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Wheat lines derived from trigeneric hybrids of wheat–rye–*Psathyrostachys huashanica*, the potential resources for grain weight improvement

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Abstract

Grain weight is regarded as one of two major components of wheat yield; however, it has not been improved greatly in recent decades. In this study, a cross of wheat–*Psathyrostachys huashanica* amphiploid 'PHW-SA' (2*n* = 56, AABBDDNsNs) and hexaploid triticale 'Zhongsi 828' (2*n* = 42, AABBRR) was carried out in order to produce novel germplasms for larger grain weight. Consequently, 15 derivatives were selected from 239 F_3 lines because they had 63–150% higher 1000-grain weight than the mean of two parents under natural field conditions. Cytological analysis showed that the chromosome number of these lines ranged from 41 to 44, and two-thirds of them had 42 chromosomes. Using Giemsa C-banding and genomic *in situ* hybridization, there were 12–14 rye chromosomes characterized in 14 lines, of which nine had the complete R genome. Ten rye chromosomes and a translocation between 2RS and 6DS were identified in the remaining line 951-13. Additionally three lines (938-1, 940-6 and 944-6) contained 1–2 chromosomes of *P. huashanica*. Meiotic analysis revealed that the averaged chromosome configuration across all was 3.35 univalents, 19.34 bivalents, 0.06 trivalents and 0.02 tetravalents per pollen mother cell. Two combinations bearing complete rye chromosomes were cytologically stable during meiosis and may therefore be considered as new hexaploid triticale. The 15 lines were also highly resistant to stripe rust. Thus, they will be utilized as useful genetic resources for improving wheat grain weight and stripe rust resistance.

Keywords: Common wheat; Genomic *in situ* hybridization; Giemsa C-banding; Grain weight; Meiosis; Mitosis; *Psathyrostachys huashanica*; *Secale cereale*; Stripe rust resistance.
Abbreviations: ASS- averaged seed set; CS- Chinese Spring wheat; GISH- genomic *in situ* hybridization; GPS- grains per spikelet; HN- hairy neck; LR- lodging resistance; MI- metaphase I; PHW-SA- wheat–*Psathyrostachys huashanica* amphiploid; PMC- pollen mother cell; SH- straw height; SL- spike length; SPP- spikes per plant; SPS- spikelets per spike; TGW- 1000-grain weight; WP- waxy peduncle.

Introduction

Wheat is one of the most crucial cereals in the world and provides approximately one-fifth of the total calorific input for all human (Reynolds et al*.,* 2009). Increasing wheat production has had a huge contribution to feeding the burgeoning World's population since the Green Revolution. As a critical trait, the yield *per se* is rather complex and determined by wheat growth and development, and many environmental factors (Slafer, 2003). To improve wheat yield efficiently, physiologists often divide this complicated trait into two major numerical components: the number of grains per unit land area (four sub-components: plants per unit area, spikes per plant, spikelets per spike, and grains per spikelet) and average individual grain weight. The two components were herein termed the sink strength during reproductive phase (Slafer, 2007). Currently, it has been widely recognized that the sink strength is the predominant determinant of final wheat yield rather than the source strength during grain filling (Miralles and Slafer, 2007; Acreche and Slafer, 2009). For the grain number, it has been augmented greatly due to the utilization of semidwarf cultivars (Fischer and Stockman, 1986), which moderates the sink limitation to some extent. However, the average grain weight has not been increased correspondingly (Miralles and Slafer, 1995). To break the sink limitation, the strategies have been proposed by the international Wheat Yield Consortium (WYC), mainly including increasing partitioning of dry mass to grains, and introducing new desirable traits and genes via wide crosses with relatives of common wheat (Foulkes et al., 2011). For the latter aspect, the relatives of wheat in Triticeae are highly potential because of the existing diverse alleles. With regard to grain weight, some wild species such as *Leymus arenarius* and *L. mollis* (Anamthawat-Jónsson et al., 1997), *L. racemosus* (Zhang et al., 2000), *Thinopyrum ponticum* (Jauhar, 1995), etc. have been proved to be important resources. But up to the present, only few studies

were conducted with the wild relatives of wheat to generate novel genetic resources for grain weight improvement.

