Cytogenetic study and stripe rust response of the derivatives from a wheat–Thinopyrum intermedium – Psathyrostachys huashanica trigenetic hybrid

Journal:	<i>Genome</i>
Manuscript ID	gen-2016-0135.R2
Manuscript Type:	Article
Date Submitted by the Author:	17-Oct-2016
Complete List of Authors:	Houyang, Kang; Triticeae Research Institute, Sichuan Agricultural University Tang, Lin; Triticeae Research Institute, Sichuan Agricultural University Li, Daiyan; Triticeae Research Institute, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, 611130, Diao, Chengdou; Triticeae Research Institute, Sichuan Agricultural University Zhu, Wei; Triticeae Research Institute, Sichuan Agricultural University Tang, Yao; Triticeae Research Institute, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, 611130, Wang, Yi; Triticeae Research Institute, Sichuan Agricultural University Fan, Xing; Triticeae Research Institute, Sichuan Agricultural University, Xu, Lili; Triticeae Research Institute, Sichuan Agricultural University Zeng, Jian; Resources College of Sichuan Agricultural University, Wenjiang, Lina, Sha; Triticeae Research Institute, Sichuan Agricultural University Yu, Xiaofang; College of Landscape Architecture, Sichuan Agricultural University Haiqin, Zhang; Triticeae Research Institute, Sichuan Agricultural University Zhou, Yong-Hong; Triticeae Research Institute
Keyword:	

SCHOLARONE™
Manuscripts

Cytogenetic study and stripe rust response of the derivatives from a wheat–*Thinopyrum intermedium* – *Psathyrostachys huashanica* trigenetic hybrid

Hou-Yang Kang¹, Lin Tang¹, Dai-Yan Li¹, Cheng-Dou Diao¹, Wei Zhu¹, Yao Tang¹, Yi Wang¹, Xing Fan¹, Li-Li Xu¹, Jian Zeng², Li-Na Sha¹, Xiao-Fang Yu³, Hai-Qin Zhang¹, Yong-Hong Zhou¹

¹Triticeae Research Institute, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, Chengdu 611130, Sichuan, China.

²College of Resources, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, Chengdu 611130, Sichuan, China.

³College of Landscape Architecture, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, Chengdu 611130, Sichuan, China.

Corresponding author:

Yonghong Zhou, E-mail: zhouyh@sicau.edu.cn,

Tel: 086-028-86291005,

Fax: 086-028-82650350

Conflict of interest statement: We declare that we have no conflict of interest.

Abstract: To transfer multiple desirable alien genes into common wheat, we previously reported a new trigeneric hybrid synthesized by crossing a wheat–*Thinopyrum intermedium* partial amphiploid with wheat–*Psathyrostachys huashanica* amphiploid. Here, the meiotic behavior, chromosome constitution, and stripe rust resistance of F₅ derivatives from the wheat–*Th. intermedium*–*P. huashanica* trigeneric hybrid were studied. Cytological analysis indicated the F₅ progenies had chromosome numbers of 42–50 (average 44.96). The mean meiotic configuration was 1.28 univalents, 21.74 bivalents, 0.04 trivalents, and 0.02 tetravalents per pollen mother cell. In 2n = 42 lines, the average pairing configuration was 0.05 I + 19.91 II (ring) + 1.06 II (rod) + 0.003 IV, suggesting these lines were cytologically stable. Most lines with 2n = 43, 44, 46, 48 or 50, bearing a high frequency of univalents or multivalents, showed abnormal meiotic behavior. Genomic *in situ* hybridization karyotyping results revealed that 25 lines contained 1–7 *Th. intermedium* chromosomes, but no *P. huashanica* chromosomes were found among the 27 self-pollinated progenies. At meiosis, univalents (1–5) possessing *Th. intermedium* hybridization signals were detected in 19 lines. Bivalents (1–3) expressing fluorescence signals were observed in 12 lines. Importantly, 21 lines harbored wheat–*Th. intermedium* chromosomal translocations with various alien translocation types. Additionally, two homozygous lines, K13-668-10 and K13-682-12, possessed a pair of wheat–*Th. intermedium* small fragmental translocations. Compared with the recurrent parent Zhong 3, most lines showed high resistance to the stripe rust (*Puccinia striiformis* f. sp. *tritici*) pathogens prevalent in China, including race V26/Gui22. This paper reports a highly efficient technical method for inducing alien translocation between wheat and *Th. intermedium* by trigeneric hybridization. These lines might be potentially valuable germplasm resources for further wheat improvement.

Keywords: Genomic *in situ* hybridization, Meiosis, *Thinopyrum intermedium*, translocation chromosome, Trigeneric hybrid.

Introduction

Wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD), one of the most important cereal crops worldwide, has been intensively bred over the past decades. However, the genetic variability of cultivated wheat has been dramatically decreased by domestication, modern cultivation, and thousands of years of selection, resulting in vulnerability to biotic and abiotic stresses (Friebe et al. 1996; Dubcovsky and Dvorak 2007). The ongoing improvement of wheat cultivars is dependent on a continuous supply of genetic variation. Wild relatives of wheat, representing a large reservoir of superior genes, have therefore become an important genetic resource, and it is becoming increasingly urgent to widen the genetic diversity of wheat by alien introgression (Mujeeb-Kazi et al. 2013). A typical and important step in alien gene transfer is the generation of wheat–alien trigeneric hybrids (Jiang et al. 1994; Ji et al. 2012; Kang et al. 2012a, b). To date, many trigeneric hybrids containing desirable genes, involving the genera *Triticum*, *Secale*, *Aegilops*, *Hordeum*, *Agropyron*, *Thinopyrum*, *Leymus*, *Dasyphyrum*, *Lophopyrum*, and *Psathyrostachys*, have been produced by crossing intergeneric hybrids or amphiploids with other species, or by crossing different amphiploids (Gupta and Fedak 1986; Orellana et al. 1989; Cabrera and Martin 1992; Li and Dong 1993; Kosina and Heslop-Harrison 1996; Mujeeb-Kazi 1996; Li et al. 2006; Ji et al. 2012; Kang et al. 2012a, b).

