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*Full Length Research Paper*

# Cytogenetic, genomic *in situ* hybridization (GISH) and agronomic characterization of alien addition lines derived from wheat-*Psathyrostachys huashanica*

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**Alien chromosome lines were developed and identified from the $BC_1F_2$ and $BC_1F_3$ generations of a wheat-*Psathyrostachys huashanica* intergeneric cross. Their agronomic traits were evaluated in the field and their meiotic behaviors and chromosome composition were analyzed by cytogenetic and GISH (genomic *in situ* hybridization) techniques. Five lines with 2n = 44 and one line with 2n = 46 showed regular meiosis and were cytologically stable. Based on chromosome pairing, C-banding and GISH analysis, lines 156-4, 160-12, 160-13, 173-2 and 197-16 (2n = 44) were alien disomic addition lines with the addition of one pair of the *P. huashanica* 5Ns chromosomes. Line 165-2, which had each pair of the *P. huashanica* chromosomes 3Ns and 5Ns being added, was an alien double addition line. Nine lines with the *P. huashanica* chromosome 1Ns, 2Ns, 4Ns or 5Ns were alien monosomic addition lines. Robertsonian translocation was observed in lines 241-2 and 241-10. Some chromosomal abnormalities were observed such as telosomics, lagging of telosomics and telosomic sister chromatids that moved to one pole at anaphase I and asynchronous chromosome separation at telophase II. These results indicated that, the presence of alien chromosomes from *P. huashanica* had influenced the meiosis. Meanwhile, by comparing the series of wheat-*P. huashanica* chromosome addition lines, the gene(s) for awns were mapped on the *P. huashanica* 5Ns chromosome.**

**Key words:** Alien addition lines, giemsa C-banding, genomic *in situ* hybridization, *Psathyrostachys huashanica* Keng ex Kuo, bread wheat.

## INTRODUCTION

Due to its narrow genetic background and genetic diversity, bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) yield is often subject to significant losses from biotic and/or abiotic stresses (Sears, 1956; Gale and Miller, 1987; Jiang et al., 1994; Friebe et al., 1996). An efficient strategy to broaden the genetic base of wheat is to introgress the stress resistance gene from its wild relatives in the tertiary gene pool into wheat through distant crosses (Lin et al., 2007). A number of desirable traits in its wild relatives have been and are being incorporated into wheat. Alien chromosome lines have been developed in wheat and other crops and have been used as genetic sources of valuable traits for the recipient species (Islam, 1983; Ananiev et al., 1997; Rubiales et al., 1999; Islam and Shepherd, 2000; Jauhar et al., 2009). Alien chromosome lines have also been used to map genes on the introgressed chromosomes and to isolate individual chromosomes and genes of interest (Okagaki et al., 2001; Cho et al., 2006; Mica et al., 2006). Normally, characterization of individual alien chromosome or chromosomal segment for its effects on cytological and agronomic traits

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is a prerequisite for efficient utilization of the introduced alien genes in alien addition, substitution and translocation lines.

*Psathyrostachys* Nevski is a small tertiary gene pool of the tribe Triticeae (Poaceae). It consists of eight species (Baden, 1991; Petersen et al., 2004), which possess abundant valuable genes, such as early maturity, tolerance to salinity and drought and resistance to barley yellow dwarf virus, dwarfing, stripe rust and take-all fungus diseases (Dewey, 1984; Plourde et al., 1990; Chen et al., 1991; Wang and Shang, 2000; Cao et al., 2005; Kang et al., 2009). To date, only *Psathyrostachys juncea* (Fish) Nevski (2n = 4x = 28, NsNs) and *Psathyrostachys huashanica* Keng ex Kuo (2n = 2x = 14, NsNs) have been used for gene introgression into wheat (Chen et al., 1991; Plourde et al., 1990; Sun et al., 1992; Mujeeb-Kazi et al., 1995; Zhou et al., 1997, 1998; Wang and Shang, 2000; Fu et al., 2003; Cao et al., 2005; Kang et al., 2009; Zhao et al., 2009). The distribution of *P. huashanica* is restricted to the Huashan pass of Qinling Mountains, Shaanxi Province, China (Kuo, 1987; Baden, 1991). *P. huashanica* has attracted the attention of wheat breeders because of its early maturity, drought and salinity tolerance, resistance to stripe rust, dwarfing, wheat take-all fungus and powdery mildew diseases (Chen et al., 1991; Jing et al., 1999; Wang and Shang, 2000; Cao et al., 2005; Kang et al., 2009; Zhao et al., 2009). Due to its limited distribution and invaluable genetic variability, *P. huashanica* has been listed as an endangered and imperatively protected wild species in China (Yue et al., 2001; Hang et al., 2004) and has been used for hybridization with wheat since the end of 1980s (Chen et al., 1991; Sun et al., 1992). Some monosomic addition lines and substitution lines involving the chromosomes 5Ns and 6Ns of *P. huashanica* as well as amphiploids were reported (Zhao et al., 2004a, b; Kang et al., 2009). Disomic addition lines involving the *P. huashanica* chromosome 1Ns were also obtained (Zhao et al., 2009). However, monosomic or disomic addition and substitution lines involving the chromosomes 2Ns, 3Ns, 4Ns and 7Ns of *P. huashanica* and wheat-*P. huashanica* partial amphiploids have not been developed. Development of wheat-*P. huashanica* addition lines and wheat- *P. huashanica* partial amphiploids would provide useful tools for locating genes of interest in *P. huashanica* and for further utilization of *P. huashanica* in wheat improvement. In addition, translocation lines between wheat and *P. huashanica* and a complete set of wheat- *P. huashanica* alien lines would be very useful if developed.

The $F_1$ hybrid between *Triticum aestivum* cv. Chinese spring *ph2b* mutant (CS*ph2b*) and *P. huashanica* was successfully obtained by our research team in 2004 (Kang et al., 2008). The objectives of this study were: (1) To develop and identify alien addition lines and translocation lines from the $BC_1F_2$ and $BC_1F_3$ generations of the wheat- *P. huashanica* using cytogenetic techniques and GISH; (2) to evaluate some of the their agronomic traits in the field; and (3) to map genes of interest on the introgressed chromosome.

