Identification and Characterization of Wheat - Aegilops Comosa 7M/7A Disomic Substitution Lines with Stripe Rust Resistance

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Abstract

Aegilops comosa (MM, 2*n* = 2*x* = 14), an important diploid species belonging to wheat tertiary gene pools, contains many excellent genes/traits, including disease resistance for wheat breeding. In this study, three sister lines, NAL-32, NAL-33, and NAL-34, were identified from a wheat - *Ae. comosa* distant cross using fluorescence *in situ* hybridization (FISH) combined with single nucleotide polymorphism (SNP) microarray analysis. Genetically, NAL-32 contained neither an alien nor translocation chromosome, whereas NAL-33 and NAL-34 had disomic 7M/7A substitution chromosomes but differed in the absence (NAL-33) or presence (NAL-34) of 1BL/1RS translocation chromosomes. The substitution of 7M/7A in NAL-33 and NAL-34 was verified using wheat 55 K SNP arrays but 1BL/1RS translocation in NAL-34 was not. The two 7M/7A substitution lines, NAL-33 and NAL-34, had similar stripe rust resistance, and both showed higher stripe rust resistance than NAL-32 and their parents, suggesting that stripe rust resistance in NAL-33 and NAL-34 was derived from the 7M of *Ae. comosa* and that their resistance was likely irrelevant to 1BL/1RS translocation. Meanwhile, the three NAL lines also showed higher grain weights (grams per 50 grains) than one to three of their three wheat parents, and the two 7M/7A substitution lines, NAL-33 and NAL-34, had larger seed size-related traits than NAL-32, suggesting that both the 7M and 1BL/1RS chromosomes had positive effects on seed size-related traits. The results provide important bridge materials that can potentially be used for transferring stripe rust resistance, as well as seed size-related traits from *Ae. comosa* to wheat.

1. Introduction

The introduction of excellent genes/traits from wild relatives into wheat may provide new insights into the use of wild genetic resources for breeding purposes. *Aegilops comosa* Sm. in Sibth. et Sm. (2*n* = 2*x* = 14, MM) is a diploid wheat wild relative species that harbors genes closely related to stripe rust, leaf rust, and powdery mildew resistance (Gill et al. 1985; Liu et al. 2019), as well as salt tolerance (Xu et al. 1993). The creation of wheat - *Ae. comosa* amphidiploids and chromosome introgression lines, such as addition, substitution, and translocation lines, are the first step for transferring desirable genes from *Ae. comosa* to common wheat (Liu et al. 2019). Several wheat - *Ae. comosa* amphidiploids and introgression lines, including 4M/4D and 6M/6A substitutions as well as 2M – 7M additions, have been developed and identified (Weng et al. 1997; Liu et al. 2019; Zuo et al. 2020). Of these introgression lines, the 2M and 7M chromosomes of *Ae. comosa* were found to carry genes for resistance to stripe rust (Riley et al. 1968) and powdery mildew (Liu et al. 2019), respectively. *Aegilops geniculata* Roth is an annual self-fertilizing tetraploid species (2*n* = 4*x* = 28) with genomic formula $U^gU^gM^gM^g$, where the M^g genome originated from *Ae. comosa* (Friebe et al. 1999). A $7M^g$ disomic addition line and a $7M^g$/7A disomic substitution line W 16998 derived from *Ae. geniculata* were found to be resistant to powdery mildew (Wang et al. 2016, 2020). There are no other reports describing the 7M/7A substitution derived from diploid *Ae. comosa* and polyploid *Aegilops* sharing the M genome and showing disease resistance, especially stripe rust resistance.

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a devastating disease threatening common wheat (*Triticum aestivum* L.) production around the world (Dean et al. 2012). Among strategies to control the epidemic of disease, breeding cultivars with resistance is considered to be a safe, economical, and effective approach (Li et al. 2011). Approximately 70% of wheat cultivars in southwestern and 55% in northern China from the 1980s to 1990s carried the wheat-rye 1BL/1RS translocation chromosomes (Zhou et al. 2004) due to harbored genes related to disease resistant, such as *Yr9* (Zeller 1973) and *Pm8* (Singh et al. 1990), high yield potential, wide adaptation to different environments, and tolerance to abiotic stresses (Villareal et al. 1991; Kumlay et al. 2003; Lelley et al. 2004). However, stripe rust resistance genes from 1RS lost their effectiveness against new virulent biotypes in the 1990s (Ren et al. 2009), owing to the coevolution of pathogen virulence and host resistance. Consequently, cultivars with 1RS via 1BL/1RS translocation have lost effectiveness against the *Pst* pathogens (Mcdonald and Linde 2002). Therefore, it is necessary to exploit and transfer new genes related to stripe rust resistance from variable germplasm sources, including wheat wild species, into wheat to enrich the genetic background and improve its disease resistance.