Psathyrostachys huashanica Keng ex Kuo (2*n* = 14, NsNs) and *Secale cereale* L. (2*n* = 14, RR) have been considered as two useful related species over recent years. *P. huashanica* is only found on the rocky Huashan Mountain, Shanxi Province, China (Baden, 1991) and therefore a member of the endangered and imperatively protected wild species (The State Forestry Administration and Ministry of Agriculture, 1999). To date, it was reported that *P. huashanica* has agronomic advantages in the genetic improvement of wheat, including high resistance to *Gaeumannomyces graminis* (Wang and Shang, 2000), *Puccinia striiformis* f. sp. *tritici* (Jing et al., 1999) and *Rhopalosiphum padi* (Li et al., 2004), tolerance to drought (Du et al., 2010), winter hardiness (Kishii et al., 2010). Further, many new germplasms have been produced by the wide cross with common wheat, and some valuable traits (e.g. resistance to stripe rust and take-all fungus) have been introduced into wheat (Kishii et al*.,* 2010; Zhao et al*.,* 2010; Kang et al*.,* 2011a; Wang et al*.,* 2011). *S. cereale*, a small-scale crop grown, has been a popular resource for a long time in wheat improvement. A famous wheat–rye translation line, namely 1RS.1BL, has been widely adopted in wheat breeding programs worldwide due to its contribution to higher yield, grain weight and spikelet fertility (Carver and Rayburn, 1994). An attempt to introduce both genetic resources into wheat for grain weight improvement was initiated in this study. The major objectives of the present study were: (i) to obtain wheat lines with larger grain weight from 239 F_3 segregating progenies derived from the cross of wheat–*P. huashanica* amphiploid (PHW-SA) with wheat–*S. cereale* amphiploid ('Zhongsi 828'), and (ii) to determine the genomic constitutions and stability of these germplasms using Giemsa C-banding and genomic *in situ* hybridization on the chromosomes during mitosis and meiosis.

Results

Screening for derivative lines with large grain weight in F_3 generation

Analysis of variance indicated that there was no significant difference in 1000-grain weight between PHW-SA and Zhongsi 828, but the latter performed better than PHW-SA in grain number, including more spikes per plant, more spikelets per spike and more grains per spikelet (Table 1). Of 239 candidate lines in F_3 generation, 15 (6.3%) were isolated on the basis of their apparently higher 1000-grain weight than the mean of two parents. The 1000-grain weight varied from 26.6–40.7 g, outperforming the mean of two parents by 63–150% (Table 1). These lines often had larger spikes and grain volume (Fig. 1). Table 1 presents the variation in the other traits. Minimum and maximum values were: 4–16 fertile spikes per plant, 30–36 spikelets per spike, 1.0–2.3 grains per spikelet, 17.5–23.0 cm for spike length, 33.3–77.4% for averaged seed set, and 117–152 cm for straw height, respectively. Each line was resistant to lodging under field conditions. It is universally recognized that the waxy peduncle and hairy neck are the rye-specific traits (Badaev et al., 1985). The two characters existed in all derivatives, implying the presence of rye chromatin. Correlative analysis showed that the 1000-grain weight was not significantly correlated with any other trait investigated, including three sub-components of grain number (i.e. spikes per plant, spikelets per spike and grains per spikelet). Similarly, there was no close correlation between spikes per plant and the remaining characters. However, spike length was significantly correlated with spikelet number per spike (r^2 = 0.54, $P < 0.05$) and grain number per spikelet (r^2 = 0.49, $P < 0.05$) while straw height was positively correlated with spike length (r^2 = 0.50, $P < 0.05$), grain number per spikelet (r^2 = 0.59, $P < 0.01$) and averaged seed set (r^2 = 0.61, $P < 0.01$).

Cytological analyses

Chromosome number in the meristem cells from the root tips of the 15 lines with large grain weight was counted (Table 2). The data indicated that chromosome number ranged from 41 to 44, and two-thirds of the total lines had 42 chromosomes. Next, 50 pollen mother cells of each line were analyzed for understanding chromosome pairing patterns at the first meiotic metaphase (Table 2, Fig. 2a–e). The results showed that chromosome pairing at MI was abnormal in 13 derivatives but normal in two lines (928-6 and 953-3). In the 13 lines, at least 15.74 bivalents (mostly rings) were formed regularly (Table 2, Fig. 2a–d). A number of univalents were also found in many cells, and the average number of univalents per cell varied greatly, from 1.24 to 10.26 (Table 2, Fig. 2a–c). Meanwhile, multivalents (i.e. trivalents and tetravalents) appeared at a low frequency (Table 2). For lines 928-6 and 953-3, in contrast, the regular chromosome pairing was observed (20–21 bivalents), with rare univalents (0–2) and no multivalent (Table 2, Fig. 2d). At meiotic anaphase I and telophase, no lagging chromosome and micronucleus were found in the two lines (Fig. 2e). Across all the lines, the averaged chromosome configuration was 3.35 univalents, 15.85 ring bivalents, 3.49 rod bivalents, 0.06 trivalents and 0.02 tetravalents per PMC.

Giemsa C-banding identification of alien chromosomes

The characteristic knobs on the short arms of rye chromosomes are usually massive, which allows the recognition of each rye chromosome in wheat background. Compared with rye chromosomes, *P. huashanica* possesses similar C-banding pattern in the terminal positions of short arms but the other bands differ in size, intensity and spatial distribution, especially interstitial bands on the long arms. In this study, identification was made successfully by C-banding patterns and arm ratios as well as chromosome size. All the lines selected previously contained the partial or complete genome of rye while only three lines were proved to include 1–2 chromosomes of *P. huashanica* (Table 3, Fig. 2f–i). Specifically, of 15 lines, nine (60%) had each pair of 1R–7R of rye chromosomes (Fig. 2f, i), and four (27%) had 13 rye chromosomes with absence of 4R in line 925-3, 5R in line 939-10 (Fig. 2g), 1R in line 947-8 and 3R in line 955-1. In addition, 12 rye chromosomes were characterized in line 943-3 with the loss of single 4R and 6R. Especially, in the line 951-13, ten rye chromosomes and a translocation between wheat and rye chromosomes were observed (Fig. 2h). A careful identification was done, and implied that it was a 2RS.6DS translocation line. However, only three lines (20%), namely 938-1, 940-6 and 944-6, were distinguished to carry 1Ns and 3Ns (Fig. 2f), 3Ns, 5Ns and 7Ns of *P. huashanica*, respectively.

