# Molecular Cytogenetic Identification of Wheat-\textit{Thinopyrum ponticum} Substitution Line with Stripe Rust Resistance

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Molecular Cytogenetic Identification of Wheat-\textit{Thinopyrum ponticum} Substitution Line with Stripe Rust Resistance

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Abstract: *Thinopyrum ponticum* (*Th. ponticum*) (*2n = 10x = 70*) is an important breeding material with excellent resistance and stress tolerance. In this study, we characterized the derivative line *CH1113*-B13-1-1-2-1 (CH1113-B13) through cytological, morphological, Genomic in situ hybridization (GISH), Fluorescence in situ hybridization (FISH), expressed sequence tag (EST), and PCR-based landmark unique gene (PLUG) marker analysis. The GISH analysis revealed that CH1113-B13 contained 20 pairs of common wheat chromosomes and one pair of *J*\(^{St}\) genomic chromosomes. Linkage analysis of *Th. ponticum* using seven EST and seven PLUG markers indicated that the pair of alien chromosomes belonged to the seventh homeologous group. Nulli-tetrasomic and FISH analysis revealed that wheat 7B chromosomes were absent in CH1113-B13; thus, CH1113-B13 was identified as a 7\(^{J}\)\(^{St}\) (7B) substitution line. Finally, adult-stage CH1113-B13 exhibited immunity to wheat stripe rust. This substitution line is therefore a promising germplasm resource for wheat breeding.

Keywords: *Thinopyrum ponticum*, GISH, FISH, Stripe rust resistance, Molecular markers, *Triticum aestivum*

Introduction

Tall wheatgrass, *Thinopyrum ponticum* (*Th. ponticum*) (Podp.) Barkworth
and D. R. Dewey [syn. *Agropyron elongatum* ssp. *ruthenicum* Beldie; *Elytrigia pontica* (Podp.) Holub; *Lophopyrum ponticum* (Podp.) Á Löve], is an important material for wheat (*Triticum aestivum* L.) breeding. This species is very resistant to diseases of wheat, is tolerant of extreme environmental conditions, and is easy to hybridize with common wheat (Tsitsin 1965; Chen 2005; Li et al. 2008; Kuzmanovic et al. 2014; Qin and Qin 2016). Many wheat cultivars with stripe rust resistance and high stress tolerance derived from *Th. ponticum* are proving to be advantageous for agricultural production (Tsitsin 1965; Li et al. 2008; Hu et al. 2011; Qin and Qin 2016). The complex genetic background and strong wheat disease resistance of *Th. ponticum* has provided researchers with many opportunities to develop novel intermediate breeding materials and genetic germplasm showing excellent resistance to wheat diseases and superior environmental adaption.

Stripe rust is one of the destructive disease of wheat caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*). This disease can be dispersed by wind over thousands of kilometers across continents and oceans, resulting in serious yield losses as high as 75% in susceptible varieties (Kolmer 2005; Hu et al. 2011). As stripe rust races have arisen and diversified, most cultivated wheat varieties have lost their resistance. The first known loss of resistance involved the *Yr2* gene, which was ineffective against an introduced stripe rust race in eastern Australia. In
New Zealand, South Africa, Western Europe, and North America, resistance genes \(YrA\), \(Yr5\), \(Yr6\), \(Yr7\), and \(Yr8\) have similarly lost their function with the appearance of 15 novel stripe rust races over 10 years (Kolmer 2005). From the 1950s to the 2010s, \(Yr1\), \(Yr2\), \(Yr3\), \(Yr9\), \(Yr10\), \(Yr24\), and \(Yr26\) gradually lost their resistance to changeable stripe rust races in China (Kang et al. 2015). Desirable genetic and breeding resources are thus needed to improve wheat resistance to stripe rust.