## MATERIALS AND METHODS

### Plant materials

The $F_1$ hybrid, which was derived from a cross between the CS*ph2b* and *P. huashanica,* was obtained in 2004. The $BC_1F_3$ lines were derived from the $F_1$ hybrid through using the CS*ph2b* to backcross the $F_1$ to obtain $BC_1$ lines, which were then selfed for 3 generations to obtain the $BC_1F_3$ lines. The $BC_1F_2$ lines were derived from the $F_1$ hybrid that was crossed to *T. aestivum* cv. Zhengmai 9023 (Zhengmai 9023) to get $F_1$ lines, which were backcrossed to Zhengmai 9023 to obtain $BC_1$ lines that were selfed for 2 generations to get the $BC_1F_2$. The *P. huashanica* (2n = 2x = 14, NsNs) was collected from the Huashan Mountain, Shaanxi Province, China by Prof. C Yen and JL Yang of Sichuan Agricultural University. The lines 237, 239 and 241 were $BC_1F_2$, and the others were $BC_1F_3$ lines. Both CS*ph2b* and Zhengmai 9023 were used as parental controls for cytological and agronomical evaluations. All of the plant materials were grown in Chengdu, China, under normal growing conditions. These plant materials are currently deposited in the Triticeae Research Institute of Sichuan Agricultural University, Sichuan, China.

### Chromosome behavior in meiosis

The chromosome behaviors in the pollen mother cells (PMC) of the $BC_1F_2$ and $BC_1F_3$ lines at meiosis were investigated. The Carnoy's II solution (six parts 95% ethanol: three parts chloroform: one part glacial acetic acid) was used to fix the spikes at room temperature for 24 h. The fixed spikes were then stored at 4°C. Immature anthers were squashed in a drop of modified carbon fuchsine stain and observed under Olympus BX-51 microscope with a Photometrics SenSys CCD camera (Olympus, Tokyo, Japan).

### Karyotyping and identification of *P. huashanica* chromosomes with Giemsa C-banding

Giemsa C-banding slides were prepared as follows: root tips were fixed at room temperature in Carnoy's I solution (three parts 95% ethanol: one part glacial acetic acid) for 24 h and then stored at 4°C. Each fixed root tip was squashed on a slide in a drop of 45% acetic acid. The slide was frozen in liquid nitrogen and cover slide was removed with a razor blade. The slides were dehydrated in 100% ethanol for 12 h.

The Giemsa C-banding procedures of Gill et al. (1991) were followed with slight modification. The Ns chromosomes of *P. huashanica* were recognized according to the methods described by Wang et al. (1998). The slides were placed in 0.2 M solution of HCl at 60°C for 2.5 min and then treated in saturated barium hydroxide solution at room temperature for 9 min. After washing, the slides were stained in 5% Giemsa dye at 37°C for 1 h. Metaphase cells with a complete chromosome complement were photographed.

### Genomic *in situ* hybridization (GISH)

Chromosomes for GISH were prepared as follows: each fixed root-tip was squashed on a slide in a drop of 45% acetic acid. The slide was frozen in liquid nitrogen and the cover slide was removed with a razor blade. The slide was then dehydrated in 100% ethanol for about 5 min and stored at -20°C until use.

Genomic DNA of *P. huashanica* and CS*ph2b* were isolated from fresh leaves by the CTAB (cetyltrimethylammonium bromide) method of Doyle and Doyle (1990). Genomic DNA of *P. huashanica* was labeled with Dig-Nick translation mix according to the manufacturer's manual (Roche 11 745 816 910) and used as probe.

Slide pre-treatment, hybridization, signal amplification and detection of the fluorescent signals for *in situ* hybridization were conducted according to the procedures described by Han et al. (1998a, b) with some modifications. Briefly, the slide was denatured in 70% formamide 2X SSC at 80°C for 2 min and then dehydrated in an alcohol series (70, 95 and 100%). The hybridization mixture was prepared as 7.5 µl formamide, 1.5 µl 2X SSC, 1.5 µl (1 µg/µl) herring sperm DNA, 50% (w/v) dextran sulfate, 1 µl (3 µg/µl) genomic DNA from CS*ph2b* as a blocker and 1µl (0.05 µg/µl) Dig-labeled probe. The hybridization mixture was denatured at 80°C for 10 min immediately quenched in ice for at least 5 min and then dropped onto the slide. After 12 h of hybridization at 37°C, post-hybridization washes were carried out in 2X SSC at room temperature for 4 min, at 42°C for 10 min and then at room temperature for 4 min, respectively. The slide was washed in 1X PBS at room temperature for 3 min. A 100 µl BSA (1% BSA, 4X SSC, 0.1% Tween-20) and 2 µl anti-digoxigenin fluorescence fab fragments (Roch 11 207 741 910) mixture solution was then added to the slide, which was then kept at 37°C for 45min. The slide was washed in 1X PBS three times at room temperature and the chromosomes were counterstained with 10 µl propidium iodide (PI, 0.5 µg/µl). Imaging was captured with CCD camera (Photometrics CoolSNAP fx: Roper Scientific) using a fluorescence microscope (Olympus BX51). To construct the karyotype of a line, 3 images from the same karyotype were observed in a GISH experiment.

#### Evaluation of agronomic traits in the field

Five agronomic traits including plant height, number of tillers, length of spike, number of spikelets per spike, awns were evaluated in the field at maturity stage. The maturity seeds weight of each plant was weighed and thousand seeds weight was calculated by the formula:

$$\text{Thousand seeds weight} = \frac{\text{The weight of plant seeds}}{\text{The number of plant seeds}} \times 1000$$

## RESULTS

### Meiotic chromosome behaviors in pollen mother cells (PMC)

The meiotic chromosome behaviors of 95 $BC_1F_2$ or $BC_1F_3$ lines were observed. The chromosome numbers of the lines ranged from 2n = 41 to 2n = 47. The numbers of lines with 2n = 41, 42, 43, 44, 45, 46 and 47 chromosomes were 6, 59, 13, 8, 6, 2 and 1, respectively.

