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

Yuanyuan Zuo
Sichuan Agricultural University

Shoufen Dai
Sichuan Agricultural University - Chengdu Campus

Zhongping Song
Sichuan Agricultural University - Chengdu Campus

Qin Xiang
Sichuan Agricultural University - Chengdu Campus

Wenjia Li
Sichuan Agricultural University - Chengdu Campus

Gang Liu
Sichuan Agricultural University - Chengdu Campus

Jian Li
Sichuan Agricultural University - Chengdu Campus

Donghe Xu
Japan International Research Center for Agricultural Sciences

Zehong Yan (✉ zhyan104@163.com)
Sichuan Agricultural University - Chengdu Campus
https://orcid.org/0000-0001-9285-9389

Research Article

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Abstract

*Aegilops comosa* (MM, 2n = 2x = 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. (2n = 2x = 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 (2n = 4x = 28) with genomic formula U*M*U*M*, where the M* genome originated from *Ae. comosa* (Friebe et al. 1999). A 7M* disomic addition line and a 7M*/7A disomic substitution line W16998 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 CSph2a (non-1BL/1RS translocation line, -1BL/1RS), and were backcrossed with another two hexaploid wheat lines, Bozi1313 (BZ1313, -1BL/1RS) and Chuannong16 (CN16, 1BL/1RS translocation line, +1BL/1RS), from a complex cross STM4/CSph2a//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, $\text{(CTT)}_{12}$, 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 NAL-33 and NAL-34 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)<sup>a</sup> | NAL-34 (+ 7M/7A, +1BL/1RS)<sup>b</sup> |
|------------|-----------------------------|----------------------------------------|----------------------------------------|
|            | 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 <sup>a</sup> and <sup>b</sup> 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 CSph2a (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 CSph2a. 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) |
|-------------------------------------------|------------------------|-------------------|-------------|--------------|------------------|-----------------|
| CSph2a                                    | 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)                  | 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)                  | 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)                  | 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) |
|-------------------------------------------|---------------------------|-------------------|-------------------|------------------|-------------------|-----------------------------------|
| CSph2a                                    | 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)                  | 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.
### Table 1

| 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 CSph2a, 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 CSph2a. 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 CSph2a 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 CSph2a. 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 of *Ae. geniculata* (Kuraparthy et al. 2007; Liu et al. 2011), respectively, and a powdery mildew gene was derived from 7M 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 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/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.
References

An DG, Zheng Q, Zhou YL, Ma PT, Lv ZL, Li LH, Li B, Luo QL, Xu HX, Xu YF (2013) Molecular cytogenetic characterization of a new wheat-rye 4R chromosome translocation line resistant to powdery mildew. Chromosome Res 21:419–432

Cui F, Zhang N, Fan XL, Zhang W, Zhao CH, Yang LJ, Pan RQ, Chen M, Han J, Zhao XQ, Ji J, Tong YP, Zhang HX, Jia JZ, Zhao GY, Li JM (2017) Utilization of a Wheat 660 K SNP array derived high-density genetic map for high-resolution mapping of a major QTL for kernel number. Sci Rep 7:3788

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13:414–430

Fao (2017) Online statistical database: Food balance [Online]. FAOSTAT. Available: http://www.fao.org/faostat/en/ [Accessed 27 February 2017]

Friebe BR, Tuleen NA, Gill BS (1999) Development and identification of a complete set of Triticum aestivum-Aegilops geniculata chromosome addition lines. Genome 42:374–380

Gill BS, Sharma HC, Raupp WJ, Browder LE, Hatchett JH, Harvey TL, Moseman JG, Waines JG (1985) Evaluation of Aegilops species for resistance to wheat powdery mildew and wheat leaf rust, Hessian fly and greenbug. Plant Dis 69(4):314–316

Han FP, Liu B, Fedak G, Liu ZH (2004) Genomic constitution and variation in five partial amphiploids of wheat-Thinopyrum intermedium as revealed by GISH, multicolor GISH and seed storage protein analysis. Theor Appl Genet 109(5):1070–1076