Genomic in situ hybridization (GISH) analysis

To confirm the results of the identification by Giemsa C-banding, the alien chromosomes from rye and *P. huashanica* in the 15 lines were further detected by GISH on the chromosomes at mitotic metaphase, and meiotic metaphase I

Table 1. Comparisons of the means of agronomic traits between the 15 lines.

Lines	TGW[a]	SPP	SPS	GPS	SL	ASS	SH	LR	WP	HN
PHW-SA	17.0 e[b]	4 c	24 d	1.3 d	16.7 d	41.8 h	117 c	Y	N	N
Zhongsi 828	15.6 e	5 c	38 a	2.3 a	20.0 b	76.1 a	133 b	Y	Y	Y
925-3	31.8 cd	11 b	33 b	2.3 a	18.0 cd	77.4 a	136 b	Y	Y	Y
926-1	32.6 cd	9 b	34 b	2.2 ab	19.0 bc	73.6 b	150 a	Y	Y	Y
928-6	31.2 cd	6 bc	34 b	2.3 a	19.3 bc	75.9 ab	148 a	Y	Y	Y
934-3	33.8 bc	9 b	33 b	2.3 a	20.3 b	76.1 a	150 a	Y	Y	Y
936-7	36.3 b	12 ab	32 bc	1.8 c	20.3 b	62.5 de	137 b	Y	Y	Y
938-1	33.9 bc	8 bc	35 ab	1.6 cd	17.7 cd	54.6 f	125 bc	Y	Y	Y
939-10	28.1 d	6 bc	31 bc	1.7 c	18.3 cd	58.1 ef	134 b	Y	Y	Y
940-6	36.2 b	4 c	34 b	2.2 ab	19.3 bc	62.8 de	146 a	Y	Y	Y
941-1	38.5 ab	13 a	31 bc	1.0 e	17.5 cd	33.3 i	117 c	Y	Y	Y
943-3	34.3 bc	5 c	30 c	1.4 d	18.7 bc	47.2 g	135 b	Y	Y	Y
944-6	40.6 a	9 b	33 b	1.7 c	20.0 b	55.9 f	148 a	Y	Y	Y
947-8	26.6 d	16 a	32 bc	1.5 d	21.0 b	48.1 g	147 a	Y	Y	Y
951-13	36.9 b	10 b	36 ab	2.3 a	23.0 a	64.0 cd	131 b	Y	Y	Y
953-3	40.7 a	8 bc	32 bc	2.0 b	18.7 bc	62.5 de	138 b	Y	Y	Y
955-1	32.6 cd	9 b	32 bc	2.1 b	20.3 b	68.7 c	152 a	Y	Y	Y

[a] Characters are denoted as abbreviations, TGW: 1000-grain weight (g), SPP: spikes per plant, SPS: spikelets per spike, GPS: grains per spikelet, SL: spike length (cm), ASS: averaged seed set (%), SH: straw height (cm), LR: lodging resistance, WP: waxy peduncle, HN: hairy neck, Y: yes, N: no. [b] Means followed by the same letter(s) within a column do not differ significantly at $P < 0.05$ probability level according to LSD test.

