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ORIGINAL PAPER

# Putative *Thinopyrum intermedium*-derived stripe rust resistance gene *Yr50* maps on wheat chromosome arm 4BL

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**Abstract** Stripe rust-resistant wheat introgression line CH223 was developed by crossing the resistant partial amphiploid TAI7047 derived from *Thinopyrum intermedium* with susceptible cultivars. The resistance is effective against all the existing Chinese stripe rust races, including the most widely virulent and predominant pathotypes CYR32 and CYR33. Cytological analyses using GISH detected no chromosomal segments from *Th. intermedium*. It was presumed that the segment was too small to be detected. Normal bivalent pairing at meiosis in CH223 and its hybrids confirmed its stability. Genetic analysis of the $F_1$, $F_2$, $F_3$ and $BC_1$ populations from crosses of CH223 with susceptible lines indicated that resistance was controlled by a single dominant gene. The resistance gene was mapped using an $F_{2:3}$ population from Taichung 29/CH223. The gene was linked to five co-dominant genomic SSR markers, *Xgwm540*, *Xbarc1096*, *Xwmc47*, *Xwmc310* and *Xgpw7272*, and flanked by *Xbarc1096* and *Xwmc47* at 8.0 and 7.2 cM, respectively. Using the Chinese Spring nullitetrasomic and ditelosomic lines, the polymorphic markers and the resistance gene were assigned to chromosome arm 4BL. As no permanently named stripe rust resistance genes had been assigned to chromosome 4BL, this new resistance gene is designated *Yr50*. The gene, together with the identified closely linked markers, could be used in marker-assisted selection to combine two or more resistance genes in a single genotype.

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## Introduction

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a major constraint to wheat production in many regions of the world. China represents the largest stripe rust epidemiologic region in the world in terms of wheat area affected by the disease, and survey data from the last 10 years showed that the area affected annually by stripe rust was on average about 4 million hectares. For example, stripe rust affected 6.6, 4.9 and 4.08 million hectares in 2002, 2003 and 2009, respectively (Kang et al. 2010). It is considered that the stripe rust epidemic in 2002, caused by the widely virulent Chinese *Pst* race CYR32 (Chinese yellow rust race 32), was the most widespread in the past three decades, resulting in yield loss of 1.31 million metric tons (Li and Zeng 2002; Wan et al. 2004). The frequencies of CYR32 and CYR33 were the highest among all detected races from 1997 to 2007 (Wan et al. 2007;

Chen et al. 2009). These races are virulent on seedlings of almost all Chinese wheat cultivars except those with resistance genes *Yr5*, *Yr10*, *Yr15*, *Yr18*, *Yr24/Yr26*, *Yr36*, *Yr39* and *Yr41* (Yang et al. 2003; Li et al. 2006a, b; Kang et al. 2010). Because of their virulence attributes, these two races are used in selection for stripe rust resistance by most breeding programs.

Host resistance is the most-cost effective, environmentally sound, and consistently used method of controlling stripe rust (Chen 2005). To date, 52 resistance alleles at 45 loci (*Yr1-Yr49*) and more than 40 temporarily designated genes have been identified on all wheat chromosomes except 1A, 4A, 4B and 7A (McIntosh et al. 2008, 2011). Some of them have been widely used in different areas of the world (Chen 2005). When resistance to an obligate parasite such as rust is determined by a single gene, it is common to observe the increase of a new race capable of overcoming the resistance within about 5 years (Wang et al. 1988). This inevitably leads to epidemic 'boom–bust' cycles such as those that have occurred in China in the past few years (Wang et al. 1988; Li and Zeng 2002; Wan et al. 2007). For example, wheat lines with *Yr9* were widely used in wheat breeding programs throughout China from the 1960s, and more than 80 % of the released cultivars contained *Yr9* by the late 1980s (Wan et al. 2004, 2007). *Pst* race CYR29 (Chinese yellow rust 29) with virulence to *Yr9* was detected in 1985. This race overcame resistance in many wheat cultivars carrying *Yr9*, resulting in yield losses of 2.65 million tons in 1990. A similar situation occurred in 2002 with the appearance of races CYR31 and CYR32 virulent to wheat variety Fan 6 and its derivatives believed to have genes *Yr3b* and *Yr4b* (Wan et al. 2004). Maintaining sustained genetic control of stripe rust is dependent on discovering and deploying new resistance genes.

Alien gene transfer is a valuable means of increasing the amount of genetic diversity available to wheat breeders (Jiang et al. 1994). Stripe rust resistance genes that have been transferred to hexaploid wheat from its relatives include *Yr15*, *Yr35*, *Yr36* and *YrH52* from *T. dicoccoides*, and *Yr8*, *Yr28*, *Yr37*, *Yr38*, *Yr40* and *Yr42* from different *Aegilops* species (McIntosh et al. 2008; Marais et al. 2009). Among these genes, only *Yr9* and *Yr24/Yr26* have been widely deployed in China (Chen 2005), and the deployment was only partly based on stripe rust resistance.

