wheat varieties in the United States possessed *Rht-B1* and *Rht-D1* alleles. Most semi-dwarf wheat cultivars from Europe and Asia contain *Rht8*, another widely used height-reducing gene which was introduced from the Japanese landrace 'Akakomugi' (Borojevic and Borojevic 2005). *Rht8* is located on chromosome 2D (Korzun *et al.* 1998; Worland *et al.* 1998).

Keywords. Dwarf; introgression line; *Thinopyrum ponticum*; wheat

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Thinopyrum ponticum (Podp.) Barkw. and D. R. Dewey [syn *Lophopyrum ponticum* (Podp.) Löve, syn *Elytrigia pontica* (Podp.) Holub. and syn *Agropyron elongatum* (Host) Beau.] (2n =70, StStStStEeEeEbEbExEx) is a tertiary gene pool carrying many potentially favourable traits for wheat improvement (Jiang *et al.* 1993, 1994; Friebe *et al.* 1996; Oliver *et al.* 2006). A number of disease-resistant genes, including *Lr19*, *Lr24* and *Lr29* (Knott 1968; Sears 1973, 1977), *Sr25* and *Sr26* (McIntosh *et al.* 1977; Jin *et al.* 2007), *Cmc2* (Whelan *et al.* 1986), *Qfhs.pur-7EL* (Shen and Ohm 2007), as well as the genes controlling salt tolerance (Chen *et al.* 2004), yield and biomass (Reynolds *et al.* 2001; Monneveux *et al.* 2003), were transferred from the *Th. ponticum* to wheat.

A wheat-*Th. ponticum* addition line 31504, with reduced plant height, was developed from a cross between wheat cultivar Lumai 5 and wheat-*Th. ponticum* partial amphiploid Xiaoyan 7631. Genetic analysis showed that a reduced height gene in line 31504 was probably derived from *Th. ponticum* (Li *et al.* 1996). And a substitution line 31505 was obtained among selfed derivatives of 31504.

In this study, a probable translocation line, 31505-1, was obtained after backcrossing 31505 to Lumai 5. Cytological study, genomic *in situ* hybridization (GISH), fluorescence *in situ* hybridization (FISH) and molecular marker analysis were conducted to identify the genomic composition of the dwarf line 31505-1.

2. Materials and methods

2.1 *Plant materials*

Materials included wheat lines 31504, 31505, 31505-1, Lumai 5, Chinese Spring and wheat-*Th. ponticum* partial amphiploid Xiaoyan 7631; *Th. ponticum* (2n=70). Semi-dwarf line 31504 (2n=44) was developed from a cross between wheat cv. Lumai 5 and Xiaoyan 7631. Semi-dwarf line 31505 was obtained in the selfed progeny of 31504, and line 31505-1 was selected from BC_1F_4 population of 31505/Lumai 5. All three lines are about 50 cm in height.

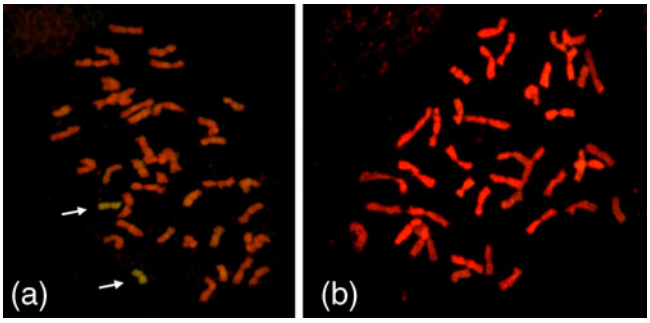

Figure 1. GISH of mitotic metaphase cells of 31505 (**a**) and 31505-1 (**b**), using labeled genomic DNA of *Th. ponticum* as probe and genomic DNA of 'Chinese Spring' for blocking. A GISH-labelled pair of chromosomes (arrows) is clear in (**a**), but not in (**b**).

2.2 *Mitotic and meiotic analyses*

Seeds were germinated at 25°C on moist filter paper in Petri dishes for 1–2 days, then transferred to 4°C for approximately 24 h, before returning to 25°C. Roots 1–2 cm in length were cut and treated in ice water for approximately 24 h before fixation in Carnoy's solution. After fixation, they were stained and squashed in carbol fuchsin and mitotic chromosomes were observed under an optical microscope. Spikes at the booting stage were sampled and anthers at Meiotic metaphase I were fixed in Carnoy's solution. For MI examination anthers were placed in 1 mol/L HCl at 60°C for 6–8 min, and squashed in 1% acetocarmine.