as well as anaphase I (Table 3, Fig. 3). For the chromosomes at mitotic metaphase, we firstly used the R-genome DNA of rye as the probe, and the ABD-genomes DNA of CS as the blocker. Consequently, 14 chromosomes fluoresced with yellow-green signals in nine lines (Fig. 3a, d), and 13 chromosomes in four lines (Fig. 3b), and 12 chromosomes in one line. The remaining line 951-13 had ten chromosomes and an extra chromosomal segment showing yellow-green fluorescence, indicating that a translocation indeed took place (Fig. 3c). Secondly, when Ns-genome DNA from *P. huashanica* was adopted as the probe, two chromosomes in lines 938-1 (Fig. 3e) and 944-6, one chromosome in line 940-6 produced yellow-green fluorescence. No yellow-green hybridization signal was seen in the other lines. For understanding the behavior and stability of these alien chromosomes during meiosis, three experiments were successively carried out by using different probes (Fig. 3f–j). In the first experiment, all lines were detected only by R-genome DNA as the probe (Fig. 3f–i). It was observed that a number of univalents (0–12) with hybridization signals appeared in the majority of lines (Fig. 3f), with the exception of lines 928-6 and 953-3, where rare univalent was hybridized by the probe. The bivalents emitting fluorescent signals were found in each of lines, ranging from 1 to 7 (Fig. 3f–h). For line 951-13, besides five pairs of bivalents, a chromosomal segment showed yellow-green fluorescence (Fig. 3g), which further confirmed the presence of a translocation in this line. Interestingly, in the lines 928-6 and 953-3, there were always seven pairs of bivalents with yellow-green hybridization signals (Fig. 3h), and regular segregation of each bivalent at the first anaphase I was also found (Fig. 3i). It seems that the complete rye genome in the two lines was likely to be stably transmitted into the next generation. Additionally, multivalents having one univalent hybridized by the probe were also found. In the second experiment, Ns-genome DNA from *P. huashanica* was used as the probe; however, only three lines (i.e. 938-1, 940-6 and 944-6) were identified to contain 1–2 chromosomes of *P. huashanica*, which is in agreement with the results from C-banding. That is, two univalents of *P. huashanica* were in lines 938-1 and 944-6, and one univalent in line 940-6, respectively. In the third experiment, we used the mixture of R- and Ns-genomes DNA as probes. The resulting number of chromosomes giving yellow-green fluorescent signals was identical with that of rye chromosomes annotated previously in the first experiment, but excluding lines 938-1, 940-6 and 944-6. In the three lines, seven bivalents and two univalents in lines 938-1 (Fig. 3j) and 944-6, and seven bivalents and one univalent in line 940-6 emitted yellow-green fluorescent signals in the most PMCs. This means that they possess both chromatins from rye and *P. huashanica*. Also, we found that some univalents in many lines (e.g. 925-3, 939-10, 943-3, 947-8 and 951-13) could not be hybridized by the mixed probes.

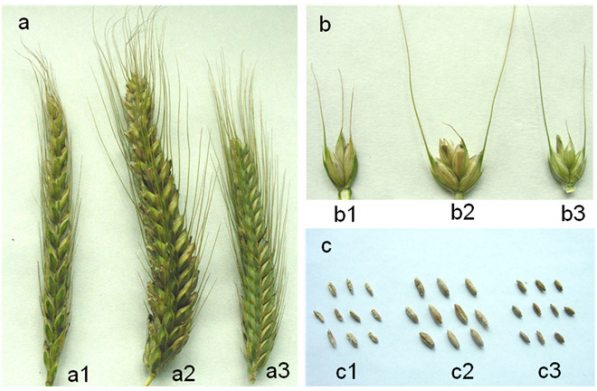

Fig 1. The spike (a), spikelet (b) and grains (c) of the lines selected from F_3 plants. (a1, b1, and c1) PHW-SA. (a2, b2, and c2) The lines selected from F_3 generation, showing larger spikes, spikelets and grains. (a3, b3, and c3) Zhongsi 828.

Stripe rust resistance

Evaluation of stripe rust resistance showed that the control Mingxian 169 was highly susceptible with infection of type 4 (leaf area covered with abundant linear pustules), whereas all of the female parent plants (PHW-SA) were resistant with infection type 0 or 1 (Fig. 4). Partial plants of the male parent (Zhongsi 828) were resistant with infection of type 2 whilst the remaining plants were susceptible with infection of type 3 or 4. For the 15 lines previously selected, all of them were

Table 2. Chromosome number at mitotic metaphase and chromosome pairing at MI in the 15 lines.

Lines	2*n*	Means and ranges of chromosome configurations					
		I	Total II	Ring II	Rod II	III	IV
925-3	42	1.24 (0–6)[a]	20.38 (18–21)	17.65 (15–20)	2.73 (1–5)	–	–
926-1	42	10.26 (5–12)	15.74 (13–18)	13.55 (10–17)	2.19 (0–5)	0.02 (0–1)	0.05 (0–1)
928-6[b]	42	0.16 (0–2)	20.92 (20–21)	15.46 (13–19)	5.46 (2–8)	–	–
934-3	42	7.36 (3–12)	17.21 (15–19)	13.00 (9–16)	4.21 (2–7)	0.07 (0–1)	–
936-7	42	6.10 (2–10)	17.70 (15–20)	13.55 (8–16)	4.15 (2–7)	0.10 (0–1)	0.05 (0–1)
938-1	44	2.70 (2–4)	20.65 (14–21)	17.18 (14–21)	3.47 (0–6)	–	–
939-10	42	2.82 (2–4)	19.59 (19–20)	15.17 (13–17)	4.42 (3–7)	–	–
940-6	43	2.92 (1–7)	20.04 (18–21)	17.37 (13–20)	2.67 (0–6)	–	–
941-1	42	3.00 (0–12)	19.39 (17–21)	16.56 (11–19)	2.83 (0–6)	–	0.06 (0–1)
943-3	42	3.58 (1–6)	19.00 (17–20)	14.97 (12–19)	4.03 (1–7)	0.14 (0–1)	–
944-6	44	1.41 (0–5)	21.10 (17–22)	16.92 (12–20)	4.18 (1–8)	0.09 (0–1)	0.03 (0–1)
947-8	42	3.42 (2–6)	19.26 (18–20)	15.86 (13–19)	3.40 (1–6)	0.02 (0–1)	–
951-13	41	3.08 (1–7)	18.90 (15–20)	16.37 (13–19)	2.53 (1–5)	–	0.03 (0–1)
953-3	42	0.30 (0–2)	20.85 (20–21)	18.11 (16–20)	2.74 (0–5)	–	–
955-1	41	1.96 (1–5)	19.44 (17–20)	16.06 (13–18)	3.38 (1–6)	–	0.04 (0–1)