More recently, molecular markers that are independent of environmental and developmental factors were developed, and these facilitate selection of genes controlling traits such as disease resistance. Microsatellites, or simple sequence repeats (SSRs), are favored due to their high variability, genomic abundance and co-dominance. Microsatellite markers linked with the stripe rust resistance genes *Yr5* (Sun et al. 2002), *Yr17* (Jia et al. 2011), *Yr18* and *Yr29* (Lillemo et al. 2008), *Yr24/Yr26* (Li et al. 2006a), *Yr36* (Uauy et al. 2005), *Yr41* (Luo et al. 2005), *Yr43* and *Yr44* (Cheng and Chen 2010), *Yr45* (Li et al. 2011), *Yr46* (Herrera-Foessel et al. 2011), *Yr47* (Bansal et al. 2011) and *Yr48* (Lowe et al. 2011) have been reported. Moreover, some molecular markers closely linked to such genes have facilitated their utilization in breeding programs (Murphy et al. 2009) and in map-based cloning (Fu et al. 2009).

At present, wheat cultivars widely grown in China have a relatively narrow genetic base and many are derivatives of 'Lovrin 10' with the ineffective *Yr9* resistance gene, 'Fan 6' or 'Shuiyuan 11'. *Thinopyrum intermedium* (Host) Barkworth and Dewey ($2n = 6x = 42$, $JJ^sSt$) have been hybridized extensively with wheat and proven to be a valuable source of genes for disease resistance. Partial wheat–*Th. intermedium* amphiploids are potential tertiary gene pools with a wide range of attributes of potential value for wheat improvement, such as resistance to wheat streak mosaic virus (WSMV) and its wheat curl mite vector, and barley yellow dwarf virus (BYDV) (Friebe et al. 1996; Fedak and Han 2005). Genes for resistance to leaf rust and stem rust were incorporated into wheat and tagged with molecular markers (Autrique et al. 1995; Fedak 1999). More recently, resistance to Chinese *Pst* races CYR31 and CYR32 was found on a *Th. intermedium* chromosome that was substituted for wheat chromosome 1D (Hu et al. 2011), but there is no report indicating that stripe rust resistance genes from *Thinopyrum* species have been transferred to wheat chromosomes. CH223, a *Th. intermedium*-derived wheat introgression line was resistant to stripe rust and powdery mildew in field nurseries in Chengdu, Sichuan Province and under greenhouse conditions in Taiyuan, Shanxi Province, from 2007 to 2010 (Yan et al. 2010; Bai et al. 2011). We investigated the inheritance of stripe rust resistance in CH223 and identified the chromosome location and flanking markers of a single gene for resistance.

## Materials and methods

### Plant material and populations

The materials used in this study were CH223 and TAI7047. The former is a homogeneous $BC_2F_6$-derived resistant wheat line from Jinmai 33/TAI7047//2*Jing 411, and the latter is the resistant parent of CH223, and is a partial amphiploid developed by crossing common wheat cultivars with *Th. intermedium* accession Z1141 with pedigree Taiyuan 768/Z1141//Jinchun 5. Wheat genotypes 'Taiyuan 768', 'Jinchun 5', 'Jing 411', 'Jinmai 33', 'Taichung 29', 'SY95-71', 'Chinese Spring' (CS) and various 'Chinese Spring' nulli-tetrasomic (NT) and ditelosomic (Dt) stocks were also included in the study.

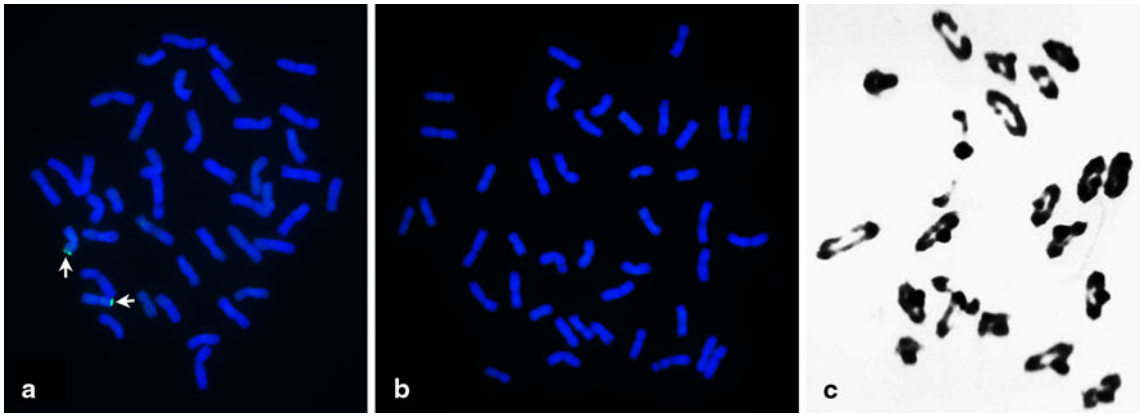


**Fig. 1** GISH pattern of line 29-12-1-2, the wheat–*Thinopyrum intermedium* recombinant control (**a**) and CH223 (**b**) at mitosis using total genomic DNA of *Th. intermedium* as probe. CH223 displayed normal bivalent pairing at metaphase I (**c**). *Arrows* indicate alien chromatin

CH223 was crossed to susceptible cultivars Taichung 29 and SY95-71 to yield segregating populations. The $F_2$, $F_3$ and $BC_1$ were tested for genetic analysis of stripe rust response. An $F_2$ population and derived $F_3$ families from CH223/Taichung 29 were further used for screening microsatellite marker to map the stripe rust resistance gene(s). The mapping population comprised 221 $F_2$ plants and the 211 derived $F_3$ families, the difference being due to loss of 10 susceptible $F_2$ plants.