Fluorescence *in situ* hybridization (FISH) is an efficient and accurate technique to directly and precisely detect alien chromosomes or introgression segments (An et al. 2013). All 14 M chromosomes of *Ae. comosa* can be distinguished by FISH probes pSc119.2, pAs1/pTa-535 combined with 5S and 35S rDNA, and $(GAA)_8$ (Kwiatek et al. 2013; Liu et al. 2019). The polymorphism of FISH signals among 48 *Ae. comosa* accessions were discovered using probes pSc119.2, $(CTT)_{12}$ and pTa 71, which allowed precise identification of M chromosomes from different accessions (Song et al. 2020). Single nucleotide polymorphisms (SNPs) are one of the most abundant molecular markers in plant (Lai et al. 2012; Cui et al. 2017). At present, several kinds of SNP microarrays, such as the 35 K (King et al. 2017), 90 K (Wang et al. 2014), and 660 K (Cui et al. 2017), have been reported in wheat. The wheat 55 K SNP microarray was jointly designed by the Chinese Academy of Agricultural Sciences and Affymetrix, based on the 660 K SNP microarray, and its price was as cheap as nearly 1/3 of the 660 K SNP array (Liu et al. 2018). It keeps loci that are associated with important agronomic traits identified in the 660 K SNP array, which allows identification of alien chromosomes through the distribution of missing marker rates on each chromosome (Hao et al. 2019).

Ae. comosa is one of the most important *Aegilops* species that harbors genes for wheat disease resistance. Currently, its resistance genes have been incompletely exploited. In the present study, we aimed to exploit and transfer genetic materials from *Ae. comosa* into wheat and identify introgression lines with desirable traits to be potentially used for wheat breeding. The objectives of this study were: (1) to identify disomic substitution lines with M chromosomes by FISH analysis; (2) to validate M chromosome substitutions by the 55 K SNP array; and (3) to evaluate stripe rust resistance, morphological characteristics, and seed size-related traits of disomic substitution lines. As a result, three wheat lines were distinguished from each other by the presence and/or absence of the 7M/7A substitution, and 1BL/1RS translocation chromosomes were identified. Of them, the two wheat lines carried the 7M/7A substitution but differed in 1BL/1RS translocation, showing no difference in stripe rust

resistance. They showed better resistance than the wheat line without the 7M/7A substitution or 1BL/1RS translocation. Furthermore, the 7M/7A substitution also had positive effects on grain weight, which was additive to the 1BL/1RS translocation.

2. Materials And Methods

2.1 Plant materials

Wheat - *Ae. comosa* introgression lines were identified from F_4 generations of wheat-distant hybridization between *Triticum turgidum* – *Ae. comosa* amphidiploids STM4 (Langdon/PI 551070) with hexaploid wheat CS*ph2a* (non-1BL/1RS translocation line, -1BL/1RS), and were backcrossed with another two heaxploid wheat lines, Bozi1313 (BZ1313, -1BL/1RS) and Chuannong16 (CN16, 1BL/1RS translocation line, +1BL/1RS), from a complex cross STM4/CS*ph2a*//BZ1313///CN16 in 2013–2020 (Fig. S1). Three wheat lines, NAL-32, NAL-33, and NAL-34, differing in the 7M/7A substitution and 1BL/1RS translocation chromosomes, were identified in 2019. Of them, neither the alien M chromosome nor the 1BL/1RS translocation chromosome were found in NAL-32 (-7M/7A, -1BL/1RS), whereas the 7M/7A disomic substitution chromosomes occurred in both NAL-33 and NAL-34 (+ 7M/7A), and the 1BL/1RS translocation chromosome was absent in NAL-33 (-1BL/1RS) but present in NAL-34 (+ 1BL/1RS). The wheat line SY95-71 was used as a spreader and contrast for wheat stripe rust as it was highly susceptible to this disease in a previous investigation (Song et al. 2019).

2.2 Cytological observation and FISH analysis

Root tips were recovered from germinated seeds by placement on moistened filter in petri dishes at 23°C for approximately 1 day until they reached 1–3 cm in length after a soaking step in water at 4°C for 1–2 days. The root tips were pretreated with nitrous oxide for 4 h, followed by a washing step with 70% ethanol (Kato 1999). The drop method was applied to prepare slides for FISH analysis after enzymolysis with 1% pectinase and 2% cellulose at 37°C for 42 min (Han et al. 2004).

Four oligonucleotide probes, namely Oligo-pSc119.2-1 (pSc119.2), Oligo-pTa535-1 (pTa-535), Oligo-pTa71-2 (pTa71) (Tang et al. 2014), and $(CTT)_{12}$ (Song et al. 2020), were used for FISH analysis. These probes were synthesized by Tsingke in Chengdu, China. FISH was performed as described by Hao et al. (2011). We used DAPI (4', 6-diamidino-2-phenylindole) as a counterstain for chromosomal observation. An Olympus BX-51 microscope coupled with a Photometric SenSys CCD camera was used for observation and documentation of chromosomes.

2.3 SNP genotyping

Total genomic DNA was extracted from fresh leaves by the hexadecyl trimethyl ammonium bromide method (Han et al. 2004). Chip-based genotyping was carried out by China Golden Marker Technology in Beijing, China (http://www.cgmb.com.cn) using a wheat 55 K SNP microarray containing 53,063 markers, following the Affymetrix Axiom 2.0 assay manual workflow protocol. The flanking sequences for each

locus were mapped to each site on the bread wheat reference sequence (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0/) by imposing a BLASTN E-value threshold of 10^{-10} and allowing a maximum mismatch of one base. The ratios of missing markers within neighboring 10 Mb intervals along individual chromosomes were calculated for the identification of alien introgression chromosomes, as described by Hao et al. (2019). A graphical representation of these ratios was obtained using Excel 2010. According to the ratios between the observed and expected numbers of markers on a given chromosome, the chromosome had an abnormally higher number of missing marker rates than others, which could be inferred as the one to be substituted.