Species in the genus *Thinopyrum* Dewey (also placed in genera including *Agropyron*, *Elytrigia*, and *Lophopyrum*) have been described as potential sources of resistance to biotic stress (diseases and insects) and tolerance to abiotic stress (Friebe et al. 1992; King et al. 1997). Since 1930, breeders have exploited different *Thinopyrum* species to transfer these resistance genes into common wheat (Friebe et al. 1992; Fedak and Han 2005; Patokar et al. 2015). Hexaploid wheatgrass, *Thinopyrum intermedium* (Host) Barkworth and Dewey (= *Elytrigia intermedia* (Host) Nevski, *Agropyron intermedium* (Host) P. B., *Agropyrum glaucum* (Desf. ex DC) Roem. et Schult.), with the genome formula EEEstEstStSt (Wang 2011) or JJJsJsStSt (Chen et al. 1998) is a valuable source of genetic variability for improving cultivated wheat that harbors many desirable characteristics, such as high grain protein content, resistance to leaf rust, stripe rust, stem rust, powdery mildew, fusarium head blight, eyespot, smut, leaf blight, root rot, and barley yellow dwarf, and tolerance to low temperature, drought, waterlogging, and salt stress (Li and Wang 2009). Because of the high crossability of *Th. intermedium* with *Triticum* species and its agronomically interesting traits, many derivatives have been produced from wheat–*Th. intermedium* hybrids, including partial amphiploids (Sun et al. 1981; Han et al. 2004; Fedak and Han

2005; Zeng et al. 2013; Bao et al. 2014), alien addition lines (Schulz-Schaeffer and Haller 1988; Larkin et al. 1995; Garg et al. 2014), substitution lines (Fu et al. 2012; Li et al. 2015) and translocation lines (Liu et al. 2011; Tang et al. 2014; Zhan et al. 2015; Li et al. 2016).

Psathyrostachys huashanica Keng ($2n = 2x = 14$, NsNs) is a distant wild relative of common wheat native to the Huashan Mountains, Shaanxi Province, China. It possesses many desirable traits, such as early maturity, tolerance to drought and salt, and resistance to wheat stripe rust, leaf rust, powdery mildew, and take-all fungus (Kang et al. 2009). Previously, to transfer desirable traits from *Th. intermedium* and *P. huashanica* into wheat, trigeneric germplasms consisting of genomic material from *Triticum*, *Thinopyrum* and *Psathyrostachys* were successfully produced by hybridizing a wheat–*P. huashanica* amphiploid (PHW-SA, $2n = 8x = 56$, AABBDDNsNs) with a wheat–*Th. intermedium* partial amphiploid (Zhong 3, $2n = 8x = 56$) (Kang et al. 2012a). The objectives of this work were to develop F_5 derivatives from the wheat–*Th. intermedium*–*P. huashanica* trigeneric hybrid, and characterize their meiotic behavior, chromosomal constitution and stripe rust resistance.

Materials and methods

Plant materials

A wheat–*Th. intermedium* partial amphiploid, Zhong 3 ($2n = 8x = 56$), was originally developed by Sun (1981) and karyotyped by Han et al. (2004). Seeds of Zhong 3 were kindly provided by Dr. Z. J. Chang, Shanxi Academy of Agricultural Sciences, China. The intergeneric amphiploid of wheat cultivar “J-11” and *P. huashanica* (PHW-SA, $2n = 8x = 56$, AABBDDNsNs) was originally produced in our laboratory (Kang et al. 2009). It is resistant to stripe rust and powdery mildew diseases, and has many interesting yield characteristics, including higher fertility, longer spikes, and more spikelets per spike (Kang et al. 2009). PHW-SA and Zhong 3 were crossed in 2008 (Kang et al. 2012a), and the seeds selected from the F_1 plants were bulked and advanced to the F_5 generation by single seed descent. Twenty-seven derivative lines were isolated from the F_5 generation. Wheat line SY95-71 was used as a susceptible check during stripe rust resistance tests. For genomic *in situ* hybridization (GISH) analysis, the wheat cultivar “J-11” ($2n = 6x = 42$, AABBDD) was used as blocking DNA, while the probe consisted of the entire genomic DNA of *Th. intermedium* and *P. huashanica*.

Meiosis analysis

Analyses of meiotic pairing were completed as described by Kang et al. (2009). Stages of meiotic division were determined in acetocarmine squashes of one of three anthers per flower. If appropriate stages were present, the remaining two anthers were fixed in Carnoy's fixative (ethanol: chloroform: glacial acetic acid, 6: 3: 1, v/v/v) and kept in a refrigerator for 24 h, and then stored at 4°C in 70% alcohol until used. Slides of pollen mother cells (PMCs) were made in 1% iron acetocarmine. At least 50 PMCs were observed for each plant. Images were captured with an Olympus BX-51 (Olympus Corporation, Tokyo, Japan) fluorescence microscope coupled to a Photometric SenSys Olympus DP70 CCD camera.