CH223, the $F_1$ hybrid involving CH223×Taichung 29, the original rust resistant donor accession (Z1141) of *Th. intermedium* and CS were also used for cytogenetic analysis to determine the size of alien introgression and to analyze the chromosome pairing in CH223.

## Cytogenetic analysis

Genomic in situ hybridization (GISH) was used to determine the presence and size of the alien introgression in the stripe rust resistant line CH223. Line 29-12-1-2, with a small distal translocation from *Th. intermedium* (Fig. 1a), was used as a positive control. Seeds were germinated on moistened filter papers in Petri dishes. Actively growing root tips were removed from seedlings and treated with nitrous oxide gas for 2 h, then fixed in 90 % acetic acid and stored in 70 % v/v ethanol at −20 °C (Kato et al. 2004). Chromosome preparation and GISH were performed according to Han et al. (2006) using *Th. intermedium* genomic DNA as probe and CS genomic DNA as blocker. The DNA was labeled with fluorescein-12-dUTP by the nick-translation procedure; the probe-to-blocker ratio was about 1:110.

For meiotic chromosome preparations, anthers from the emerging spikes containing pollen mother cells (PMCs) at MI were fixed in ethanol:acetic acid (3:1) for 1 day, transferred to 70 % ethanol and kept at 4 °C in a refrigerator for about 2 weeks. Anthers were then stained in 1 % acetocarmine and squashed in 45 % acetic acid.

## Disease rating

Seedlings of *Th. intermedium*, one partial amphiploid and seven wheat cultivars/lines (Table 1) were evaluated in the greenhouse based on the methods described by Li and Zeng (2002) using nine Chinese *Pst* races (CYR23, SY11-4, SY11-5, SY11-7, CYR29, CYR31, CYR32, CYR33 and v26). Among these *Pst* races, v26 is a new *Yr24/Yr26* virulent pathotype and different from currently known pathotypes in China (Liu et al. 2010), the others are the most widely virulent and/or predominant isolates collected during 2003 through 2007 from wheat growing areas in 15 provinces in China (Chen et al. 2009). Seedlings of each cultivar/line were grown in 70 × 45 × 18 cm flat plastic trays. The highly susceptible cv. Mingxian 169 was used as a control. Inoculations were performed when the first leaves were fully expanded. Inoculated seedlings were kept in a dew chamber at 10 °C for about 24 h and then moved to an environmentally controlled greenhouse with a daily cycle of 16 h of light at 18 °C and 8 h of darkness at 10 °C. Plant reactions were scored on a 0–4 scale, 18–21 days after inoculation when the susceptible checks were heavily infected (Table 1).

Race CYR32, which is avirulent to CH223, but virulent to Taichung 29 and SY95-71, was used to test $F_1$, $F_2$ and $BC_1$ populations derived from Taichuang 29/CH223 and SY95-71/CH223 (Table 2). Seeds from the parents, $F_1$, $F_2$, $F_3$ and $BC_1$ populations were planted in the greenhouse. Twenty seeds for each parent and $F_1$, 221 seeds of the $F_2$, 104 seeds of the $BC_1$ and 15 seeds for each of the $F_2$-derived $F_3$ families were planted in a randomized design with 13–15 plants in a 1.2 m row, 25 cm apart. Susceptible spreaders of cv. Mingxian 169 or SY95-71 were planted in every tenth row for each population. The predominant *Pst* race CYR32 was used for adult plant testing and the spreaders were artificially inoculated two to three times at the seedling stage. The stripe rust epidemics

**Table 1** Seedling infection types on selected donor lines, parents and controls to nine Chinese races of *Puccinia striiformis* f. sp. *tritici* (*Pst*)

| Line | Chromosome number | Genomic formula | *Pst* races | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CYR23 | CYR29 | CYR31 | CYR32 | CYR33 | SY11-4 | SY11-5 | SY11-7 | v26 |
| *Th. intermedum* | | | | | | | | | | | |
| Z1141 | 42 | $JJ^sSt$ | 0[a] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TAI7047 | 56 | ABD[b] + J + $J^s$ + St[c] | 0; | 0 | 0 | 0, 0; | 0 | 0; | 0; | 0 | 0 |
| CH223 | 42 | ABD | 0; | 0 | 0 | 0, 0; | 0, 0; | 0; | 0 | 0; | 0; |
| Taiyuan (TY) 768[d] | 42 | ABD | 1 | 4 | 4 | 4 | 4 | 0; | 0; | 0; | 4 |
| Jinchun (JC) 5[d] | 42 | ABD | 0 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 |
| Jinmai (JM) 33[e] | 42 | ABD | 1 | 4 | 4 | 4 | 4 | 1 | 4 | 4 | 3 |
| Jing 411[e] | 42 | ABD | 0 | 4 | 4 | 4 | 4 | 2 | 4 | 3 | 4 |
| Taichung 29 | 42 | ABD | 1 | 4 | 4 | 4 | 4 | 4 | 3 | 0; | 0; |
| SY 95-71 | 42 | ABD | 0; | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 |

