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Research Paper

Cytological identification of an *Aegilops variabilis* chromosome carrying stripe rust resistance in wheat

Laibin Zhao†, Shunzong Ning†, Jianjun Yu, Ming Hao, Lianquan Zhang, Zhongwei Yuan, Youliang Zheng and Dengcai Liu*

Triticeae Research Institute, Sichuan Agricultural University, Chengdu, Sichuan 611130, China

Aegilops variabilis (UUS^vS^v), an important sources for wheat improvement, originated from chromosome doubling of a natural hybrid between *Ae. umbellulata* (UU) with *Ae. longissima* (S^lS^l). The *Ae. variabilis* karyotype was poorly characterized by fluorescent in situ hybridization (FISH). The FISH probe combination of pSc119.2, pTa71 and pTa-713 identified each of the 14 pairs of *Ae. variabilis* chromosomes. Our FISH ideogram was further used to detect an *Ae. variabilis* chromosome carrying stripe rust resistance in the background of wheat lines developed from crosses of the stripe rust susceptible bread wheat cultivar Yiyuan 2 with a resistant *Ae. variabilis* accession. Among the 15 resistant BC_1F_7 lines, three were $2S^v + 4S^v$ addition lines (2n = 46) and 12 were $2S^v$(2B) or $2S^v$(2D) substitution lines that were confirmed with SSR markers. SSR marker *gwm148* can be used to trace $2S^v$ in common wheat background. Chromosome $2S^v$ probably carries gametocidal(*Gc*) gene(s) since cytological instability and chromosome structural variations, including non-homologous translocations, were observed in some lines with this chromosome. Due to the effects of photoperiod genes, substitution lines $2S^v$(2D) and $2S^v$(2B) exhibited late heading with $2S^v$(2D) lines being later than $2S^v$(2B) lines. $2S^v$(2D) substitution lines were also taller and exhibited higher spikelet numbers and longer spikes.

Key Words: additional line, *Aegilops variabilis*, FISH, *Puccinia striiformis*, substitution line, translocation line.

Introduction

Triticeae within the Pooideae subfamily of grasses is a large tribe that contains over 500 species and about 30 genera depending on the opinions of taxonomists (Wang and Lu 2014, Yen *et al.* 2005, Yen and Yang 2009). These species provide a vast gene pool for the genetic improvement of common wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD genome). The *Aegilops* genus consists of 10 diploid, 10 tetraploid, and 2 hexaploid species (van Slageren 1994). The genus is closely related to *Triticum* and played an important role in the evolution of common wheat. The ancestor of the D-genome of wheat is *Ae. tauschii* (Kihara 1944, McFadden and Sears 1944), whereas the B-genome is thought to be a differentiated S-genome from *Ae. speltoides* or a closely related species (Kilian *et al.* 2007, Petersen *et al.* 2006). In addition to *Ae. speltoides* the S-genome is also shared by the diploid species *Ae. longissima* (S^l), *Ae. sharonensis* (S^{sh}), *Ae. searsii* (S^s), and *Ae. bicornis* (S^b) in the *Sitopsis* section of genus *Aegilops* (van Slageren 1994).

Ae. variabilis Eig [syn. *Ae. peregrina* (Hack.) Maire and Weiller; 2n = 4x = 28, S^vS^vUU] was derived from hybridization of the diploid species *Ae. umbellulata* (UU) and *Ae. longissima* (S^lS^l) (Kihara 1954, Yu and Jahier 1992). *Ae. variabilis* is a test genotype widely used in detection of homeologous chromosome pairing genes (Sears 1976). *Ae. variabilis* also contains desirable traits for common wheat improvement, such as high concentrations of iron and zinc in the grain (Neelam *et al.* 2011), resistance to *Meloidogyne naasi* (root knot nematode) and *Heterodera avenae* (cereal cyst nematode) (Barloy *et al.* 2007, Yu *et al.* 1990, 1992), powdery mildew (Spetsov *et al.* 1997), leaf rust (Marais *et al.* 2008), stripe rust (Liu *et al.* 2011), spot blotch, and Karnal bunt (Mujeeb-Kazi *et al.* 2007).

Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most serious wheat diseases worldwide (Chen 2005). In China, the disease is more prevalent in southwest and northwest regions due to favorable climatic conditions (Wan *et al.* 2007). Following the emergence of *Pst* races CYR32 detected in 1994 and CYR33 detected in

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*Corresponding author (e-mail: dcliu7@yahoo.com)
† These authors contributed equally to this work

1997 most wheat cultivars in southwest China became susceptible, except those with resistance gene(s) *Yr24/Yr26* (Chen *et al.* 2009, Liu *et al.* 2013, Wan *et al.* 2004). *Yr24/Yr26* was the most frequently used resistance source in wheat breeding lines and currently grown cultivars in the region; but a new Pst race (or races) known as v26 (also called CH42) was first reported in Sichuan province in 2008–2009 (Liu *et al.* 2010). During 2014 and 2015, most of the commercial wheat cultivars in Sichuan province were susceptible and epidemics of v26 led to significant yield losses. Therefore, it is urgent to identify effective stripe rust resistance genes for deployment in new cultivars to prevent future stripe rust epidemics.

Fluorescence in situ hybridization (FISH) is widely used in identification of alien chromosomes in wheat. Although *Ae. variabilis* was cytologically characterized using FISH markers (Badaeva *et al.* 2004); however, some *Ae. variabilis* chromosomes could not be identified. The objective of this study was to identify fluorescence in situ hybridization (FISH) patterns of *Ae. variabilis* chromosomes using different repetitive sequences, with the aim of identifying each of the 14 pairs of chromosomes. The resulting FISH patterns were further used to detect the *Ae. variabilis* chromosome carrying stripe rust resistance in wheat BC_1F_7 lines that were derived from a cross of *Ae. variabilis* with susceptible bread wheat cultivar Yiyuan 2 and backcross to Yiyuan 2.

Materials and Methods

Plant materials

The plant genotypes used in this study included *Ae. variabilis* AS116 (2n = 4x = 28, S^vS^vUU), common wheat line Yiyuan 2 (2n = 6x = 42, AABBDD), and 26 homozygous BC_1F_7 derivatives with the cytoplasm of *Ae. variabilis* were generated by selection for fertility, stripe rust resistance and desirable agronomic traits. Chinese Spring (CS) nullisomic-tetrasomic (NT) lines for homoeologous group 2 were used for molecular marker location. Stripe rust susceptible wheat line SY95-71 was used as a disease spreader.

