



## RESEARCH ARTICLE

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# Possible origin of *Triticum petropavlovskyi* based on cytological analyses of crosses between *T. petropavlovskyi* and tetraploid, hexaploid, and synthetic hexaploid (SHW-DPW) wheat accessions

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## Abstract

Intraspecific hybridization between *Triticum petropavlovskyi* Udacz. et Migusch., synthetic hexaploid wheat (SHW-DPW), and tetraploid and hexaploid wheat, was performed to collect data on seed set, fertility of F<sub>1</sub> hybrid, and meiotic pairing configuration, aiming to evaluate the possible origin of *T. petropavlovskyi*. Our data showed that (1) seed set of crosses *T. petropavlovskyi* × *T. polonicum* and *T. petropavlovskyi* × *T. aestivum* cv. Chinese Spring was significantly high; (2) fertility of hybrids *T. petropavlovskyi* × *T. polonicum* and *T. petropavlovskyi* × *T. aestivum* ssp. *yunnanense* was higher than that of the other hybrids; (3) fertility of F<sub>1</sub> hybrids SHW-DPW × *T. dicoccoides* and SHW-DPW × *T. aestivum* ssp. *tibetanum* was significantly high; and (4) c-value of *T. petropavlovskyi* × *T. polonicum* and *T. petropavlovskyi* × *T. aestivum* cv. Changning white wheat was also significantly high. The results indicate that the probable origin of *T. petropavlovskyi* is divergence from a natural cross between *T. aestivum* and *T. polonicum*, via either spontaneous introgression or breeding effort.

**Additional key words:** tetraploid wheat; hexaploid wheat; seed set; fertility of hybrids; c-value; meiotic pairing configuration

**Abbreviations used:** AFLP (amplified fragment length polymorphism); DPW (dwarfing Polish wheat); RFLP (restriction fragment length polymorphism); SAUTI (Triticaceae Research Institute of Sichuan Agricultural University); SHW (Synthetic hexaploid wheat).

**Authors' contributions:** Conceived and designed the experiments: QC, LYX and GRY. Performed the experiments and analysed the data: QC, JS and WPD. Contributed reagents/materials/analysis tools: QC. Improved the manuscript: LYX and GRY.

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## Introduction

Xinjiang rice wheat (*Triticum petropavlovskyi* Udacz. et Migusch.), known as 'Daosuimai' or rice-head wheat, is one of the four unique Chinese endemic wheat landraces, which also include the Sichuan white wheat complex (*Triticum aestivum* L.), Tibetan weederace (*T. aestivum* ssp. *tibetanum* Shao), and Yunnan hulled wheat (*T. aestivum* ssp. *yunnanense* King) (Shao *et al.*, 1980; Dong *et al.*, 1981; Yao *et al.*, 1983; Yen *et al.*, 1988).

Numerous studies on morphology and cytogenetics indicated that these landraces have the primitive and stable chromosomal constitution AABBDD (Riley *et al.*, 1967; Shao *et al.*, 1980; Yao *et al.*, 1983; Chen *et al.*,

1985; Yang *et al.*, 1992). However, taxonomic classifications based on morphological traits, chromosome pairing patterns, eco-geographical origins and RFLP analysis suggested that *T. petropavlovskyi* is distinct from the other three Chinese landrace groups (Ward *et al.*, 1998). Furthermore, previous studies pointed out that *T. petropavlovskyi* has some primitive traits that distinguish it from *Triticum spelta* L. and the common wheat of East-Mediterranean origin (Yao *et al.*, 1983; Chen *et al.*, 1988; Yen *et al.*, 1988). Due to *T. petropavlovskyi* features of very long glumes with a straw-like constituency, long lemmas, and well-marked knobs on the rachis under the glume, which are absent in other wheat species except for *Triticum polonicum* L.; Jakubtsiner (1959) hy-

pothesized that *T. petropavlovskyi* was a mutant of *T. polonicum*. Genomic analysis showed that *T. petropavlovskyi* might have originated in China independently from the other Chinese endemic wheat landraces (Yang *et al.*, 1992). Phylogenetic analyses have indicated that *T. petropavlovskyi* originated from *T. polonicum* in Xinjiang and from the exotic landraces of *T. aestivum* via either spontaneous introgression or breeding effort (Kang *et al.*, 2010; Chen *et al.*, 2013).

Despite decades of intensive studies, the origin of *T. petropavlovskyi* is still under discussion. According to previous studies, which included the analysis of plant morphology, cytology, and DNA sequences, three hypotheses haven't been raised regarding the species origin: (1) the species divergence was caused by a single mutation in *T. aestivum* (Efremova *et al.*, 2000; Akond & Watanabe, 2005); (2) *T. petropavlovskyi* is an independent species formed by hybridization and allopolyploidization between *T. polonicum* and *Aegilops tauschii* Cosson (Yen *et al.*, 1983; Yang *et al.*, 1992; Chen, 1999; Goncharov, 2005); and (3) the species originated via either a natural crossing or backcrossing between *T. polonicum* and *T. aestivum* (Jakubtsiner, 1959; Dorofeev *et al.*, 1979; Chen *et al.*, 1985; Watanabe & Imamura, 2002; Akond *et al.*, 2008). To verify the hypothesis that *T. petropavlovskyi* originated from a hybridization between *T. polonicum* and *Ae. tauschii*, Kang *et al.* (2008, 2009) performed intergeneric hybridization between a dwarf accession of *T. polonicum* from Xinjiang and *Ae. tauschii*. The hybrid they obtained was called synthetic hexaploid wheat (SHW-DPW). Morphologically, the spike of SHW-DPW is quite similar to that of *T. petropavlovskyi*.