\(Th.\ ponticum\) is usually considered to possess genomes similar to the E (\(E^e\) or \(J^e\)) genome of \(Th.\ elongatum\), the J (\(E^b\) or \(J^b\)) genome of \(Th.\ bessarabicum\), and sometimes the S or St genome of \(Pseudoroegneria\ spicata\) (\(P.\ spicata\)) (Kruppa and Molnár-Láng 2016). Genomic in situ hybridization (GISH) analysis using probes based on DNAs of \(Th.\ elongatum\), \(Th.\ bessarabicum\), and \(P.\ spicata\) is a very useful technique to identify the genomic origin of alien chromosomes in \(Th.\ ponticum\) derivatives.

In this study, substitution line CH1113-B13 was developed from the progeny of a cross of common wheat and \(Th.\ ponticum\). Characterization of this line revealed a novel stripe rust resistance gene(s) derived from \(Th.\ ponticum\) with excellent stripe rust resistance. CH1113-B13 is thus a new material useful for wheat breeding.

**Materials and methods**
Plant materials

Materials used in this study included common wheat \((2n = 6x = 42,\ AABBD\) varieties 7182, Zhongmai 895, Chinese Spring (CS), and Huixianhong (HXH) as well as \(Th.\ ponticum, Th.\ elongatum (2n = 2x = 14, E^eE^e \text{ or } J^fJ^f), Th.\ bessarabicum (2n = 2x = 14, E^bE^b \text{ or } J^bJ^b), P.\ spicata (2n = 4x = 28, StStStSt \text{ or } SSSS)\) (Zhang et al. 1996; Chen et al. 1998), and nulli-tetrasomic materials based on a CS background. The wheat-\(Th.\ ponticum\) substitution line CH1113-B13 was derived from the F\(_6\) progeny of the cross of 7182/\(Th.\ ponticum//Zhongmai 895\). The materials 7182, Zhongmai 895, and \(Th.\ ponticum\) were used as controls in electrophoretic analysis and agronomic trait assessment. HXH was used as the susceptible control in a field evaluation of stripe rust resistance at the adult stage. Genomic DNA of \(Th.\ elongatum, Th.\ bessarabicum,\) and \(P.\ spicata\) was extracted and used for GISH and electrophoretic analysis. All plant materials were provided and maintained with strict selfing in the field at the College of Agronomy, Northwest A & F University, Shaanxi, China.

Cytological characterization
Roots and young spikes growing in the field were sampled at suitable stages and used for observations, chromosome counting, and GISH analysis according to Yang et al. (2014). Root tip cells (RTCs) and pollen mother cells (PMCs) in approximately 30 or more cells with a complete set of chromosomes at metaphase I (MI) were observed, identified, and then photographed with a Photometrics SenSys CCD camera attached to an Olympus BX-43 microscope (Japan).

DNA extraction

Total genomic DNAs of CS, nulli-tetrasomic materials, CH1113-B13, and parents of CH1113-B13 were extracted according to the CTAB method with slight modification. DNAs of Th. ponticum and CS were purified to a level sufficient for GISH analysis (Allen et al. 2006). The DNAs of Th. ponticum, Th. elongatum, Th. bessarabicum, and P. spicata were used for GISH probe labeling. The DNA of CS was used as a block. All these DNAs were also used for electrophoretic analysis.

GISH and Fluorescence in situ hybridization (FISH) analysis

GISH analysis was used to detect alien chromosomes in CH1113-B13. Seeds were germinated on moist filter paper at 23 °C until the root tips
reached 1 to 3 cm. The roots were removed and pretreated with N\textsubscript{2}O for 2 h and then fixed in 90% acetic acid. The root tips were digested in an enzyme mixture (1% pectinase and 2% cellulose) at 37 °C for 52–60 min depending on the material; the drop method was used to prepare slides for GISH and FISH analysis (Han et al. 2004).

GISH and probe labeling were performed as described previously with slight modification (Lukaszewski et al. 2005; Fu et al. 2012). In the FISH analysis, oligonucleotide probes Oligo-pSc119.2 (green) and Oligo-pTa535 (red) were used to identify all 42 chromosomes of common wheat (Tang et al. 2014). The slides were washed in 2× SSC and counterstained with 4,6-diamidino-2-phenylindole (DAPI) prior to observation and photography.