GISH analysis

Total genomic DNA of *Th. intermedium* and *P. huashanica* was isolated using the CTAB method (Allen et al. 2006). *Th. intermedium* and *P. huashanica* genomic DNA samples were labeled with digoxigenin-11-dUTP using the nick translation mix (Roche, Mannheim, Germany) and used as probes. Root tips from the 27 F₅ derivatives germinating seeds were pretreated in ice-cold water for 24–28 h, fixed in ethanol: acetic acid (3:1 v/v) for 24 h at room temperature and then stored in refrigerator. Each root tip was squashed in a drop of 45 % acetic acid. After freezing with liquid nitrogen, the squashed slides were uncovered, air-dried, and stored at -20 °C until used. The labeling of probes and *in situ* hybridizations were conducted using a slightly modified version of the method described by Han et al. (2004). The hybridization solution, containing 2× SSC, 10% dextran sulfate, 0.2% sodium dodecyl sulphate, and 1 ng/μL labeled denatured DNA probe together with the competitor DNA, was loaded onto the slide (30 μL final volume), which was then incubated at 37 °C for 12 h. The chromosomes were counterstained with propidium iodide solution (Vector Laboratories, Inc., Burlingame, USA). The *in situ* hybridization images were obtained using an Olympus BX-51 fluorescence microscope coupled to a Photometric SenSys Olympus DP70 CCD camera.

Stripe rust resistance screening

Twenty-seven F₅ derivatives as well as their parents, PHW-SA and Zhong 3, and SY95-71 were evaluated for seedling and adult plant responses to a mixture of *Puccinia striiformis* f. sp. *tritici* (*Pst*) races CYR-32, CYR-33, V26/Gui22-9, V26/Gui22-14, Su4, and Su5. Urediniospores of the *Pst* races

were supplied by Prof. Q. Z. Jia from the Plant Protection Institute of Gansu Academy of Agricultural Sciences, Gansu Province, China. Stripe rust infection types (ITs) were scored using the following scale: 0, 0₊, 1, 2, 3, and 4, where 0 = immunity, 0₊ = necrotic flecks, and 1–4 = increasing sporulation and decreasing necrosis or chlorosis. Plants with an IT score 2 or lower were considered resistant while plants with an IT score 3 or 4 were considered susceptible. Infections were recorded three times when uniform severity levels were observed on the susceptible check SY95-71 at the booting, flowering, and milky stages (Liu 1988).

Results

Meiotic pairing analysis of F₅ progenies of the trigeneric hybrid

The chromosomal behaviors at meiosis of F₅ derivatives from the PHW-SA/Zhong 3 trigeneric hybrid were observed. Twenty-seven lines were selected randomly; the chromosome pairing frequency data are presented in Table 1. The results showed that these lines had 42–50 chromosomes, with an average of $2n = 44.96$. Most lines (59.3%) had $2n = 42$ –44, and nine plants (33.3%) had $2n = 42$. The average chromosomal configuration at meiotic metaphase I (MI) of PMCs of the F₅ lines was 1.28 univalents, 21.74 bivalents, 0.04 trivalents and 0.02 tetravalents. The mean number of univalents varied greatly per cell, from 0 to 4.52. The number of bivalents ranged from 20.75 to 24.38, with an average of 21.74 per cell. Trivalents or tetravalents were observed in 6.1% of the cells.

Among the lines with $2n = 42$, the average pairing configuration was 0.05 I + 19.91 II (ring) + 1.06 II (rod) + 0.003 IV per PMC. Most of the cells had 21 bivalents at MI, with low frequencies of univalents and/or multivalents occurring in the other cells (Table 1). The average univalent frequency ranged from 0 to 0.14. Only one line, K13-680-7, contained a tetravalent. Four lines showed complete chromosome pairing at MI (Fig. 1A), indicating that these lines were cytologically stable.

Two lines with $2n = 43$ had an average meiotic configuration of 1.28 I + 18.31 II (ring) + 2.55 II (rod) per PMC (Fig. 1B). The mean pairing configuration of five lines with $2n = 44$ was 1.12 univalents, 19.71 ring bivalents, 1.69 rod bivalents and 0.02 tetravalents (Fig. 1C). Similarly, the PMCs of lines with $2n = 46$ showed abnormal meiotic behavior, possessing approximately two univalents, with chromosome pairing configurations of 1.92 I + 19.47 II (ring) + 2.36 II (rod) + 0.14 III (Fig. 1D). Two lines containing 48 chromosomes had very high frequencies of univalents (2.48–4.52), trivalents (0–0.12) and tetravalents (0–0.23) per cell (Fig. 1E). On average, 2.29 univalents, 0.09 trivalents, and 0.02

tetravalents were observed in five lines with $2n = 50$ (Fig. 1F). In addition, lagging chromosomes, chromosome bridges, and micronuclei were observed at anaphase I and telophase I in most of the analyzed cells of these lines (Fig. 1G–I).