2.4 Assessment of morphological characteristics and stripe rust resistance

All wheat materials, including NALs and their three common wheat parents, were planted in the Wenjiang farm of Sichuan Agricultural University in Chengdu of Sichuan, China, in the planting season of 2019–2020. After random identification of FISH consistent karyotypes for each NAL, the morphological characteristics, such as tiller number, plant height, and spike length of three sister lines, NAL-32, NAL-33, and NAL-34, and their three common wheat parents were investigated in the field based on at least five individual plants. Seed size-related traits, including grain length and width, aspect ratio, and grain weight, were assessed for three plants of each material. A total of 30 randomly selected grains of each plant, in total 90 grains of each material, were assessed using an Epson Expression 11000 XL scanner. Data were analyzed with the aid of Win SEEDLETM 2012a (Regent Instruments, Canada). Analysis of variance (ANOVA) was applied for morphological characteristics and the least significant difference was used for a Post-hoc test of significant differences.

All wheat materials were inoculated with the mixture race of seven prevalent Chinese *P. striiformis* f. sp. *tritici* isolates of CYR32, CYR33, CYR34, G22-14, Su11-4, Su11-5, and Su11-7 at the three-leaf young seedling stage. At the adult-plant stage, stripe rust resistance was evaluated under field conditions in 2020. More than 30 plants of every common wheat parent and 100 plants of every NAL line (NAL-32, NAL-33, and NAL-34) were investigated for stripe rust resistance. Stripe rust resistance was recorded according to the six scales of Pei et al. (2018), where the highly susceptible wheat line SY95-71 was highly injured and used as a control. The evaluation results were scored according to the standard classification system of 6 scales from 0 to 4 as follows: 0 for no visible symptoms; 0, for necrotic flecks without sporulation; and 1, 2, 3, and 4 for highly resistant, resistant, susceptible, and highly susceptible, respectively.

3. Results

3.1 FISH identification

Using four probes, pSc119.2, pTa-535, $(CTT)_{12}$, and pTa71, three sister lines, NAL-32, NAL-33, and NAL-34, were identified as euploid for their chromosomes, which were $2n = 42$ (Fig. 1). The probe signals of

pSc119.2 and $(CTT)_{12}$ were mainly located on B chromosomes, and the pTa-535 signals were mainly on the A and D chromosomes. Probe pTa71 had signals only on the chromosome arms of 1BS and 6BS. When compared with the FISH patterns of three common wheat (Fig. S2) and *Ae. comosa* parents (Zuo et al. 2020), wheat lines NAL-33 and NAL-34 (Figs. 1b1–c2) were different from NAL-32 (Figs. 1a1, a2) by two 7M substitutions for two 7A chromosomes, as revealed by the pSc119.2 signals localized on telomeres and the $(CTT)_{12}$ signal near the centromeres. Further, NAL-34 was distinguished from NAL-32 and NAL-33 by the presence of 1BL/1RS translocations that showed more pSc119.2 signals and weaker pTa71 signals in 1BS, which were derived from the common wheat parent CN16 in comparison to normal 1B chromosomes (Figs. 1 and S2. c1, c2). Therefore, NAL-32 contained neither an alien nor translocation chromosome, whereas NAL-33 and NAL-34 had two 7M/7A disomic substitution chromosomes but differed in the absence (NAL-33) or presence (NAL-34) of 1BL/1RS translocation chromosomes.

3.2 SNP genotyping

Two wheat lines, NAL-33 and NAL-34, were subjected to SNP genotyping to validate the 7M/7A substitution chromosomes identified by FISH analysis. Of the 53,063 markers detected in the wheat 55 K SNP microarray, 33,266 (62.69%) reliable markers showed hybridization signals on the 21 chromosome pairs of wheat lines NAL-33 and NAL-34, with the numbers of SNPs identified on each chromosome ranging from 640 (6A) to 2,091 (5A) for both of them. A total of 1,268 and 1,179 SNP markers showed negative signals on all chromosomes of NAL-33 and NAL-34, respectively, with the number of markers absent on each chromosome varying from 3–1,036 and 1–978, respectively. Both NAL33 and NAL34 had higher marker deletion rates in 7A (56.74 and 53.56%, respectively) than in other chromosomes (all below 4%, Table 1). The deletion markers were distributed throughout the 7A chromosome (Fig. 2), suggesting that chromosome 7A of NAL-33 and NAL-34 was most likely replaced by the alien 7M chromosome from *Ae. comosa*. Although NAL-34 was different from NAL-33 by the occurrence of 1BL/1RS translocations, as revealed by FISH analysis, no significant difference in marker deletion rates were found between the 1B chromosomes (all below 0.40%).