[a] Seedling infection types (IT) described by Li and Zeng (2002). 0 = no visible symptoms; 0; = hypersensitive flecks or necrotic areas without sporulation; 1 = necrotic and chlorotic areas with restricted sporulation; 2 = moderate sporulation with necrosis and chlorosis; 3 = sporulation with chlorosis; 4 = abundant sporulation without chlorosis. Scores of 0–2 were classified as resistant and 3–4 as susceptible reactions

[b] Wheat genome

[c] Mixed genome with chromosomes from three different genomic sets

[d] The wheat parents of TAI7047

[e] The wheat parents of CH223

**Table 2** Adult plant segregation ratios of stripe rust response in $F_1$, $F_2$ and $BC_1$ plants, and $F_3$ lines when inoculated with *Pst* race CYR32

| IT | Parent | | | $P_2/P_1$[a] | | | | | $P_3/P_1$ | | | | | $P_3/P_1//P_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of plant | | No. of line | | | No. of plant | | No. of line | | | No. of plant |
| | $P_1$ | $P_2$ | $P_3$ | $F_1$ | $F_2$ | $F^b_{2:3}$ | | | $F_1$ | $F_2$ | $F_{2:3}$ | | | $BC_1$ |
| | | | | | | *HR* | *Seg* | *HS* | | | *HR* | *Seg* | *HS* | |
| 0 | 4 | | | 2 | 17 | 17 | | | 11 | 46 | 40 | 6 | | 8 |
| 0; | 9 | | | 12 | 43 | 36 | 7 | | 3 | 22 | 14 | 8 | | 28 |
| 1 | | | | | 64 | | 64 | | | 71 | 3 | 68 | | 11 |
| 2 | | | | | 38 | | 37 | 1 | | 18 | 1 | 17 | | 6 |
| 3 | | | | | 20 | | 3 | 15[c] | | 21 | | 14 | 7 | 24 |
| 4 | | 14 | 12 | | 39 | | | 31[d] | | 37 | | | 37 | 21 |
| **Total** | **13** | **14** | **12** | **14** | **221** | **53** | **111** | **47 + 10** | **14** | **215** | **58** | **113** | **44** | **98** |
| | | | | $\chi^{2e}_{(3:1)} = 0.34$ | | $\chi^2_{(1:2:1)} = 0.92$ | | | $\chi^2_{(3:1)} = 0.45$ | | $\chi^2_{(1:2:1)} = 2.39$ | | | $\chi^2_{(1:1)} = 0.65$ |
| | | | | $P = 0.56$ | | $P = 0.63$ | | | $P = 0.50$ | | $P = 0.30$ | | | $P = 0.42$ |

[a] $P_1$ = CH223, $P_2$ = Taichung 29, $P_3$ = SY95-71

[b] *HR*, *Seg* and *HS*: homozygous resistant, segregating and homozygous susceptible

[c] Two plants died in the field. They were assumed to be *HS*

[d] Eight plants died in the field. They were assumed to be *HS*

[e] Values for significance at $P = 0.05$ are 3.83 for 1 *df* and 5.99 for 2 *df*

were initiated by spraying aqueous suspensions of urediniospores of CYR32, to which a few drops of Tween 20 (0.03 %) had been added, onto the spreader rows at tillering. Adult plant reactions were scored at the soft dough stage using the 0–4 scale previously described. To determine the genotypes of $F_2$ plants from Taichung 29/CH223 and SY95-71/CH223, the $F_2$-derived $F_3$ families were tested with the same race.

### Bulk segregant analysis

The parents and 211 $F_2$ plants corresponding to their $F_3$ families from Taichung 29/CH223 were used for SSR analysis. Total DNA was extracted from seedling tissues following Stein et al. (2001). Resistant (*Br*) and susceptible (*Bs*) bulks were made from equal amounts of DNA from ten resistant and ten susceptible $F_2$ plants, respectively. DNA bulks of ten homozygous resistant and ten homozygous susceptible $F_{2:3}$ lines were similarly prepared for bulk segregant analysis (BSA). Markers polymorphic between the resistant and susceptible parents and bulks were used to genotype the entire $F_2$ population.