Genomic analysis is an important tool for determining genome constitution of Triticeae species (Kihara & Nishiyama, 1930; Alonso & Kimber, 1981). Genome affinity is usually determined by observation of the chromosome pairing behavior at meiotic metaphase I (MI) of interspecific or intergeneric hybrids. In this study, we aimed to (1) verify if hypothesis no. 2 by analyzing seed set, fertility of F<sub>1</sub> hybrid, and meiotic pairing configuration between *T. petropavlovskyi* and SHW-DPW; and (2) elucidate the possible origin of *T. petropavlovskyi* by analyzing the seed set, fertility of F<sub>1</sub> hybrid, and meiotic pairing configuration of the hybridizations between *T. petropavlovskyi* and its possible tetraploid and hexaploid *Triticum* ancestors.

## Material and methods

### Plant materials

Twenty-nine accessions were used in this study (Table 1), which included: nine accessions of *T. pe-*

*trhopavlovskyi*; six accessions of the other three unique Chinese endemic wheat landraces; one accession of *T. carthlicum*, *T. dicoccoides*, and *T. turanicum*; two accessions of *T. durum*, *T. turgidum*, *T. polonicum*, and *T. compactum*; *T. aestivum* cv. Norin-10; and the synthetic hexaploid wheat (SHW-DPW). The artificial synthetic amphiploid between the dwarfing Polish wheat *T. polonicum* from Xinjiang and *Aegilops tauschii* (AS60) was produced and named SHW-DPW by Kang *et al.* (2008), to simulate the hypothesis no. 2 of origin of *T. petropavlovskyi*. The tetraploid *T. polonicum* cv. dwarfing Polish wheat was collected from Tulufan, Xinjiang, China. It is the only dwarf mutant of *T. polonicum* in China. *Aegilops tauschii* (AS60) originated in the Middle East. Voucher specimens were deposited in the Triticeae Research Institute herbarium, at Sichuan Agricultural University (SAUTI).

### Artificial hybridization

Crosses were made in the field at the Triticeae Research Institute, SAUTI. *T. petropavlovskyi* (AS360) and SHW-DPW were used as maternal plants to cross with tetraploid and hexaploid wheat plants. Florets were emasculated and covered with a cellulose bag. Hand-emasculated spikes were pollinated two days later, and maternal stigmas were brushed with freshly broken anthers from the paternal species. Hybrid seeds were counted, germinated on filter paper in petri-dishes, and then transplanted in pots at the two-leaf stage.

### Meiotic analysis

For cytological procedures, spikes were fixed in Carnoy's II solution (absolute ethanol: chloroform: glacial acetic acid, 6:3:1, v/v) for 24 h, transferred to 70% ethanol and stored in a refrigerator. Pollen mother cells (PMCs) at metaphase I (MI) were squashed and stained with 1.5% carbol fuchsin solution. Sixty cells at MI were observed from each hybrid, and the calculation of mean pairing frequency (c-value: the mean frequency with which two related chromosome arms pair) was made according to Alonso & Kimber (1981). Micrographs were taken from permanent meiosis preparations using the Olympus BX-51 camera system.

### Statistical analysis

The percentage of seed set and fertility of F<sub>1</sub> hybrids were converted to angle by arcsine transformation, and the transformed data was then subjected to analysis of

**Table 1.** Plant materials used in this study.

Ploidy level	Species	Genome	Accession No. <sup>[1]</sup>	Origin
<b>Tetraploid</b>	<i>Triticum carthlicum</i> Nevski	AB	PI532494	Kars, Turkey
	<i>Triticum dicoccoides</i> (Koern. ex Aschers. et Graeb.) Schweinf.	AB	AS838	
	<i>Triticum turanicum</i> Jakubz.	AB	AS2279	Xinjiang, China
	<i>Triticum durum</i> Desf.	AB	AS2349	Xinjiang, China
	<i>Triticum durum</i> Desf. cv. Langdon	AB	LDN	USA
	<i>Triticum turgidum</i> L.	AB	AS2233	Xinjiang, China
		AB	AS2277	Xinjiang, China
	<i>Triticum polonicum</i> L.	AB	PI190951	Xinjiang, China
	<i>Triticum polonicum</i> L. cv. dwarfing Polish wheat	AB	AS304	Xinjiang, China
	<b>Hexaploid</b>	<i>Triticum compactum</i> Host	ABD	PI352299
		ABD	PI124299	Switzerland
<i>Triticum aestivum</i> L. cv. Norin-10		ABD	N-10	Sichuan, China
<b>Synthetic hexaploid wheat</b>		ABD	SHW-DPW	
Sichuan white wheat complex				
<i>Triticum aestivum</i> L. cv. Changning white wheat		ABD	CBZ	Sichuan, China
<i>Triticum aestivum</i> L. cv. Chinese Spring		ABD	CS	Sichuan, China
<i>Triticum aestivum</i> L. cv. Kaixian luohan mai		ABD	KLM	Sichuan, China
<i>Triticum aestivum</i> L. cv. Yinong white wheat		ABD	J-11	Sichuan, China
<b>Tibetan weedrace</b>				
<i>Triticum aestivum</i> L. ssp. <i>tibetanum</i> Shao		ABD		Tibet, China
<b>Yunnan hulled wehat</b>				
<i>Triticum aestivum</i> L. ssp. <i>yunnanense</i> King		ABD		Yunnan, China
<b>Xinjiang rice wheat</b>				
<i>Triticum petropavlovskyi</i> Udacz. et Migusch.		ABD	AS350	Xinjiang, China
	ABD	AS356	Xinjiang, China	
	ABD	AS358	Xinjiang, China	
	ABD	AS359	Xinjiang, China	
	ABD	AS360	Xinjiang, China	
	ABD	AS362	Xinjiang, China	
	ABD	AS363	Xinjiang, China	
	ABD	AS364	Xinjiang, China	
	ABD	AS365	Xinjiang, China	