Electrophoretic screening and analysis

Polymerase chain reaction (PCR) assays with EST and PLUG markers were used to detect alien chromosomes and homeologous group relationships in the wheat-\textit{Th. ponticum} substitution line CH1113-B13. DNAs of 7182, Zhongmai 895, CS, nulli-tetrasomic materials based on a CS background, \textit{Th. ponticum}, \textit{Th. elongatum}, \textit{Th. bessarabicum}, and \textit{P. spicata} were used for controls. These controls were used to confirm the genomic origin of alien chromosomes in CH1113-B13 and to verify the
results of GISH and FISH analysis. The EST and PLUG markers, which were selected from the Grain Genes database (GrainGenes, http://wheat.pw.usda.gov/GG2/index.shtml; PCR Primers of GrainGenes, http://wheat.pw.usda.gov/SNP/new/pcr_primers.shtml) or published articles (Ishikawa et al. 2009; Hu et al. 2011; Li et al. 2015a), were synthesized by AuGCT DNA-SYN Biotechnology Co. (Beijing, China). DNA amplifications were carried out in 10.0 µl reaction volumes consisting of 1.0 µl of 10× PCR buffer (Mg$^{2+}$ plus), 0.8 µl dNTP mixture (2.5 mM each), 0.06 µl rTaq DNA polymerase (2.5 U µl$^{-1}$, R001WZ; Takara), 0.5 µl of each primer, 1.0 µl template DNA (100–200 ng µl$^{-1}$), and 6.14 µl double-distilled water. All amplifications were performed on a Gene Amp9700 Thermo Cycler (ABI, USA) or a S1000 Thermal Cycler (Bio-Rad, CA, USA). EST markers amplifications were carried out using the following protocol: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 50–65 °C for 45 s (based on the primer annealing temperature), and 72 °C for 50–90 s (based on the length of target fragments), with a final extension of 72 °C for 10 min and then cooling to 4 °C. The PCR protocol used for amplifications of PLUG markers were as follows: 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 s, 55–60 °C for 45 s (based on primer information), and 72 °C for 2 min, with a final extension of 72 °C for 10 min before cooling to 4 °C. The amplification products and ESTs were electrophoresed on 8% non-denaturing
polyacrylamide gels and photographed after silver staining (0.1% Silver nitrate) and development. To improve levels of polymorphism, the PLUG PCR products were treated with TaqI at 65 °C for 2 h or HaeIII at 37 °C for 3 h and visualized on 2% agarose gels with DuRed nucleic acid gel stain (Fanbo Biochemicals, Beijing, China).

Evaluation of agronomic performance and adult-plant stripe rust resistance

To evaluate morphological traits of line CH1113-B13 and its parents, including plant height, plant type, spikes per plant, spike length, spikelets per spike, kernels per spikelet, thousand-kernel weight, kernel length, kernel width, awnedness, seed setting ([kernels per spike / spikelets] * 100) (Hao et al. 2013), and maturity (Li and Yang 2006), plants were sampled randomly and investigated at suitable stages during two wheat growing periods (2014–2015 and 2015–2016).

To evaluate stripe rust resistance, we used a mixture of *Pst* races CYR32 and CYR33 provided by the College of Plant Protection, Northwest A & F University. Adult-stage common wheat 7182, Zhongmai 895, CH1113-B13, and *Th. ponticum* were investigated for stripe rust resistance when the susceptible control HXH was fully infected under field conditions at the College of Agronomy, Northwest A & F University.
To quantify stripe rust resistance, plants were scored by infection type (IT) according to published methods (Ma et al. 1995) on a 0–4 scale as follows: 0 and 0, immune and nearly immune, respectively; 1, highly resistant; 2, middle resistant; 3 and 4, susceptible.