### Microsatellite marker analysis

For the initial polymorphic marker survey, one marker from each of the *Xgwm*, *Xwmc*, *Xbarc*, *Xcfd*, *Xcfa* and *Xgdm* microsatellite (SSR) marker series was chosen approximately every 10 cM along the chromosomes according to the reported consensus map (Somers et al. 2004) and was used in BSA. Additional markers from the above series and *Xgpw* markers located on 4B showing polymorphism between resistant and susceptible bulk were used in genotyping individual plants.

PCR for each SSR marker was performed in a PTC200 Peltier Thermal Cycler (Bio-Rad Inc., Hercules, USA) in a total volume of 20 µl containing 2 µl 10× buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 1 unit *Taq* DNA polymerase, 0.25 µM of each primer and 80–100 ng total genomic DNA. Each PCR amplification was performed at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 50, 55 or 60 °C (based on primer annealing temperature) for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min before cooling to 4 °C. After amplification, 12 µl loading buffer (0.4 g/ml sucrose, 1 mg/ml bromophenol blue and 1 mg/ml xylene cyanol) was added to the PCR products; 4–6 µl of each sample was loaded on 8 % non-denaturing polyacrylamide gels (Acr:Bis = 29:1) and separated at 150 V for approximately 2 h, then visualized by silver staining.

### Chromosome assignment and linkage analysis

Chromosomal locations of linked microsatellite markers were confirmed using Chinese Spring nullisomic 4B tetrasomic 4A (N4BT4A), nullisomic 4A tetrasomic 4B (N4AT4B), nullisomic 4D tetrasomic 4B (N4DT4B) and ditelosomic 4BS (Dt4BS) lines kindly provided by the Wheat Genetic and Genomic Resources Center, Kansas State University. Chi-squared ($\chi^2$) tests for goodness of fit were used to evaluate deviations of observed data from theoretically expected segregation ratios. Linkages between markers and the resistance gene were determined using Mapmaker 3.0, with an LOD score threshold of 3.0 (Lincoln et al. 1993).

## Results

### Likely origin of the stripe rust resistance

CH223, TAI7047 and the *Th. intermedium* parent were resistant to all nine Chinese *Pst* races tested at the seedling stage, whereas among the various wheat parents, Jinchun 5 was susceptible to all races tested except CYR23, Jinmai 33 and Jing 411 that were only resistant to CYR23 and SY11-4, and Taiyuan 768 that was resistant to races CYR23, SY11-4, SY11-5 and SY11-7 (Table 1). These results demonstrated that CH223 conferred resistance to stripe rust similar to its putative donor TAI7047 and wild parent.

### GISH identification and chromosomal pairing in CH223

When somatic cells of both the resistant line CH223 and the recombinant control (line 29-12-1-2) were probed by genomic DNA of *Th. intermedium* and blocked with CS genomic DNA, a pair of chromosomes revealed green fluorescence signals at the distal regions of their short arms in the positive control (Fig. 1a). No translocation was visible in CH223 (Fig. 1b), suggesting that if a translocation was present, its size was too small to be detected by GISH.

Metaphase I chromosome pairing in PMCs of CH223 and its hybrids with susceptible wheat genotype Taichung 29 was 21 bivalents (Fig. 1c).

### Inheritance of the adult plant resistance to stripe rust in CH223

The infection type data for the parents, $F_1$ plants and $F_2$ and $BC_1$ populations are summarized in Table 2. When inoculated with race CYR32 at the adult plant stage, $F_1$ plants from both crosses showed infection types (IT) similar to the resistant parent, indicating that resistance was dominant. Segregation in the $F_2$ and $BC_1$ populations (Table 2) included many plants with intermediate responses. When the numbers of $F_2$ plants with IT 0–2 and those with IT 3–4 were pooled as separate resistant and susceptible groups, the ratios were consistent with those expected for segregation at a single locus. When tested with the same race, the $F_3$ lines from Taichung 29/CH223 and SY95-71/CH223 segregated 1 homozygous resistant (*HR*):2 segregating (*Seg*):1 homozygous susceptible (*HS*), confirming the single gene segregation (Table 2). In addition, the pooled numbers of resistant and susceptible plants in segregating