Evaluation of agronomic traits

All lines were planted at Wenjiang Experimental Station, Sichuan Agricultural University. In the 2013–2014 cropping season, 20 plants of each BC_1F_7 selection and parental lines were space-planted in 2.0 m rows, with 30 cm between rows. This experiment consisted of three replicates. In the 2014–2015 season, each line was grown in a five-row plot with 20 plants per row. The highly susceptible spreader line SY95-71 was planted on both sides of each experimental row.

All materials were inoculated with mixed urediniospores of races CYR32, CYR33, Gui22-9, Gui22-14, Su11-4, and Su11-5 in 2014 and CYR32, CYR33, Gui22-9, Gui22-14, Gui22-8, Su11-4, and Su11-5 in 2015, provided by the Research Institute of Plant Protection, Gansu Academy of Agricultural Sciences. Infection types on individual plants after heading were recorded three times at 10-day intervals on a 0–9 scale (McNeal *et al.* 1971) when the spreader line SY95-71 was fully infected.

Heading time was recorded in both years when approximately one-half of the spikes in each line had emerged. Waxiness, a morphological marker associated with variation in homoeologous group 2 chromosomes, was also recorded at anthesis in 2015. Other agronomic traits (plant height, tiller number per plant, spike length and spikelet number) were evaluated at maturity from 10 randomly selected plants from each plot. The three tallest tillers of the selected plant were measured for plant height, spike length and spikelet number. Plant height was calculated as the average height from the soil surface to the tip of the spike (awns excluded). The average value for each trait was then calculated.

Fluorescence in situ hybridization (FISH)

Root tips excised from germinating seeds were treated for 2 hours with nitrous oxide and then placed in 70% ethanol (Kato 1999). Root tips were treated with cellulase and pectinase and the suspension was dropped onto slides (Komuro *et al.* 2013). The slides were prepared for FISH as previously described by Hao *et al.* (2011, 2013). Before chromosomal observations, DAPI was applied to the slides. After capturing images coverslips were removed, the slides were washed gently with 70% ethanol, then submerged in boiling 2× SSC buffer (100°C) for 5 min to remove probes, washed with distilled water, briefly rinsed with 70% ethanol, and air dried for the next FISH (Komuro *et al.* 2013). Probes pSc119.2, pAs1, pTa-535, pTa71 (Tang *et al.* 2014), $(GAA)_5$ (Cuadrado *et al.* 2008, Dennis *et al.* 1980), and pTa-713 (FAM or TAM 5′ AGACGAGCACGTGACACCA TTCCCACCCTGTCTTAGCGTAACGCGAGTCG 3′) designed according to Komuro *et al.* (2013) were used. All the probes are oligonucleotides and were synthesized by TSINGKE (Chengdu, China).

SSR analysis

To identify substituted chromosomes, 62 wheat microsatellite (SSR) markers on 2B and 2D were used based on genetic-physical maps of Sourdille *et al.* (2004) and GrainGenes website (http://wheat.pw.usda.gov/GG3/maps). Genomic DNA from plant materials were extracted from young leaves using a plant genomic DNA kit (Tiangen Biotech (Beijing) Co. Ltd). PCR amplifications were performed in a GeneAmp PCR System 9700 (Applied Biosystems, Singapore) with the following conditions: 95°C for 4 min, 35 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. Amplification products were separated on 3% agarose gels in TAE buffer and visualized under UV light with ethidium bromide.

Results

Identification of parent chromosomes by FISH

Out of six FISH probes used, $(GAA)_5$, pSc119.2, pAs1,

pTa535, pTa71, and pTa-713, $(GAA)_5$, pSc119.2, pTa71, and pTa-713 had desirable fluorescence signals. The four probes were used to differentiate individual chromosomes of *Ae. variabilis* (**Fig. 1a–1c**). Probe pSc119.2 had fluorescence signals on U- and S-genome chromosomes except 6U, pTa71 had signals on 1U and 5U, and $(GAA)_5$ had signals on all chromosomes of the U- and S-genomes, similar to those in corresponding chromosomes in other *Aegilops* species (Badaeva *et al.* 2004, Molnár *et al.* 2011, Schneider *et al.* 2005, Zhang *et al.* 2013). Most of the pTa-713 signals appeared in intermediate positions of chromosome arms, thus enhancing ability to recognise of *Ae. variabilis* chromosomes. The probe combination of pSc119.2, pTa71 and pTa-713 clearly differentiated all 14 pairs of *Ae. variabilis* chromosomes from each other (**Fig. 1a**, **1b**).

Although signals of pSc119.2 and pTa-535 can differentiate all 21 pairs of Chinese Spring chromosomes (Tang *et al.* 2014), signals on 5A, 2B and 7B were difficult to distinguish from each other in common wheat Yiyuan 2 (**Fig. 1d**). With probes pTa-713 and $(GAA)_5$, these three chromosomes were clearly identified in Yiyuan 2 (**Fig. 1e**).

Chromosome identification of hybrid progenies

In 2014, 26 BC_1F_7 lines were tested for stripe rust infection. Fifteen lines exhibited resistance and 11 were susceptible. All 15 resistant lines and one randomly selected susceptible line (NZ309) were subjected to chromosome identification (**Table 1**). Alien chromosomes were identified in all resistant lines using pTa-535, pSc119.2, $(GAA)_5$, and pTa-713. An example of chromosome identification in line NZ311 is shown in **Fig. 2**. NZ309 had 42 common normal wheat chromosomes whereas all 15 resistant lines had a pair of $2S^v$ chromosomes (**Table 1**).