Table 1
Distribution of single nucleotide polymorphisms (SNPs) on the chromosomes of two 7M/7A disomic substitution lines

Chromosome	Total no. of SNPs identified	NAL-33 (+ 7M/7A, -1BL/1RS)[a]		NAL-34 (+ 7M/7A, +1BL/1RS)[b]	
		Deletion number	Deletion ratio	Deletion number	Deletion ratio
1A	1830	14	0.77%	23	1.26%
1B	1791	6	0.34%	4	0.22%
1D	1440	6	0.42%	5	0.35%
2A	2016	5	0.25%	6	0.30%
2B	1799	18	1.00%	8	0.44%
2D	1412	6	0.42%	6	0.42%
3A	1664	8	0.48%	11	0.66%
3B	2012	25	1.24%	23	1.14%
3D	1096	4	0.36%	6	0.55%
4A	1566	3	0.19%	6	0.38%
4B	2066	3	0.15%	1	0.05%
4D	667	13	1.95%	10	1.50%
5A	2091	10	0.48%	8	0.38%
5B	1966	3	0.15%	8	0.41%
5D	1081	9	0.83%	10	0.93%
6A	640	7	1.09%	3	0.47%
6B	1770	4	0.23%	7	0.40%
6D	1196	8	0.67%	6	0.50%
7A	1826	1036	56.74%	978	53.56%
7B	1611	26	1.61%	22	1.37%
7D	1726	54	3.13%	28	1.62%

Note: The superscript [a] and [b] indicate the two lines shared the 7M/7A disomic substitution but differed in absence (-) or presence (+) of 1BL/1RS translocation chromosomes, respectively.

3.3 Variations of morphological characteristics and stripe rust resistance

The major morphological traits of three NAL lines and their parents were assessed and compared (Table 2). The three NAL lines, NAL-32, NAL-33, and NAL-34, showed better morphological traits than one to three of their three common wheat parents. For example, all three NAL lines (81.07-88.00 cm) had significantly lower plant height than CS*ph2a* (130.56 ± 1.48 cm), although they showed a similar or higher plant height than CN16 and BZ1313 (67.18–75.66 cm). Of the three NAL lines, NAL-32 had a higher plant height than NAL-34. NAL-33 (7.53 ± 2.63) had more tillers than parent BZ1313 (4.40 ± 0.49), and all three NAL lines had a significantly longer spike length than parent CS*ph2a*. Besides, the seed-set rate of the three NAL lines was very similar to the three wheat parents.

Table 2
Comparison of morphological characteristics, seed size-related traits, and stripe rust resistance of three NAL lines and their common wheat parents

Material	Stripe rust resistance	Plant height (cm)	No. tillers	No. spikelet	Spike length (cm)	Awn length (cm)
CS*ph2a*	3	130.56 ± 1.48a	10.40 ± 1.74a	23.80 ± 0.75a	7.90 ± 0.77c	0
CN16	3	67.18 ± 0.74e	5.80 ± 0.98bc	18.40 ± 0.49b	9.60 ± 0.09b	5.76 ± 0.38b
BZ1313	3	75.66 ± 3.58de	4.40 ± 0.49c	20.20 ± 0.98b	11.00 ± 0.57a	4.74 ± 0.45c
NAL32 (-7M/7A, -1BL/1RS)[a]	3	88.00 ± 7.49b	4.87 ± 1.45c	19.80 ± 1.33b	11.31 ± 1.00a	6.93 ± 0.65a
NAL33 (+ 7M/7A, -1BL/1RS)[b]	1	83.99 ± 5.25bc	7.53 ± 2.63b	16.07 ± 1.24c	9.21 ± 0.62b	6.16 ± 0.47b
NAL34 (+ 7M/7A, +1BL/1RS)[c]	1	81.07 ± 10.46cd	4.93 ± 1.53c	14.80 ± 1.87d	9.62 ± 1.01b	6.24 ± 0.53b
SY95-71	4	-	-	-	-	-
Material	Length/width of flag leaf	Seed-set rate (%)	Grain length (mm)	Grain width (mm)	Grain width/ length	Grain weight(grams per 50 grains)
CS*ph2a*	20.67 ± 2.69a	69.90 ± 12.16a	6.12 ± 0.06d	3.10 ± 0.05c	0.51 ± 0.01a	1.55 ± 0.16cd
CN16	13.64 ± 1.70de	67.44 ± 5.82a	6.92 ± 0.11b	2.96 ± 0.34c	0.43 ± 0.05b	1.44 ± 0.34d
BZ1313	17.30 ± 1.22b	68.11 ± 14.81a	6.56 ± 0.15c	3.39 ± 0.08b	0.52 ± 0.02a	1.82 ± 0.23bc
NAL32 (-7M/7A, -1BL/1RS)[a]	15.68 ± 1.14bc	71.49 ± 7.23a	6.53 ± 0.32c	3.34 ± 0.17b	0.51 ± 0.01a	1.57 ± 0.21cd

Note: The different lowercase letters indicate a significant difference at $P < 0.05$ level. The superscript letters [a], [b], and [c] in material column indicate that these lines distinguished from each other by the presence (+) and/or absence (-) of 7M/7A disomic substitution and 1BL/1RS translocation chromosomes, respectively. The stripe rust resistance of flag leaves were evaluated according to the 6 scale standards as described by Pei et al. (2018).-: Not investigated. Post-hoc test was made among NAL lines and their common wheat parents.