Five lines (156-4, 160-12, 160-13, 173-2 and 197-16) with 2n = 44 chromosomes and one line (165-2) with 2n = 46 chromosomes (Figure 1a,b; Table 1) had nearly regular meiosis with very low frequencies of either unpaired chromosomes or multivalent configurations. The frequency of univalent chromosomes ranged from 0 to 4 in these lines with averages ranging from 0 to 0.4 per cell. Either one or none of the trivalent or quadrivalent was observed in their respective meiocytes, which indicated that these lines may be disomic or double disomic addition lines.

Of the 13 lines with 2n = 43 chromosomes, 9 lines had 1 univalent and 21 bivalents per PMC, suggesting that they may be monosomic addition lines (Figure 1c). Line 173-1 with 2n = 43 had 20 bivalents and 1 trivalent per PMC (Figure 1d). Line 241-3 (2n = 43) had averages of 2.5 univalents (included 2 telosomics per cell), 0.3 trivalent and 0.04 pentavalent per PMC (Figure 1e).

In five of the six (2n = 45) lines, 22 bivalents and 1 univalent per PMC were observed (Figure 1f), suggesting that these lines have 42 wheat chromosomes plus three alien chromosomes. Four lines (241-2, 241-6, 241-9 and 241-10) with 2n = 42 chromosomes had, at least, an average of 0.88 univalent per PMC. However, in line 241-2, averages of 2.12 univalents, 0.12 trivalent and 0.12 quadrivalent per PMC (Figure 1g) were observed, suggesting that alien chromosome or alien chromosome fragment may be included in this line. The chromosome configuration of line 189-2 (2n = 47) was 22.85 bivalents and 1.3 univalents.

Chromosome abnormalities were observed at meiosis in most of the cases. Telosomics were observed at metaphase I of meiosis in lines 160-3 (2n = 46), 160-14 (2n = 45), 169-3 (2n = 43), 172-1 (2n = 41), 239-2 (2n = 44) and 241-3 (2n = 43) (Figures 1e,h). Lagging chromosome, lagging chromosome of telosomic and the telosomic sister chromatids moved to one pole at anaphase I were observed in line 172-1 (Figures 1i,j). A high frequency of micronuclei at both dyads and tetrads stage was observed in line 241-3 (Figure 1k), which resulted in the high frequency of unpaired chromosomes at metaphase I of meiosis. Asynchronous chromosomes separation was observed in line 160-13 at telophase II (Figure 1i).

### Giemsa C-banding of the alien chromosomes

Giemsa C-banding analyses was carried out for 15 $BC_1F_2$ and $BC_1F_3$ lines. The results are shown in Table 1 and Figure 2. The banding patterns of *P. huashanica* chromosomes are illustrated in Figure 2a. The chromosomes 1Ns and 5Ns displayed telomeric bands at the short arm. On the contrary, the 4Ns displayed telomeric bands at the long arm. The 2Ns, 3Ns, 6Ns and 7Ns showed sharp telomeric bands at both arms. The bands of the long arm were stronger than that of the short arm on both 3Ns and 6Ns and the 3Ns were middle centromeric chromosomes. The 7Ns were the shortest among these fourteen chromosomes.

Of the 15 lines analyzed, 9 lines were monosomic alien addition lines with a single *P. huashanica* chromosome added to the wheat genome. These lines were divided into four groups, 1Ns (163-1 and 189-22), 2Ns (187-1), 4Ns (177-2 and 177-5) and 5Ns (156-1, 160-2, 160-10 and 173-1) (Table 1, Figure 2b,e). A pair of 5Ns of the *P. huashanica* chromosomes and 21 pairs of the wheat chromosomes were detected in lines 156-4, 160-12, 160-13, 173-2 and 197-6 (Figure 2f), indicating that these lines were alien disomic addition lines. Line 165-2 which