The 15 resistant lines had different chromosome constitutions. Compared to line NZ309 (**Fig. 3a**), three lines, NZ272 (**Fig. 3b**), NZ281, and NZ283, were addition lines containing two $2S^v$ and two $4S^v$ chromosomes (2n = 46). The other 12 resistant lines carried $2S^v$(2B) (**Fig. 3c**) or $2S^v$(2D) substitutions (**Fig. 2**, **Fig. 3d**). Among them, six lines (50%) showed cytological abnormalities and variable chromosome constitutions (**Table 1**). For instance, NZ286 had two cytotypes, 40W + II $2S^v$(2B) and 38W + II $2S^v$(2B) + I 3AS·1BL + I 1BS·3AL (**Fig. 3e**). In addition to $2S^v$(2B) substitution, some cells in line NZ292 carried 5BS·5DS and 5BL·5DL translocation chromosomes (**Fig. 3f**); NZ266 had a changed 6B plus a $5S^v$ addition (**Fig. 3g**); and NZ294 had a pair of changed 6B chromosomes plus an added $4S^v$ chromosome (**Fig. 3h**). In addition to $2S^v$(2D), some cells in lines NZ306 and NZ323 (**Fig. 3i**) had a $4S^v$ addition. This chimeric behavior was attributed to *Gc* gene(s) activity of chromosome $2S^v$.

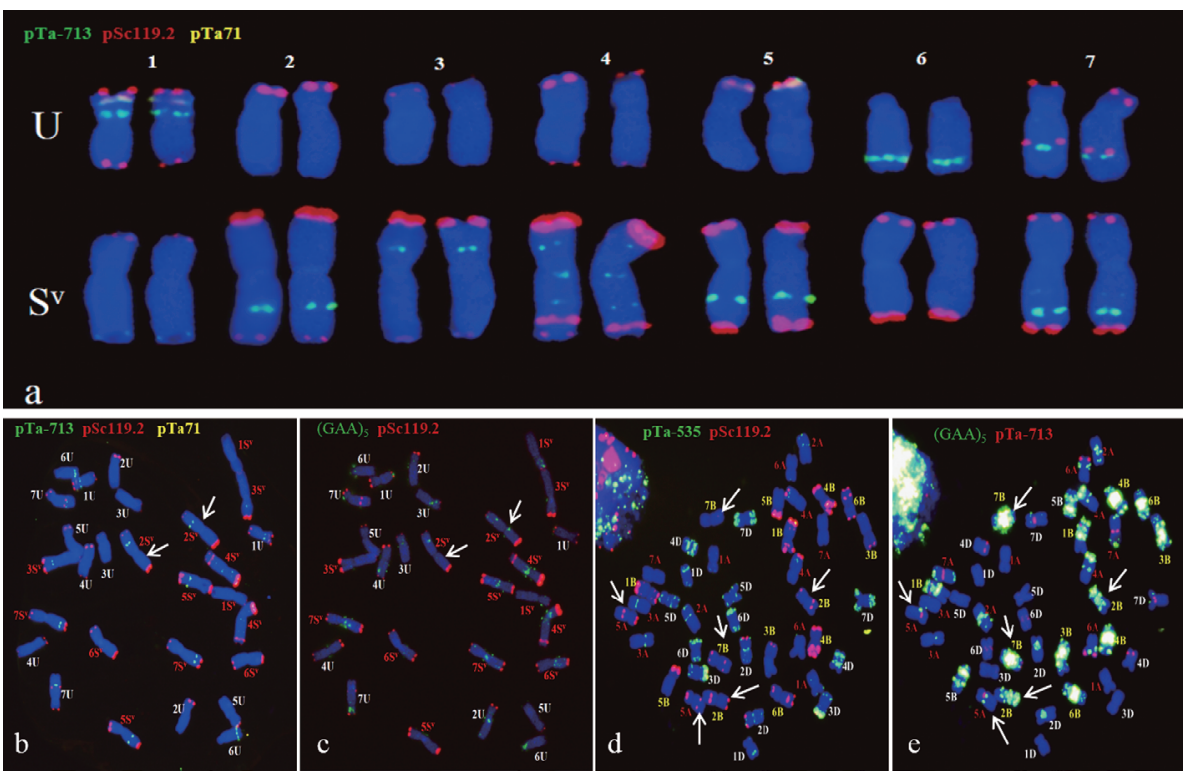

Fig. 1. FISH identification of parent chromosomes. (a) FISH patterns of individual *Ae. variabilis* chromosomes. (b, c) *Ae. variabilis* chromosomes from a same mitotic cell, but using different probe combinations. Arrows indicate $2S^v$. (d, e) Chromosomes of common wheat Yiyuan 2 from the same mitotic cell. (d) Probes pTa535 and pSc119.2 failed to differentiate chromosomes 5A, 2B, 7B from each other (arrows). (e) Probes $(GAA)_5$ and pTa-713 differentiated the three pairs chromosomes (arrows).