Hao M, Luo JT, Yang M, Zhang LQ, Yan ZH, Yuan ZW, Zheng YL, Zhang HG, Liu DC (2011) Comparison of homoeologous chromosome pairing between hybrids of wheat genotypes Chinese Spring ph1b and Kaixian-luohanmai with rye. Genome 54(12):959–964

Hao M, Zhang LQ, Zhao LB, Dai SF, Li AL, Yang WY, Xie D, Li QC, Ning SZ, Yan ZH, Wu BH, Lan XJ, Yuan ZW, Huang L, Wang JR, Zheng K, Chen WS, Yu M, Chen XJ, Chen MP, Wei YM, Zhang HG, Kishii M, Hawkesford MJ, Mao L, Zheng YL, Liu DC (2019) A breeding strategy targeting the secondary gene pool of bread wheat: introgression from a synthetic hexaploid wheat. Theor Appl Genet 132:2285–2294

Kato A (1999) Air drying method using nitrous oxide for chromosome counting in maize. Biotech Histochem 74(3):160–166

King J, Grewal S, Yang CY, Hubbart S, Scholefield D, Ashling S, Edwards KJ, Allen AM, Burridge A, Bloor C, Davassi A, Silva GJ, Chalmers K, King IP (2017) A step change in the transfer of interspecific variation into wheat from Amblyopyrum muticum. Plant Biotechnol J 15:217–226
Kuchel H, Williams KJ, Langridge P, Eagles HA, Jefferies SP (2007) Genetic dissection of grain yield in bread wheat. I. QTL analysis. Theor Appl Genet 115:1029–1041

Kumlay AM, Baenziger PS, Gill KS, Shelton DR, Graybosch RA, Lukaszewski AJ, Wesenberg DM (2003) Understanding the effect of rye chromatin in bread wheat. Crop Sci 43:1643–1651

Kuraparthy V, Chhuneja P, Dhaliwal HS, Kaur S, Bowden RL, Gill BS (2007) Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance gene *Lr57* and *Yr40* in wheat. Theor Appl Genet 114:1379–1389

Kwiatek M, Wiśniewska H, Apolinarska B (2013) Cytogenetic analysis of *Aegilops* chromosomes, potentially usable in triticale (*X Triticosecale* Witt.) breeding. J Appl Genet 54(2):147–155

Lai KT, Duran C, Berkman PJ, Lorenc MT, Stiller J, Manoli S, Hayden MJ, Forrest KL, Fleury D, Baumann U, Zander M, Mason AS, Batley J, Edwards D (2012) Single nucleotide polymorphism discovery from wheat next-generation sequence data. Plant Biotechnol J 10:743–749

Lelley T, Eder C, Grausgruber H (2004) Influence of 1BL.1RS wheat-rye chromosome translocation on genotype by environment interaction. J Cereal Sci 39:313–320

Li T, Zhang ZY, Hu YK, Duan XY, Xin ZY (2011) Identification and molecular mapping of a resistance gene to powdery mildew from the synthetic wheat line M53. J Appl Genet 52:137–143

Liu C, Gong WP, Han R, Guo J, Li GR, Li HS, Song JM, Liu AF, Cao XY, Zhai SN, Cheng DG, Li GY, Zhao ZD, Yang ZJ, Liu JJ, Reader SM (2019) Characterization, identification and evaluation of a set of wheat-*Aegilops comosa* chromosome lines. Sci Rep 9:4773

Liu JJ, Luo W, Qin NN, Ding PY, Zhang H, Yang CC, Mu Y, Tang HP, Liu YX, Li W, Jiang QT, Chen GY, Wei YM, Zheng YL, Liu CJ, Lan XJ, Ma J (2018) A 55 K SNP array-based genetic map and its utilization in QTL mapping for productive tiller number in common wheat. Theor Appl Genet 131:2439–2450

Liu WX, Rouse M, Friebe B, Jin Y, Gill B, Pumphrey MO (2011) Discovery and molecular mapping of a new gene conferring resistance to stem rust, *Sr53*, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. Chromosome Res 19:669–682

McDonald BA, Linde C (2002) The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124:163–180