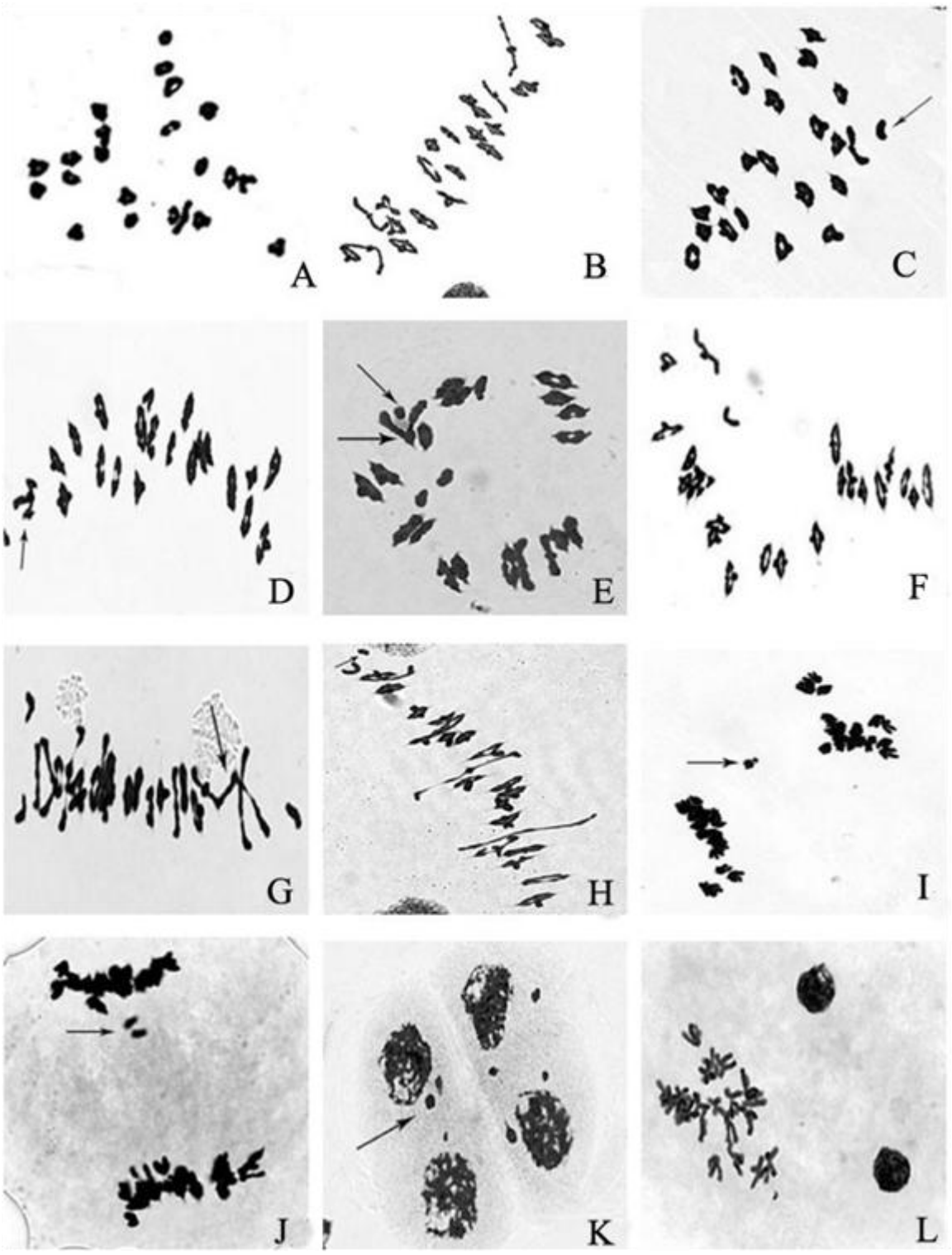


**Figure 1.** The meiotic behaviors of the $BC_1F_2$ and $BC_1F_3$ lines (L). (A) L 156-4, 2n = 44 = 2 II (rods) + 20 II (rings); (B) L 165-2, 2n = 46 = 4 II (rods) + 19 II (rings); (C) L 160-10, 2n = 43 = 1 I+ 1 II (rods) + 20 II (rings) (Arrow pointed to univalent); (D) L 173-1, 2n = 43 = 20 II (rings) + 1III (Arrow pointed to trivalent) (E) L 241-3, 2n =43 = $2^t$ + 2 I+2 II (rods) + 16 II (rings) + 1 III (Arrow pointed to trivalent and telosomic); (F) L 160-1, 2n = 45 = 1 I+ 1 II (rods) + 21 II (rings); (G) L 241-2, 2n = 42 = 2 I+7 II (rods) + 11 II (rings) + 1 IV (Arrow pointed to quadrivalent); (H) L 160-3, 2n = 46 = $1^t$ + 1 I+3 II (rods) + 19 II (rings) (Arrow pointed to telosomic); (I) L 172-1, a telosomic lagging chromosome (Arrow pointed to telosomic); (J) L 172-1, two sister chromatids of telosomic moved to one pole (Arrow pointed to telosomic); (K) L 241-3, micronuclei and L) L 160-13, chromosomes separated asynchronously at telophase II.

**Table 1.** Chromosome pairing at metaphase I in the pollen mother cells of the $BC_1F_2$ and $BC_1F_3$ generations progenies derived from CS*ph2b* × *P. huashanica.*

| Line | Number of cells observed | 2n | Chromosome pairing | | | | | | | Alien chromosome | GISH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | I | II | | | β | IV | V | | |
| | | | | Total | Ring | Rod | | | | | |
| 156-1 | 50 | 43 | 1 | 21 | 19.72 | 1.28 | | | | 5Ns | √ |
| | | | | | 15-21 | 0-6 | | | | | |
| 156-4 | 50 | 44 | | 22 | 20.94 | 1.06 | | | | 5Ns | √ |
| | | | | | 17-22 | 0-5 | | | | | |
| 160-1 | 50 | 45 | 1 | 22 | 19.36 | 2.64 | | | | | √ |
| | | | | | 16-22 | 0-6 | | | | | |
| 160-2 | 50 | 43 | 1.2 | 20.9 | 20.04 | 0.86 | | | | 5Ns | √ |
| | | | 0-3 | | 18-21 | 0-3 | | | | | |
| 160-3 | 50 | 46 | 2.2 | 21.9 | 20.05 | 1.85 | | | | Telosomic | |
| | | | 0-4 | | 17-22 | 0-4 | | | | | |
| 160-10 | 50 | 43 | 1 | 21 | 19.08 | 1.92 | | | | 5Ns | √ |
| | | | | | 16-21 | 0-5 | | | | | |
| 160-12 | 50 | 44 | | 22 | 20.52 | 1.48 | | | | 5Ns | √ |
| | | | | | 19-22 | 0-3 | | | | | |
| 160-13 | 50 | 44 | | 22 | 20.74 | 1.26 | | | | 5Ns | √ |
| | | | | | 18-22 | 0-4 | | | | | |
| 160-14 | 50 | 45 | 1.14 | 21.93 | 19.93 | 2 | | | | Telosomic | |
| | | | 1-3 | | 16-22 | 0-6 | | | | | |
| 163-1 | 50 | 43 | 1 | 21 | 19.6 | 1.4 | | | | 1Ns | √ |
| | | | | | 16-21 | 0-5 | | | | | |
| 165-2 | 50 | 46 | 0.24 | 22.88 | 19.74 | 3.14 | | | | 3Ns+5Ns | √ |
| | | | 0-2 | | 16-22 | 0-7 | | | | | |
| 169-3 | 50 | 43 | 1.24 | 20.88 | 18.5 | 2.38 | | | | Telosomic | |
| | | | 1-3 | | 13-21 | 0-7 | | | | | |
| 172-1 | 50 | 41 | 1 | 20 | 18.92 | 1.08 | | | | Telosomic | |
| | | | | | 17-20 | 0-3 | | | | | |
| 173-1 | 50 | 43 | | 20 | 17.4 | 2.6 | 1 | | | 5Ns | √ |
| | | | | | 16-20 | 0-4 | 1 | | | | |
| 173-2 | 50 | 44 | 0.26 | 21.62 | 19.39 | 2.23 | 0.06 | 0.08 | | 5Ns | √ |
| | | | 0-2 | | 16-22 | 0-6 | 0-1 | 0-1 | | | |
| 177-2 | 50 | 43 | 1 | 21 | 19.06 | 1.94 | | | | 4Ns | √ |
| | | | | | 16-21 | 0-5 | | | | | |
| 177-5 | 50 | 43 | 1 | 21 | 19.8 | 1.2 | | | | 4Ns | √ |
| | | | | | 18-21 | 0-3 | | | | | |
| 187-1 | 50 | 43 | 1.4 | 20.8 | 18.8 | 2 | | | | 2Ns | √ |
| | | | 1-3 | | 17-21 | 0-4 | | | | | |
| 189-2 | 50 | 47 | 1.3 | 22.85 | 20.45 | 2.4 | | | | | |
| | | | 1-3 | | 13-23 | 0-8 | | | | | |
| 189-22 | 50 | 43 | 1 | 21 | 19.84 | 1.16 | | | | 1Ns | √ |
| | | | | | 17-21 | 0-4 | | | | | |
| 197-16 | 50 | 44 | 0.4 | 21.6 | 19.14 | 2.46 | | 0.1 | | 5Ns | √ |
| | | | 0-4 | | 15-21 | 1-7 | | 0-1 | | | |
| 237-2 | 50 | 42 | | 21 | 18.6 | 2.4 | | | | | √ |
| | | | | | 17-21 | 0-4 | | | | | |
| 239-1 | 50 | 45 | 1.24 | 21.84 | 19.66 | 2.18 | | 0.04 | | | |
| | | | 1-3 | | 17-21 | 0-5 | | 0-1 | | | |