Table 1. Measurement and description of agronomic traits

Line	Chromosomes composition	Days to heading		Rust reaction		Plant height[a]	Tiller no.[a]	Spike length[a]	Spikelet number[a]	Waxiness[a]
		2014	2015	2014	2015					
Ae. variabilis	UUS^{v}S^{v}	165	150	0~1	0~1	83.0 ± 6.1	131.8 ± 44.5	5.5 ± 0.5	5.3 ± 0.8	Weak
Yiyuan 2	42W	149	133	7~9	7~9	70.7 ± 3.0	5.0 ± 0.7	7.7 ± 0.9	16.9 ± 2.1	Weak
NZ309	42W	148	135	7~9	7~9	74.6 ± 2.9	7.5 ± 1.5	8.2 ± 0.6	19.1 ± 1.4	Weak
NZ298	40W + II 2S^{v}(2B)	158	146	0~1	0~1	81.6 ± 3.1**	5.6 ± 1.5*	9.1 ± 0.9	18.6 ± 0.8	Weak
NZ300	40W + II 2S^{v}(2B)	158	145	0~1	0~1	79.6 ± 3.5	5.5 ± 1.1*	9.1 ± 0.7	18.8 ± 1.1	Weak
NZ286	40W + II 2S^{v}(2B) 38W + II 2S^{v}(2B) + I 3AS·1BL + I 1BS·3AL	159	144	0~1	0~1	86.9 ± 5.1**	7.6 ± 1.1	9.3 ± 0.9*	19.6 ± 0.8	Weak
NZ292	40W + II 2S^{v}(2B) 36W + II 2S^{v}(2B) + II 4S^{v}(4B) + I 5BS·5DS + I 5BL·5DL	158	146	0~1	0~1	74.5 ± 5.2	7.0 ± 1.6	8.9 ± 1.2	18.7 ± 1.1	Weak
NZ266	40W^{+} + II 2S^{v}(2B) 40W^{+} + II 2S^{v}(2B) + I 5S^{v}	157	140	0~1	0~1	83.6 ± 1.9**	6.9 ± 1.1	9.6 ± 1.2*	18.6 ± 1.3	Weak
NZ294	40W^{++} + II 2S^{v}(2B) 40W^{++} + II 2S^{v}(2B) + I 4S^{v}	158	147	0~1	0~1	72.4 ± 4.9	5.7 ± 1.3	9.2 ± 0.8	19.3 ± 0.7	Weak
NZ304	40W + II 2S^{v}(2D)	166	158	0~1	0~1	97.9 ± 5.2**	8.7 ± 2.7	11.8 ± 1.5**	23.1 ± 1.7**	Strong
NZ307	40W + II 2S^{v}(2D)	162	150	0~1	0~1	82.3 ± 6.5**	6.8 ± 2.4	10.5 ± 1.2**	22.0 ± 2.1**	Strong
NZ311	40W + II 2S^{v}(2D)	164	152	0~1	0~1	93.7 ± 1.5**	8.8 ± 0.4	10.8 ± 0.6**	21.7 ± 0.4**	Strong
NZ321	40W + II 2S^{v}(2D)	164	152	0~1	0~1	96.5 ± 1.2**	6.6 ± 1.1	11.9 ± 0.9**	22.2 ± 1.5**	Strong
NZ323	40W + II 2S^{v}(2D) 40W + II 2S^{v}(2D) + I 4S^{v}	164	155	0~1	0~1	89.6 ± 6.1**	7.7 ± 2.3	11.3 ± 1.0**	21.9 ± 1.4**	Strong
NZ306	40W + II 2S^{v}(2D) 40W + II 2S^{v}(2D) + I 4S^{v}	166	159	0~1	0~1	77.5 ± 2.3	9.5 ± 0.9*	11.9 ± 0.9**	23.9 ± 1.7**	Strong
NZ272	42W+ II 2S^{v} + II 4S^{v}	161		0~1						
NZ281	42W + II 2S^{v} + II 4S^{v}	164		0~1						
NZ283	42W + II 2S^{v} + II 4S^{v}	163		0~1						

*,**, significantly different from common wheat line NZ309 at P = 0.05 and P = 0.01, respectively (t-test).

$^{+}$, including one changed 6B. $^{++}$, including two changed 6B chromosomes.

[a] traits measured in 2015.

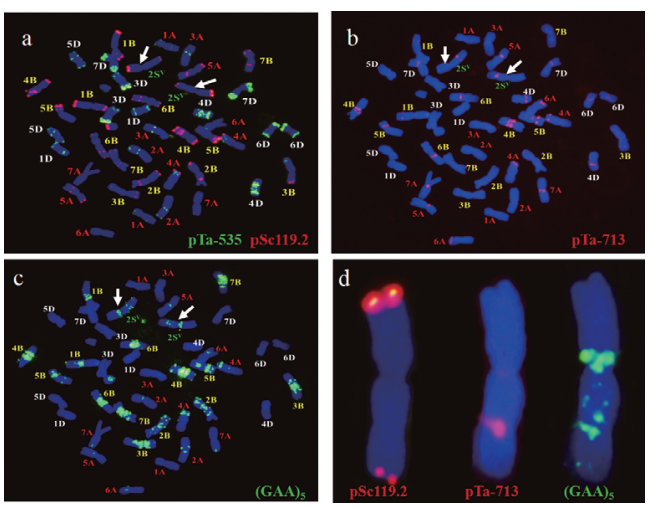

Fig. 2. Identification of alien chromosomes 2S^{v} from *Ae. variabilis* and chromosome constitution in NZ311. (a), (b) and (c) were from a same mitotic cell. (d) Chromosome 2S^{v} from a, b and c. Based on the signals of pSc119.2, pTa713 and $(GAA)_5$, chromosome 2S^{v} (arrows) was identified in wheat background. Based on signals of pTa-535, pSc119.2, pTa713 and $(GAA)_5$, individual wheat chromosomes were recognized.

SSR confirmation of substitution lines

Of 62 SSR markers analyzed, 30 were specific to chromosome 2B or 2D as confirmed by CS NT lines, including 15 on 2B (*barc55*, *barc18*, *gwm630*, *gwm257*, *gwm374*, *barc200*, *barc13*, *barc160*, *wmc257*, *gwm319*, *gwm455*, *cfd2278*, *gwm388*, *gwm120*, and *barc167*) and 15 on 2D (*gwm148*, *gdm107*, *gdm35*, *gdm77*, *cfd51*, *cfd56*, *cfd53*, *cfd77*, *barc159*, *cfd233*, *barc228*, *gpw1184*, *gwm320*, *cfd239*, and *gwm301*). These markers showed polymorphism between *Ae. variabilis* and Yiyuan 2. The primers for specific markers on 2B and 2D did not amplify PCR products from the 2S^{v}(2B) or 2S^{v}(2D) substitution lines, confirming absence of 2B or 2D (**Fig. 4**). Marker *gwm148* was amplified as co-dominant bands from 2D and 2S^{v}, the band from 2S^{v} was easily differentiated by size from that of 2D (**Fig. 4**).

Evaluation of agronomic traits

The stripe rust resistance was evaluated in both 2014 and 2015 by inoculation with mixed urediospores. Yiyuan 2 was highly susceptible whereas *Ae. variabilis* was resistant (**Fig. 5a**). The 15 derivatives with 2S^{v} all exhibited resistance (infection type 0–1) whereas line NZ309 lacking 2S^{v} was susceptible (7–9) (**Fig. 5a**, **Table 1**). This result indicated that a resistance gene(s) was present on chromosome 2S^{v}.

Ae. variabilis cytoplasm had effects on agronomic traits compared to its wheat parent, for example, line NZ309 had significantly more tillers than Yiyuan 2. Compared to NZ309 the 2S^{v}(2D) and 2S^{v}(2B) substitution lines of exhibited delayed heading, with 2S^{v}(2D) lines generally being later (**Table 1**, **Fig. 5b**). The difference between 2S^{v}(2D) and 2S^{v}(2B) indicated that deletion of the 2D and 2B chromosomes had different effects on heading time.

