**Characterization of wheat-Psathyrostachys huashanica
small segment translocation line with enhanced kernels per
spike and stripe rust resistance**

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Draft

Characterization of wheat-*Psathyrostachys huashanica* small segment translocation line with enhanced kernels per spike and stripe rust resistance

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Abstract: *Psathyrostachys huashanica* Keng ($2n = 2x = 14$, NsNs), a distant wild relative of common wheat, possesses rich potentially valuable traits, such as disease resistance and more spikelets and kernels per spike, that could be useful for wheat genetic improvement. Development of wheat-*P. huashanica* translocation lines will facilitate its practical utilization in wheat breeding. In the present study, a wheat-*P. huashanica* small segmental translocation line K-13-835-3 was isolated and characterized from the BC_1F_5 population of a cross between wheat-*P. huashanica* amphiploid PHW-SA and wheat cultivar CN16. Cytological studies showed that the mean chromosome configuration of K-13-835-3 at meiosis was $2n = 42 = 0.10 \text{ I} + 19.43 \text{ II (ring)} + 1.52 \text{ II (rod)}$. GISH analyses indicated that chromosome composition of K-13-835-3 included 40 wheat chromosomes and a pair of wheat-*P. huashanica* translocation chromosomes. FISH results demonstrated that the small segment from an unidentified *P. huashanica* chromosome was translocated into wheat chromosome arm 5DS, proximal to the centromere region of 5DS. Compared with the cultivar wheat parent CN16, K-13-835-3 was highly resistant to stripe rust pathogens prevalent in China. Furthermore, spikelets and kernels per spike in K-13-835-3 were significantly higher than those of CN16 in two season years. These results suggested that the desirable genes from *P. huashanica* were successfully transferred into CN16 background. This translocation line could be used as novel germplasm for high-yield and eventually resistant cultivars breeding.

Keywords: *Psathyrostachys huashanica*, Translocation line, Stripe rust, Kernels per spike

Abbreviations

<i>Pst</i>	<i>Puccinia striiformis</i> f. sp. <i>tritici</i>
GISH	Genome in situ hybridization
FISH	Fluorescence in situ hybridization
CN16	Chuannong16
PMC	Pollen mother cell

Draft

Introduction

The relatively narrow range of genetic variation of wheat is the primary factor that has hampered the improvement of crop yield in recent years (Mujeeb-Kazi et al. 2013). Widening the diversity of the wheat genetic pool is an important means of improving its yield and quality, and enhancing the capacity of resistance to biotic and abiotic stresses for wheat-breeding programs (Tester and Langridge 2010). The wild relatives of wheat harbor superior agronomic traits, such as wide adaptability, resistance to diseases, better quality and more numbers of spikelets and kernels per spike, which confer ample genetic diversity for wheat improvement (Dong et al. 1992; Mujeeb-Kazi et al. 2013). Developing wheat-alien species translocation lines and elucidating their chromosome constitutions are key steps for effective transfer of desirable genes into common wheat (Jiang et al. 1994; Gill et al. 2011). Some translocation lines, including 1RS of *Secale cereale*, 6VS of *Dasypyrum villosum*, 7Ag of *Lophopyrum elongatum*, and 6P of *Agropyron cristatum* chromosome, are the most successful examples of the introgression of elite genes from the wild relatives in wheat improvement (Chen et al. 1995, 2013; Tester and Langridge 2010; Niu et al. 2014; Ye et al. 2015; Zhang et al. 2015).

Psathyrostachys huashanica Keng ($2n=2x=14$, NsNs) is a distant wild relative of common wheat native from Huashan Mountains, Shaanxi Province, China. It possesses many desirable traits, such as early maturity, more spikelets and kernels per spike, tolerance to drought and salt, and resistance to wheat stripe rust, powdery mildew and take-all fungus (Chen et al. 1991; Jing et al. 1999; Wang and Shang 2000; Kang et al. 2008, 2009; Du et al. 2010). Therefore, *P. huashanica* Ns genome can be used as donor to provide genes for the genetic improvement of wheat crops. In order to transfer desirable traits from *P. huashanica* into wheat, wide crosses between *P. huashanica* and common wheat began in the 1980s (Chen et al. 1991). Progeny lines with single *P. huashanica* chromosomes incorporated into