GISH analysis of F_5 progenies of the trigeneric hybrid

GISH was carried out to determine the chromosomal constitution of the 27 F_5 lines. Labeled *P. huashanica* genomic DNA was used as a probe and J-11 total genomic DNA as the blocking DNA. No *P. huashanica* chromosomes (segments) were detected in any of the lines. When *Th. intermedium* genomic DNA was used as the probe, we observed 1–7 *Th. intermedium* chromosomes in 25 lines (Fig. 2A–C). At meiotic MI, 1–5 univalents with yellow-green hybridization signals appeared in 19 lines (Fig. 2D). Bivalents (1–3) expressing fluorescence signals, derived from the pairing of *Th. intermedium* chromosomes, were observed in 12 lines. Interestingly, 21 (77.8%) of the 27 lines harbored wheat–*Th. intermedium* chromosomal translocations, including whole-arm translocations (Fig. 2A), whole-arm and small fragmental translocations (Fig. 2B), large fragmental translocations (Fig. 2E, F), large and small fragmental translocations (Fig. 2G), two-point terminal translocations (Fig. 2H, I), and small fragmental translocations (Fig. 2J). Two homozygous lines, K13-668-10 and K13-682-12, possessing a pair of wheat–*Th. intermedium* small terminal fragmental translocation chromosomes were identified (Fig. 2J). Some lagging chromosomes with *Th. intermedium* fragments were detected at early anaphase I, and then underwent an equational division (Fig. 2K). Portions of these chromosomes were unable to reach the poles and were eventually merged into other chromosome groups and then formed the micronuclei at telophase I (Fig. 2L).

Stripe rust resistance evaluation

Twenty-seven derivative lines, PHW-SA, Zhong 3, and SY95-71 were evaluated for stripe rust resistance using a mixture of *Pst* races CYR-32, CYR-33, V26/Gui22-9, V26/Gui22-14, Su4, and Su5 at Chengdu, Sichuan, China. At the seedling and adult plant stages, Zhong 3, SY95-71, and six derivative lines were susceptible to these races, showing IT scores of 4, 4, and 3, respectively. In contrast, PHW-SA and 21 derivative lines were highly resistant to the races, all showing IT scores of 0 (Fig. 3).

Discussion

Developing wheat–alien species translocation lines is an effective approach for introducing alien chromosome segments or superior genes from wild relatives into wheat (Qi et al. 2007). Strategies for producing wheat–alien translocations include the use of the *Ph* mutation, chromosome misdivision and centric break-fusion, tissue culture, *Aegilops* gametocidal chromosomes, irradiation, and somatic cell hybridization (Masoudi-Nejad et al. 2002; Wang et al. 2004; Qi et al. 2007). However, previous reports have indicated that the translocation frequency is generally low (Jiang et al. 1994). For example, the frequency of translocation induced by *Ph* gene mutants or tissue culture is usually less than 1%, while that induced by gametocidal chromosomes or irradiation is generally no more than 10% (Friebe et al. 2000; Song et al. 2013). Therefore, developing a more efficient method to induce wheat–alien translocations is highly desirable to speed up the gene exchanges between cultivated wheat and its related species. Recently, when wheat–alien amphiploids, addition lines or translocation lines were irradiated with ^{60}Co - γ rays at the flowering stage, a high frequency (37.83–62.5%) of alien chromosome translocations with different structural types were produced in the M_1 and M_2 generations (Chen et al. 2013; Song et al. 2013). Hao et al. (2013) also showed that high frequencies of wheat–rye translocation chromosomes were produced from a cross between synthetic hexaploid wheat with unreduced gametes and rye. In this study, we identified 21 lines containing wheat–*Th. intermedium* translocation chromosomes in the selfed derivatives from a wheat–*Th. intermedium*–*P. huashanica* trigeneric hybrid, and the frequency of alien translocation was 77.8%, much higher than in previous reports. Moreover, various translocation types were identified, including whole-arm, whole-arm and small fragmental, large fragmental, large and small fragmental, two-point terminal, and small terminal fragmental translocations.

The high frequency of translocations produced in this study might be a result of multiple chromosome recombination between homoeologous pairs in the wheat–*Th. intermedium*–*P. huashanica* trigeneric hybrids (Jiang and Gill 1993; Fu et al. 2012) or centric breakage-fusion when some chromosomes exist as univalents (Lukaszewski 1993). Centric misdivision followed by the fusion of broken centromeres often results in Robertsonian translocations (Robertson 1916). Recombination between homoeologous chromosomes is generally confined to the distal regions of chromosome arms (Lukaszewski 1995). The advantage of homoeologous recombination is that the alien segment replaces a homoeologous wheat chromosome segment, thereby producing genetically compensating

translocations. Further work involving the observation of different meiotic stages using FISH and GISH will elucidate the production mechanism of mass intergeneric translocation.

Th. intermedium possesses genes enabling it to survive high levels of salinity, drought and low temperature, as well as genes for resistance against various fungal and viral diseases (Hohmann et al. 1996; Li and Wang 2009). Translocations between wheat and *Th. intermedium* have provided the starting point for a number of attempts to transfer some of these genes into wheat (Li and Wang 2009). Numerous chromosomal translocation lines have been obtained and widely used for the improvement of wheat (Liu et al. 2011; Tang et al. 2014; Zhan et al. 2015). We previously reported that the wheat–*Th. intermedium*–*P. huashanica* trigeneric F₁ hybrid had seven *P. huashanica* chromosomes and seven *Th. intermedium* chromosomes. *P. huashanica* chromosomes were not detected in any of the 32 F₄ plants, while some wheat–*Th. intermedium* chromosomal translocations occurred in F₄ progenies by GISH analysis (Kang et al. 2012a). In this work, 21 lines contained wheat–*Th. intermedium* translocation chromosomes among 27 self-pollinated F₅ progenies. Most of the plants with translocated chromosomes grew normally and produced seeds by self-pollination because the translocated chromosomes segregated normally at anaphase and telophase I of meiosis. However, some lines with the wheat–*Th. intermedium* translocations had abnormal meiotic behavior due to the *Th. intermedium* and/or wheat univalent chromosomes. Homozygous translocation lines may be obtained from these lines via crossing and backcrossing with recipient wheat and chromosome engineering. Two homozygous wheat–*Th. intermedium* small fragmental translocation lines, K13-668-10 and K13-682-12, were identified and characterized. At MI, the chromosome pairing association of K13-668-10 was similar to that of K13-682-12 with high frequencies of bivalents and very low univalent formation. These meiotic configurations indicated that the two novel translocation lines had basic cytological stability with a vigorous growth habit and high fertility. Therefore, the present study suggests that new wheat–*Th. intermedium* translocation lines can be produced by the wheat–*Th. intermedium*–*P. huashanica* trigeneric hybrid method.