Results

Cytological characterization of CH1113-B13

As shown in Fig. 1a, 42 chromosomes were present in mitotic RTCs of CH1113-B13. At PMC MI, 342 meiotic cells were observed and identified, which revealed a 21 II chromosome pairing configuration within them (Fig. 1b). The average chromosome configuration observed at PMC MI was 0.06 univalent, 20.97 bivalents, and no trivalents or quadrivalents. At meiotic anaphase I, the 42 chromosomes were equally distributed at the two poles of PMCs with no lagging chromosomes (Fig. 1c). CH1113-B13 was thus confirmed to be cytogenetically stable.

GISH and FISH analysis of CH1113-B13

GISH analysis using *Th. ponticum* total genomic DNA as a probe and DNA of CS as a block revealed the presence of two clear green
hybridization signals in every whole cell (Fig. 2b). FISH analysis
combined with a published FISH karyotype of common wheat was used
to identify all 42 chromosomes of common wheat and to distinguish alien
chromosomes (Tang et al. 2014). In addition, a pair of 7B chromosomes
was found to be absent in the RTCs of CH1113-B13. Two chromosomes
displayed similar red Oligo-pTa535 signals on both ends and no green
Oligo-pSc119.2 signal (Fig. 2a); these two alien chromosomes were
regarded as one pair of chromosomes.

More interestingly, GISH analysis conducted with *Th. elongatum*, *Th.
bessarabicum*, or *P. spicata* total genomic DNAs as probes and CS DNA
as a block resulted in two green signals in every integrated somatic cell of
CH1113-B13 (Fig. 2c–e). As shown in Fig. 2c–e, however, different types
of hybridization signals were detected on the pair of alien chromosomes
depending on which probe DNA was used. When *Th. elongatum* or *Th.
bessarabicum* was used as a probe, two hybridization signals were
observed at both ends of the alien chromosomes. When *P. spicata* was
used as a probe, hybridization signals were detected in the centromeric
area of the two alien chromosomes rather than their flanking regions. The
alien chromosome pair was thus inferred to have originated from the J<sup>st</sup>
genome of *Th. ponticum*.

Molecular marker screening and nulli-tetrasomic electrophoretic
A total of 207 primer pairs (72 EST and 135 PLUG primers; Supplementary Table 1) covering all 21 pairs of common wheat chromosomes were used to compare homeologous group relationships of CH1113-B13 and its parents. Of these, 14 markers (seven EST and seven PLUG markers) amplified fragment length polymorphism among CH1113-B13 and its common wheat parents (Supplementary Figs. 1 and 2; Table 1). This screening indicated that the two alien chromosomes were derived from the seventh homeologous group of *Th. ponticum*.

The results of an electrophoretic analysis of nulli-tetrasomic materials in a CS background using EST and PLUG markers are shown in Figs. 3 and 4. Common wheat specific bands on chromosomes 7A, 7B, and 7D were amplified using seventh homeologous group EST and PLUG markers. Significant regions present in CS on chromosomes 7A, 7B, and 7D were amplified with three PLUG markers (*TNAC1888*-TaqI/HaeIII, *TNAC1826*-TaqI, and *TNAC1821*-HaeIII) and five EST markers (*CD452422*, *BE637663*, *BQ168298*, *BF482530*, and *BE591737*). Analysis of the amplification products revealed that *Th. ponticum* specific bands were present in CH1113-B13, while chromosome 7B specific bands were absent. The pair of 7B chromosomes was thus found to be absent in CH1113-B13, while a pair
of seventh homeologous group chromosomes was donated to CH1113-B13 from *Th. ponticum*.

When EST markers were used to amplify DNAs of *Th. elongatum*, *Th. bessarabicum*, and *P. spicata* (Fig. 4), an even more interesting result was obtained: some marker regions were amplified only in *Th. elongatum* (EST markers *CD452422*, *BE637663*, and *BF482530*), some were amplified in both *Th. elongatum* and *Th. bessarabicum* (EST marker *BQ168298*), and some were amplified in both *Th. elongatum* and *P. spicata* (EST marker *BE591737*). The pair of alien chromosomes in CH1113-B13 was thus found to contain genetic material similar to that from J or E genomes.