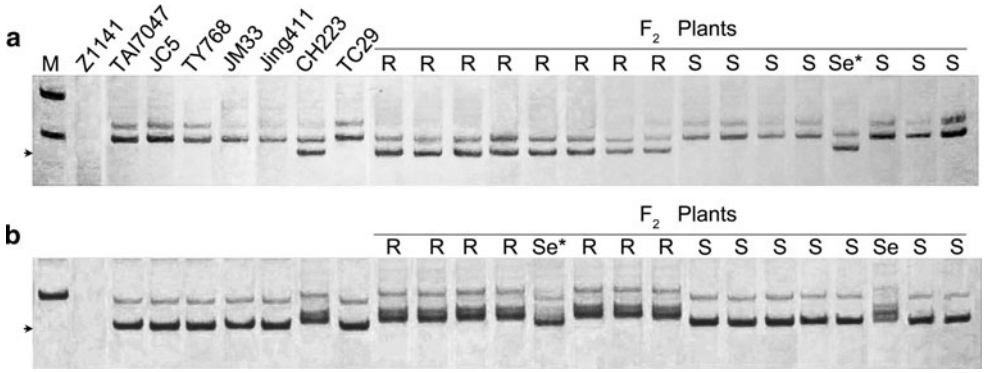


**Fig. 2** Silver-stained polyacrylamide gels showing simple sequence repeat (SSR) markers *Xbarc1096* (**a**) and *Xwmc47* (**b**) flanking the *Yr50* locus. Z1141, the accession of the wild parent *Th. intermedium* accession; TAI7047, partial amphiploid and the resistant parent of CH223; JC5 and TY768, the wheat parents of TAI7047; and JM33 and Jing 411, the wheat parents of CH223. Selected homozygous resistant (*R*), homozygous susceptible (*S*), segregating (*Se*) and recombinant (*Se**) $F_2$ plants from Taichung (TC) 29/CH223 were determined by the phenotypic data of $F_{2:3}$ lines. M, 100-bp DNA ladder; *arrows* on the *left side* indicate the fragment linked to the resistance gene

$F_3$ lines from Taichung 29/CH223 were 1072 resistant:341 susceptible ($\chi^2_{(3:1)} = 0.57$, $P_{1df} = 0.45 > 0.25$). These results suggested that a stripe rust resistance gene, provisionally designated *YrCH223*, was present in CH223, presumably having been transferred from *Th. intermedium* accession Z1141.

All 17 plants scored IT 0 in the $F_2$ population from Taichung 29/CH223 were homozygous resistant (*HR*) in tests on the $F_3$ lines. Of the 102 plants scored IT 1 or 2, 101 produced segregating (*Seg*) $F_3$ lines and 1 was homozygous susceptible (*HS*). In SY95-71/CH223, 40 of the 46 plants scored IT 0 were *HR* and 6 segregated in $F_3$ lines, while among the 89 plants with IT 1 or 2, 85 segregated and 4 were *HR*. In both populations, $F_3$ distributions for *HR*, *Seg* and *HS* showed a good fit with a 1:2:1 ratio, indicating the resistance in CH223 could be under control of a single dominant gene. Again, low proportions of plants that scored IT 3 in the two crosses were shown to be heterozygous rather than homozygous susceptible on progeny testing (Table 2).

## Mapping YrCH223

### *Identification of microsatellite markers linked to YrCH223*

The $F_{2:3}$ lines of Taichung 29/CH223 were used for mapping the resistance gene. Of the 581 GWM, WMC, BARC, CFD, CFA and GDM microsatellite primers chosen for initial screening, 338 (58.2 %) were polymorphic between the parental lines. Only *Xgwm540* and *Xwmc47* were polymorphic between the contrasting parents and bulks, and fragments of about 120 and 100 bp, respectively, were associated with *YrCH223* (Fig. 2).

Because *Xwmc47* and *Xgwm540* were previously mapped to the long and short arm of chromosome 4B (Somers et al. 2004), 89 additional SSR primer pairs for chromosome 4B including the above series and the GPM series were tested. Three polymorphic markers, *Xbarc1096*, *Xwmc310* and *Xgpw7272*, were associated with resistance in both the bulk segregant pools and the parents (Fig. 4), and were used to genotype all 211 surviving plants of the $F_2$ population. Linkage analyses confirmed the genetic associations of the five SSR markers with stripe rust resistance. The $F_2$ population segregated 1:2:1 for all five markers (Table 3). Analyses with MAPMAKER/EXP also showed linkage between the markers, and *YrCH223*; *Xgwm540* and *Xbarc1096* were proximal to the resistance gene and *Xwmc47*, *Xwmc310* and *Xgpw7272* were distal (Fig. 3).

### *Chromosomal assignment*

Based on the reported chromosomal locations of the five linked microsatellite markers (Somers et al. 2004), *YrCH223* was putatively assigned to the long arm of chromosome 4B, and the order of these SSR loci agreed well with established SSR maps of chromosome 4B (http://wheat.pw.usda.gov/cgi-bin/graingenes). However, microsatellite markers are not always chromosome specific (Plaschke et al. 1996). Two of the SSR markers linked with *YrCH223* were reported to occur at more than one location in the genome; for example, *Xwmc47* was assigned to 4BL, 5AS and 5BS, and *Xgwm540* to 5BS and 4BS/4BL (Paillard et al. 2003; Somers et al. 2004; http://wheat.pw.usda.gov/cgi-bin/graingenes). *Xwmc310*, *Xbarc1096* and *Xgpw7272* were mapped only to 4BL. The locations of the linked microsatellite loci were also verified using CS nullitetrasomic and ditelosomic lines. Four of the five microsatellite primer pairs, BARC1096, WMC310, GPW7272 and WMC47, amplified products of the expected size in CS