the wheat genome were obtained either as chromosome additions or substitutions lines (Wu et al. 2007; Wei et al. 2009; Kishii et al. 2010; Zhao et al. 2010). Recently, a complete set of wheat-*P. huashanica* (1Ns-7Ns) disomic addition lines with disease resistance and good agronomic traits were produced (Du et al. 2013a, b, c; Du et al. 2014a, b, c, d). We previously reported that a new wheat-*P. huashanica* amphiploid (PHW-SA, $2n=8x=56$, AABBDDNsNs) between *Triticum aestivum* cv. J-11 and *P. huashanica* was successfully synthesized (Kang et al. 2009). PHW-SA is resistant to stripe rust and powdery mildew diseases, and has many interesting yield characteristics, including higher fertility, longer spikes, more spikelets and kernels per spike (Kang et al. 2009, 2010). The amphiploid production serves not only to maintain the germplasm, but also to provide an effective and rapid way of introgressing desirable traits from related species into cultivated wheat (Jiang et al. 1994).

The objectives of this study were to characterize the chromosome constitution of a wheat-*P. huashanica* translocation line and to evaluate their effect on stripe rust resistance and agronomic traits.

Materials and methods

Plant materials

Chuannong 16 (CN16) is a native wheat cultivar possessing good comprehensive characters, such as higher spike number per plant and superior weak-gluten character, which is an ideal recurrent parent for wheat breeding program of southwestern China. However, with the appearance of new *Puccinia striiformis tritici* (*Pst*) race, the cultivar CN16 has become susceptible. PHW-SA ($2n=8x=56$, AABBDDNsNs) is an intergeneric amphiploid of common wheat and *P. huashanica*, which was originally produced in our laboratory (Kang et al. 2009). To transfer desirable traits from *P. huashanica*

into CN16, the F₁ hybrids between PHW-SA and CN16 were backcrossed with CN16, and then seeds selected from the BC₁F₁ plants were bulked and advanced to the BC₁F₃ generation by single seed descent. Wheat line K-13-835-3, with highly resistant response to stripe rust over several years of observation, was isolated from the BC₁F₃ generation. Wheat line SY95-71 was used as a susceptible check in the tests to determine stripe rust resistance.

For GISH analysis, wheat cultivar J-11 (2n = 6x = 42, AABBDD) was used as blocking DNA, and the entire genomic DNA of *P. huashanica* was used as a probe.

Evaluation of the agronomic traits

Evaluation of agronomic traits was conducted in a field trial in Wenjiang, Sichuan Province, China with three replications in 2013-2014 and 2014-2015 seasons, respectively. For each replication, 20 grains of each line were evenly planted in 2.0 m rows, spaced 0.3 m apart. At maturity stage, plant height, spike length, spikelet per spike, kernels per spike and thousand-grain weight were evaluated from 10 randomly selected translocation line and parent plants of each replication. Statistical analyses were conducted using the SAS 8.2 system (SAS Institute Inc., Cary, NC, USA), and a *t* test was used to test the difference of the agronomic traits between the translocation plants and the parents.

Mitosis and meiosis analyses

Somatic chromosome analysis followed the procedures described by Kang et al. (2009). For meiosis analysis, young spikes at metaphase I stage were fixed in the Carnoy's fixative (ethanol: chloroform: glacial acetic acid, 6: 3: 1, v/v/v) for 24h and stored in 70% ethanol until use. The macerated root tips and anthers were squashed in 1% acetocarmine. At least 50 PMCs were observed

for each plant.

GISH and FISH analyses

Total genomic DNA of *P. huashanica* was isolated using the CTAB method (Allen et al. 2006). *P. huashanica* Genomic DNA was labeled with digoxigenin-11-dUTP by a nick translation mix (Roche, Mannheim, Germany) and used as a probe. Unlabeled J-11 DNA sheared to 200-400 bp fragments by autoclaving at 120°C for two minutes was used as a competitor at 50 times the quantity of probe in order to block common sequences from hybridization. Slide pre-treatment, hybridization, signal amplification and detection of the fluorescent signals were carried out as described by Han et al. (2004), with slight modifications. 30µl of denatured hybridization solution containing 2× SSC, 10% dextran sulphate, 0.2% sodium dodecyl sulphate, 1ng/µl labeled probe DNA together with the competitor DNA, were loaded per slide and incubated for 12h at 37°C. Finally, the chromosomes were counterstained with the propidium iodide (PI) solution (Vector Laboratories, Inc., Burlingame, USA). The in situ hybridization images were obtained using an Olympus BX-51 microscope coupled to a Photometric SenSys Olympus DP70 CCD camera.