[a] Means are given with minimum and maximum values in parentheses. [b] The lines denoted in bold showed regular meiosis.

immune or highly resistant to stripe rust with infection of type 0 or type 1.

Discussion

Grain weight is one of two major components of wheat yield. There is no doubt that a substantial increase in grain weight is of paramount importance for raising the yield threshold of wheat in the immediate future. From the perspective of empirical breeding, identification of some secondary traits that are closely correlated with grain weight is critical because they can be used for indirect selection of larger grain weight (Araus et al., 2008). To date a number of secondary traits related to grain weight, for example, carpel weight at anthesis (Calderini et al., 2001), the rate and duration of grain filling (Nass and Reiser, 1975) and the content of stem water soluble carbohydrate (Shearman et al., 2005), have been identified. In this study, correlative analysis was implemented between 1000-grain weight and the other parameters (i.e. spikes per plant, spikelets per spike, grains per spikelet, spike length, averaged seed set and straw height) based on the 15 lines selected from the F_3 segregating generation. As a consequence, the correlation coefficients between them were not significant, suggesting that they cannot be used as the secondary traits of grain weight. Also, 1000-grain weight was not significantly correlated with three sub-components of grain number (spikes per plant, spikelets per spike and grains per spikelet), which is in line with the previous hypothesis that grain number and grain weight may be independent of strong competition (Miralles and Slafer, 2007; Slafer, 2007). Thus, the increase of either grain number or grain weight would almost unequivocally boost the yield potential. Moreover, there were significant correlation coefficients between spike length and two sub-components (spikelet number per spike and grain number per spikelet), indicating that spike length could be considered as a desirable secondary trait of grain number; that is to say, moderate increase of spike length may give rise to incremental grain number. To understand the genetic basis of these lines with large grain weight, their chromosome constitutions were carefully identified by Giemsa C-banding, and further confirmed by genomic *in situ* hybridization. Consequently, 10–14 rye chromosomes and 0–2 *P. huashanica* chromosomes were found existing in the 15 lines, which

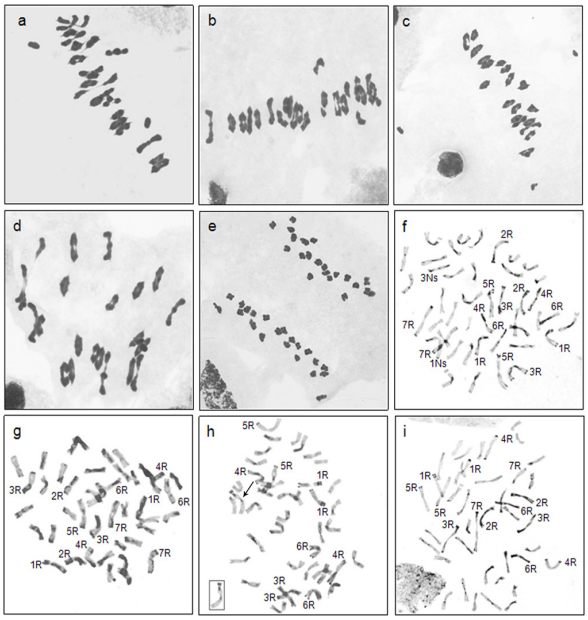

Fig 2. Chromosome pairing at the first metaphase and anaphase (a–e), and Giemsa C-banding patterns of mitotic metaphase chromosomes (f–i) of partial lines obtained. a Chromosome pairing of line 938-1, 2*n* = 44 = 2 I + 6 II (*rod*) + 15 II (*ring*). b Chromosome pairing of line 939-10, 2*n* = 42 = 2 I + 4 II (*rod*) + 16 II (*ring*). c Chromosome pairing of line 951-13, 2*n* = 41 = 3 I + 2 II (*rod*) + 17 II (*ring*). d–e Chromosome pairing of line 953-3, 2*n* = 42 = 7 II (*rod*) + 14 II (*ring*) (d), and normal chromosome segregation at the first anaphase (e). f C-banding pattern of line 938-1, showing rye chromosomes including 1R–7R and *P. huashanica* chromosomes consisting of 1Ns and 3Ns. g C-banding pattern of line 939-10, showing complete genome of rye except single 5R. h C-banding pattern of line 951-13, showing five pairs of rye chromosomes (1R, 3R, 4R, 5R, and 6R) and a speculative 2RS.6DS translocation (*arrow* and *inset*). i C-banding pattern of line 953-3, showing complete genome of rye.