Morphology and adult-stage stripe rust resistance identification of CH1113-B13

As shown in Fig. 5 and Table 2, the plant type, spike length, spikelets per spike, and awnedness of CH1113-B13 were similar to those of its common wheat parents 7182 and Zhongmai 895. The number of florets per spikelet and spikes per plant of CH1113-B13 were similar to those of its parent 7182 and significantly different from those of its wheat parent Zhongmai 895. The thousand kernel weight of CH1113-B13 was greater than that of its wheat parent 7182 and less than that of Zhongmai 895; the
opposite was true for plant height \((P < 0.01)\).

The reaction of adult-stage stripe rust resistance of CH1113-B13 and its parents was investigated after inoculation with a mixture of \(Pst\) races CYR32 and CYR33 in the field. While the susceptible control HXH was highly susceptible to the mixed \(Pst\) races in the field \((IT = 4)\), \(Th. ponticum\) was immune \((IT = 0)\), 7182 and Zhongmai 895 were middle susceptible \((IT = 3)\), and CH1113-B13 was immune \((IT = 0)\) at the adult stage (Fig. 6).

**Discussion**

In this study, CH1113-B13 was identified and characterized through cytological, morphological, GISH, FISH, and molecular marker analysis. These analyses identified CH1113-B13 as a wheat-\(Th. ponticum\) substitution line with stripe rust resistance. \(Th. ponticum\), an important genetic resource in wheat breeding, is a decaploid. During the 1920s and 1930s, \(Th. ponticum\) was crossed with common wheat to yield the first hybrid offspring (Tsitsin 1965). In China, Li et al. (2008) created a series of novel wheat cultivars, including Xiaoyan No. 4, 5, and 6, derived from the cross of \(Th. ponticum\) with bread wheat. Although \(Th. ponticum\) has been studied for approximately 90 years because of its importance as a distant hybridization material, its genomic constitution is still
controversial. *Th. ponticum* has been proposed to possess JJJJJJJJJJ (Wang et al. 1991), J1JJ1JJ2JJ3JJ4JJ5J5 (Muramatsu 1990), JJJJJJJJJJ*JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
different types of fragments specific to *Th. ponticum*, *Th. elongatum*, *Th. bessarabicum*, *P. spicata*, and CH1113-B13 (Fig. 4). These results are similar to the results of our GISH analysis, in which the use of *Th. elongatum*, *Th. bessarabicum*, and *P. spicata* probes resulted in different types of green signals on alien chromosomes.

FISH analysis, an economical and easy method to identify wheat hybrids using oligonucleotide probes, can be used to characterize all 42 chromosomes of common wheat with probes Oligo-pSc119.2 and Oligo-pTa535 (Tang et al. 2014). FISH analysis has been used to identify many addition (Li et al. 2015b; Wang et al. 2016; Yang et al. 2016), substitution (Pang et al. 2014; Wang et al. 2015; Yang et al. 2015), translation (Zheng et al. 2006; Patokar et al. 2016), and introgression lines (Wang et al. 2005; Zheng et al. 2006; Zhan et al. 2014). By combining FISH analysis we identified 20 pairs of chromosomes of common wheat and a pair of alien chromosomes in CH113-B13 and determined that a pair of 7B chromosomes was absent. We also detected a few Oligo-pTa535 signals on both ends of the alien chromosomes, similar to the results of Zheng et al. (2014).