Material	Stripe rust resistance	Plant height (cm)	No. tillers	No. spikelet	Spike length (cm)	Awn length (cm)
NAL33 (+ 7M/7A, -1BL/1RS)[b]	15.12 ± 1.60cd	79.58 ± 9.81a	7.19 ± 0.22b	3.56 ± 0.10ab	0.50 ± 0.01a	1.90 ± 0.20b
NAL34 (+ 7M/7A, +1BL/1RS)[c]	12.95 ± 1.78e	74.52 ± 15.38a	7.68 ± 0.38a	3.68 ± 0.13a	0.48 ± 0.04a	2.37 ± 0.29a

Note: The different lowercase letters indicate a significant difference at $P < 0.05$ level. The superscript letters [a], [b], and [c] in material column indicate that these lines distinguished from each other by the presence (+) and/or absence (-) of 7M/7A disomic substitution and 1BL/1RS translocation chromosomes, respectively. The stripe rust resistance of flag leaves were evaluated according to the 6 scale standards as described by Pei et al. (2018).-: Not investigated. Post-hoc test was made among NAL lines and their common wheat parents.

In addition to the morphological traits, NAL-33 and NAL-34 also showed better stripe rust resistance with a score of 1 for highly resistant (Fig. 3), compared with a score of 3 for wheat parents CS*ph2a*, BZ1313, and CN16 and the non-7M/7A substitution line NAL-32, as well as a score of 4 for control spreader SY95-71 with high susceptibility to stripe rust.

Furthermore, the three NAL lines also showed excellent seed size-related traits compared to their three parents (Table 2). For example, NAL-32 had a longer grain length and larger grain width than CS*ph2a*. NAL-33 had a longer grain length and larger grain width and grain weight than two of the three parents ($P < 0.05$), whereas NAL-34 showed higher values in all of these traits than all three wheat parents. In contrast, the grain width/length of NAL-32, NAL-33, and NAL-34 were similar to that of parents CS*ph2a* and BZ1313 but higher than that of CN16 ($P < 0.05$). Moreover, the three NAL lines, differing in 7M/7A substitution and 1BL/1RS translocation chromosomes, also showed a significant difference in three (except for grain width/length) of the four seed size-related traits. NAL-34 (+ 7M/7A, +1BL/1RS), showing significantly higher grain length, grain width, and grain weight (grams per 50 grains) than the control sister line NAL-32 (-7M/7A, -1BL/1RS), whereas NAL-33 (+ 7M/7A, -1BL/1RS) showed significantly higher grain length and grain weight (grams per 50 grains) than the control NAL-32. Furthermore, NAL-34 had a higher grain length and grain weight than NAL-33.

4. Discussion

The introgression lines of wheat - *Aegilops* spp. could be utilized as valuable germplasm sources for the genetic improvement of wheat in disease resistance and other agronomically important traits. *Ae. comosa* contains many disease resistant genes, such as resistance to stripe rust, leaf rust, and powdery mildew of wheat, that may be potentially used for wheat breeding (Riley et al. 1968; Gill et al. 1985; Liu et al. 2019). In this study, three wheat - *Ae. comosa* introgression lines, NAL-32, NAL-33, and NAL-34, were identified by FISH analysis and the latter two lines were further verified by SNP microarrays. The three

lines were different from each other by the presence or absence of a 7M/7A disomic substitution and/or 1BL/1RS translocation chromosomes, with two of the three lines (NAL-33 and NAL-34) having a disomic substitution of 7M/7A and one of the three lines (NAL-34) with the 1BL/1RS translocation chromosomes. NAL-32 lacked both the alien 7M and 1BL/1RS translocation chromosomes. SNP genotyping verified the replacement of two 7A by two 7M chromosomes in lines NAL-33 and NAL-34, whereas no significant difference in the 1B chromosome was revealed, although NAL-34 was different from NAL-33 by the existence of 1BL/1RS translocations as revealed FISH analysis. The actual reason for no significant difference between 1B of NAL-33 and 1BL/1RS of NAL-34 by SNP markers is not clear at present. We speculated that that SNP markers are not as sensitive to translocation of partial chromosomes, such as 1BL/1RS, as they are to the substitution of the whole 7M alien chromosome by 7A. Another reason could be that the collinearity between 7M of *Ae. comosa* and 7A of wheat is weaker than that between 1BL/1RS of NAL-34, which was derived from the wheat parent, and 1B of NAL-33.

All these materials, including three introgression lines (NAL lines) and the related parents and controls, were inoculated with a mixture of race seven prevalent Chinese *P. striiformis* f. sp. *tritici* isolates and their stripe rust resistance levels were evaluated. The results indicated that the two disomic 7M/7A substitution lines (NAL-33 and NAL-34), whether present (NAL-34, with 1BL/1RS translocation instead of normal 1B) or absent (NAL-33, with normal 1B) with 1BL/1RS translocation chromosomes, showed similar stripe rust resistance, and both of them showed higher resistant than all three parents and controls, including the sister line NAL-32 (non-7M/7A substitution and non-1BL/1RS translocation) (Fig. 3). 1BL/1RS translocation in NAL-34 can be traced to wheat parent CN16 but not BZ1313 and CS*ph2a*. Owing to no significant difference in stripe rust resistance between the two 7M/7A substitution lines, differing in the presence or absence of 1BL/1RS translocation, both of them showed better resistance than their sister line, NAL-32, without the 7M/7A substitution and 1BL/1RS translocation chromosomes. We deduced that the stripe rust resistance of NAL-33 and NAL-34 may be contributed to the introduction of the 7M chromosome through the replacement of 7A rather than from the transfer of 1BL/1RS translocation chromosomes from wheat parent CN16.