<sup>[1]</sup>The accessions with PI and AS numbers were kindly provided by the American National Plant Germplasm System (Pullman, Washington, USA) and the Triticeae Research Institute (Sichuan Agricultural University, China), respectively.

variance using the DPS (Data Processing System) 3.01 computer package (<http://www.statforum.com/>). Seed set, fertility of F<sub>1</sub> hybrids and c-value means were compared using the Duncan's multiple range test (Seraj *et al.*, 1997; Pitkanen, 2000). Differences in seed set and fertility were analyzed at 1% probability threshold.

## Results

### Interspecific hybridizations

*T. petropavlovskyi* was used as maternal parent and crossed with *Triticum* species and the synthetic hexaploid wheat (SHW-DPW). The results are shown in Table 2. All crosses produced seeds and resulted in mature hybrid plants. The seed sets for two combinations of *T. petropavlovskyi* × *T. polonicum* were 34.0% and

43.3%. Statistical analysis indicated that seed sets of crosses between *T. petropavlovskyi* and two *T. polonicum* accessions were the highest among the crosses between *T. petropavlovskyi* and tetraploid wheat ( $p < 0.01$ ). Among the crosses between *T. petropavlovskyi* and hexaploid wheat, the seed set of *T. petropavlovskyi* × *T. aestivum* cv. Chinese Spring was the highest ( $p < 0.01$ ).

Using SHW-DPW as maternal parent, nine crosses with tetraploid and twelve crosses with hexaploid wheat were made (Table 2), all of which produced seeds. In hybrids between SHW-DPW and tetraploid wheat, the seed set of SHW-DPW × *T. polonicum* was 11.3%, which was at the ordinary level comparing with the other crosses between SHW-DPW and tetraploid wheat. Statistical analysis suggested that the seed set of SHW-DPW × *T. carthlicum* was significantly higher than that of the other crosses ( $p < 0.01$ ). Considering hybrids between SHW-DPW and hexaploid wheat, the seed set of