After GISH analysis, the slides were washed with 2 × SSC (saline sodium citrate) and 70% (v/v) ethanol for 5 min, respectively. Fluorescence in situ hybridization (FISH) analysis was subsequently used to identify the constitution of the translocation chromosomes, using pSc119.2, pTa535 and pAs1 as probes. Probe pSc119.2 was used to identify the B-genome chromosomes, pTa535 discriminates between the A- and D-genome chromosomes and pAs1 hybridizes preferentially to D-genome chromosomes. The three oligonucleotide probes were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China). Probe labeling was operated according to Tang et al. (2014).

The FISH procedure was performed according to Han et al. (2004), with slight modifications. The probe mixture (4 ng μl^{-1} of each probe in $2 \times \text{SSC}$ and $1 \times \text{TE}$ buffer) and chromosomes were denatured together by heating for 5 minutes at 80°C . The chromosomes were finally counterstained with DAPI (4, 6-diamidino-2-phenylindole) solution (Vector Laboratories, Inc., Burlingame, USA). The detection and visualization of FISH patterns were the same as the aforementioned GISH protocol.

Stripe rust resistance screening

K-13-835-3, its parental species PHW-SA and CN16, and SY95-71 were evaluated for seedling and adult plant responses to stripe rust at the experimental station of Sichuan Agricultural University, Chengdu, Sichuan, China. Individual plants of each line were grown rows at 10 cm space in 30 cm wide beds and 2 m in length. The plots were surrounded by the susceptible wheat line SY95-71. Artificial inoculation was made by spraying the SY95-71 rows at the two-leaf stage with a mixture of *Puccinia striiformis* f. sp. *tritici* (*Pst*) races CYR-32, CYR-33, V26/Gui22-9, V26/Gui22-14, Su4 and Su5 suspended in light weight mineral oil. The *Pst* races were supplied by Prof. Qiu-Zhen Jia, Plant Protection Institute of Gansu Academy of Agricultural Sciences, Gansu, China. Stripe rust infection types (IT) based on a scale of 0, 0₊, 1, 2, 3 and 4, where 0 = immunity, 0₊ = necrotic flecks, and 1–4 = increasing sporulation and decreasing necrosis or chlorosis, considered highly resistant, resistant, susceptible and highly susceptible, respectively. These values were recorded three times when uniform severity levels were observed on susceptible check SY95-71 at booting, flowering and milky stages (Liu 1988).

Results

Morphology of K-13-835-3

We observed that the plants from the same translocation line K-13-835-3 displayed similar phenotypes in two growing seasons, indicating that the genetic constitution was the major factor controlling these phenotypes (Table 1). K-13-835-3 displayed typical morphologic traits similar to the parent CN16 (Fig. 1a). Spike length in K-13-835-3 was higher than the parent CN16, but lower than that of wheat-*P. huashanica* amphiploid PHW-SA (Fig. 1b). Spikelets per spike of K-13-835-3 were significantly higher than that of CN16, and were similar to the PHW-SA (Fig. 1c). Compared with the parents, K-13-835-3 had significant multikernel characteristics (high numbers of kernels per spikelet). For plant height, tiller number and thousand-kernel weight, no significant differences were observed among K-13-835-3 and CN16.

Mitosis and meiosis analyses

The root tip cells of K-13-835-3 had 42 chromosomes (Fig. 2a). Chromosome pairing at meiotic metaphase I (MI) of pollen mother cells (PMCs) of K-13-835-3 was high, with average chromosome configuration 0.10 univalents + 19.43 ring bivalents+ 1.52 rod bivalents scored in about 50 PMCs per plant (Table 2; Fig. 2b). No lagging chromosomes or bridges were observed at anaphase I and II. These results show that K-13-835-3 was cytologically stable.