**Table 1.** Continued.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 239-2 | 50 | 44 | 2<br> | 21 | 18.8<br>18-20 | 2.2<br>1-3 | | | | Telosomic | |
| 241-2 | 50 | 42 | 2.12<br>0-6 | 19.52 | 14.02<br>6-18 | 5.5<br>3-10 | 0.12<br>0-1 | 0.12<br>0-1 | | | √ |
| 241-3 | 50 | 43 | 2.5<br>0-7 | 19.4 | 15.6<br>9-19 | 3.8<br>2-11 | 0.3<br>0-1 | | 0.04<br>0-1 | Telosomic | |
| 241-6 | 50 | 42 | 2<br>0-6 | 20 | 13.84<br>9-16 | 6.16<br>3-10 | | | | | √ |
| 241-9 | 50 | 42 | 0.88<br>0-4 | 20.56 | 16.6<br>12-20 | 3.96<br>1-9 | | | | | √ |
| 241-10 | 50 | 42 | 1.08<br>0-4 | 20.46 | 14.46<br>9-19 | 6<br>1-11 | | | | | √ |

√These lines were detected by GISH.

is an alien double disomic addition line (2n = 42 + 2 + 2 = 46) carrying two pairs (3Ns and 5Ns) of the *P. huashanica* chromosomes and 21 pairs of wheat chromosomes (Figure 2g).

### GISH characterization of the alien chromosomes

In total, 21 lines were analyzed by GISH (Table 1). The Dig-labeled genomic DNA of *P. huashanica* was used as a probe and CS*ph2b* genomic DNA was used as a blocker. Each of the 2n = 43 lines showed one strong yellowish-green hybridization signal (Figure 3a), suggesting that these lines were monosomic addition lines. Two yellowish-green hybridization signals were observed in each of the five lines with 2n = 44 (Figure 3b), indicated that these five lines were alien disomic addition lines. Line 165-2 had four strong yellowish-green hybridization signals (Figure 3c) and the other 42 chromosomes were red, suggesting that this line had four *P. huashanica* chromosomes and was an alien double disomic addition line. Line 241-2 exhibited an entire short arm with strong yellowish-green signal (Figure 3d) and line 241-10 showed an entire long arm with strong yellowish-green signal (Figure 3e), which indicated that, both 241-2 and 241-10 had a single wheat- *P. huashanica* chromosome Robertsonian translocation. Three strong yellowish-green signals were detected in plant 160-1 (Figure 3f), suggesting that this line had three *P. huashanica* chromosomes.

### Agronomic characterization of the alien lines

Six agronomic traits of these lines and their parents, that is, plant height, number of tillers, length of spike, number of spikelets per spike, awn and thousand seeds weight were evaluated in the field trials (Table 2). The agronomic traits exhibited great variations in mature lines. Plant height ranged from 78 to 139 cm. The number of tillers varied from 5 to 40. In most of the lines, both the length of spike and the number of spikelets per spike were higher than those of the parents. Thousand seeds weighted ranged from 12.21 to 37.96 g.

CS*ph2b* is awnless and the *P. huashanica* has awns. However, ten of the $BC_1F_3$ lines exhibited awns. It was interesting to note that only lines with the 5Ns alien chromosomes had regular awns, except plant 156-1 (5Ns monosomic alien addition line) and plant 165-2 (3Ns and 5Ns double disomic alien addition line) had shorter awns.