Furthermore, most of the derivative lines are highly resistant to prevalent Chinese *Pst* races, including V26/Gui 22. Of their parents, PHW-SA is resistant to all tested races, while Zhong3 is susceptible. We previously concluded that the resistance of PHW-SA was likely derived from *P. huashanica* (Kang et al. 2009). Therefore, this pedigree provides the only evidence that these lines carry the stripe rust resistance gene from *P. huashanica*. Interestingly, *in situ* hybridization signals were

undetectable when *P. huashanica* genomic DNA was used as a probe. The stripe rust resistance gene in these lines is likely associated with a cryptic translocation involving a small *P. huashanica* chromosomal segment. Such cryptic translocation is alien introgression that can be undetected by cytological methods and rarely a few molecular markers (Kuraparthi et al. 2007). Similar cryptic introgressions have also been reported for hybrids involving wheat and *Ae. geniculata*, *Ae. triuncialis*, *Dasyphyrum villosum*, *Thinopyrum intermedium*, and *Thinopyrum ponticum* (Kuraparthi et al. 2007; Caceres et al. 2012), as well as for wheat–rye–*P. huashanica* (Kang et al. 2016; Qi et al. 2016). These materials are potentially interesting as sources of improved resistance to prevalent races of stripe rust in wheat. The priority now will be to develop more homozygous translocation lines with the desirable genes relevant to wheat improvement, to evaluate their agronomic performance, and to monitor the expression of donor genes in the translocation plants.

Acknowledgments

This work was funded by the National Key Research and Development Program of China (No. 2016YFD0102000), the National Natural Science Foundation of China (No. 31501311), and the Science and Technology Bureau of Sichuan Province (No. 2016HH0048). We also thank the anonymous reviewers for their very useful comments regarding the manuscript.

References

- Allen, G.C., Flores-Vergara M.A., Krasynanski S., Kumar S., and Thompson W.F. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nat. Protoc.* **1**: 2320–2325.
- Bao, Y.G., Wu X., Zhang C., Li X.F., He F., Qi X.L., et al. 2014. Chromosomal constitutions and reactions to powdery mildew and stripe rust of four novel wheat–*Thinopyrum intermedium* partial amphiploids. *J Genet. Genomics*, **41**: 663–666.
- Cabrera, A., and Martin A. 1992. A trigeneric hybrids between *Hordeum*, *Aegilops*, and *Secale*. *Genome*, **35**: 647–649.
- Caceres, M.E., Pupilli F., Ceccarelli M., Vaccino P., Sarri V., De Pace C., et al. 2012. Cryptic introgression of *Dasyphyrum villosum* parental DNA in wheat lines derived from intergeneric hybridization. *Cytogenet. Genome Res.* **136** :75–81.
- Chen, P.D., You C.F., Hu Y., Chen S.W., Zhou B., Cao A.Z., et al. 2013. Radiation-induced translocations with reduced *Haynaldia villosa* chromatin at the *Pm21* locus for powdery mildew resistance in wheat. *Mol. Breed.* **31**: 477–484.
- Chen, Q., Conner R.L., Laroche A., and Thomas J.B. 1998. Genome analysis of *Thinopyrum intermedium* and *Th. ponticum* using genomic in situ hybridization. *Genome*, **41**: 580–586.
- Dubcovsky, J., and Dvorak J. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*, **316**: 1862–1866.