Molecular markers are simple and powerful tools to trace alien chromosomes and ascertain homeologous group relationships in a wheat background (Yang et al. 2015). By combining GISH, FISH, and molecular marker analysis, many substitution and addition lines have
been identified, such as addition lines 1V, 5V addition line (Li et al. 2015b), 1Y addition line, 1St addition line (Dou et al. 2012), 1R addition line (Yang et al. 2016), 7Ns (7D) substitution line (Yang et al. 2015), and 6St (6A) substitution line (Wang et al. 2015). In the present study, seven EST and seven PLUG markers, all located on the seventh homeologous chromosome group, were able to amplify different length fragments between CH1113-B13 and its common wheat parents and identical-sized fragments between CH1113-B13 and Th. ponticum. We thus conclude that the pair of alien chromosomes in CH113-B13 is from the seventh homeologous group of Th. ponticum. To further characterize the substitution line CH1113-B13, nulli-tetrasomic analysis was conducted to identify the substituted wheat chromosomes in CH1113-B13. Taken together, the results of the FISH and GISH analysis confirmed that the substitution line CH1113-B13 carries a pair of 7JSt chromosomes from Th. ponticum and 40 chromosomes from common wheat.

Th. ponticum, a superior material and potential genetic source for wheat breeding, is resistant to and can confer resistance to various wheat diseases and unfavorable growth conditions, including leaf rust (Li et al. 2003), stripe rust (Li et al. 2008; Hu et al. 2011), stem rust (Ayala-Navarrete et al. 2013), powdery mildew (Wang et al. 2015), wheat streak mosaic virus (Li and Wang 2009), Barley yellow dwarf virus (Li et al. 2008), salinization (Yuan and Tomita 2015), and drought stress (Qin
Substitution line CH1113-B13 is immune to stripe rust (IT = 0) at the adult stage, whereas its common wheat parents Zhongmai 895 and 7182 are middle susceptible to stripe rust (IT = 3; Fig. 6) at the same stage. CH1113-B13 may thus carry a novel resistance gene(s) to wheat stripe rust on the pair of alien chromosomes. Unfortunately, our molecular markers were not correlated with the phenotype of stripe rust resistance. In order to confirm the origin of stripe rust resistance in CH1113-B13, we plan to conduct a genetic analysis based on previous research (Hu et al. 2011).

In summary, cytogenetic, morphological, GISH, FISH, molecular marker, and nulli-tetrasomic analyses were used to characterize the substitution line CH1113-B13, the offspring from the cross of T. aestivum 7182/Th. ponticum//Zhongmai 895. The selfed seed setting of CH1113-B13 was 158.1%, which suggests that all of its chromosomes underwent normal pairing and separation. This substitution line, which carried a pair of 7J^{st} chromosomes and all 40 common wheat chromosomes except a pair of 7B chromosomes, had a higher thousand kernel weight than 7182 and more spikes per plant and florets per spikes than Zhongmai 895. Furthermore, the stripe rust resistance of CH1113-B13 is significantly different from those of its common wheat parents. No stripe rust resistance genes have been reported on the 7J^{st} chromosomes of Th. ponticum, which suggests that we could have
uncovered one or more novel genes from *Th. ponticum*. The observed characteristics of CH1113-B13 may protect this substitution line and its progeny from stressful environments and contribute to yield improvements. These characteristics may be transferrable to common wheat using wide cross breeding techniques. CH1113-B13 thus represents a new germplasm resource that can be exploited as an important intermediate breeding material to confer disease resistance and enhance yields.

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**Author contributions:** W. J., C. Z. and C. W. designed the experiments; C. Z., Y. W., C. C., A. Z., N. P., and Y. W. performed the experiments; C. Z., Y. W., C. W., Y. W., H. Z., and X. L. analyzed the data; C. Z., C. W. and W. J. wrote the paper.

**Disclosure of potential conflict of interest:** The authors declare no conflict of interest.

**References**


Dou, Q.W., Lei, Y., Li, X., Mott, I.W., and Wang, R.R. 2012. Characterization of alien chromosomes in backcross derivatives of
*Triticum aestivum* × *Elymus rectisetus* hybrids by using molecular markers and sequential multicolor FISH / GISH. Genome 55(5): 337-347. doi: 10.1139/g2012-018.