## DISCUSSION

### Selection and identification of the wheat-*P. huashanica* introgression lines

In most cases, stable incorporation of genes from wild species into wheat requires normal meiotic pairing and recombination between their corresponding genomes in the $F_1$ hybrids or in their subsequent selfing or backcrossing progenies (Cifuentes and Benavente, 2009). Based on chromosome pairing analyzed in this study, five lines with 2n = 44 and one line with 2n = 46 showed regular meiosis, indicating that they were cytologically stable. Nine lines (2n = 43) had 1 univalent and 21 bivalents per PMC, suggesting that these lines were monosomic addition lines. The presence of univalents, trivalents, telosomics, and micronuclei in high frequencies, as well as the asynchronous separation of chromosome in some lines, such as 241-2, 241-3, 172-1, 239-2 and 160-13, indicated that the presence of the alien chromosomes influenced the chromosome behaviors. Since chromosome pairing just provided the basic information for

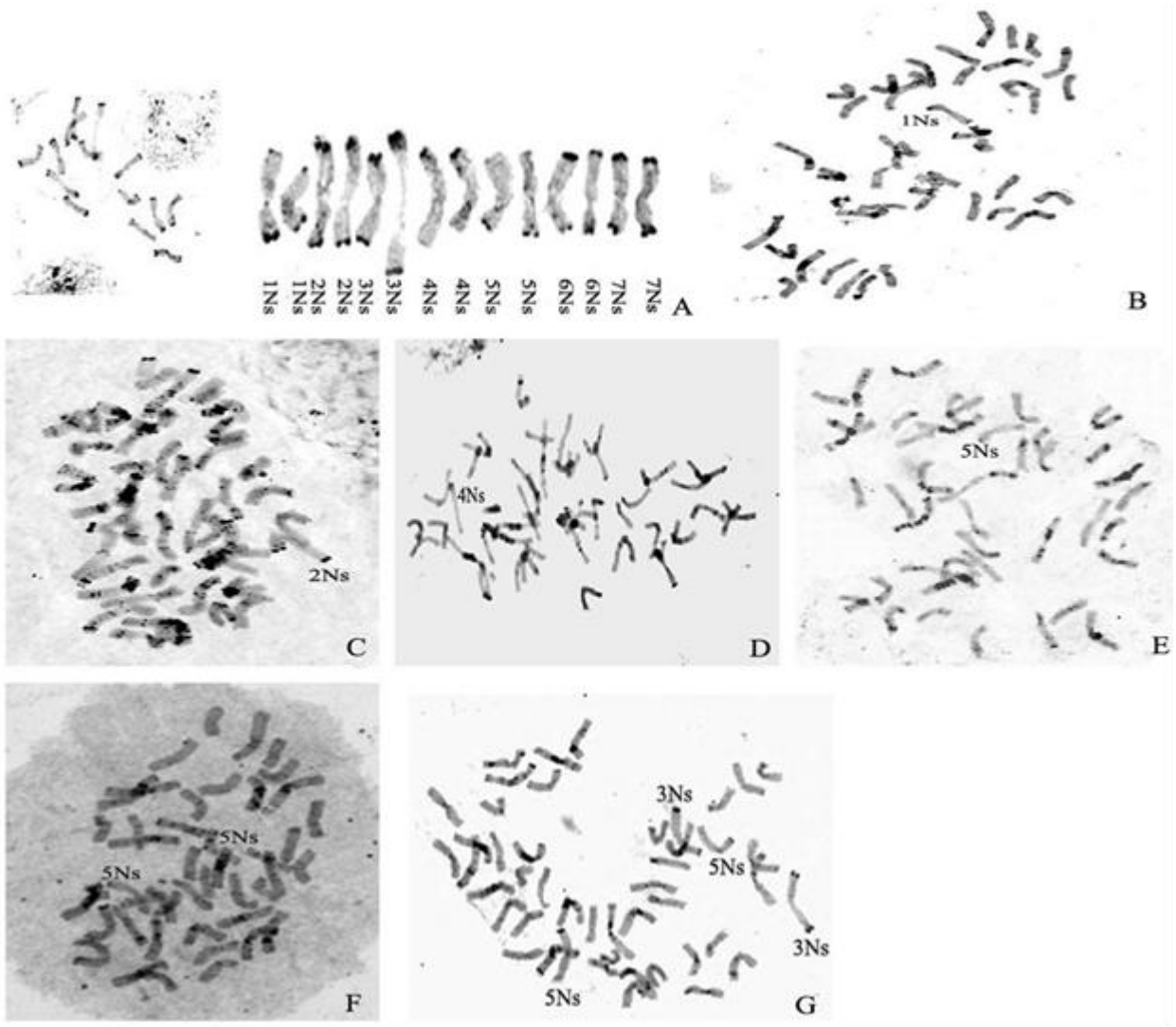


**Figure 2.** Giemsa C-banding of the somatic chromosomes of *P. huashanica* (*Ph*) and the $BC_1F_2$ and $BC_1F_3$ lines (L). (A) C-banding of the mitotic metaphase chromosomes and karyotype of *Ph*. (B-K) C-banding of the mitotic metaphase chromosomes of the $BC_1F_2$ and $BC_1F_3$ lines; (B) L 163-1 with 2n = 43, addition of 1Ns; (C) L 187-1 with 2n = 43, addition of 2Ns; (D) L 177-2 with 2n = 43, addition of 4Ns, (E) L 160-2 with 2n = 43, addition of 5Ns; (F) L 160-13 with 2n = 44, addition of one pair of 5Ns, and G) L 165-2 with 2n = 46, addition of each pair of 3Ns and 5Ns.

chromosome constitution, it is hard to identify the introgressed chromosomes.