Diploid *Ae. comosa* and its derivate *T. turgidum* - *Ae. comosa* amphidiploids were immune to stripe rust without any visible symptoms (Zuo et al. 2020). Several genes related to disease resistance were exploited in diploid *Ae. comosa* and polyploid *Aegilops* spp. sharing the M chromosomes donated from *Ae. comosa*. For example, two (*Yr8* and *Sr34)* and three (*Lr57*, *Yr40*, and *Sr53*) rust-related resistance genes were localized on 2M of *Ae. comosa* (Nasuda et al. 1998) and $5M^g$ of *Ae. geniculata* (Kuraparthy et al. 2007; Liu et al. 2011), respectively, and a powdery mildew gene was derived from $7M^g$ of *Ae. geniculata* (Wang et al. 2020). Because stripe rust resistance in the 7M/7A substitution line was a little weaker than that of the diploid *Ae. comosa* parent PI 551070 and its derivate *T. turgidum* - *Ae. comosa* amphidiploids STM4, it is most likely that the stripe rust resistance of *Ae. comosa* and accession PI 551070, used in present study, could be localized on chromosomes 7M and other chromosomes. Previous studies suggested that chromosome 2M of *Ae. comosa* has good stripe resistance for the existence of *Yr8* (Nasuda et al. 1998; Liu et al. 2019), we do not know whether the stripe rust resistance of

Ae. comosa was derived from 2M or even other M chromosomes. In contrast, $7M^g$ from polyploid species sharing the M chromosomes were reported to have powdery mildew resistance (Wang et al. 2016, 2020), so it would be worthwhile to test whether the two wheat - *Ae. comosa* 7M/7A disomic substitution lines also had powdery mildew resistance.

Wheat (*Triticum aestivum*) accounts for over 20% of the calorific intake for human beings among the major crops and provides more protein (23%) than all animal sources (FAO 2017). Grain weight is one of the most pivotal yield components, which is stably inherited (Kuchel et al. 2007). In our study, both the two wheat - *Ae. comosa* 7M/7A disomic substitution lines showed a similar seed set rate to the three common wheat parents and had even better seed size traits than one to three of the parents. For example, both of the two lines, NAL-33 and NAL-34, showed higher (23–65%) grain weight (grams per 50 grains) than common wheat parents. This result was consistent with the $7M^g$/7A disomic substitution line W 16998 derived from *Ae. geniculata*, which was 33% higher than CS in thousand kernel weight (Wang et al. 2020). Although the 1BL/1RS translocation chromosome in NAL-34 did not significantly increase stripe rust resistance, the higher grain weight of NAL-34 in comparison to that of NAL-33 and other parents was not solely ascribed to the replacement of 7A by 7M, but also contributed by the 1BL/1RS translocation chromosomes, which is also perceived to confer increased yield and perhaps adaptive advantages over a wide range of environments (Villareal et al. 1991; Lelley et al. 2004). Thus, the two wheat - *Ae. comosa* 7M/7A disomic substitution lines could serve as potential bridge materials for further development of introgression lines, such as translocation lines for mining the chromosome or fragments involving the resistance gene of *Ae. comosa* in a common wheat background.

Declarations

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Author contributions ZHY and DHX designed the experiment and revised this paper. SFD provided suggestions. YYZ and ZPS performed the experiment. QX and WJL analyzed the data. GL planted the materials. JL participated in the investigation of agronomic traits. YYZ and ZHY wrote the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval There are no studies with human participants or animals performed by any of the authors in this article.