Kruppa, K., and Molnár-Láng, M. 2016. Simultaneous visualization of different genomes (J, JSt and St) in a *Thinopyrum intermedium* × *Thinopyrum ponticum* synthetic hybrid (Poaceae) and in its parental species by multicolour genomic in situ hybridization (mcGISH). Comp Cytogenet 10(2): 283-293. doi: 10.3897/CompCytogen.v10i2.7305.


mapping of the blue-grained gene(s) from *Thinopyrum ponticum* by GISH and FISH in a set of translocation lines with different seed colors in wheat. Genome 49(9): 1109-1114. doi: 10.1139/g06-073.

Legends to Figures

Fig. 1 Characteristics of mitotic (a), meiotic I (b), and anaphase I (c) chromosomes of CH1113-B13. (a) 2n = 42; (b) 2n = 21 II; (c) 2n = 21+21.

Fig. 2 Fluorescence in situ hybridization (FISH) analysis using (a) Oligo-pTa535 (red), Oligo-pSc119.2 (green) oligonucleotides as probes to identify common wheat chromosomes on root tip metaphase chromosomes of CH1113-B13, (b) Genomic in situ hybridization (GISH) analysis using Th. ponticum genomic DNA, (c) Th. bessarabicum genomic DNA, (d) Th. elongatum genomic DNA, and (e) P. spicata genomic DNAas probes (green) on root tip metaphase chromosomes of CH1113-B13. Chromosomes were counterstained with DAPI (blue). The white arrows indicate introduced Th. ponticum chromosomes in CH1113-B13.

Fig. 3 Null-tetrasomic analysis of CH1113-B13 with PLUG markers. The white arrow indicates a Th. ponticum specific band; the red arrow indicates a 7B chromosomes specific band. Lanes are as follows: M, DL2000 (3427A, Takara); 1, Th. ponticum; 2, CH1113-B13; 3, Zhongmai 895; 4, 7182; 5, CS; 6, CSN7AT7D; 7, CSN7BT7A; 8, CSN7DT7B; a,
Fig. 4 Amplification of EST markers in nulli-tetrasomic materials, *Th. bessarabicum*, *Th. elongatum*, and *P. spicata*. Black and red arrows indicate a *Th. ponticum* specific band and a 7B chromosomes specific band, respectively; the blue arrow indicates a band that is common only to CH1113-B13 and *Th. ponticum*. Lanes are as follows: M, DL2000; 1, *Th. ponticum*; 2, CH1113-B13; 3, Zhongmai 895; 4, 7182; 5, CS; 6, CSN7AT7D; 7, CSN7BT7A; 8, CSN7DT7B; 9, *Th. bessarabicum*; 10, *Th. elongatum*; 11, *P. spicata*. 

Fig. 5 Morphological comparison of adult plants, spikes, spikelets, and seeds from the wheat-*Th. ponticum* derivative line CH1113-B13 and its parents: *Th. ponticum*, common wheat 7182, and Zhongmai 895. (a) Adult plants, (b) spikes, and (c) spikelets and seeds. 1, Zhongmai 895; 2, CH1113-B13; 3, 7182; 4, *Th. ponticum*.

Fig. 6 Stripe rust resistance of susceptible control variety HXH (5), *Th. ponticum* (1), CH1113-B13 (2), Zhongmai 895 (3), and 7182 (4) at the
adult stage.