After transferring an alien chromosome or chromosome segment into wheat, the next step is to identify the actual alien chromatin or to localize the translocation breakpoint(s) (Schneider et al., 2008). Giemsa C-banding is a faster, reliable and economical technique that allows the identification of alien chromosomes (Lukaszewski and Gustafson, 1987; Jiang et al., 1994). Characteristic C-bands obtained on different chromosomes can be employed to identify individual chromosomes in various plant groups or genetic stocks (Friebe and Gill, 1994; Friebe et al., 1996; Jauhar et al., 2009). Due to low content of constitutive heterochromatin in *Psathyrostachys fragilis* (Boiss) Nevski (2n = 2x = 14, NsNs) and *P. lanuginosa* (Trin.) Pilger (2n = 2x = 14, NsNs) and the considerable polymorphism and structure modification in *P. juncea*, it was difficult to identify individual chromosome within and among those species using Giemsa C-banding (Linde-Laursen and Bothmer, 1984, 1986; Endo and Gill, 1984; Linde-Laursen and Baden, 1994). However, all of the *P. huashanica* chromosomes exhibited strong bands in their terminal regions (Wang et al., 1998). In this study, all *P. huashanica* chromosomes were completely distinguishable from wheat chromosomes, and nine monosomic addition lines, five disomic addition lines, one double disomic addition line and one double disomic addition-substitution line were identified by Giemsa C-banding in the $BC_1F_2$ and $BC_1F_3$ generations. The lines identified in this study covered almost the complete genome of *P.*

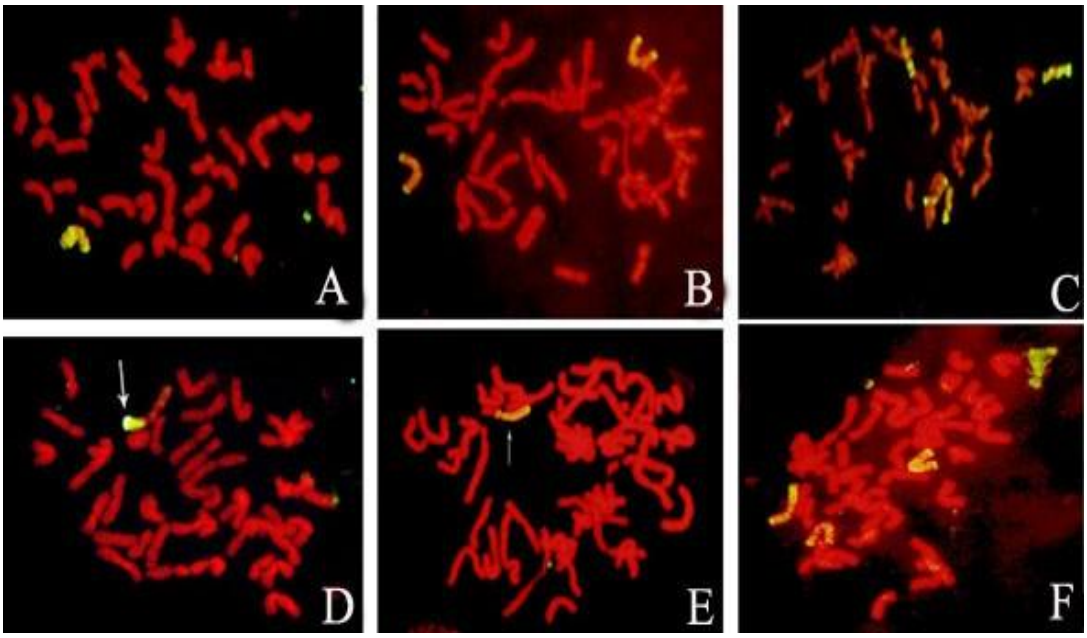


**Figure 3.** GISH of the mitotic metaphase chromosomes of the $BC_1F_2$ and $BC_1F_3$ lines (L) using *Psathyrostachys huashanica* (*Ph*) genome DNA as a probe that is shown as yellowish-green signal. (A) L 163-1 with 2n = 43, addition of one *Ph* chromosome; (B) L 160-13 with 2n = 44, addition of one pair of *Ph* chromosomes; (C) L 165-2 with 2n = 46, addition of two pairs of *Ph* chromosomes; (D) L 241-2 with 2n = 42, arrow pointed to the wheat- *Ph* translocated chromosome on short arm; (E) L 241-10 with 2n = 42, arrow pointed to the wheat- *Ph* translocated chromosome on long arm, and F) L 160-1 with 2n = 45, addition of three *Ph* chromosomes.

*huashanica* except the 6Ns and 7Ns.

GISH has been used to characterize genomes and chromosomes in hybrid polyploids, hybrid lines, partial allopolyploids and recombinant breeding lines and to localize and detect the amount of introgressed alien chromatin (Schwarzacher et al., 1992; Castilho et al., 1996; Chen et al., 1998; Raina and Rani, 2001). In the present study, GISH using *P. huashanica* DNA as a probe provided information about the numbers of the introgressed *P. huashanica* chromosomes and the chromosomal breakage and translocation sites occurred in some of the lines. Some of the information reported here represented novel genomic constitutions and rearrangements. The results of GISH were in agreement with those from the Giemsa C-banding and the chromosome pairing analysis.

The use of Giemsa C-banding and GISH enabled us to describe the genome constitution of wheat- *P. huashanica* hybrid derivatives. GISH revealed the numbers of the introgressed *P. huashanica* chromosomes, whereas, Giemsa C-banding allowed the identification of *P. huashanica* chromosomes in a wheat background.

## Mapping genes of *P. huashanica* on the introgressed chromosomes

Recently, alien chromosome lines were used to map genes on the introgressed chromosomes according to the presence or absence of the genes on either the introgressed chromosomes or the recipient chromosomes (Cho et al., 2006). Lammer (2004) used a series of wheat- *Thinopyrum elongatum* chromosome addition lines to demonstrate that *T. elongatum* chromosome 4E conferring a polycarpic life history to annual Chinese spring wheat. In the present study, the chromosomal locations for awn were examined using the series of the wheat-*P. huashanica* alien chromosome lines in the awnless wheat background. Because all of the disomic and monosomic addition lines with the 5Ns chromosome(s) conferred the awns trait to the awnless C*Sph2b*, it was concluded that, the gene(s) for awns in *P. huashanica* are located on the 5Ns chromosome.