**Table 3** $F_2$ genotypes of Taichung 29/CH223 inferred from reactions of $F_3$ families and genotypes of five closely linked SSR markers and the level of association between the resistance and each SSR marker

| Genotype | Total | *Xgpw7272* | | | *Xwmc310* | | | *Xwmc47* | | | *Xbarc1096* | | | *Xgwm540* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | Aa | aa | AA | Aa | aa | AA | Aa | aa | AA | Aa | aa | AA | Aa | aa |
| *YrYr* | 53 | 42[a] | 9 | 2 | 44 | 7 | 2 | 44 | 8 | 1 | 41 | 9 | 3 | 36 | 10 | 7 |
| *Yryr* | 111 | 5 | 100 | 6 | 6 | 102 | 3 | 6 | 99 | 6 | 8 | 101 | 2 | 11 | 93 | 7 |
| *yryr* | 47 | 4 | 5 | 38 | 2 | 3 | 42 | 1 | 5 | 41 | 3 | 1 | 43 | 5 | 7 | 35 |
| Total | 211 | 51 | 114 | 46 | 52 | 112 | 47 | 51 | 112 | 48 | 52 | 111 | 48 | 52 | 110 | 49 |
| $\chi^{2b}_{(1:2:1)}$ | 0.915 | | 1.607 | | | 1.038 | | | 0.886 | | | 0.725 | | | 0.469 | |
| *P* value | | | 1.17E−38 | | | 5.19E−45 | | | 1.77E−40 | | | 5.78E−37 | | | 5.37E−23 | |
| *r* | | | 0.752 | | | 0.790 | | | 0.765 | | | 0.739 | | | 0.623 | |

[a] *AA* homozygous for the CH223 allele, *aa* homozygous for the Taichung 29 allele, *Aa* heterozygous

[b] Table value of $\chi^2$ at $P = 0.05$ and 2 *df* is 5.99

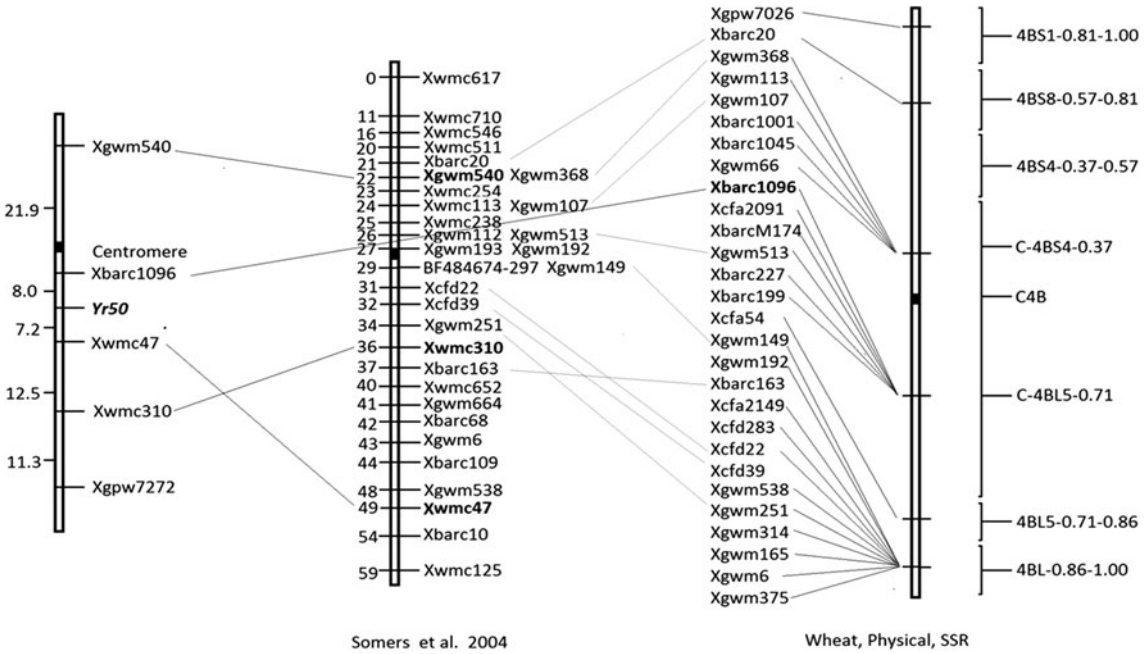


**Fig. 3** Map position of *Yr50* on chromosome 4BL and comparison of the order of SSR markers with those in the consensus (Somers et al. 2004) and physical (http://wheat.pw.usda.gov/cgi-bin/cmap/) maps for hexaploid wheat

and the CS nulli-tetrasomic lines N4AT4B and N4DT4B, but no PCR product was observed in the nulli-tetrasomic N4BT4A and Dt4BS lines for any of them (Fig. 4). The absence of PCR products in the N4BT4A and Dt4BS lines further confirmed the assignment of the linked microsatellite markers to the long arm of chromosome 4BL. Based on its origin and map location, the dominant allele *YrCH223* was apparently new and was therefore designated *Yr50*.