- Fedak, G., and Han F. 2005. Characterization of derivatives from wheat-*Thinopyrum* wide crosses. *Cytogenet. Genome Res.* **109**: 360–367.
- Friebe, B., Zeller F.J., Mukai Y., Forster B.P., Bartos P., and McIntosh R.A. 1992. Characterization of rust-resistant wheat *Agropyron intermedium* derivatives by C-Banding, in situ hybridization and isozyme analysis. *Theor. Appl. Genet.* **83**: 775–782.
- Friebe, B., Jiang J., Raupp W.J., McIntosh R.A., and Gill B.S. 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica*, **91**: 59–87.
- Friebe, B., Kynast R.G., and Gill B.S. 2000. Gametocidal factor-induced structural rearrangements in rye chromosomes added to common wheat. *Chromosome Res.* **8**: 501–511.
- Fu, S.L., Lv Z.L., Qi B., Guo X., Li J., Liu B., et al. 2012. Molecular cytogenetic characterization of wheat- *Thinopyrum elongatum* addition, substitution and translocation lines with a novel source of resistance to wheat fusarium head blight. *J. Genet. Genomics*, **39**: 103–110.
- Garg, M., Yanaka M., Tanaka H., and Tsujimoto H. 2014. Introgression of useful genes from *Thinopyrum intermedium* to wheat for improvement of bread-making quality. *Plant Breed.* **133**: 327–334.
- Gupta, P.K., and Fedak G. 1986 Intergeneric hybrids between *Triticosecale* cv. Welsh (2n = 42) and three genotypes of *Agropyron intermedium* (2n = 42). *Can. J. Genet. Cytol.* **28**: 176–179.
- Han, F.P., Liu B., Fedak G., and Liu Z. 2004. Genomic constitution and variation in five partial amphiploids of wheat-*Thinopyrum intermedium* as revealed by GISH, multicolor GISH and seed storage protein analysis. *Theor. Appl. Genet.* **109**: 1070–1076.
- Hao, M., Luo J.T., Zhang L.Q., Yuan Z.W., Yang Y.W., Wu M., et al. 2013. Production of hexaploid triticale by a synthetic hexaploid wheat-rye hybrid method. *Euphytica*, **193**: 347–357.
- Hohmann, U., Busch W., Badaeva K., Friebe B., and Gill B.S. 1996. Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. *Genome*, **39**: 336–347.
- Ji, J., Zhang A.M., Wang Z.G., Wang J., Zhang W., Liu D.C., et al. 2012. A wheat-*Thinopyrum ponticum*-rye trigenic germplasm line with resistance to powdery mildew and stripe rust. *Euphytica*, **188**: 199–207.
- Jiang, J.M., Friebe B., and Gill B.S. 1994. Recent advances in alien gene transfer in wheat. *Euphytica*, **73**: 199–212.
- Jiang, J.M., and Gill B.S. 1993. A “zebra” chromosome arising from multiple translocations involving non-homologous chromosomes. *Chromosoma*, **102**: 612–617.
- Kang, H.Y., Wang Y., Sun G.L., Zhang H.Q., Fan X., and Zhou Y.H. 2009. Production and characterization of an amphiploid between common wheat and *Psathyrostachys huashanica* Keng ex Kuo. *Plant Breed.* **128**: 36–40.
- Kang, H.Y., Zeng J., Xie Q., Tao S., Zhong M.Y., Zhang H.Q., et al. 2012a. Molecular cytogenetic characterization and stripe rust response of a trigenic hybrid involving *Triticum*, *Psathyrostachys*, and *Thinopyrum*. *Genome*, **55**: 383–390.
- Kang, H.Y., Zhong M.Y., Xie Q., Zhang H.Q., Fan X., Sha L.N., et al. 2012b. Production and cytogenetics of trigenic hybrid involving *Triticum*, *Psathyrostachys* and *Secale*. *Genet. Resour. Crop Evol.* **59**: 445–453.
- Kang, H.Y., Wang H., Huang J., Wang Y.J., Li D.Y., Diao C.D., et al. 2016. Divergent development of hexaploid triticale by a wheat-rye-*Psathyrostachys huashanica* trigenic hybrid method. *PLoS ONE*, **11**: e0155667.
- King, I.P., Forster B.P., Law C.C., Cant K.A., Orford S.E., Gorham J., et al. 1997. Introgression of salt-tolerance genes from *Thinopyrum bessarabicum* into wheat. *New Phytologist*, **137**: 75–81.

- Kosina, R., and Heslop-Harrison J.S. 1996. Molecular cytogenetics of an amphiploid trigeneric hybrid between *Triticum durum*, *Thinopyrum distichum* and *Lophopyrum elongatum*. *Ann. Bot.* **78**: 583–589.
- Kuraparthi, V., Sood S., Chhuneja P., Dhaliwal H.S., Kaur S., Bowden R.L., et al. 2007. A cryptic wheat-*Aegilops triuncialis* translocation with leaf rust resistance gene *Lr58*. *Crop Sci.* **47**: 1995–2003.
- Larkin, P.J., Banks P.M., Lagudah E.S., Apple R., Chen X., Xin Z.Y., et al. 1995. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. *Genome*, **38**: 385–394.
- Li, G.R., Lang T., Dai G., Li D.H., Li C.H., Song X.J., et al. 2015. Precise identification of two wheat-*Thinopyrum intermedium* substitutions reveals the compensation and rearrangement between wheat and *Thinopyrum* chromosomes. *Mol. Breed.* **35**: 1 DOI 10.1007/s11032-015-0202-z.
- Li G.R., Wang H.J., Lang T., Li J.B., La S.X., Yang E.N., et al. 2016. New molecular markers and cytogenetic probes enable chromosome identification of wheat-*Thinopyrum intermedium* introgression lines for improving protein and gluten contents. *Planta*, DOI 10.1007/s00425-016-2554-y.
- Li, H., and Wang X. 2009. *Thinopyrum ponticum* and *Th. intermedium*: the promising source of resistance to fungal and viral diseases of wheat. *J. Genet. Genomics*, **36**: 557–565.
- Li, L.H., and Dong Y.C. 1993. A self-fertile trigeneric hybrid, *Triticum aestivum* × *Agropyron michnoi* × *Secale cereale*. *Theor. Appl. Genet.* **87**: 361–368.
- Li, X.F., Song Z.Q., Liu S.B., Gao J.R., and Wang H.G. 2006. Cytogenetic study of a trigeneric (*triticales* × *tritileymus*) hybrid. *Euphytica*, **150**: 117–122.
- Liu X.K. 1988. Study on the yellow rust resistance to common wheat (*T. aestivum*). *Plant Prot.* **15**: 33–39.
- Liu, W., Seifers D.L., Qi L.L., Friebe B., and Gill B.S. 2011. A compensating wheat-*Thinopyrum intermedium* robertsonian translocation conferring resistance to wheat streak mosaic virus and *Triticum* mosaic virus. *Crop Sci.* **51**: 2382–2390.
- Lukaszewski, A.J. 1993. Reconstruction in wheat of complete chromosomes 1B and 1R from the IRS.1BL translocation of 'Kavkaz' origin. *Genome*, **36**: 821–824.
- Lukaszewski, A.J. 1995. Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticales. *Theor. Appl. Genet.* **90**: 714–719.
- Masoudi-Nejad, A., Nasuda S., McIntosh R.A., and Endo T.R. 2002. Transfer of rye chromosome segments to wheat by a gametocidal system. *Chromosome Res.* **10**: 349–357.
- Mujeeb-Kazi, A. 1996. Apomixia in trigeneric hybrids of *Triticum aestivum*/*Leymus racemosus*//*Thinopyrum elongatum*. *Cytologia*, **61**: 15–18.
- Mujeeb-Kazi, A., Kazi A.G., Dundas I., Rasheed A., Ogonnaya F., Kishi M., et al. 2013. Genetic diversity for wheat improvement as a conduit for food security. *In* *Advances in agronomy*. Vol. 122. Edited by D. Sparks. Academic Press, Burlington. pp. 179–257.
- Orellana, J., Vazquez J.F., and Carrillo J.M. 1989. Genome analysis in wheat-rye-*Aegilops caudate* trigeneric hybrids. *Genome*, **32**: 169–172.
- Patokar, C., Seps A., Schwarzacher T., Kishii M., and Heslop-Harrison J.S. 2015. Molecular cytogenetic characterization of novel wheat-*Thinopyrum bessarabicum* recombinant lines carrying intercalary translocations. *Chromosoma*, **125**: 1–10.
- Qi, L.L., Friebe B., Zhang P., and Gill B.S. 2007. Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res.* **15**: 3–19.