2.3 *GISH*

Total genomic DNA from *Th. ponticum* to be used as a probe was labelled with digoxigenin-11-dUTP by the nick translation. Sheared genomic DNA from Chinese Spring was used for blocking. Detailed procedures of chromosome preparation and hybridization were described by Bao *et al.* (2009). GISH signals were detected with fluorescein-conjugated anti-digoxigenin antibodies, and the slides were finally mounted within a thin layer of Vectrashield antifade solution containing propidium iodide (PI). Photographs were captured with an Olympus BX-61 fluorescence microscope equipped with a CCD (charge-coupled device) camera.

2.4 *FISH*

Multicolour FISH was carried out following the protocols of GISH with two probes, pAs1 labelled with digoxigenin-11-dUTP and pSc119.2 labeled with biotin-11-dUTP. Two probes were mixed 1:1 before hybridization. After hybridization, anti-digoxigenin-FITC and avidin-rhodamine were used for simultaneous detection of the two probes. The slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI).

2.5 *Molecular marker analyses*

SSR, EST-SSR and STS markers, located on 2D chromosome, were used to identify the genomic composition of 31505-1. Relevant information regarding G-SSR markers, including those with BARC, CFA, CFD, CFT, GWM, GDM, GPW, WMC and PSP codes, as well as PCR-based STS markers with

the MAG code, were taken from the GrainGenes Website (*http://wheat.pw.usda.gov)*.

Each PCR was conducted in a total volume of 25 µl in a Bio-Rad 9600 thermal cycler, following the proportion described by Röder *et al.* (1998). Amplifications were performed using a touchdown PCR protocol detailed by Hao *et al.* (2008). PCR products were run under standard conditions on 6% polyacylamide gel and visualized by silver staining.

3. Results

3.1 *Cytological study of dwarf lines 31505 and 31505-1*

The plant height of Lumai 5 was 85–90 cm, whereas 31504, 31505, 31505-1 were 50–55 cm. Multi-year and location experiments showed that the semi-dwarf phenotype was stable, and that 31505-1 also exhibited similar agronomic traits and equivalent fertility to its parent Lumai 5.