The 12 BC_1F_8 2S^{v}(2D) or 2S^{v}(2B) lines were evaluated for plant height, number of tillers, main spikelet length,

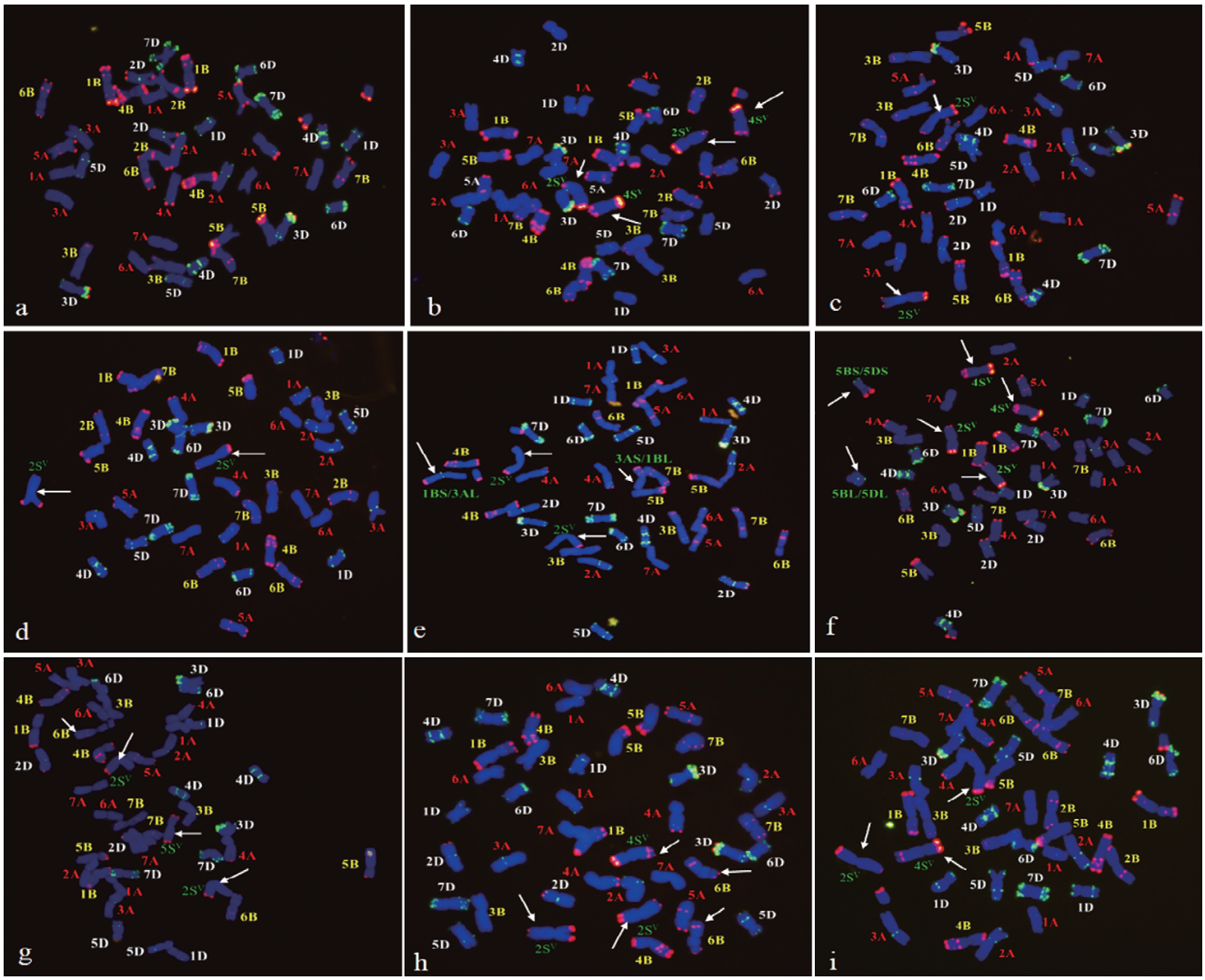

Fig. 3. Chromosome constitutions of progenies. Green signals for pTa535 and red for pSc119.2, counterstained with DAPI. (a) Line NZ309 with 42 common wheat chromosomes (42W). (b) Line NZ272, 42W + II $2S^v$ + II $4S^v$. (c) Line NZ300, 40W + II $2S^v$(2B). (d) Line NZ304, 40W + II $2S^v$(2D). (e) Line NZ286, 38W + II $2S^v$(2B) + I 3AS·1BL + I 1BS·3AL. (f) Line NZ292, 36W + II $2S^v$(2B) + II $4S^v$(4B) + I 5BS·5DS + I 5BL·5DL. (g) Line NZ266, $40W^+$ + II $2S^v$(2B) + I $5S^v$. (h) Line NZ294, $40W^{++}$ + II $2S^v$(2B) + I $4S^v$. (i) Line NZ323, 40W + II $2S^v$ (2D) + I $4S^v$. $^+$, one changed 6B chromosome. $^{++}$, two changed 6B chromosomes.

main spikelet number, and plant waxiness. Compared to NZ309, $2S^v$(2B) was less affected than $2S^v$(2D). The $2S^v$(2D) substitution lines were taller, and had more spikelets and longer spikes (**Table 1**). The loss of 2B and 2D caused different effects on waxiness. *Ae. variabilis*, Yiyuan 2, NZ309 and the $2S^v$(2B) substitution line were weakly waxy, but the $2S^v$(2D) substitution line was strongly waxy (**Fig. 5c**).