Cereal awns have been regarded as rudimentary leaves because of the presence of stomata on them (Vercelde, 1953). Awns play a dual role in wheat, one is manifested as a means to protect against animals and as a mechanism for seed dispersal; another role is the physiological contribution for the formation of the seed (Grundbacher, 1963). Yield in wheat was influenced by the presence or absence of awns (Teich, 1982). In common wheat, there are three awn inhibitor genes, *B1*, *B2* and *Hd*, which are located on chromosomes 5A, 6B and 4A, respectively (Sears, 1954; Tsunewaki, 1966). In the present study, the lines with the *P. huashanica* 5Ns chromosome showed awns, it means that there are awns

**Table 2.** Agronomic traits of the $BC_1F_2$ and $BC_1F_3$ generations derived from CS*ph2b* × *P. huashanica.*

| Material | Height (cm) | Number of tillers | Length of spike | No. of spikelets per spike | Awn [a] | Thousand seeds weight (g) | Alien chromosome |
|---|---|---|---|---|---|---|---|
| 156-1[b] | 120 | 19 | 10 | 26 | + | 15.62 | 5Ns |
| 156-4 | 139 | 11 | 11 | 16 | + | 15.21 | 5Ns |
| 160-1 | 101 | 13 | 11 | 19 | - | 12.21 | |
| 160-2 | 132 | 23 | 13 | 22 | + | 17.85 | 5Ns |
| 160-10 | 80 | 8 | 8 | 20 | + | 18.99 | 5Ns |
| 160-12 | 111 | 10 | 11 | 21 | + | 17.3 | 5Ns |
| 160-13 | 108 | 7 | 11 | 20 | + | 12.73 | 5Ns |
| 160-14 | 121 | 10 | 10 | 20 | - | 15.14 | |
| 163-1 | 114 | 9 | 9 | 21 | - | 20.49 | 1Ns |
| 165-2[b] | 103 | 8 | 12 | 23 | + | 19.42 | 3Ns+5Ns |
| 169-3 | 133 | 40 | 11.5 | 24 | - | 20.9 | |
| 172-1 | 136 | 16 | 10 | 25 | - | 21.2 | |
| 173-1 | 117 | 21 | 10 | 24 | + | 18.57 | 5Ns |
| 173-2 | 120 | 15 | 9.5 | 21 | + | 19.82 | 5Ns |
| 177-2 | 113 | 5 | 11 | 22 | - | 18.21 | 4Ns |
| 177-5 | 99 | 10 | 8 | 16 | - | 21.9 | 4Ns |
| 187-1 | 112 | 14 | 10 | 21 | - | 17.34 | 2Ns |
| 189-22 | 124 | 7 | 8 | 22 | - | 20.41 | 1Ns |
| 197-16 | 117 | 9 | 10 | 24 | + | 12.38 | 5Ns |
| 237-2 | 80 | 8 | 10 | 21 | + | 37.96 | |
| 239-2 | 95 | 8 | 12 | 20 | + | 23.61 | |
| 241-2 | 78 | 15 | 12 | 19 | + | 13.44 | |
| 241-3 | 78 | 23 | 12 | 17 | + | 28.4 | |
| 241-6 | 70 | 8 | 12 | 17 | + | 34.36 | |
| 241-10 | 80 | 7 | 11 | 19 | + | 27.7 | |
| CS*ph2b* | 125 ± 4.39 | 15 ± 4.24 | 9 ± 1.28 | 21 ± 5.97 | - | 19.99 | |
| *P. huashanica* | 76 ± 3.45 | limitless | 9 ± 1.43 | 29 ± 2.12 | + | 7.44 | |
| Zhengmai 9023 | 82 ± 3.988 | 6 ±1.02 | 8 ± 1.47 | 14 ± 3.56 | + | 43 | |

[a] -, no awn; +, awn. [b] These two lines had shorter awns. No., Number.

gene(s) in *P. huashanica* 5Ns chromosome which can overcome partial of inhibitor genes of wheat.

## The Robertsonian translocation between wheat and *P. huashanica* chromosomes

Robertsonian translocations usually result from recombination that occurred on the short arms of two acrocentric chromosomes and followed by the loss of the acentric fragment (Holmquist and Dancis, 1979; Schubert and Rieger, 1985; Slijepcevic, 1998). In the present study, GISH revealed that line 241-2 had a wheat- *P. huashanica* translocation on the short arm and line 241-10 had a wheat- *P. huashanica* translocation on the long arm. A strong yellowish-green signal on the long arm was also observed in the selfed progenies of line 241-3 (Data not shown). Chromosome pairing studies showed that, the line 241-3 had averages of 19.4 bivalents, 2.5 univalents (including 2 telosomics per cell), 0.3 trivalent and 0.04 pentavalent per pollen mother cells (PMC). These results indicated that, Robertsonian translocations in these materials may be resulted from the simultaneous breakage at the centromere of the two univalent chromosomes and fused the arms from different chromosomes to form the translocated chromosome, which was similar to the results of Zhou et al. (1998) and Friebe et al. (2005). The presence of wheat- *P. huashanica* translocated chromosomes explained the large number of univalents and high frequency of multivalent at metaphase I in the lines 241-2 and 241-10. The telosomics which were observed at metaphase I of some lines (160-3 160-14, 169-3, 239-2 and 241-3) may be resulted from the centric misdivision of univalents.

Kang et al. (2010) reported that, there was a high frequency of wheat- *P. huashanica* chromosomal recombination in PHW-SA that is a synthetic amphiploid of *T. aestivum* cv. J-11- *P. huashanica*. The low frequency (2%)

of wheat-*P. huashanica* chromosome translocations detected in the present study suggests that, little homology exists between *P. huashanica* and *T. aestivum* chromosomes. The recombination arising from the exchange of homoeologous chromosome pairing between wheat and *P. huashanica* were limited in the $BC_1F_2$ and $BC_1F_3$ generations. However, the absence of exchanged chromosome segments did not exclude the cryptic intergenomic introgression and genomic changes at the DNA sequence level (Han et al., 2004), it can be used to demonstrate why there were not *P. huashanica* chromosome or chromosome fragment in 237-2 and 241-6 and their agronomic traits expressed better than their parent of Zhengmai 9023.

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