## Discussion

Alien introgressions represent rich sources of genes for crop improvement. *Th. intermedium* is an important perennial *Triticeae* species with considerable potential for wheat improvement. As they are readily crossable with wheat, *Th. intermedium*-derived partial amphiploids have been widely used in attempts to transfer useful traits into wheat, including resistance to viral diseases. Transfer of *Thinopyrum* chromatin conferring resistance to barley yellow dwarf virus, wheat streak mosaic virus, leaf rust, stem rust (Friebe et al. 1996; Fedak and Han 2005), scab (Han et al. 2003) and powdery mildew (He et al. 2009; Luo et al. 2009) into wheat has been reported, and some genes were incorporated into wheat and tagged with molecular markers (Fedak and Han 2005; He et al. 2009; Qi et al. 2007; Luo et al. 2009).

Certain wheat–*Th. intermedium* derivatives have high resistance to Chinese *Pst* races, including CYR32 and

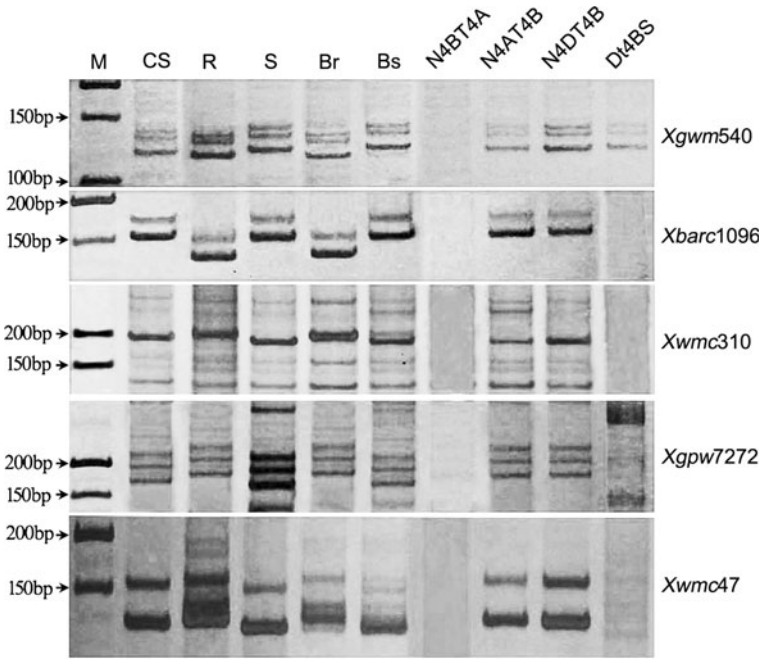


**Fig. 4** Chromosomal localizations of five linked microsatellite markers in CH223 (*R*), Taichung 29 (*S*), Chinese Spring (CS), resistant bulk (*Br*), susceptible bulk (*Bs*), nulli-tetrasomic and ditelosomic 4BS (Dt4BS) lines of homoeologous group 4. No PCR products were generated by nullisomic 4B (N4BT4A) and Dt4BS individuals (except for *Xgwm540*)

CYR33. A resistance gene was recently found in partial amphiploids and in the substitution line 1St(1D), in which a St-chromosome was substituted for wheat chromosome 1D (Yang et al. 2006; Chang et al. 2010; Hu et al. 2011). However, there is no published report of transfer of stripe rust resistance from this species to a wheat chromosome. In the present study, a novel stripe rust resistance gene was presumably transferred from *Th. intermedium* into common wheat, using a resistant partial amphiploid as a bridging parent in crosses with susceptible wheat lines. After selection in the $BC_1F_2$ for fertility and resistance to stripe rust, followed by a second backcross with a susceptible wheat cultivar, and further selfing and selection of resistant plants, stable hexaploid introgression lines, including CH223, with good agronomic appearance and resistance to stripe rust were obtained.

In the present study, $F_2$ plants in the most resistant and most susceptible groups were predominantly homozygous, whereas those with the intermediate IT 1–2 predominantly segregated in $F_3$. The few discrepancies indicated that $F_2$ phenotypes were not always predictive of genotypes. The intermediate phenotypes of some heterozygous $F_2$ plants were most probably caused by background effects commonly observed in stripe rust testing (McIntosh et al. 1995).

CH223 was produced by crossing and backcrossing TAI7047 with susceptible wheat cultivars and selection for stripe rust resistance. However, based on GISH and chromosome pairing of $F_1$ hybrids of CH223 and Taichung 29, we found no cytological evidence for an alien translocation. The gene on chromosome 4BL named *Yr50* must either be present in a cryptic translocation involving a small alien segment from *Th. intermedium*, or a wheat gene derived from an unknown source. Cryptic alien transfers have been reported in other studies (Dong et al. 2004; Kuraparthy et al. 2007). More studies are needed to determine the source of *Yr50*.

Molecular markers closely linked to (preferably flanking) resistance genes permit marker-assisted selection enabling transfer of resistance genes without performing disease tests. Introgression of disease resistance genes from related species or genera into wheat has become crucial to the continuing need for sources of resistance to stripe rust in wheat. Because resistance to stripe rust in many Chinese cultivars has a relatively narrow genetic base, the availability of *Yr50* in wheat and identification of flanking markers should be beneficial for increasing the overall diversity available for wheat breeding.

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