**Table 2.** Hybridizations between *T. petropavlovskiyi*, SHW-DPW and tetraploid, hexaploid wheat.

Hybridization combinations	No. of plants studied/ (No. of spikes/plant emasculated)	No. of emasculated florets	Seed set (%)	Mean separation <sup>[1]</sup>	Plants	Fertility of F <sub>1</sub> hybrid (%) <sup>[2]</sup>	Mean separation <sup>[1]</sup>
<i>T. petropavlovskiyi</i> (AS360) × <i>T. carthlicum</i> (PI532494)	10 (2)	190	12.8	K	8	19.3	E
<i>T. petropavlovskiyi</i> (AS360) × <i>T. dicoccoides</i> (AS838)	10 (2)	170	9.09	L	4	14.6	E
<i>T. petropavlovskiyi</i> (AS360) × <i>T. turanicum</i> (AS2279)	10 (2)	148	15.0	JK	4	0	F
<i>T. petropavlovskiyi</i> (AS360) × <i>T. durum</i> (AS2349)	10 (2)	136	3.10	M	3	0	F
<i>T. petropavlovskiyi</i> (AS360) × <i>T. durum</i> cv. Langdon	10 (2)	168	3.06	M	4	51.9	D
<i>T. petropavlovskiyi</i> (AS360) × <i>T. turgidum</i> (AS2233)	10 (2)	184	24.4	I	16	21.4	E
<i>T. petropavlovskiyi</i> (AS360) × <i>T. turgidum</i> (AS2277)	10 (2)	178	23.7	I	16	67.9	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. polonicum</i> (PI190951)	10 (2)	110	34.0	G	16	43.7	D
<i>T. petropavlovskiyi</i> (AS360) × <i>T. polonicum</i> (AS304)	10 (2)	192	43.3	F	16	45.7	D
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Changning baimaizi	10 (2)	157	64.7	C	16	51.5	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Chinese Spring	10 (2)	182	78.9	A	16	74.5	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Kaixian luohan mai	10 (2)	145	60.6	D	16	74.0	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Yinong white wheat	10 (2)	142	75.0	B	16	89.9	AB
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> ssp. <i>tibetanum</i>	10 (2)	146	28.9	H	8	69.6	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> ssp. <i>yunnanense</i>	10 (2)	166	52.3	E	16	96.6	A
<i>T. petropavlovskiyi</i> (AS360) × <i>T. compactum</i> (PI352299)	10 (2)	128	75.5	B	16	75	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. compactum</i> (PI124299)	10 (2)	145	76.9	AB	16	80.4	BC
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Norin-10	10 (2)	174	65.0	C	16	0	F
<i>T. petropavlovskiyi</i> (AS360) × Synthetic hexaploid wheat (SHW-DPW)	10 (2)	186	16.7	J	8	75.7	C
<i>T. petropavlovskiyi</i> (AS360) × <i>T. petropavlovskiyi</i> (AS358)	10 (2)	154	83.2		10	93.2	
<i>T. petropavlovskiyi</i> (AS360) × <i>T. petropavlovskiyi</i> (AS360)	10 (2)	166	88.6		16	98.6	
SHW-DPW × <i>T. carthlicum</i> (PI532494)	10 (2)	127	39.4	EF	8	26.2	IJK
SHW-DPW × <i>T. dicoccoides</i> (AS838)	10 (2)	194	31.8	G	16	79.8	AB
SHW-DPW × <i>T. turanicum</i> (AS2279)	10 (2)	162	10.0	J	4	0	L
SHW-DPW × <i>T. durum</i> (AS2349)	10 (2)	134	6.9	JK	2	25.2	JK
SHW-DPW × <i>T. durum</i> cv. Langdon (LDN)	10 (2)	143	4.0	KL	1	0	L
SHW-DPW × <i>T. turgidum</i> (AS2255)	10 (2)	188	36.2	FG	16	34.6	H
SHW-DPW × <i>T. turgidum</i> (AS2277)	10 (2)	162	18.2	I	8	0	L
SHW-DPW × <i>T. polonicum</i> (PI190951)	10 (2)	116	11.7	J	8	0	L
SHW-DPW × <i>T. polonicum</i> (AS304)	10 (2)	108	11.3	J	8	40.6	G
SHW-DPW × <i>T. aestivum</i> cv. Changning baimaizi	10 (2)	153	62.5	A	16	31.0	HI
SHW-DPW × <i>T. aestivum</i> cv. Chinese Spring	10 (2)	158	41.1	EF	16	21.3	K
SHW-DPW × <i>T. aestivum</i> cv. Kaixian luohan mai	10 (2)	141	46.5	CD	16	28.6	IJ
SHW-DPW × <i>T. aestivum</i> cv. Yinong white wheat	10 (2)	118	44.3	CD	8	0	L
SHW-DPW × <i>T. aestivum</i> ssp. <i>tibetanum</i>	10 (2)	128	52.7	B	8	84.5	A
SHW-DPW × <i>T. aestivum</i> ssp. <i>yunnanense</i>	10 (2)	136	24.5	H	8	65.7	D
SHW-DPW × <i>T. petropavlovskiyi</i> (AS356)	10 (2)	189	16.2	I	8	60.8	E
SHW-DPW × <i>T. petropavlovskiyi</i> (AS358)	10 (2)	179	51.6	BC	16	78	B
SHW-DPW × <i>T. petropavlovskiyi</i> (AS359)	10 (2)	196	50.0	BC	16	43.9	G
SHW-DPW × <i>T. petropavlovskiyi</i> (AS360)	10 (2)	120	42.0	DE	16	71.6	C
SHW-DPW × <i>T. petropavlovskiyi</i> (AS362)	10 (2)	117	16.7	I	16	80.1	AB
SHW-DPW × <i>T. petropavlovskiyi</i> (AS363)	10 (2)	112	47.7	BC	16	55.6	F
SHW-DPW × <i>T. petropavlovskiyi</i> (AS364)	10 (2)	198	61.4	A	16	51.9	F
SHW-DPW × SHW-DPW	10 (2)	133	85.3		16	93.6	

<sup>[1]</sup> Letters represent significant difference between the means, according to Duncan's multiple range test ( $p < 0.01$ ). <sup>[2]</sup> "0" means that the hybrid plants failed to produce seeds.

SHW-DPW  $\times$  *T. aestivum* cv. Changning white wheat was 62.5%, the highest among such accessions ( $p < 0.01$ ).

Among the orthogonal and reciprocal crosses between SHW-DPW and *T. petropavlovskyi*, the seed set of *T. petropavlovskyi*  $\times$  SHW-DPW was 16.7%, the lowest one among crosses between *T. petropavlovskyi* and hexaploid wheat. The seed set of SHW-DPW  $\times$  *T. petropavlovskyi* was non-significant in relation to the crosses between SHW-DPW and hexaploid wheat not obvious (Table 2).

### Fertility in F<sub>1</sub> hybrids

Fertility of all F<sub>1</sub> hybrids is shown in Table 2. The F<sub>1</sub> hybrid plants between *T. petropavlovskyi* and tetraploid and hexaploid wheat grew well. However, the hybrids *T. petropavlovskyi*  $\times$  *T. turanicum* (AS2279), *T. petropavlovskyi*  $\times$  *T. durum* (AS2349), and *T. petropavlovskyi*  $\times$  *T. aestivum* cv. Norin-10 failed to produce seeds. Fertility of hybrids *T. petropavlovskyi*  $\times$  *T. turgidum* (AS2277) and *T. petropavlovskyi*  $\times$  *T. aestivum* ssp. *yunnanense* was significantly higher than that of the other hybrids between *T. petropavlovskyi* and tetraploid and hexaploid wheat ( $p < 0.01$ ).

The F<sub>1</sub> hybrids between SHW-DPW and tetraploid and hexaploid wheat also grew well (Table 2). However, five hybrid plants failed to produce seeds: SHW-DPW  $\times$  *T. durum* cv. Langdon, SHW-DPW  $\times$  *T. dicoccoides* (AS847), SHW-DPW  $\times$  *T. turgidum* (AS2277), SHW-DPW  $\times$  *T. turanicum* (AS2279) and SHW-DPW  $\times$  *T. aestivum* cv. Yinong white wheat. Fertility of hybrids from the SHW-DPW  $\times$  *T. dicoccoides* (AS838) was 79.8%, the highest one among hybrids between SHW-DPW and tetraploid wheat ( $p < 0.01$ ). Statistical analysis indicated that the fertility of SHW-DPW  $\times$  *T. aestivum* ssp. *tibetanum* was the highest among all hybrids between SHW-DPW and hexaploid wheat ( $p < 0.01$ ).