Discussion

Alien genetic resources are important for improving agronomic traits in wheat. The identification of alien chromosomes in wheat backgrounds is a critical step in utilizing alien genetic resources. FISH probes of repetitive sequences, such as pAs1, pSc119.2, pTa71, pTa-86, and pTa-535, have been widely used to identify individual chromosomes of the wheat A-, B-, and D-genomes (Hao *et al.* 2013, Komuro *et al.* 2013, Langridge 1997, Pedersen and Sepsi *et al.* 2008). FISH technology has been also used for chromosome identification in *Aegilops* species, including U- or/and S-genome chromosomes (Badaeva *et al.* 2004, Kwiatek *et al.* 2013, Molnár *et al.* 2011, Salina *et al.* 2006, Schneider *et al.* 2005, Zhang *et al.* 2013). However, the fluorescence signals of most probes appear in terminal regions, leading to uncertain identification of alien U- and S-chromosomes of *Ae. variabilis*, especially in wheat backgrounds. In this study we found that most of the pTa-713 signals were in the middle regions of chromosome arms, allowing better identification and easier identification of chromosomes from

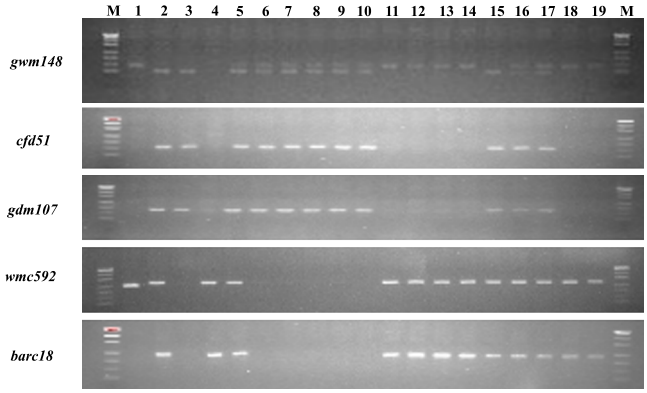

Fig. 4. PCR amplification patterns generated by SSR markers. M 500 bp marker, 1 *Ae. variabilis*, 2 Yiyuan 2, 3 N2BT2D, 4 N2DT2B, 5 NZ272, 6 NZ266, 7 NZ286, 8 NZ292, 9 NZ294, 10 NZ298, 11NZ304, 12 NZ306, 13 NZ307, 14 NZ311, 15 NZ309, 16 NZ281, 17 NZ283, 18 NZ321, 19 NZ323. 5, 16 and 17 were 2S^{v} addition lines; 6, 7, 8, 9 and 10, 2S^{v}(2B) substitution lines; 11, 12, 13, 14, 18 and 19, 2S^{v}(2D) substitution lines; 15 common wheat without an alien chromosome. Markers *gwm148*, *cfd51* and *gdm107* were the specific markers for 2D; markers *wmc592* and *barc18* were specific for 2B.

Ae. variabilis. The probe combination of pSc119.2, pTa71 and pTa-713 easily differentiated all 14 pairs of *Ae. variabilis* chromosomes. In addition, the co-dominant SSR marker gwm148 can be used in tracing chromosome 2S^{v} in common wheat background (**Fig. 4**).

Liu *et al.* (2011) reported transfer of stripe rust resistance from *Ae. variabilis* accession 13E to wheat, but the chromosome location of the gene(s) was unknown. The present study shows that resistance from *Ae. variabilis* accession AS116 is in chromosome 2S^{v}. It is unclear whether the genes from the two accessions are the same. The gene on 2S^{v} must now be transferred to wheat before it can be used in breeding. This should be possible through the use of a *ph1* genetic stock to allow chromosome 2S^{v} to synapse and recombine with a wheat homoeolog.

Photoperiod affects both vegetative and reproductive development in wheat (Miralles *et al.* 2000). The strongest genes affecting photoperiod response in wheat and presumably closely related species are located in Group II chromosomes. *Ppd-D1* on 2D is the most photoperiod insensitive locus followed by *Ppd-B1* on 2B and *Ppd-A1* on 2A (Worland 1996). In this study, 2S^{v}(2D) substitution lines were later flowering than 2S^{v}(2B) lines and euploid wheat. The extended growth period of the 2S^{v}(2D) line may have caused more spikelets and longer spikes. In addition, 2S^{v}(2D) or 2S^{v}(2B) lines affected other agronomic traits, such as waxiness and plant height.

Chromosome structural aberrations, including non-homologous translocations, were observed at relatively high frequency in a number of lines. This may be due to the presence of so-called gametocidal genes that have been reported in various 2S chromosomes (Endo 1985, 1990, Knight *et al.* 2015, Kota and Dvorak 1988, Miller *et al.* 1982, Tsujimoto 2005). There are *Gc* genes on S-genome chromosomes, such as 2S and 6S of *Ae. speltoides*, 2S^{l} and 4S^{l} of *Ae. longissima*, 2S^{sh} and 4S^{sh} of *Ae. sharonensis*. *Ae. longissima* is the S-genome donor species of *Ae. variabilis* (Kihara 1954, Yu and Jahier 1992). Hence *Ae. variabilis* may have inherited Gc gene(s) from *Ae. longissima*. Although wheat lines with 2S^{v} or 4S^{v} can be used as tools to induce novel chromosome structural rearrangements, this is not the preferred method as such recombination events appear to be random and therefore less likely to be compensatory.

Acknowledgments

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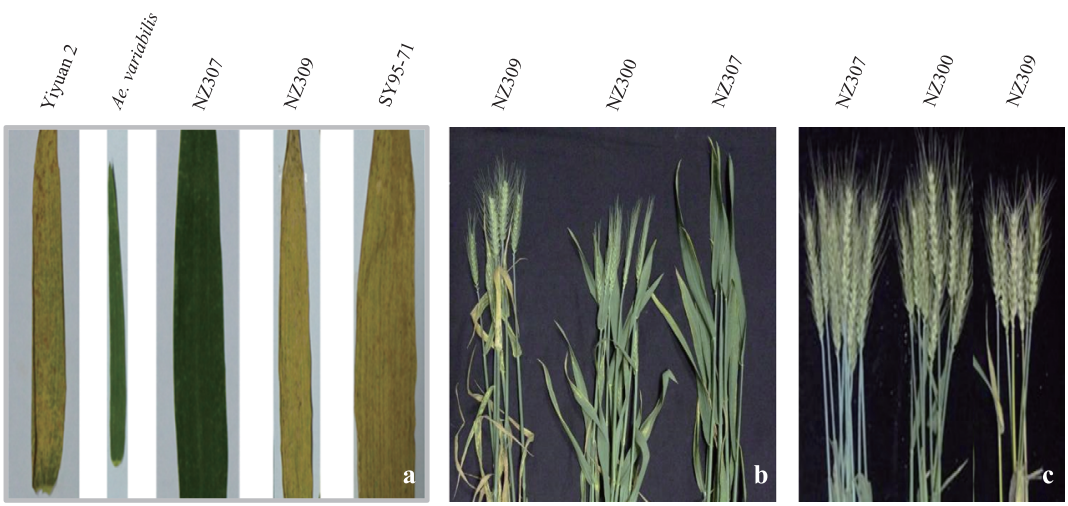

Fig. 5. Field performances. (a) Stripe rust responses on flag leaves. *Ae. variabilis* AS116 and NZ307 (2S^{v}(2D)) were resistant; NZ309 and YY2 were susceptible. Wheat SY95-71 was the disease spreader. (b) NZ309 showed earlier heading than NZ307 (2S^{v}(2D)) and NZ300 (2S^{v}(2B)). (c) NZ309 and NZ300 (2S^{v}(2B)) were weakly waxy, whereas NZ307 (2S^{v}(2D)) was strongly waxy.