Table 3. Chromosome constitutions of the 15 lines with large grain weight.

Lines	No. of plants examined	Genome constitutions			
		A+B+D	R	Ns	Total
925-3	3	29	13 (single of 4R absent)	0	42
926-1	3	28	14	0	42
928-6	3	28	14	0	42
934-3	3	28	14	0	42
936-7	3	28	14	0	42
938-1	3	28	14	2 (1Ns, 3Ns)	44
939-10	3	29	13 (single of 5R absent)	0	42
940-6	3	28	14	1 (3Ns)	43
941-1	3	28	14	0	42
943-3	3	30	12 (single of 4R and 6R absent)	0	42
944-6	3	28	14	2 (5Ns, 7Ns)	44
947-8	3	29	13 (single of 1R absent)	0	42
951-13	3	31	10 (2R and 7R absent, plus 2RS.6DS)	0	41
953-3	3	28	14	0	42
955-1	3	28	13 (single of 3R absent)	0	41

means that rye and *P. huashanica* were successfully introgressed. Apart from this, attention should be paid to two facts (i) that the total chromosome number of these lines differs slightly from 41 to 44, mainly being 42 (two-thirds of the total lines), almost equivalent to the chromosome number of common wheat, and (ii) that there are still 10–14 rye chromosomes in these lines (complete R genome in three-fifths of the total lines). It may be speculated that the chromosomes of R genome from rye are being accumulated but some chromosomes from wheat are missing. The further details can be obtained from the developmental process of the plant materials. Firstly, the female parent PHW-SA is an amphiploid ($2n = 8x = 56$, AABBDDNsNs) (Kang et al., 2009) while the female parent Zhongsi 828 was identified as a true hexaploid triticale ($2n = 6x = 42$, AABBRR) (data not shown). The hybrids of PHW-SA × Zhongsi 828 were expected to have the genomic constitution of AABBDNsR (49 chromosomes) (Kang et al., 2011b). After three generations, it may be assumed that the genomes AABB would be still present in their progenies and chromosome pairing at MI should be normal. By contrast, the chromosomes of D, Ns and R genomes would form univalents, and be randomly distributed between the poles; and ultimately they may be lost gradually because of the lack of their homologous chromosomes. Actually, it is certain that AABB genomes are still contained in these lines because at least 15.74 bivalents that failed to be hybridized by both probes (Ns and R genomes) were found over them. Most of chromosomes of genome Ns may be lost so we only observed 1–2 chromosomes of *P. huashanica* in three lines. The chromosomes of genome D have the similar fate so that a few univalents could be seen but not labeled by both probes. However, there are still 10–14 rye chromosomes and most of them could pair normally, for example, in lines 928-6 and 953-3, although R genome should have been also destined to be eliminated as the Ns and D genomes. It appears that rye chromosomes tend to survive compared with its counterparts of D genome from wheat and Ns genome from *P. huashanica*. Similar phenomenon occurs in octoploid triticale. In the progenies of octoploid triticale, a high frequency of aneuploids was observed as a result of the meiotic disturbances. Initially, however, it was deemed that the rye chromosomes were the primary ones that were finally eliminated (Pieritz, 1970), or that the chromosomes eliminated were in proportional to the number of genomes from the two parental species (Weimarck, 1974). After that, Nakata et al. (1984) characterized some hexaploid lines from

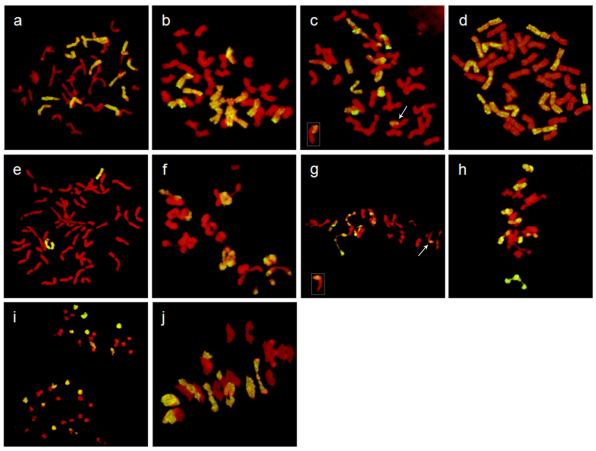

Fig 3. GISH on chromosome preparations at mitotic metaphase (a–e), and meiotic metaphase I and anaphase I (f–j) of partial lines with large grain weight. The DNA of rye was used as probe in a–d and f–i, while the DNA of *P. huashanica* was adopted as probe in e. The mixture of both genomic DNA (R+Ns) was used as probes in j. The genomic DNA of CS was autoclaved as the blocker in all the experiments. (a, e and j) GISH pattern of line 938-1, showing 14 rye chromosomes (yellow-green signals) (a) and two *P. huashanica* chromosomes (yellow-green signals) (e), or two univalents and seven bivalents at MI using the mixed probes (R+Ns) (j). (b and f) GISH pattern of line 939-10, showing 13 rye chromosomes (b), or one univalent and six bivalents at MI (f). (c and g) GISH pattern of line 951-13, showing ten chromosomes and a chromosomal segment (*arrow* and *inset*) from rye (c), or five bivalents and a chromosomal segment (*arrow* and *inset*) at MI (g). (d, h and i) GISH pattern of line 953-3, showing 14 rye chromosomes (d), or seven bivalents at MI (h); and regular segregation at the first anaphase (i).