### Meiotic pairing in hybrids between *T. petropavlovskyi* and tetraploid and hexaploid wheat

In the seven hybrids ( $2n = 5x = 35$ ) between *T. petropavlovskyi* and tetraploid wheat, the meiotic configuration patterns in *T. petropavlovskyi*  $\times$  *T. dicoccoides* and *T. petropavlovskyi*  $\times$  *T. durum* cv. Langdon were similar, with a low frequency of trivalents (Table 3; Fig. 1A, 1B). Chromosome pairing at MI in *T. petropavlovskyi*  $\times$  *T. polonicum* (PI190951), however, was the highest, with an average 13.70 bivalents per cell, the most frequent configurations being 7 I + 14 II (Fig. 1C). In *T. petropavlovskyi*  $\times$  *T. polonicum*

(AS304), an average 13.07 bivalents and 60% of cells with 13 or 14 bivalents were observed (Fig. 1D). The c-value of *T. petropavlovskyi*  $\times$  *T. polonicum* (PI190951) was the highest among all crosses with tetraploid wheat ( $p < 0.01$ ) (Table 3).

In all nine hexaploid hybrids ( $2n = 6x = 42$ ), mean chromosome pairing ranged from 18.50 to 20.85 bivalents (Table 3). In the combinations between *T. petropavlovskyi* and the Chinese endemic wheat landraces, mean pairing configuration ranged from 19.85 to 20.85 bivalents per cell (Fig. 1E). The c-value of *T. aestivum* cv. Changning white wheat was higher than that of the other combinations ( $p < 0.01$ ) (Table 3). An average 18.65 bivalents per cell was observed at MI in hybrids of *T. petropavlovskyi*  $\times$  SHW-DPW, most cells containing 18 or 19 bivalents (Fig. 1F).

### Meiotic pairing in the hybrids between SHW-DPW and tetraploid, hexaploid wheats

Seventeen hybrids were produced with tetraploid and hexaploid wheat plants having SHW-DPW as female parent. Between SHW-DPW and tetraploid wheat, mean chromosome pairing ranged from 10.70 to 13.85 bivalents per cell (Table 3). Trivalents were observed only in the SHW-DPW  $\times$  *T. durum* combination (Fig. 1G). The highest number of bivalents was observed in the SHW-DPW  $\times$  *T. polonicum* combination, with a mean pairing configuration of 7.30 I + 13.85 II and c-value of 0.88 (Fig. 1H). The c-value of SHW-DPW  $\times$  *T. polonicum* was significantly higher than that of the other crosses ( $p < 0.01$ ) (Table 3).

Chromosome pairing at MI in SHW-DPW  $\times$  *T. aestivum* ssp. *tibetanum* showed an average 19.35 bivalents per cell with a c-value of 0.76 (Fig. 1I). In the crosses between SHW-DPW and the Sichuan white wheat complex, a large number of univalents (average of 10.25 per cell) was observed in hybrids in SHW-DPW  $\times$  *T. aestivum* cv. Chinese Spring (Fig. 1J).

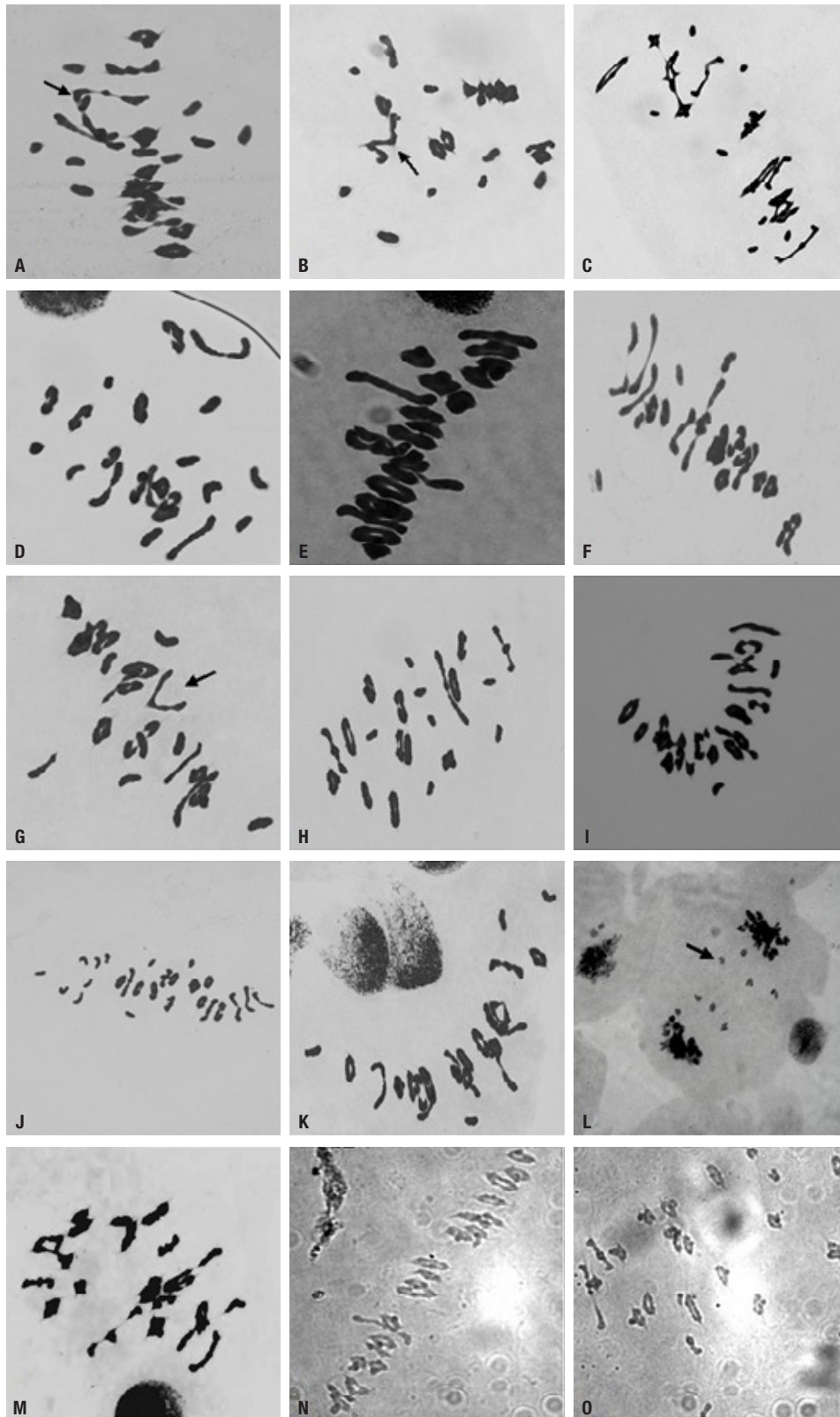
Mean chromosome pairing in SHW-DPW  $\times$  *T. petropavlovskyi* ranged from 17.20 to 19.30 bivalents. Nearly 20 bivalents were observed in the combination between SHW-DPW and *T. petropavlovskyi* (AS362) (Fig. 1K). The meiotic configuration of this hybrid was 3.40 I + 19.3 II. The c-value of SHW-DPW  $\times$  *T. petropavlovskyi* (AS362) was significantly higher than that of the other combinations ( $p < 0.01$ ) (Table 3).