GISH and FISH analyses

The GISH analysis was performed to determine the chromosome constitution of line K-13-835-3 using total genomic DNA of *P. huashanica* as the probe and J-11 total genomic DNA as the block.

K-13-835-3 was found to have a pair of wheat-*P. huashanica* small segment translocation chromosomes (Fig. 3a). To further determine the identity of the wheat chromosomes involved in the translocation, dual-color FISH was performed on the translocation using pSc119.2, pTa535 and pAs1 probes, which were used to distinguish all 21 pairs of wheat chromosomes (Tang et al. 2014). As illustrated in Fig. 3b, a pair of translocation chromosomes had strong pTa535 signals in the terminal and intercalary region of the long arm, and a faint red pTa535 signal in the terminal region of the short arm. Assuming that the FISH pattern of Chinese Spring pSc119.2 and pTa535 probes corresponds to the wheat cultivar used in this work, the small translocated fragment is situated on wheat chromosome 5DS. Similarly, FISH karyotypes of K-13-835-3 were established by employing Oligo-pAs1 probe (Fig. 3c, d). Therefore, it is concluded that a pair of *P. huashanica* chromosomes small segments were translocated onto wheat chromosome arm 5DS, near to the centromere region of 5DS.

Stripe rust resistance evaluation

K-13-835-3, PHW-SA, CN16 and SY95-71 were evaluated with mixture of *Pst* races CYR-32, CYR-33, V26/Gui22-9, V26/Gui22-14, Su4 and Su5 at Chengdu, Sichuan, China. At seedling and adult plant stages, CN16 and SY95-71 were susceptible to these races, showing infection of types 3 and 4, respectively. K-13-835-3 and PHW-SA were highly resistant to the races, both showing 0 infection types (Table 3, Figure 4). These results indicate that the stripe rust resistance gene(s) derived from *P. huashanica* is expressed in the wheat-*P. huashanica* translocatin line K-13-835-3.

Discussion

Alien genetic resources are important for improving agronomic traits in wheat. Translocations are

preferred to transfer the alien genes and used directly by wheat breeders because of the smaller amount of alien genetic material, less linkage drag, and regular meiotic behavior (Falke et al. 2009). The conventional chromosomal manipulation by crossing between common wheat and the amphiploid carrying alien desirable genes is an effective method to induce chromosome translocation (Jiang et al. 1994; Friebe et al. 1996; Qi et al. 2007). The amphiploids between wheat and species from genera *Secale*, *Thinopyrum*, *Agropyron*, *Dasypyrum*, *Leymus* and *Psathyrostachys* have been synthesized to produce many translocation genotypes in wheat breeding (Jiang et al. 1994; Lukaszewski 1995; Qi et al. 2007; Kang 2011; Ochoa et al. 2015; Zhang et al. 2015). However, it has been a challenge to transfer a small amount of alien chromatin containing the gene of interest from one genome to another non-homologous genome (Niu et al. 2011). In our study, the translocation line K-13-835-3 was identified from the BC₁F₅ progenies of a cross between wheat - *P. huashanica* amphiploid PHW-SA and the wheat parent CN16. GISH and FISH analyses on somatic metaphase chromosome confirmed the presence of a wheat- *P. huashanica* small segment translocation on wheat chromosome arm 5DS. Generally, translocations with smaller alien segments are genetically more stable and less likely to have deleterious effects (Faris et al. 2008). Above results, together with the high cytological stability and fertility of the K-13-835-3, indicated that the fragment that has been transferred from *P. huashanica* compensated well with the lack of wheat 5DS chromosome segment in the K-13-835-3 line. This line provides the bridge breeding-usable material of alien excellent gene introgression for wheat improvement. Similar spontaneous chromosomal rearrangements have also been reported in wheat-rye and wheat-*A. cristatum* translocation line (Ren et al. 1991; Ochoa et al. 2015).