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Literature Cited

Badaeva, E.D., A.V. Amosova, T.E. Samatadze, S.A. Zoshchuk, N.G. Shostak, N.N. Chikida, A.V. Zelenin, W.J. Raupp, B. Friebe and B.S. Gill (2004) Genome differentiation in *Aegilops*. 4. Evolution of the U-genome cluster. Plant Syst. Evol. 246: 45–76.

Barloy, D., J. Lemoine, P. Abelard, A.M. Tanguy, R. Rivoal and J. Jahier (2007) Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. Mol. Breed. 20: 31–40.

Chen, W.Q., L.R. Wu, T.G. Liu, S.C. Xu, S.L. Jin, Y.L. Peng and B.T. Wang (2009) Race dynamics, diversity, and virulence evolution in *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust in China from 2003 to 2007. Plant Dis. 93: 1093–1101.

Chen, X.M. (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can. J. Plant Pathol. 27: 314–337.

Cuadrado, A., M. Cardoso and N. Jouve (2008) Increasing the physical markers of wheat chromosomes using SSRs as FISH probes. Genome 51: 809–815.

Dennis, E.S., W.L. Gerlach and W.J. Peacock (1980) Identical polypyrimidine-polypurine satellite DNAs in wheat and barley. Heredity 44: 349–366.

Endo, T.R. (1985) Two types of gametocidal chromosomes of *Aegilops sharonensis* and *Ae. longissima*. Jpn. J. Genet. 60: 125–135.

Endo, T.R. (1990) Gametocidal chromosomes and their induction of chromosome mutations in wheat. Jpn. J. Genet. 65: 135–152.

Hao, M., J.T. Luo, M. Yang, L.Q. Zhang, Z.H. Yan, Z.W. Yuan, Y.L. Zheng, H.G. Zhang and D.C. Liu (2011) Comparison of homoeologous chromosome pairing between hybrids of wheat genotypes Chinese Spring *ph1b* and Kaixian-luohanmai with rye. Genome 54: 959–964.

Hao, M., J.T. Luo, L.Q. Zhang, Z.W. Yuan, Y.W. Yang, M. Wu, W.J. Chen, Y.L. Zheng, H.G. Zhang and D.C. Liu (2013) Production of hexaploid triticale by a synthetic hexaploid wheat–rye hybrid method. Euphytica 193: 347–357.

Kato, A. (1999) Air drying method using nitrous oxide for chromosome counting in maize. Biotech. Histochem. 74: 160–166.

Kihara, H. (1944) Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. Agric. Hortic. 19: 889–890.

Kihara, H. (1954) Considerations on the evolution and distribution of *Aegilops* species based on the analyser-method. Cytologia 19: 336–357.

Kilian, B., H. Ozkan, O. Deusch, S. Effgen, A. Brandolini, J. Kohl, W. Martin and F. Salamini (2007) Independent wheat B and G genome origins in outcrossing *Aegilops* progenitor haplotypes. Mol. Biol. Evol. 24: 217–227.

Knight, E., A. Binnie, T. Draeger, M.D. Moscou, M. Rey, J. Sucher, S. Mehra, I. King and G. Moore (2015) Mapping the 'breaker' element of the gametocidal locus proximal to a block of sub-telomeric heterochromatin on the long arm of chromosome $4S^{sh}$ of *Aegilops sharonensis*. Theor. Appl. Genet. 128: 1049–1059.

Komuro, S., R. Endo, K. Shikata and A. Kato (2013) Genomic and chromosomal distribution patterns of various repeated DNA sequences in wheat revealed by a fluorescence in situ hybridization procedure. Genome 56: 131–137.

Kota, R.S. and J. Dvorak (1988) Genomic instability in wheat induced by chromosome $6B^S$ of *Triticum speltoides*. Genetics 120: 1085–1094.

Kwiatek, M., H. Wiśniewska and B. Apolinarska (2013) Cytogenetic analysis of *Aegilops* chromosomes, potentially usable in triticale (X *Triticosecale* Witt.) breeding. J. Appl. Genet. 54: 147–155.

Liu, D.C., Z.G. Xiang, L.Q. Zhang, Y.L. Zheng, W.Y. Yang, G.Y. Chen, C.J. Wan and H.G. Zhang (2011) Transfer of stripe rust resistance from *Aegilops variabilis* to bread wheat. Afr. J. Biotechnol. 10: 136–139.

Liu, J., Z.J. Chang, X.J. Zhang, Z.J. Yang, X. Li, J.Q. Jia, H.X. Zhan, H.J. Guo and J.M. Wang (2013) Putative *Thinopyrum intermedium* derived stripe rust resistance gene *Yr50* maps on wheat chromosome arm 4BL. Theor. Appl. Genet. 126: 265–274.

Liu, T.G., Y.L. Peng, W.Q. Chen and Z.Y. Zhang (2010) First detection of virulence in *Puccinia striiformis* f. sp. *tritici* in China to resistance genes *Yr24* (=*Yr26*) present in wheat cultivar Chuanmai 42. Plant Dis. 94: 1163.

Marais, G.F., B. McCallum and A.S. Marais (2008) Wheat leaf rust resistance gene *Lr59* derived from *Aegilops peregrina*. Plant Breed. 127: 340–345.

McFadden, E.S. and E.R. Sears (1944) The artificial synthesis of *Triticum spleta*. Rec. Genet. Soc. Am. 13: 26–27.