Meiosis in hybrids was more irregular at later stages, especially in hybrids of tetraploid wheat accessions. Lagging chromosomes and chromosome bridges along with fragments were observed at anaphases I and II in some hybrids (Fig. 1L).

**Table 3.** Meiotic associations at metaphase I in pollen mother cells of the hybrids between *T. petropavlovskiyi*, SHW-DPW and tetraploid, hexaploid wheat.

Hybrids	2n	No. of cells observed/ (No. of plants studied)	Chromosome associations					Chiasmata/ cell	C-value	Means separation <sup>1)</sup>
			I	II			III			
				Total	Ring	Rod				
<i>T. petropavlovskiyi</i> (AS360) × <i>T. carthlicum</i> (PI532494)	35	60 (5)	11.60 (9-15)	11.70 (10-13)	7.25 (6-10)	4.45 (3-7)		18.95	0.68	H
<i>T. petropavlovskiyi</i> (AS360) × <i>T. dicoccoides</i> (AS838)	35	60 (3)	9.25 (9-15)	12.40 (10-13)	8.35 (7-10)	3.05 (3-5)	0.95 (0-1)	19.94	0.71	GH
<i>T. petropavlovskiyi</i> (AS360) × <i>T. durum</i> cv. Langdon (LDN)	35	60 (2)	8.51 (7-11)	12.75 (12-14)	9.45 (9-12)	3.30 (2-5)	0.99 (0-1)	24.18	0.86	ABCD
<i>T. petropavlovskiyi</i> (AS360) × <i>T. polonicum</i> (PI190951)	35	60 (5)	7.60 (7-15)	13.70 (10-14)	10.65 (9-11)	3.05 (0-4)		24.35	0.87	ABC
<i>T. petropavlovskiyi</i> (AS360) × <i>T. polonicum</i> (AS304)	35	60 (5)	8.86 (7-15)	13.07 (10-14)	9.25 (8-11)	3.82 (1-5)		22.32	0.80	CDEF
<i>T. petropavlovskiyi</i> (AS360) × <i>T. turgidum</i> (AS2255)	35	60 (5)	9.40 (7-15)	12.80 (10-14)	8.75 (6-10)	4.05 (4-5)		21.55	0.77	EFG
<i>T. petropavlovskiyi</i> (AS360) × <i>T. turgidum</i> (AS2277)	35	60 (5)	9.70 (7-15)	12.65 (10-14)	8.05 (6-9)	4.60 (4-6)		20.70	0.73	FGH
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Changning baimaizi (CNB)	42	60 (5)	1.30 (0-8)	20.35 (17-21)	16.90 (14-17)	3.45 (3-5)		37.25	0.89	AA
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Chinese Spring	42	60 (5)	2.00 (0-4)	20.00 (19-21)	14.05 (11-16)	5.95 (4-7)		34.05	0.81	BCDE
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Kaixian luohan mai (KLM)	42	60 (5)	0.50 (0-6)	20.75 (18-21)	16.35 (14-17)	4.40 (2-5)		37.10	0.88	AB
<i>T. petropavlovskiyi</i> (AS360) × <i>T. aestivum</i> cv. Yinong white wheat	42	60 (5)	2.30 (2-6)	19.85 (18-20)	15.25 (13-17)	4.60 (3-6)		35.10	0.84	ABCDE
<i>T. petropavlovskiyi</i> (AS360) × <i>T. compactum</i> (PI24299)	42	60 (5)	5.00 (4-8)	18.50 (17-19)	10.60 (9-12)	7.90 (5-10)		29.10	0.69	H
<i>T. petropavlovskiyi</i> (AS360) × <i>T. compactum</i> (PI352299)	42	60 (5)	3.10 (2-6)	19.45 (18-20)	14.60 (9-16)	4.85 (4-6)		34.05	0.81	BCDE
<i>T. petropavlovskiyi</i> (AS360) × Synthetic hexaploid wheat (SHW-DPW)	42	60 (5)	4.70 (4-6)	18.65 (18-19)	10.40 (7-13)	8.25 (6-11)		29.05	0.69	H