the octoploid triticale by acetocarmine-Giemsa banding, and found that these lines included complete A and B genomes, and a composite genome containing the chromosomes from genomes R and D (1D, 2D, 3R, 4R, 5R, 6R, 7R, and 1R, 2D, 3R, 4R, 5R, 6R, 7R). Recently, Dou et al. (2006) identified 14 hexaploid lines from octoploid triticale using fluorescence *in situ* hybridization and molecular markers, confirming the presence of a composite genome consisting of genomes R and D (1R, 2D, 3R, 4R, 5R, 6R and 7R; or 1D, 2D, 3R, 4R,

Fig 4. The leaf symptom inoculated using the mixed pathogens of stripe rust. a Zhongsi 828. b PHW-SA. c Mingxian 169. d–f The lines selected from F_3 generation, showing high resistance to stripe rust.

5R, 6R and 7R; or 1R, 2D, 3R, 4R, 5R, 6D and 7R). According to the previous findings, together with the present study, it may be inferred that genome D from wheat may be strongly affected by genome R from rye and finally is more likely to miss. In the second place, the number of rye chromosomes increased, or even doubled in F_3 generation in comparison to that in F_1 hybrids. Nine of the total derivatives have complete rye genome. Two of them (e.g. lines 928-6 and 953-3) are cytologically stable so may be regarded as new triticale. This may be due to a spontaneous process of meiotic restitution (Silkova et al., 2003). The use of wild species in Triticeae is a critical avenue to broaden the genetic basis of common wheat. In this way, novel allelic diversity from these species can be identified for some agronomically significant traits, and further introduced into common wheat, thereby overcoming the genetic bottle neck. For rye, Ehdaie et al. (2003) demonstrated that the 1RS translocations (1RS.1AL, 1RS.1BL and 1RS.1DL) in 'Pavon' spring wheat background augmented grain weight under both drought and well-watered conditions. 10-A and Lanxiaohei, two bridge materials containing 1RS.1BL translocation, were crossed with a wide range of elite cultivars, producing abundant germplasms with higher grain weight (Zheng et al., 1997; Hou et al., 2003; Du et al., 2009). In the present study, 15 lines were gained from the F_3 segregating population because they showed 63–150% better grain weight than their parents under field conditions. All of these lines obtained include 10–14 rye chromosomes. This result, coupled with previous studies, implies that rye may be a useful candidate germplasm for improvement of grain weight in common wheat.

Materials and methods

Plant materials

Materials analyzed in this study consisted of common wheat–*Psathyrostachys huashanica* amphiploid (PHW-SA, $2n = 8x = 56$, AABBDDNsNs), triticale cultivar 'Zhongsi 828' ($2n = 6x = 42$, AABBRR), wheat cultivar 'Mingxian 169' and 'Chinese Spring' wheat (CS), rye cultivar 'Qinling' (*Secale cereale*, $2n = 14$, RR), and *Psathyrostachys huashanica* ($2n = 14$, NsNs). PHW-SA was generated in 2006 and used as a bridge material because of its stability in morphology and cytology, and some considerable agronomic traits such as high resistance to stripe rust. Zhongsi 828, a triticale cultivar for feed and forage use, was developed by the Chinese Academy of Agricultural Science in 2002. It has abundant advantages, for example, large spike and grain, cold tolerance, lodging resistance, and high resistance to rust and powdery mildew. A cross between PHW-SA (female parent) and Zhongsi 828 (male parent) was carried out in 2008 (Kang et al., 2011b). Their F_1 hybrids were then selfed for three generations by bagging the heads before GS59 (wheat growth stages, Tottman and Broad, 1987), and produced a total of 239 wheat lines. Wheat cultivar Mingxian 169 was used as a susceptible control for stripe rust testing. Chinese Spring wheat provided blocking DNA, while rye cultivar Qinling and *P. huashanica* were employed as sources of the probes for genomic *in situ* hybridization. All stocks were grown under normal field conditions without fertilizers or plant growth regulators.

Screening F_3 segregating population for the lines with large grain weight

Large grain weight was indicated by 1000-grain weight (TGW) in the present study. For practical measurement, three plants of each line were selected randomly from GS92 to GS93, and threshed carefully by hand. These grains were dried in the sealed metal pail filled with desiccant (calcium oxide) for at least two months until constant weight was achieved. Three 200-grain samples parted at random were weighed and afterwards the mean was converted to 1000-grain weight. The other parameters, including spikes per plant, spikelets per spike, grains per spikelet, spike length, averaged seed set, straw height (awns inclusive), lodging resistance (45° less from the vertical), waxy peduncle and hairy neck, were recorded as well. Multiple comparisons of the means of the traits between these lines were performed for significant differences using LSD test by the statistic software GenStat (14th Edition). Pearson correlations were used to determine the relationships between variables.