- Qi, W.L., Tang Y., Zhu W., Li D.Y., Diao C.D., Xu L.L., et al. 2016. Molecular cytogenetic characterization of a new wheat-rye 1BL•1RS translocation line expressing superior stripe rust resistance and enhanced grain yield. *Planta*, **244**: 405–416.
- Robertson, W. M. R. B. 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of *Tettigidae* and *Acrididae*: V-shaped chromosomes and their significance in *Acrididae*, *Locustidae* and *Grillidae*: chromosomes and variations. *J. Morphol.* **27**: 179–331.
- Schulz-Schaeffer, J., and Haller S.E. 1988. Alien chromosome addition in durum wheat. II. Advanced progeny. *Genome*, **30**: 303–306.
- Song, L.Q., Jiang L.L., Han H.M., Gao A.N., Yang X.M., Li L.H., et al. 2013. Efficient induction of wheat-*Agropyron cristatum* 6P translocation lines and GISH detection. *PLoS ONE*, **8**: e69501.
- Sun, S.C. 1981. The approach and methods of breeding new varieties and new species from *Agrotriticum* hybrids. *Acta Agron. Sin.* **7**: 51–58.
- Tang, X.Q., Shi D., Xu J., Li Y.L., Li W.J., Ren Z.L., et al. 2014. Molecular cytogenetic characteristics of a translocation line between common wheat and *Thinopyrum intermedium* with resistance to powdery mildew. *Euphytica*, **197**: 201–210.
- Wang, L., Chen X., and Liu X. 2004. Wheat mutation breeding. *In* Inducing alien chromosome translocation and germplasm innovation. China Agricultural Science and Technology Press, China: Beijing. pp. 300–307.
- Wang, R.R.C. 2011. *Agropyron* and *Psathyrostachys*. *In* Wild crop relatives: genomic and breeding resources, cereals. *Edited by* C. Kole. Springer, Heidelberg. pp. 77–108.
- Zeng, J., Cao W., Fedak G., Sun S., Mccallum B., Fetch T., et al. 2013. Molecular cytological characterization of two novel durum- *Thinopyrum intermedium* partial amphiploids with resistance to leaf rust, stem rust and Fusarium head blight. *Hereditas*, **150**: 10-16.
- Zhan, H.X., Zhang X.J., Li G.R., Pan Z.H., Hu J., Li X., et al. 2015. Molecular characterization of a new wheat-*Thinopyrum intermedium* translocation line with resistance to powdery mildew and stripe rust. *Int. J. Mol. Sci.* **16**: 2162–2173.

Table 1. Chromosome pairing at metaphase I in pollen mother cells of the PHW-SA/Zhong 3 F₅ progenies