<i>T. petropavlovskiyi</i> (AS360) × <i>T. petropavlovskiyi</i> (AS358)	42	60 (5)	0.50 (0-2)	20.75(18-21)	16.35 (14-18)	4.40 (2-6)		37.10	0.88	
<i>T. petropavlovskiyi</i> (AS360) × <i>T. petropavlovskiyi</i> (AS360)	42	60 (5)	0.30 (0-1)	20.85 (20-21)	16.15 (15-18)	4.70 (4-5)		37.00	0.88	
Synthetic hexaploid wheat (SHW-DPW) × <i>T. carthlicum</i> (PI532494)	35	60 (5)	10.30 (9-13)	12.35 (11-13)	6.40 (6-8)	5.95 (4-6)		18.75	0.67	EFG
SHW-DPW × <i>T. dicoccoides</i> (AS838)	35	60 (5)	13.60 (11-15)	10.70 (10-12)	5.25 (5-7)	5.45 (4-6)		15.95	0.57	I
SHW-DPW × <i>T. durum</i> (AS2349)	35	60 (2)	7.30 (7-15)	12.88 (10-14)	9.70 (8-10)	3.18 (2-5)	0.65 (0-1)	23.88	0.85	A
SHW-DPW × <i>T. polonicum</i> (AS304)	35	60 (5)	7.30 (7-13)	13.85 (11-14)	10.70 (8-12)	3.15 (3-4)		24.55	0.88	A
SHW-DPW × <i>T. turgidum</i> (AS2255)	35	60 (5)	9.40 (7-13)	12.80 (11-14)	6.60 (6-8)	6.20 (5-8)		19.40	0.69	DEF
SHW-DPW × <i>T. aestivum</i> cv. Changning baimaizi (CNB)	42	60 (5)	7.00 (6-10)	17.50 (16-18)	7.45 (5-9)	10.05 (9-12)		24.95	0.59	HI
SHW-DPW × <i>T. aestivum</i> cv. Chinese Spring (CS)	42	60 (5)	10.25 (8-16)	15.90 (13-17)	5.25 (2-7)	10.65 (8-13)		21.15	0.50	J
SHW-DPW × <i>T. aestivum</i> cv. Kaixian luohan mai (KLM)	42	60 (5)	5.70 (4-12)	18.10 (15-19)	9.40 (7-11)	8.70 (7-11)		27.50	0.65	EFGH
SHW-DPW × <i>T. aestivum</i> ssp. <i>tibetanum</i> (AS1026)	42	60 (5)	3.30 (2-8)	19.35 (17-20)	12.75 (10-16)	6.60 (4-7)		32.10	0.76	BC
SHW-DPW × <i>T. aestivum</i> ssp. <i>yunnanense</i> (AS343)	42	60 (5)	3.60 (2-10)	19.15 (16-20)	10.35 (9-12)	8.80 (8-10)		29.50	0.70	CDF
SHW-DPW × <i>T. petropavlovskiyi</i> (AS356)	42	60 (5)	4.40 (4-6)	18.85 (18-19)	8.70 (5-11)	10.15 (8-14)		27.55	0.66	EFG
SHW-DPW × <i>T. petropavlovskiyi</i> (AS358)	42	60 (5)	5.90 (4-8)	18.05 (17-19)	11.85 (10-13)	6.20 (5-9)		29.90	0.71	BCDE
SHW-DPW × <i>T. petropavlovskiyi</i> (AS359)	42	60 (5)	7.60 (6-12)	17.20 (15-18)	9.10 (8-10)	8.10 (6-9)		26.30	0.63	FGHI
SHW-DPW × <i>T. petropavlovskiyi</i> (AS360)	42	60 (5)	6.30 (4-10)	17.85 (16-19)	10.05 (9-12)	7.80 (5-9)		27.90	0.66	EFG
SHW-DPW × <i>T. petropavlovskiyi</i> (AS362)	42	60 (5)	3.40 (2-8)	19.30 (17-20)	12.85 (11-14)	6.45 (4-8)		32.15	0.77	B
SHW-DPW × <i>T. petropavlovskiyi</i> (AS363)	42	60 (5)	7.30 (6-10)	17.35 (16-18)	8.35 (8-10)	9.00 (7-10)		25.70	0.62	GHI
SHW-DPW × <i>T. petropavlovskiyi</i> (AS364)	42	60 (5)	4.60 (2-6)	18.70 (18-20)	12.50 (11-14)	6.20 (5-8)		31.20	0.74	BCD
SHW-DPW × SHW-DPW	42	60 (5)	0.14 (0-2)	20.93 (16-21)	19.25 (16-21)	1.68 (0-4)		40.18	0.95	

<sup>1</sup> Letters represent significant difference between the means, according to Duncan's multiple range test ( $p < 0.01$ ).