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Figures

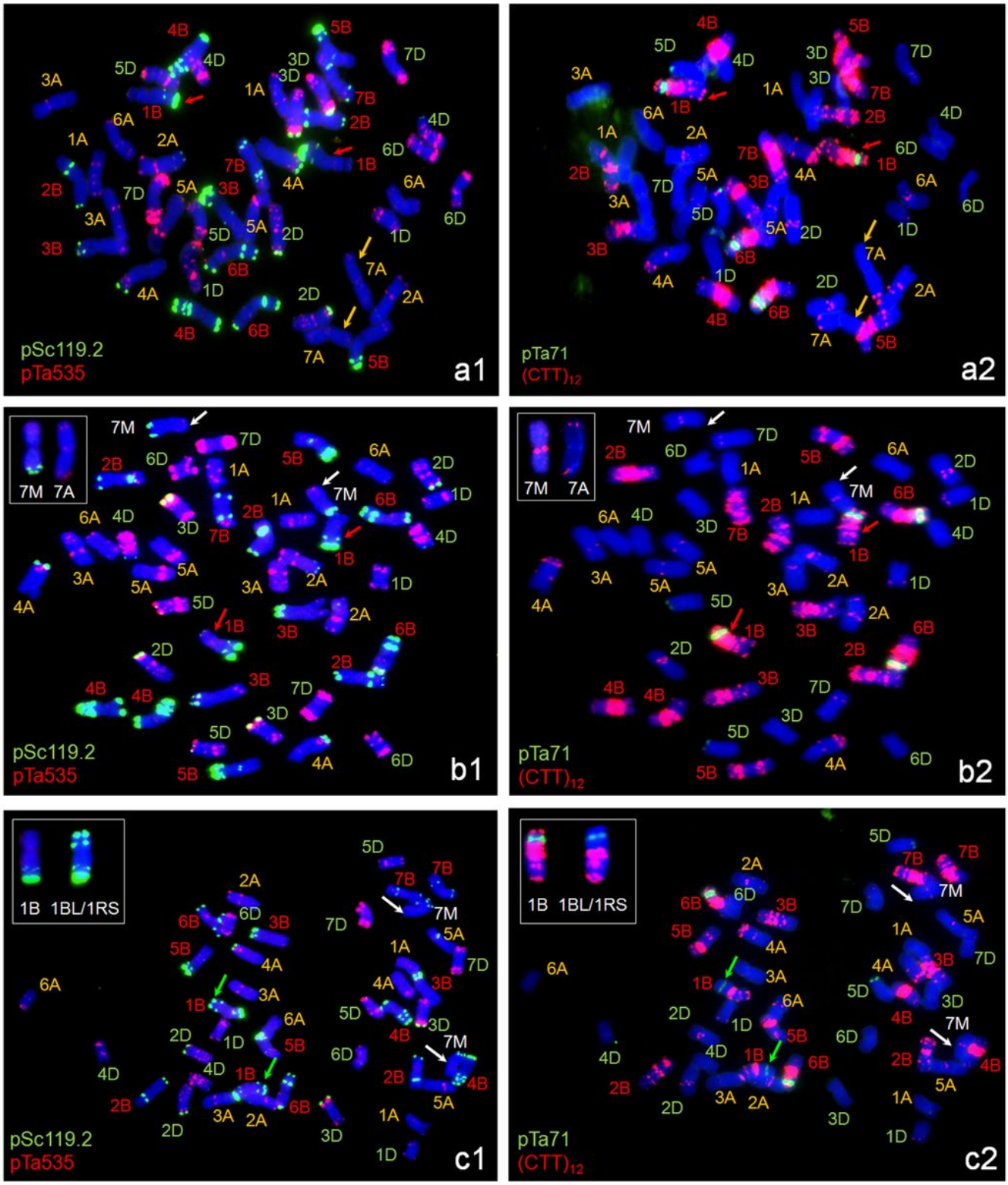

Figure 1

Non-denaturing fluorescence in situ hybridization of three sister lines using probes pSc119.2/pTa-535 (a1–c1) and (CTT)12/pTa71 (a2–c2). (a) NAL-32 (non-7M/7A disomic substitution, non-1BL/1RS translation line); (b) NAL-33 (7M/7A disomic substitution, non-1BL/1RS translation line); (c) NAL-34 (7M/7A disomic substitution, 1BL/1RS translation line). White pane in b1-b2: 7M and 7A chromosomes using probes pSc119.2/pTa-535 (b1) and (CTT)12/pTa71 (b2); c1 and c2: 1B and 1BL/1RS

chromosomes using probes pSc119.2/pTa-535 (c1) and (CTT)12/pTa71 (c2). Red arrows in a1–b2: normal 1B chromosomes. Green arrows in c1–c2: 1BL/1RS translocation chromosomes. Yellow arrows in a1–a2: normal 7A chromosomes (-7M/7A disomic substitution). White arrow in b1–c2: normal 7M chromosome (+7M/7A disomic substitution)

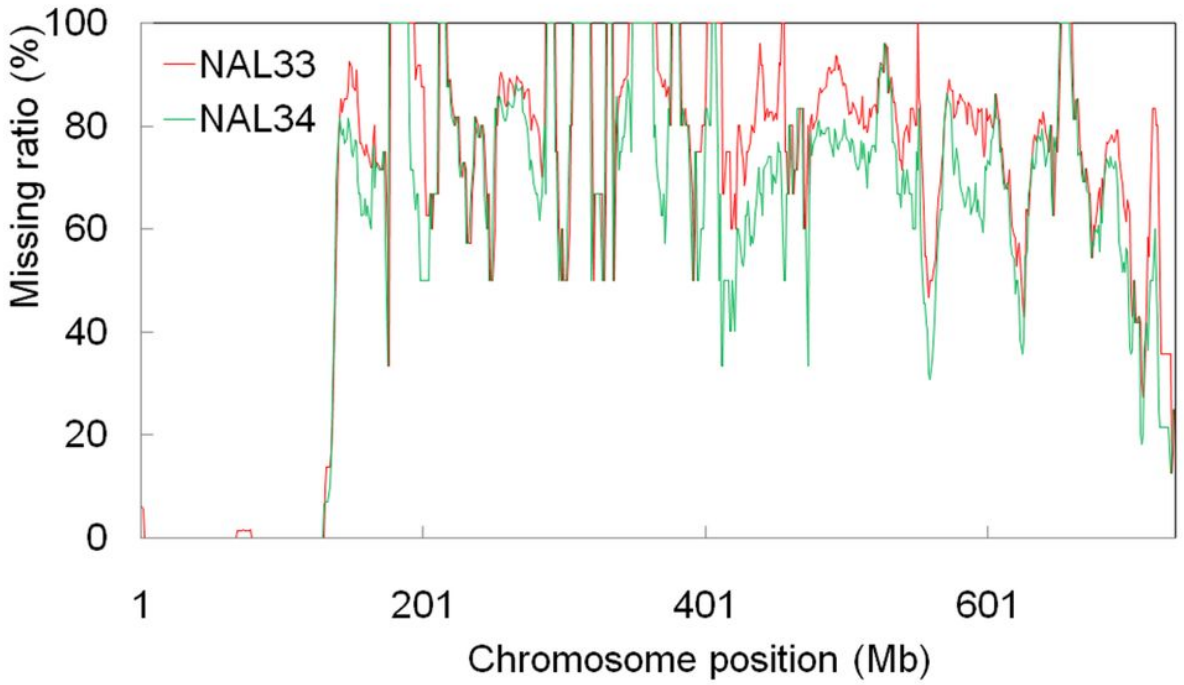

Figure 2

Map location of the deletion ratio of 7A SNPs in the two 7M/7A disomic substitution lines NAL-33 and NAL-34. The missing ratio (%) was calculated as the number of missing SNPs to the total number of SNPs detected on chromosome 7A.