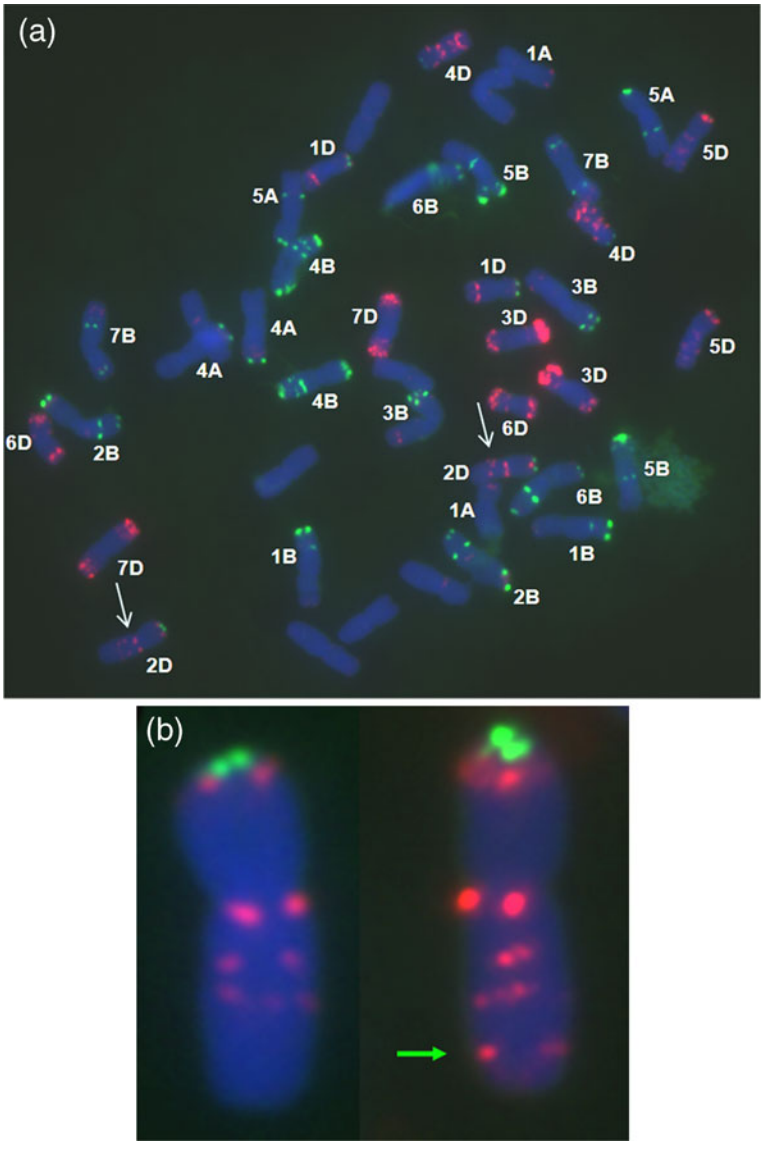

Figure 2. Multicolor fluorescence *in situ* hybridization (FISH) of mitotic metaphase cells of 31505-1 (**a**), and the hybridization signals of 2D chromosome in 31505-1 and Chinese Spring (**b**). Labelled pAs1 (red) and pSc119.2 (green) were used as probes. Arrows show the difference bands of 2D chromosome.

Table 1. Amplified results of the four mapped markers

Primers	*Xmag4059*	*Xcfd168*	*Xwmc41*	*Xmag3596*
Th. ponticum	245 bp	no bands	175 bp	273 bp
31504	245 bp	no bands	175 bp	273 bp
31505	245 bp	no bands	175 bp	273 bp
31505-1	245 bp	no bands	175 bp	273 bp
Lumai 5	no 245 bp bands	215 bp	180 bp	270 bp
Chinese Spring	no 245 bp bands	215 bp	180 bp	270 bp
N2BT2A	no 245 bp bands	215 bp	180 bp	270 bp
N2AT2B	no 245 bp bands	215 bp	180 bp	270 bp
N2DT2A	no 245 bp bands	no 215 bp bands	no 180 bp bands	no 270 bp bands

Analysis of root tip cells proved that both 31505 and 31505-1 had the chromosome number with 2n=42. Investigation of the meiosis showed that most observed cells of these two lines had 21 bivalents at meiotic MI, which indicated that these two lines were cytologically stable.

GISH analysis was conducted in order to understand the chromosome composition of 31505 and 31505-1. The results showed that among the 42 chromosomes in 31505 root-tip cells, 2 chromosomes presented greenish-yellow hybridization signals, while the remaining 40 chromosomes showed a uniform red fluorescence (figure 1a). These results indicated that the 31505 had a pair of chromosomes from *Th. ponticum* substituting for a pair of wheat chromosomes. No differential fluorescence was observed in line 31505-1 (figure 1b), indicating either that it had no chromatin from *Th. ponticum* or that an alien segment if present was too small to be visualized by GISH.

FISH analysis using pSc119.2 and pAs1 as probes was also carried out to identify the chromosome composition of 31505-1 (figure 2). Two specific pSc119.2 hybridization bands (red) were observed on the long arm of 2D chromosomes in 31505-1, while there were three bands on 2DL chromosome of Chinese Spring. A pSc119.2 hybridization bands was missing on subtelomeric region of 2DL chromosome of 31505-1. Results indicated that there was structural change happened on 2DL chromosome of 31505-1, probably included the introgressed chromatin from *Th. ponticum*.

3.2 *Molecular characterization of the translocations in 31505-1*

In order to confirm if the introgressed chromatin from *Th. ponticum* presented in wheat chromosome 2D of 31505-1, SSR, EST-SSR and STS markers located on 2D were used to test the lines 31505-1, 31505, 31504, *Th. ponticum*, Lumai 5, Chinese Spring and CS nullisomic-tetrasomic lines.

Among 67 molecular markers tested, two SSR markers (*Xwmc41* and *Xcfd168*) and two STS markers (*Xmag4059* and *Xmag3596*) amplified unique bands of *Th. ponticum* in 31505-1 (table 1; figures 3, 4 and 5). The polymorphic fragment *Xwmc41*/175 bp were presented both in *Th. ponticum*, 31504, 31505 and 31505-1, while polymorphic fragment *Xwmc41*/180 bp was observed in Chinese Spring and Lumai 5. The absence of PCR products *Xwmc41*/180 bp in the N2DT2A line and their presence in the N2BT2A and N2AT2B lines further confirmed the assignment of the linked microsatellite markers *Xwmc41* to the chromosome 2D. Amplified results of STS markers *Xmag4059* and *Xmag3596* were consistent with marker *Xwmc41*, they also amplified unique bands of *Th. ponticum* in 31505-1, 31505 and 31504. SSR