Mitotic and meiotic analyses

Root tips (2 cm long) were cut from the germinated seeds, and pretreated in distilled water in tubes on ice for 24–28 h, and then fixed in fresh Carnoy's fixative I (3 volumes of 100% ethanol + 1 volume of glacial acetic acid) for 24 h at room temperature. After being hydrolyzed in 0.2 mol/L HCl for 3–4 h, the meristem cells were squashed with a drop of carbol fuchsin. Chromosome number was established by counting 50 cells at mitotic metaphase from five seeds of each wheat line. For pollen mother cells (PMCs), the growing spikes were removed out from the leaf sheathes between GS39 and GS41, and one of three anthers in a floret at meiotic metaphase I (MI) was fixed in Carnoy's fixative II (6 volumes of 100% ethanol + 3 volumes of chloroform + 1 volume of glacial acetic acid) for 24 h at room temperature, and squashed in carbol fuchsin. The average chromosome

configuration was calculated based on the data of 50 PMCs with dispersed chromosomes. The remaining two anthers in the same floret were stored in 70% ethanol at 4°C for genomic *in situ* hybridization use.

Giemsa C-banding identification of alien chromosomes

Giemsa C-banding was implemented using the method of Gill et al. (1991). Five meristem cells from root tips of each wheat line were photographed and subsequently analyzed. Identification of *S. cereale* and *P. huashanica* chromosomes was done according to the previous studies of Gill and Kimber (1974), and Zhang et al. (2009), respectively.

Genomic in situ hybridization (GISH)

The total genomic DNA from the leaves of rye (RR), *P. huashanica* (NsNs) and CS (AABBDD) was extracted by the cetyltrimethylammonium bromide (CTAB) method (Kidwell and Osborn, 1992). The genomic DNA of rye and *P. huashanica* was then labeled with digoxigenin-11-dUTP by nick translation, and used as probes. Details of the protocol were described by the manufacturer (Roche, Mannheim, Germany). The genomic DNA of CS was autoclaved as the blocker (200–500 bp). GISH procedure was performed according to Chen et al. (1998) with minor modifications. For one slide, the hybridization solution was 30 μl, containing 50% deionized formamide, 2 × SSC, 10% dextran sulfate, 0.17 μg/μl sheared herring sperm DNA (200–500 bp), 50 ng probing DNA, 3 000 ng blocking DNA and deionized distilled water. The hybridization mixture and the slides were denatured at 80°C for 10 min and 2 min, respectively. After incubating overnight, the hybridization signal was detected by the anti-digoxigenin-fluorescein (Roche, Mannheim, Germany) in 1% BSA (2.5 μg/ml). Chromosomes were counterstained by propidium iodide (PI, 1.5 μg/ml) (Vector, California, USA). The slides were observed with Olympus BX-51 fluorescence microscope, and the images were captured using a CCD (charge-coupled device) camera operated with the software Olympus Micro DP70 (Olympus, Tokyo, Japan).

Stripe rust examination

Stripe rust inoculum was a mixture of virulent races of *Puccinia striiformis* f. sp. *tritici* (CYR-31, CYR-32, CYR-33, Shuiyuan-7 and Shuiyuan-14), and kindly supplied by Prof. Q. Z. Jia, Institute of Plant Protection, Gansu Academy of Agricultural Sciences, China. When the seedlings of the F_3 plants, their parents and the susceptible control Mingxian 169 grew at GS13, the stripe rust inoculum, mixed with talcum powder (1:50), was applied onto the misted foliage of these seedlings. The disease reactions were assessed at GS31 (the pustules on the susceptible control Mingxian 169 developed completely). Infection types on each genotype were evaluated and recorded based on a 0 (immune)–4 (highly susceptible) scale according to Bariana and McIntosh (1993).

Conclusion

In this study 15 derivative lines were obtained from 239 F_3 segregating progeny of wheat-rye-*Psathyrostachys huashanica* hybrids as their 1000-grain weight increased by 63–150% in comparison to the mean of two parents under the natural field conditions. The genome constitutions and meiotic behavior of these lines were subsequently analyzed. All of them contain almost complete R genome, and three lines have 1–2 *P. huashanica* chromosomes. This suggests that the alien chromosomes from the two related species were transmitted successfully. Production of these introgression lines may undergo a spontaneous doubling process of rye genome coinciding with the absence of D genome of wheat. As a result, most of the 15 lines have similar genomic compositions to that of hexaploid triticale (AABBRR), and two of them are cytologically stable. The candidate triticale lines will be useful for improvement of grain weight in wheat. In addition, these combinations are resistant to multiple races of *Puccinia striiformis* f. sp. *tritici*, showing their promising application in wheat breeding for stripe rust resistance.

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