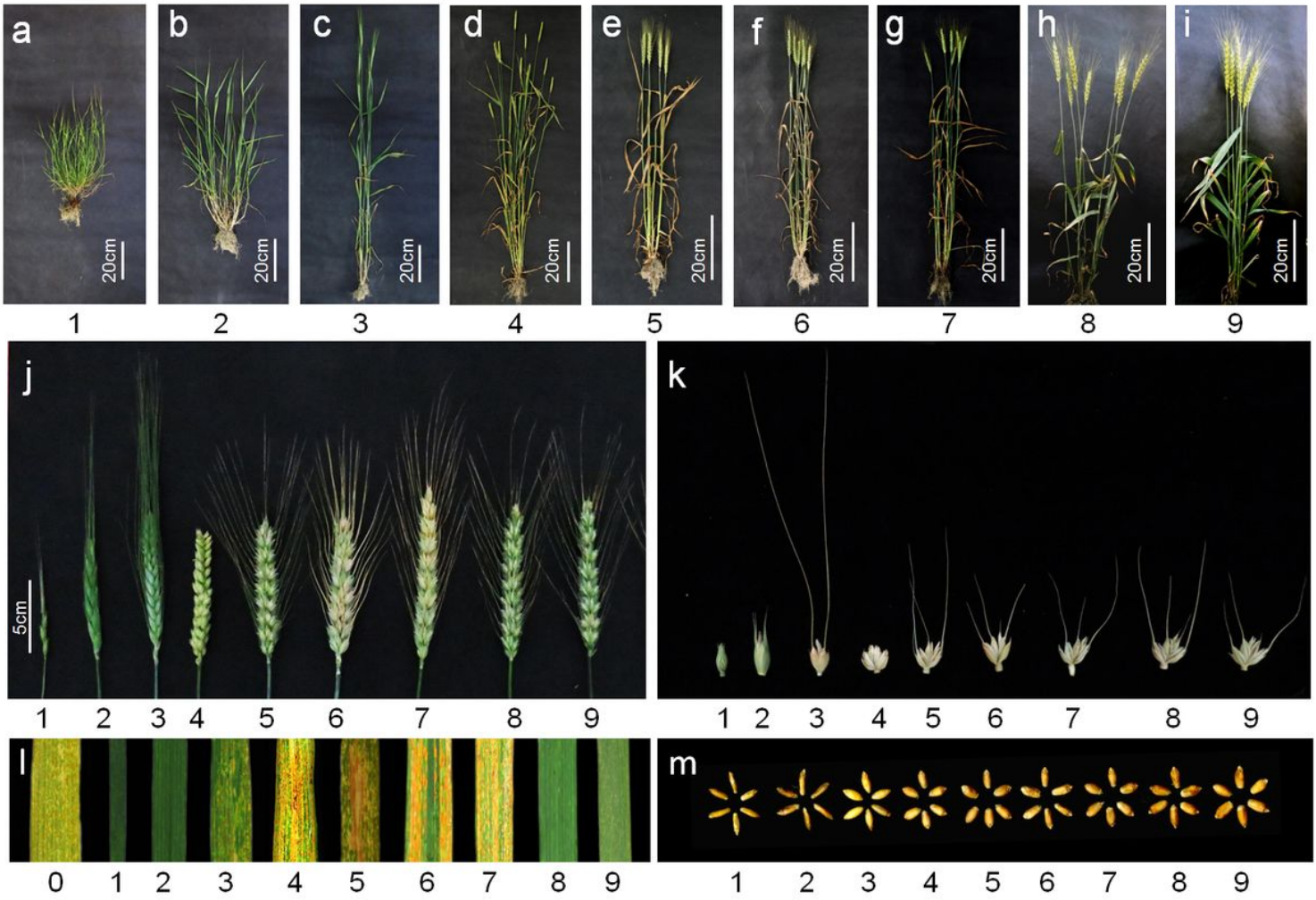

Figure 3

Comparison of three sister lines NAL-32, NAL-33, and NAL-34 with their parents in terms of plant morphology and seeds (a-k, m), and stripe rust resistance (l). 0. stripe rust spider SY95-71; 1. Ae. comosa PI 551070; 2. T. turgidum-Ae. comosa amphidiploid STM 4 (Langdon/PI 551070); 3. T. turgidum subsp. durum var. Langdon; 4. Hexaploid wheat parent CSph2a (non-1BL/1RS translation line); 5. Hexaploid wheat parent CN16 (1BL/1RS translation line); 6. Hexaploid wheat parent BZ1313 (non-1BL/1RS translation line); 7. NAL32 (non-7M/7A disomic substitution, non-1BL/1RS translation line); 8. NAL33 (7M/7A disomic substitution, non-1BL/1RS), and 9. NAL34 (7M/7A disomic substitution, 1BL/1RS translation line).

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