Nasuda S, Friebe B, Busch W, Kynast RG, Gill BS (1998) Structural rearrangement in chromosome 2M of *Aegilops comosa* has prevented the utilization of the Compair and related wheat-*Ae.comosa* translocations in wheat improvement. Theor Appl Genet 96:780–785

Pei YR, Cui Y, Zhang YP, Wang HG, Bao YG, Li XF (2018) Molecular cytogenetic identification of three rust-resistant wheat-*Thinopyrum ponticum* partial amphiploids. Mol Cytogenet 11:27
Ren TH, Yang ZJ, Yan BJ, Zhang HQ, Fu SL, Ren ZL (2009) Development and characterization of a new 1BL.1RS translocation line with resistance to stripe rust and powdery mildew of wheat. Euphytica 169:207–213

Riley R, Chapman V, Johnson ROY (1968) Introduction of yellow rust resistance of *Aegilops comosa* into wheat by genetically induced homoeologous recombination. Nature 217:383–384

Singh NK, Shepherd KW, McIntosh RA (1990) Linkage mapping of genes for resistance to leaf, stem, and stripe rusts and ω-secalins on the short arm of rye chromosome 1R. Theor Appl Genet 80:609–616

Song ZP, Dai SF, Bao TY, Zuo YY, Xiang Q, Li J, Liu G and Yan ZH (2020) Analysis of structural Genomic diversity in *Aegilops umbellulata, Ae. markgrafi*, *Ae. comosa*, and *Ae. uniaristata* by Fluorescence in situ hybridization Karyotyping. Front Plant Sci 11:710

Song ZP, Dai SF, Jia YN, Zhao L, Kang LZ, Liu DC, Wei YM, Zheng YL, Yan ZH (2019) Development and characterization of *Triticum turgidum-Aegilops umbellulata* amphidiploids. Plant Genet Resour 17(1):24–32

Tang ZX, Yang ZJ, Fu SL (2014) Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. J Appl Genet 55(3): 313–318

Villareal RL, Rajaram S, Mujeeb-Kazi A, Del Toro E (1991) The effect of chromosome 1B/1R translocation on the yield potential of certain spring wheats (*Triticum aestivum* L.). Plant Breed 106:77–81

Wang SC, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J et al (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Plant Biotechnol J 12:787–796

Wang YJ, Long DY, Wang YZ, Wang CY, Liu XL, Zhang H, Tian ZR, Chen CH, Ji WQ (2020) Characterization and evaluation of resistance to powdery mildew of wheat-*Aegilops geniculata* Roth 7Mg(7A) alien disomic substitution line W16998. Int J Mol Sci 21:1861

Wang YJ, Quan W, Peng NN, Wang CY, Yang XF, Liu XL, Zhang H, Chen CH, Ji WQ (2016) Molecular cytogenetic identification of a wheat-*Aegilops geniculata* Roth 7Mg disomic addition line with powdery mildew resistance. Mol Breed 36:40

Weng Y, Jia J, Dong Y (1997) Identification of *Aegilops comosa* substitution line in wheat using RFLP markers. J Genet Genomics 24:248–254

Xu X, Monneveux P, Damania AB, Zahavieva M (1993) Evaluation of salt tolerance in genetic resources of *Triticum* and *Aegilops* species. Plant Genet Resour News 96:11–16.
Zeller FJ (1973) 1B/1R wheat-rye chromosome substitutions and translocations. In: Sears ER, Sears LMS (eds) Proceedings of 4th international wheat genetic symptoms. Columbia USA, pp 209–221

Zhou Y, He ZH, Zhang GS, Xia LQ, Chen XM, Gao YC, Jin ZB, Yu GJ (2004) Utilization of 1BL/1RS translocation in wheat breeding in China. Acta Agron Sin 30:531-535

Zuo YY, Xiang Q, Dai SF, Song ZP, Bao TY, Hao M, Zhang LQ, Liu G, Li J, Liu DC, Wei YM, Zheng YL, Yan ZH (2020) Development and characterization of Triticum turgidum-Aegilops comosa and T. turgidum-Ae. markgrafi amphidiploids. Genome 63(5):263–273