The emergence of virulent stripe rust races, such as CYR32, CYR33 and its variants, represents a destructive serious threat to wheat production in northwestern and southwestern China (Wan et al. 2004;

Han et al. 2010). Furthermore, a new *Pst* race V26/Gui22, virulent to *Yr24/Yr26* and different from currently known races in China, was first reported in Gansu province, China in 2012 (Han et al. 2015). Most of modern cultivars or adapted germplasms of southwestern China have become susceptible to V26/Gui22 (Han et al. 2015; Ren et al. 2015). It is, therefore, urgent to identify new stripe rust resistance genes and to use more effective genes to counterbalance the continuous evolution of rust pathogens in wheat breeding programs. *P. huashanica* has many useful traits such as abiotic-stress tolerances and disease resistances for wheat improvement (Chen et al. 1991; Jing et al. 1999; Wang and Shang 2000; Kang et al. 2009). It is resistant to all prevalent Chinese *Pst* races, including V26/Gui22, at both the seedling and adult-plant stages (unpublished data). The resistance from *P. huashanica* has remained effective in Sichuan province, the forefront of stripe rust epidemics, for more than 10 years. Recently, a few stripe rust resistance genes of wheat-*P. huashanica* translocation lines were mapped and located on wheat chromosome 1AL, 1DL, 2BS (two), 2DS, and 6AL, respectively (Cao et al. 2008; Yao et al. 2010; Tian et al. 2011; Li et al. 2012; Ma et al. 2013a, b). However, these authors did not report the molecular cytogenetic results to identify the *P. huashanica* translocated segments in these lines. Kang et al. (2011) successfully produced and characterized a small segment translocation line T3BL-3NsS with resistance to stripe rust, from a cross between wheat-*P. huashanica* amphiploid PHW-SA and the wheat parent J-11. In the present study, the small segmental translocation line K-13-835-3 was selected and identified from PHW-SA/CN16 BC₁F₅ generation. Compared with the cultivar wheat parent CN16, K-13-835-3 was highly resistant to all prevalent Chinese *Pst* races, including V26/Gui22. This concludes that the chromosome fragment carrying the resistance gene of *P. huashanica* has successfully transferred into CN16 background. The new wheat line provides novel resource for improving resistance to all prevalent races of stripe rust in wheat.

The spike number per plant, kernel number per spike and 1,000- kernel weight are the main parameters that influence wheat yield. Long-term breeding practices have shown that the spikelet and kernel number per spike are the most important factors of the many potential characteristics that determine wheat yield (Zhou et al. 2007). Yen et al. (1993) obtained a common wheat line, 10-A, with multi-spikelets through crossing rye with wheat. The progenies that are derived from crosses between wheat and *Thinopyrum* spp. often display super spikes with more kernels (Li et al. 1985). Luan et al. (2010) produced the *T. aestivum*- *A. cristatum* 6P translocation lines, which had transferred the chromosome 6P fragment taking the multi-kernel gene(s) along into a wheat background and may improve kernel number, thus increasing crop yield. Du et al. (2013b) identified that a wheat-*P. huashanica* 6Ns disomic addition line with twin spikelets and multi-florets and kernels. Zhang et al. (2015) reported that gene(s) controlling the longer spikes, more spikelets and more grains per spike are located on chromosome 2VS of the *T. aestivum* - *D. villosum* translocation line. In the present study, the wheat- *P. huashanica* small segmental translocation line K-13-835-3 exhibited significantly higher numbers of spikelets and kernels per spike than the wheat parent CN16. This indicates that the introgression of chromosome segment of *P. huashanica* not only increases the spikelet number in a spike, but also improves the kernel number per spike. This line will be useful in wheat breeding programs for improving kernel numbers.

CN16 is a native wheat cultivar possessing superior comprehensive characters, such as high spike number per plant and weak-gluten character, which is an ideal recurrent parent for wheat breeding program of southwestern China. However, CN16 has some disadvantages including poor stripe rust resistance and lower kernels number per spike (Liao et al. 2007). The usefulness of an alien chromosomal translocation line in breeding is largely dependent on whether the introgressed alien

segments carry genes for deleterious traits and whether they can compensate for the replaced wheat segments (Faris et al. 2008). The results of agronomic traits evaluation showed that the small segmental translocation line K-13-835-3 has longer spikes, more spikelets and kernels per spike, but no obvious differences were found between the number of spikes and 1000-kernel weight of this line and their recurrent parent CN16. Therefore, K-13-835-3, which combined significant characteristics of high yield and stripe rust resistance and inherited more stably than the addition, substitution lines, could be used directly by wheat breeders and therefore have a high application value.