**Figure 1.** Meiotic chromosome pairing at MI in hybrids. A: *Triticum petropavlovskiyi* × *T. dicoccoides*, 10 I + 11 II + 1 III (arrowed); B: *T. petropavlovskiyi* × *T. durum* cv. Langdon, 8 I + 12 II + 1 III (arrowed); C: *T. petropavlovskiyi* × *T. polonicum*, 7 I + 14 II; D: *T. petropavlovskiyi* × *T. polonicum*, 9I + 13 II; E: *T. petropavlovskiyi* × *T. aestivum* cv. Kaixian luohan mai; F: *T. petropavlovskiyi* × Synthetic hexaploid wheat (SHW-DPW), 6 I + 18 II; G: SHW-DPW × *T. durum*, 6 I + 13 II + 1 III (arrowed); H: SHW-DPW × *T. polonicum*, 7 I + 14 II; I: SHW-DPW × *T. aestivum* ssp. *tibetanum*, 4 I + 19 II; J: SHW-DPW × *T. aestivum* cv. Chinese Spring, 10 I + 16II; K: SHW × *T. petropavlovskiyi* (AS362), 4 I + 19 II; L: Lagging chromosomes (arrowed). M: The selfing of SHW-DPW, 21 I + 16II; N: The selfing of *T. petropavlovskiyi* (AS360), 21II; O: *T. petropavlovskiyi* (AS360) × *T. Petropavlovskiyi* (AS358), 2I + 20II.

## Discussion

### Seed set, fertility and meiotic pairing behavior

In previous cytological studies, seed set, fertility, and meiotic pairing behavior indicated that *T. petropavlovskyi* is more closely related to *T. aestivum* cv. White head than to other hexaploid wheat. In addition, the relationship between *T. petropavlovskyi* and *T. polonicum* was found to be distant compared to other tetraploid wheat landraces (Yao *et al.*, 1983; Chen *et al.*, 1985). In the present study, the statistical analysis of data on seed set, fertility, and meiotic pairing behavior indicated that *T. petropavlovskyi* × *T. polonicum* and *T. petropavlovskyi* × *T. aestivum* cv. Chinese Spring were significantly higher than that with any other cross ( $p < 0.01$ ). These results indicate that the relationships between *T. petropavlovskyi* and *T. polonicum*, and Sichuan white wheat complex are closer than the other tetraploid and hexaploid wheats, which is in agreement with the results of Yao *et al.* (1983) and Chen *et al.* (1985). Moreover, based on the results of seed set, fertility and meiotic pairing behavior of the hybrid between SHW-DPW and tetraploid, and hexaploid wheat, we found that SHW-DPW might be different from *T. petropavlovskyi*.

In short, the relationship between *T. petropavlovskyi* and domestic wheat species, especially those from the Sichuan white wheat complex, is closer than that with exotic wheat landraces. Furthermore, the chromosome pairing results indicated that *T. polonicum* might have played a role in the origin of *T. petropavlovskyi*, and that SHW-DPW and *T. petropavlovskyi* are different from one another.

### The possible origin and donors of *T. petropavlovskyi*

It has been reported that the spike of *T. petropavlovskyi* is similar to that of *T. polonicum* (Udaczin & Miguschova, 1970; Kang *et al.*, 2010). The genes for long glume in *T. polonicum* and *T. petropavlovskyi* were located on the long arm of chromosome 7A and are allelic (Dorofeev *et al.*, 1979). A phylogenetic classification with molecular markers indicated that *T. petropavlovskyi* is more closely related to *T. polonicum* than *T. durum* and *T. turgidum* (Akond & Watanabe, 2005). The phylogenetic relationship analysis of *Acc-1* and *Pgk-1* gene pointed out that the *T. petropavlovskyi* and *T. polonicum* from Xinjiang are clustered in one group (Kang *et al.*, 2010; Chen *et al.*, 2013). Based on our results, the hybrids between *T. petropavlovskyi* and

tetraploid wheat showed that the bivalents, seed set, and fertility of F<sub>1</sub> hybrids were significantly higher in the cross *T. petropavlovskyi* × *T. polonicum* compared to the other cross combinations. Our findings show that *T. petropavlovskyi* is more closely related to *T. polonicum* than to any other tetraploid wheat.

Molecular analyses indicated that *T. petropavlovskyi* is genetically distinct from three other Chinese endemic wheat landraces (Wei *et al.*, 2002). UPGMA clustering, estimated from AFLP, suggested a similar genomic constitution of *T. aestivum* and *T. petropavlovskyi* (Akond & Watanabe, 2005). Phylogenetic relationship analysis of *Acc-1* sequences provided additional evidence of a close affinity between *T. petropavlovskyi* and exotic landraces of *T. aestivum* (Kang *et al.*, 2010). In contrast, our results indicate that the relationship of *T. petropavlovskyi* with native *T. aestivum* is closer than with exotic *T. aestivum*. The relationships of *T. petropavlovskyi* with the other three Chinese endemic wheat landraces and with exotic primitive wheat need further research. In addition, SHW-DPW was used as parental plant in crosses with tetraploid and hexaploid wheat and *T. petropavlovskyi*. Based on the results of seed sets, fertility of F<sub>1</sub> hybrids and chromosome pairing, we speculate that SHW-DPW is different from *T. petropavlovskyi*, and consider the possibility of hypothesis no. 2 that *T. petropavlovskyi* originated from an independent allopolyploidization event seems unlikely. Based on cytological analyses (Yao *et al.*, 1983; Chen *et al.*, 1985), agronomic and morphological studies (unpublished), and the results of the present study, we also discard the hypothesis no. 1 that *T. petropavlovskyi* is derived from a single mutation in *T. aestivum*. We consider most likely the hypothesis no. 3 that *T. petropavlovskyi* probably derives from a natural cross between *T. aestivum* and *T. polonicum* via either spontaneous introgression or breeding effort.

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