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Fig. 3
Fig. 4
Fig. 5
Fig. 6
### Table 1: EST and PLUG polymorphic markers applied to linkage analysis of the *Th. ponticum*.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Type</th>
<th>Primers (5'-3')</th>
<th>Location</th>
<th>Gel type / Restriction enzyme</th>
<th>Tm (°C) / Time of enzyme digestion (h)</th>
</tr>
</thead>
</table>
| CD452422  | EST  | F: GAAGTCTTTGAGCAGCTCCG  
R: TCAGATGCCTCACTGATGATGG | 7AL 7BL 7DL | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| BG274576  | EST  | F: AGATGAACTCTGGCCTGGAT  
R: AGCTCGATGATCTGCTTGG | 7A 7BL 7DS | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| BE637663  | EST  | F: ACTGTGTCCTCGCTCAAGT  
R: GTTCCATTTCCGATGTC | 7A 7BL 7DL | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| BE591127  | EST  | F: GCAGCTCATCTTACATGTC  
R: CGTTCGACCAATCAGTCCTA | 7A 7BL 7DS | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| BQ168298  | EST  | F: GCTTCGCTCCTCACTACAA  
R: CTCGCAATTCTGACCAAGT | 7A 7BL 7DS | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| BF482530  | EST  | F: GAAGTCTTTGAGCAGCTCCG  
R: TCAGATGCCTCACTGATGATGG | 7AL 7BL 7DL | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| BE591737  | EST  | F: AGCAGCTAGGAGGGTGTCTG  
R: TAACCGCAGCTTTCTCATCC | 7A 7BL 7DS | 8% non-denaturing polyacrylamide gel / NA | 60 / NA |
| TNAC1888  | PLUG | F: AGGATGTTGAGCAGCTCCG  
R: CACGTGACACCTCTGCTCCTT | 7A 7BL 7DL | 2% agarose gel / TaqI or HaeIII | 60 / 2 or 3 |
| TNAC1805  | PLUG | F: TCTTTTCTCTTGTTCTCTTG  
R: CACGCTAGTGAAGGACCAAT | 7A 7BS 7DS | 2% agarose gel / TaqI | 60 / 2 or 3 |
| TNAC1826  | PLUG | F: CACATATGATGATGACGGGAA  
R: GGCAGGGAGGAAACTCTACTG | 7A 7BL 7DL | 2% agarose gel / TaqI | 60 / 2 |
| TNAC1903  | PLUG | F: TCCGTCTTCTCTGCTTCTT  
R: CTCGACGACACCTCTTACTC | 7A 7BL 7DL | 2% agarose gel / TaqI | 60 / 2 |
| TNAC1926  | PLUG | F: CGTCAGCTAGGAGGGTGTCTG  
R: TAACCGCAGCTTTCTCATCC | 7A 7BL 7DL | 2% agarose gel / TaqI | 60 / 2 or 3 |
| TNAC1845  | PLUG | F: AATGAACTCTGGCTTCTGC  
R: CAGATGCTCTGATTCTCAGG | 7A 7BL 7DL | 2% agarose gel / HaeIII | 60 / 3 |

### Table 2: Agronomic traits of disomic substitution line CH1113-B13 and its parents.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Plant height (cm)</th>
<th>Plant type</th>
<th>Spike length (cm)</th>
<th>Spikes / plant</th>
<th>Spikelets / spike</th>
<th>Florets / spikelet</th>
<th>Thousand kernel weight (g)</th>
<th>Awnedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhongmai 895</td>
<td>76.5±1.5b</td>
<td>Tighten</td>
<td>9.4±0.6a</td>
<td>9±1b</td>
<td>19±2a</td>
<td>5±0a</td>
<td>57.9±1.4b</td>
<td>Long awn</td>
</tr>
<tr>
<td>Th. ponticum</td>
<td>150.0</td>
<td>Drooping</td>
<td>31.6±3.4</td>
<td>Cluster</td>
<td>23±3</td>
<td>8±1</td>
<td>NA</td>
<td>Awnless</td>
</tr>
<tr>
<td>CH1113-B13-1-1-2-1</td>
<td>78.7±2.1ab</td>
<td>Tighten</td>
<td>9.7±0.7a</td>
<td>19±3a</td>
<td>20±2a</td>
<td>4±1a</td>
<td>40.1±0.5c</td>
<td>Long awn</td>
</tr>
</tbody>
</table>

Different letter a, b and c indicates significant differences between CH1113-B13 and its wheat parents (*P* < 0.01).