Lines	2n	No of cells observed	Chromosomes pairing					
			I	II (Ring)	II (Rod)	II (Total)	III	IV
K13-668-10	42	50	–	19.77 (19-21)	1.23 (0-2)	21.00 (21-21)	–	–
K13-671-1	42	50	0.14 (0-4)	19.78 (18-20)	1.15 (0-3)	20.93 (19-21)	–	–
K13-671-8	42	50	–	19.67 (18-21)	1.33 (0-3)	21.00 (21-21)	–	–
K13-680-7	42	50	–	19.66 (17-21)	1.28 (0-4)	20.94 (19-21)	–	0.03 (0-1)
K13-682-1	42	50	0.04 (0-2)	20.43 (17-21)	0.55 (0-4)	20.98 (20-21)	–	–
K13-682-12	42	50	0.08 (0-2)	19.96 (16-21)	1.00 (0-4)	20.96 (20-21)	–	–
K13-683-10	42	50	0.12 (0-2)	20.32 (18-21)	0.62 (0-3)	20.94 (20-21)	–	–
K13-685-1	42	50	0.10 (0-2)	19.69 (19-21)	1.26 (0-2)	20.95 (20-21)	–	–
K13-688-8	42	50	–	19.89 (19-21)	1.11 (0-2)	21.00 (21-21)	–	–
K13-668-1	43	50	1.16 (1-3)	19.68 (16-21)	1.24 (0-5)	20.92 (20-21)	–	–
K13-674-4	43	50	1.40 (1-5)	16.94 (13-18)	3.86 (2-7)	20.80 (19-21)	–	–
K13-668-6	44	50	2.40 (0-6)	19.08 (16-20)	1.72 (1-6)	20.80 (19-22)	–	–
K13-670-2	44	50	0.14 (0-2)	20.58 (19-22)	1.35 (0-3)	21.93 (21-22)	–	–
K13-671-2	44	50	2.50 (0-6)	19.39 (17-20)	1.36 (1-4)	20.75 (19-22)	–	–
K13-673-9	44	50	0.28 (0-4)	19.09 (15-20)	2.59 (1-6)	21.68 (20-22)	–	0.09 (0-1)
K13-689-11	44	50	0.28 (0-2)	20.43 (19-22)	1.43 (0-3)	21.86 (21-22)	–	–
K13-668-5	46	50	1.40 (0-4)	21.18 (18-22)	1.12 (0-5)	22.30 (21-23)	–	–
K13-669-2	46	50	1.91 (0-5)	17.81 (15-20)	3.53 (2-6)	21.34 (19-23)	0.47 (0-2)	–
K13-670-3	46	50	2.05 (1-5)	19.52 (17-21)	2.38 (1-5)	21.90 (19-22)	0.05 (0-1)	–
K13-670-7	46	50	2.27 (0-6)	19.38 (17-22)	2.41 (0-6)	21.79 (20-23)	0.05 (0-1)	–
K13-693-1	48	50	2.48 (0-6)	18.45 (15-22)	3.85 (1-8)	22.30 (21-24)	–	0.23 (0-1)
K13-694-4	48	50	4.52 (2-7)	17.28 (16-20)	4.28 (2-7)	21.56 (19-23)	0.12 (0-1)	–
K13-692-5	50	50	2.38 (0-6)	18.86 (16-23)	4.95 (2-8)	23.81 (22-25)	–	–
K13-692-9	50	50	1.21 (0-4)	19.77 (15-24)	3.73 (1-8)	23.50 (21-25)	0.45 (0-2)	0.11 (0-1)
K13-695-1	50	50	2.71 (2-4)	20.69 (16-23)	2.95 (1-7)	23.64 (23-24)	–	–
K13-696-6	50	50	1.24 (0-6)	19.38 (13-22)	5.00 (2-11)	24.38 (22-25)	–	–
K13-697-9	50	50	3.92 (2-8)	17.91 (9-21)	5.13 (3-12)	23.04 (21-24)	–	–

Figure captions

Fig. 1. Meiotic behaviors of the PHW-SA/Zhong 3 F₅ lines. (A) Line K13-668-10, $2n = 42 = 1 \text{ II (rod)} + 20 \text{ II (ring)}$; (B) Line K13-668-1, $2n = 43 = 1 \text{ I} + 1 \text{ II (rod)} + 20 \text{ II (ring)}$; (C) Line K13-670-2, $2n = 44 = 2 \text{ I} + 2 \text{ II (rod)} + 19 \text{ II (ring)}$; (D) Line K13-669-2, $2n = 46 = 1 \text{ I} + 6 \text{ II (rod)} + 15 \text{ II (ring)} + 1 \text{ III (arrow)}$; (E) Line K13-693-1, $2n = 48 = 2 \text{ I} + 2 \text{ II (rod)} + 21 \text{ II (ring)}$; (F) K13-697-9, $2n = 50 = 8 \text{ I} + 12 \text{ II (rod)} + 9 \text{ II (ring)}$; (G) Line K13-670-3, lagging chromosomes at anaphase I (arrow); (H) Line K13-673-9, chromosome bridges at anaphase I (arrow); (I) Line K13-692-9, micronuclei at telophase I (arrow).

Fig. 2. GISH identification of the PHW-SA/Zhong 3 F₅ lines at mitotic metaphase (A–C), and meiotic metaphase I (D–J), anaphase I (K), and telophase I (L). Total *Th. intermedium* genomic DNA labeled with digoxigenin-11-dUTP was used as a probe for genomic in situ hybridization (GISH). Red and yellow-green in the GISH patterns represent wheat and *Th. intermedium* chromosomes, respectively. Arrows indicate the different types of wheat–*Th. intermedium* translocated chromosomes. (A) Three *Th. intermedium* and one whole-arm translocation chromosomes; (B) One *Th. intermedium*, three whole-arm and small fragmental translocation chromosomes; (C) One *Th. intermedium* smaller size chromosome (asterisk); (D) Two *Th. intermedium* chromosomes (2 I); (E) One *Th. intermedium* (1 I) and one large fragmental translocation chromosomes; (F) Two *Th. intermedium* (1 rod II) and one large fragmental translocation chromosomes; (G) Two *Th. intermedium* (2 I), one large fragmental and one small fragmental translocation chromosomes; (H) One *Th. intermedium* (1 I) and one two-point translocation chromosomes; (I) Seven *Th. intermedium* (3 I + 1 ring II + 1 rod II) and one two-point translocation chromosomes; (J) Two small fragmental translocation chromosomes; (K) lagging chromosomes with small fragmental translocation; (L) normal dyad with small fragmental translocation.

Fig. 3. Stripe rust resistance of the PHW-SA/Zhong 3 F₅ lines and the controls. (1) SY95-71; (2) Zhong 3; (3) PHW-SA; (4, 5) resistant derivative lines K13-668-10 and K13-682-12; (6, 7) susceptible derivative lines K13-674-4 and K13-696-6.

169x168mm (300 x 300 DPI)

169x232mm (300 x 300 DPI)

169x101mm (300 x 300 DPI)