McNeal, F.H., C.F. Koznak, E.P. Smith, W.S. Tate and T.S. Russell (1971) A uniform system for recording and processing cereal research data. USDA-ARS Bull. 42: 34–121.

Miller, T.E., J. Hutchinson and V. Chapman (1982) Investigation of a preferentially transmitted *Aegilops sharonensis* chromosome in wheat. Theor. Appl. Genet. 61: 27–33.

Miralles, D.J., R.A. Richards and G.A. Slafer (2000) Duration of stem elongation period influences the number of fertile florets in wheat and barley. Aust. J. Plant Physiol. 27: 931–940.

Molnár, I., M. Cifuentes, A. Schneider, E. Benavente and M. Molnár-Láng (2011) Association between simple sequence repeat-rich chromosome regions and intergenomic translocation breakpoints in natural populations of allopolyploid wild wheats. Ann. Bot. 107: 65–76.

Mujeeb-Kazi, A., A. Gul, M. Farooq, S. Rizwan and J.I. Mirza (2007) Genetic diversity of *Aegilops variabilis* (2n = 4x = 28; UUSS) for wheat improvement: morpho-cytogentic characterization of some derived amphiploids and their practical significance. Pak. J. Bot. 39: 57–66.

Neelam, K., N. Rawat, V.K. Tiwari, S. Kumar, P. Chhuneja, K. Singh, S. Randhawa and H.S. Dhaliwal (2011) Introgression of group 4 and 7 chromosomes of *Ae. peregrina* in wheat enhances grain iron and zinc density. Mol. Breed. 28: 623–634.

Pedersen, C. and P. Langridge (1997) Identification of the entire chromosome complement of bread wheat by two-colour FISH. Genome 40: 589–593.

Petersen, G., O. Seberg, M. Yde and K. Berthelsen (2006) Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). Mol. Phylogenet. Evol. 39: 70–82.

Salina, E.A., K.Y. Lim, E.D. Badaeva, A.B. Shcherban, I.G. Adonina, A.V. Amosova, T.E. Samatadze, T.Y. Vatolina, S.A. Zoshchuk and A.R. Leitch (2006) Phylogenetic reconstruction of *Aegilops* section *Sitopsis* and the evolution of tandem repeats in the diploids and derived wheat polyploids. Genome 49: 1023–1035.

Schneider, A., G. Linc, I. Molnár and M. Molnár-Láng (2005) Molecular cytogenetic characterization of *Aegilops biuncialis* and its use

for the identification of 5 derived wheats – *Aegilops biuncialis* disomic addition lines. Genome 48: 1070–1082.

Sears, E.R. (1976) Genetic control of chromosome pairing in wheat. Annu. Rev. Genet. 10: 31–51.

Sepsi, A., I. Molnár, D. Szalay and M. Molnár-Láng (2008) Characterization of a leaf rust-resistant wheat–*Thinopyrum ponticum* partial amphiploid BE-1, using sequential multicolor GISH and FISH. Theor. Appl. Genet. 116: 825–834.

Sourdille, P., S. Singh, T. Cadalen, G.L. Brown-Guedira, G. Gay, L.L. Qi, B.S. Gill, P. Dufour, A. Murigneux and M. Bernard (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct. Integr. Genomics 4: 12–25.

Spetsov, P., D. Mingeot, J.M. Jacquemin, K. Samardjieva and E. Marinova (1997) Transfer of powdery mildew resistance from *Aegilops variabilis* into bread wheat. Euphytica 93: 49–54.

Tang, Z.X., Z.J. Yang and S.L. Fu (2014) Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. J. Appl. Genet. 55: 313–318.

Tsujimoto, H. (2005) Gametocidal genes in wheat as the inducer of chromosome breakage. *In*: Tsunewaki, K. (ed.) Frontiers of Wheat Bioscience. Memorial issue, Wheat Information Service No. 100. Kihara Memorial Yokohama Foundation, Yokohama, pp. 33–48.

van Slageren, M.W. (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Agricultural University, Wageningen; International Center for Agricultural Research in Dry Areas, Aleppo, Syria.

Wan, A., Z. Zhao, X. Chen, Z. He, S. Jin, Q. Jia, G. Yao, J. Yang, B. Wang, G. Li *et al.* (2004) Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *Tritici* in China in 2002. Plant Dis. 88: 896–904.

Wan, A.M., X.M. Chen and Z.H. He (2007) Wheat stripe rust in China. Aust. J. Agric. Res. 58: 605–619.

Wang, R.R. and B. Lu (2014) Biosystematics and evolutionary relationships of perennial Triticeae species revealed by genomic analyses. J. Syst. Evol. 52: 697–705.

Worland, A. (1996) The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89: 49–57.

Yen, C., J.L. Yang and Y. Yen (2005) Hitoshi Kihara, Askell Löve and the modern genetic concept of the genera in the tribe Triticeae (Poaceae). Acta Phytotaxon. Sin. 43: 82–93.

Yen, C. and J.L. Yang (2009) Historical review and prospect of taxonomy of tribe Triticeae Dumortier (Poaceae). Breed. Sci. 59: 513–518.

Yu, M.Q., F. Person-Dedryver, J. Jahier, D. Pannetier, A.M. Tanguy and P. Abelard (1990) Resistance to root knot nematode, *Meloidogyne naasi* (Franklin) transferred from *Aegilops variabilis* Eig to bread wheat. Agronomie 6: 451–456.

Yu, M.Q. and J. Jahier (1992) Origin of S^v genome of *Aegilops variabilis* and utilization of the S^v as analyser of the S genome of the *Aegilops* species in the *Sitopsis* section. Plant Breed. 108: 290–295.

Yu, M.Q., J. Jahier and F. Person-Dedryver (1992) Genetics of two mechanisms of resistance to *Meloidogyne naasi* (FRANKLIN) in an *Aegilops variabilis* Eig. Accession. Euphytica 58: 267–273.

Zhang, H., Y. Bian, X.W. Gou, Y.Z. Dong, S. Rustgi, B.J. Zhang, C.M. Xu, N. Li, B. Qi, F.P. Han *et al.* (2013) Intrinsic karyotype stability and gene copy number variations may have laid the foundation for tetraploid wheat formation. Proc. Natl. Acad. Sci USA 110: 19466–19471.