In conclusion, by studying the chromosomal constitution, agronomic traits and stripe rust resistance of wheat- *P. huashanica* translocation line, we pinpointed the chromosomal segments of *P. huashanica* positively regulating stripe rust resistance, fertile spikelets and kernels per spike in wheat. Our work not only contributed to potential disease resistance resources, but also provided the starting materials for high yield wheat breeding.

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Table 1. The agronomic traits of the wheat- *P. huashanica* translocation line K-13-835-3 and its parents

Lines	Year	Tiller	Plant height	Spike	Spikelets	Kernels per	Thousand-kernel
		number	(cm)	length (cm)	per spike	spike	weight (g)
K-13-835-3	2013-2014	7.0±1.3 b	70.6±0.8 b	12.3±0.3 b	23.7±1.8 a	67.8±4.6 a	42.6±0.7 a
	2014-2015	4.8±1.0 c	72.2±0.6 b	11.8±0.4 b	23.0±2.1 a	65.7±1.9 a	41.3±1.1 a
CN16	2013-2014	6.6±1.1 b	69.5±0.9 b	9.2±0.3 c	17.6±2.0 b	45.3±5.8 b	41.9±0.5 a
	2014-2015	5.3±1.0 c	68.6±2.4 b	9.9±0.2 c	19.8±1.4 b	48.6±4.9 b	40.6±0.4 a
PHW-SA	2013-2014	8.2±1.7 a	137.0±4.6 a	15.1±0.1 a	24.7±1.2 a	39.3±5.5 c	41.7±0.3 a
	2014-2015	8.0±1.0 a	130.1±7.8 a	14.7±0.3 a	24.8±1.1 a	36.0±5.0 c	40.1±0.4 a

Data in the columns indicate mean ± standard error

Means followed by a, b, c, indicate significant differences at the $P < 0.05$ as determined by the least significant difference. For example, tiller number in K-13-835-3 was significantly different in the seasons 2013-2014 vs 2014-2015, whereas plant height was not.

Draft

Table 2. Chromosome pairing at MI in the PMCs of the wheat- *P. huashanica* translocation line K-13-835-3

line	2n	No of cells observed	Chromosome pairing			
			I	II		
				Total	Rings	Rods
K-13-835-3	42	50	0.10 (0-2)	20.95 (20-21)	19.43 (17-21)	1.52 (0-4)

Draft

Table 3. Infection type of the wheat- *P. huashanica* translocation line K-13-835-3 and its parents for stripe rust with a mixture of races at seedling and adult plant stages

Materials	No. of plants observed	Infection type	Resistance/susceptibility
K-13-835-3	20	0	R
PHW-SA	15	0	R
CN16	15	3	S
SY95-71	15	4	S

Wheat line “SY95-71” was used as susceptible control

R resistance, S susceptibility

Draft

Figure captions

Fig. 1. Plant morphology of wheat- *P. huashanica* translocation line K-13-835-3 and its parents. a adult plant; b spikes; c Spikelets and kernels. 1-3 in figures represent CN16, K-13-835-3, and PHW-SA respectively.

Fig. 2. Mitotic and meiotic analysis of wheat- *P. huashanica* translocation line K-13-835-3. a Mitotic metaphase showing 42 chromosomes. b Meiotic metaphase I pairing: $2n = 3 \text{ II (rod)} + 18 \text{ II (ring)}$.

Fig. 3. GISH (a, c) and FISH (b, d) metaphases of wheat- *P. huashanica* translocation line K-13-835-3. The probes used for in situ hybridization were *P. huashanica* genomic DNA (a, c); pSc119.2 and pTa535 (b); pAs1 (d). Arrows indicate the pair of wheat-*P.huashanica* translocated chromosomes. The enlarged chromosomes in figures b and d (from left to right) are the translocation chromosome by *P. huashanica* probe, translocation chromosome and Chinese Spring 5D chromosome by pTa535 (b) and pAs1(d) probe, respectively.

Fig. 4. Stripe rust resistance of wheat- *P. huashanica* translocation line K-13-835-3 and its parents. 1 SY95-71; 2 CN 16; 3 K-13-835-3; 4 PHW-SA.

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