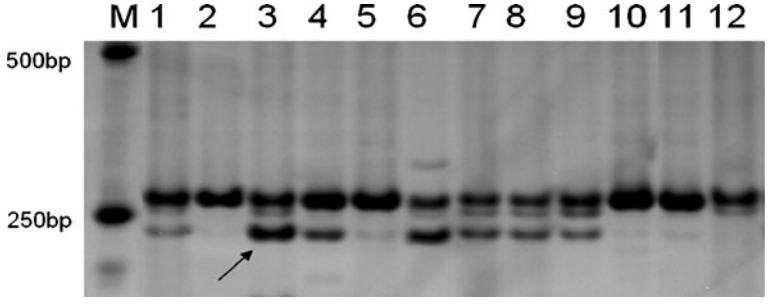

Figure 3. Amplified PCR products of SSR primer pairs *Xmag4059*. M, Marker 1, *Th. ponticum* 2, Chinese Spring 3, 31505-1 4, 31505-1/Chinese Spring F_1 5, Lumai5 6-7, 31504 8-9, 31505 10, N2D-T2A 11, N2A-T2B 12, N2B-T2A.

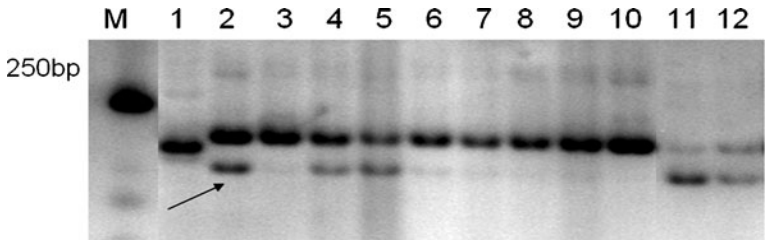

Figure 4. Amplified PCR products of SSR primer pairs *Xcfd168.* M, Marker 1, *Th. ponticum* 2, Chinese Spring 3, 31505-1 4, 31505-1/Chinese Spring F_1 5, Lumai5 6-7, 31504 8-9, 31505 10, N2D-T2A 11, N2A-T2B 12, N2B-T2A.

marker *Xcfd168* amplified the specific 215 bp fragments in Chinese Spring and Lumai 5, while not presented in *Th. ponticum*, 31505-1, 31505 and 31504. These results confirmed the replacement of 2DL chromosome by a chromosome segment introgressed from *Th. ponticum* in 31505-1.

According to the linkage map of chromosome Ta-N×W-2D (Xue *et al.* 2008), marker *Xmag3596* was mapped to the distal 176 cM of chromosome arm 2DL, marker *Xwmc41* was mapped to the distal 180.5 cM, and marker *Xcfd168* and *Xmag4059* were mapped to the distal 187 cM of 2DL (figure 6). Therefore, the introgressed *Th. ponticum* chromosome segment probably involved segment from *Xmag3596* to *Xmag4059*.

4. Discussion

Wild relatives of common wheat have been widely used as valuable sources for introgression of useful traits in wheat improvement. *Th. ponticum*, a perennial decaploid species (2n =10x=70) in the tribe Triticeae, is commonly used as a forage crop in saline land. It is known to possess a number of valuable genes for wheat improvement, such as tolerance to abiotic stresses, salinity and drought, and good resistance to leaf rust, yellow rust, stem rust, wheat curl mite, wheat streak mosaic virus (WSMV), barley yellow dwarf virus (BYDV) resistance and tan spot (Jiang *et al.* 1994; Friebe *et al.* 1996; Li and Wang 2009). All of these characters make this species a potential source of gene pool for wheat improvement. As it can be easily crossed with common wheat, a number of useful genes have been transferred into wheat in form of wheat-*Th. ponticum* chromosome translocations (Fedak and Han 2005; Li *et al.* 2008; Li and Wang 2009). But there had no reports about the reduced height gene introduced from *Th. ponticum*.

We had developed an addition line 31504, with reduced plant height than its wheat parent, from the cross between wheat cultivar Lumai 5 and wheat-*Th. ponticum* amphiploid Xiaoyan 7631. Earlier analysis showed that the reduced height characters probably controlled by gene derived from *Th. ponticum* (Li *et al.* 1996). A substitution line 31505 and an introgression line 31505-1 were then obtained in derivatives of 31504. All, 31504, 31505 and 31505-1, were 50–55 cm high, about 30 cm lower than wheat parent Lumai 5. The results indicated that these three dwarfing lines probably had the same reduce height gene from *Th. ponticum*. Since no current formally named wheat gene for reduced height is derived from this specie, it would be a novel semi-dwarfing gene.

Although no GISH signal was observed in 31505-1 when using genomic DNA from *Th. ponticum* as a probe, PCR marker *Xwmc41*, *Xmag4059*, *Xmag3596* and *Xcfd168*, which were located on 2DL chromosome, amplified unique bands of *Th. ponticum* in 31505-1 and 31505. The FISH pattern also indicated that structural change presented on 2DL chromosome of 31505-1. These results indicated that 31505-1 contained introgressed chromatin from *Th. ponticum*, the alien chromatin involved segment from marker *Xmag3596* to *Xmag4059*. However, it was very small and cannot be detected by GISH analysis.

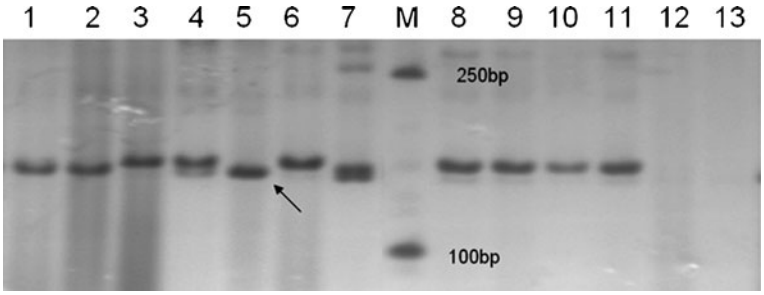

Figure 5. Amplified PCR products of SSR primer pairs *Xwmc41.* 1, 31505 2, 31504 3, Lumai5 4, 31505-1/Chinese Spring F_1 5, 31505-1 6, Chinese Spring 7, *Th. ponticum* M, Marker 7-9, N2A-T2B 10-11, N2B-T2A 12-13, N2D-T2A.

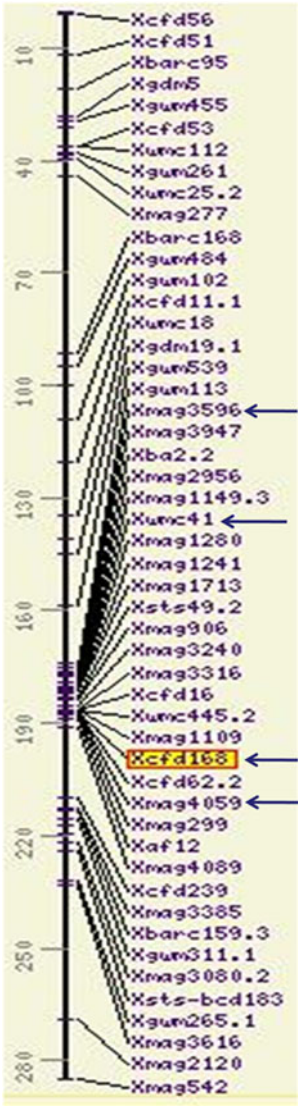

Figure 6. Linkage map of chromosome Ta-N×W-2D (Xue *et al.* 2008) showing the location of four markers.

While transfer good genes from *Thinopyrum* into wheat in form of chromosome substitution or translocations lines, spontaneous wheat-*Thinopyrum* translocations and substitutions usually happen in the D genome, some do so in the A genome and rarely in the B genome (Friebe *et al.* 1996; Han *et al.* 2003; Qi *et al.* 2007). For example, the newly designated powdery mildew resistance gene Pm43 introgressed from *Th. intermedium* was also located in 2DL and was linked with SSR marker *Xwmc41* (He *et al.* 2009). This is possibly because the *Thinopyrum* genome shares more homology with the D than with the A or B genomes (Liu *et al.* 2007).

In this study, introgressed segment from *Th. ponticum* was detected in semi-dwarf line 31505-1. Genetic analysis showed that the dwarfism phenotype of 31505-1 was controlled by a single partial dominant gene, and the introgressed segment probably associated with the dwarfing gene (results was not presented). More work needs to be done for the precise location and high-resolution mapping of it.

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