Characterization, Identification and Evaluation of Wheat-Aegilops sharonensis Chromosome Derivatives

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Aegilops sharonensis, a wild relative of wheat, harbors diverse disease and insect resistance genes, making it a potentially excellent gene source for wheat improvement. In this study, we characterized and evaluated six wheat-A. sharonensis derivatives, which included three disomic additions, one disomic substitution + monotelosomic addition and two disomic substitution + disomic additions. A total of 51 PLUG markers were developed and used to allocate the A. sharonensis chromosomes in each of the six derivatives to Triticeae homoeologous groups. A set of cytogenetic markers specific for A. sharonensis chromosomes was established based on FISH using oligonucleotides as probes. Molecular cytogenetic marker analysis confirmed that these lines were a CS-A. sharonensis 2S sh disomic addition, a 4S sh disomic addition, a 4S sh (4D) substitution + 5S sh monotelosomic addition, a 6S sh disomic addition, a 4S sh (4D) substitution + 6S sh disomic addition and a 4S sh (4D) substitution + 7S sh disomic addition line, respectively. Disease resistance investigations showed that chromosome 7S sh of A. sharonensis might harbor a new powdery mildew resistance gene, and therefore it has potential for use as resistance source for wheat breeding.

Keywords: Aegilops sharonensis, chromosome derivatives, cytogenetic identification, PLUG marker, powdery mildew resistance

INTRODUCTION

Aegilops sharonensis Eig (Sharon goatgrass, 5S 4S, 2n = 2x = 14), a wild relative of wheat, is endemic to the coastal plains of Israel and southern Lebanon (Slageren, 1994), and its genome is closely related to the B genome of common wheat (Olivera and Steffenson, 2009). A. sharonensis is a diverse source of genes for disease and insect resistance (Gill et al., 1985; Olivera et al., 2007). It has been reported that A. sharonensis carries resistance to leaf rust (Snyman et al., 2004; Olivera et al., 2007), stem rust (Valkoun et al., 1985; Olivera et al., 2007), stripe rust (Anikster et al., 2005; Olivera et al., 2007), powdery mildew (Dhaliwal et al., 1993; Olivera et al., 2007), and greenbug (Gill et al., 1985). Moreover, A. sharonensis has high tolerance to salt, drought, aluminum, boron, and...
nutrient deficiencies (Manyowa, 1989; Waines et al., 1993; Xu et al., 1993; Gorny and Garczynski, 2008). Consequently, A. sharonensis is potentially an excellent gene source for wheat improvement.

Miller et al. (1982) succeeded in producing and identifying a wheat-A. sharonensis addition line, which was due to the preferential transmission of one chromosome from A. sharonensis. Subsequently, this chromosome was identified as a gametocidal chromosome 4S<sup>sh</sup> by cytological methods such as chromosome observation, C-banding and in situ hybridization. They also produced a wheat-A. sharonensis 4S<sup>sh</sup> (4D) substitution line (Miller et al., 1982). Later, Xu et al. (1992) also reported that they had succeeded in producing a wheat-A. sharonensis 4S<sup>sh</sup> (4D) substitution line by using a nullisomic backcrossing procedure. Millet (2007) developed a tetraploid wheat-A. sharonensis amphiploid (genome AABBS<sup>1</sup>S<sup>1</sup>). Yu et al. (2017) identified two novel wheat stem resistance genes in A. sharonensis. Antonyuk et al. (2009) studied 26 wheat-A. sharonensis introgression lines. Recently, both Zhao et al. (2014) and Jiang et al. (2014) developed tetraploid wheat-A. sharonensis amphiploids. However, there are very few reports on the isolation of wheat plants carrying individual A. sharonensis chromosomes. Li X. Y. et al. (2019); Li et al. (2020) reported 24 HMW-GS homozygous lines derived from progenies of cross wheat/A. sharonensis, and produced three 1S<sup>sh</sup> (1A) substitution lines, two 1S<sup>sh</sup> (1B) substitution lines, three 1S<sup>sh</sup> (1D) substitution lines and two 1S<sup>sh</sup> (5D) substitution lines. Therefore, the set of wheat-A. sharonensis chromosome lines is still not complete, which greatly limits the mapping and utilization of excellent genes derived from this species in wheat.

In this study, six wheat-A. sharonensis chromosome derivatives, including three disomic addition lines, one disomic substitution + monotelosomic addition line, and two disomic substitution + disomic addition lines, were identified by (Polymerase Chain Reaction) PCR-based landmark unique gene (PLUG) markers and fluorescence in situ hybridization (FISH) analysis. In addition, the infection types (ITs) of disease resistance, spike and grain characteristics of these wheat-A. sharonensis chromosome lines were also investigated to provide useful information for the possible subsequent development of wheat-A. sharonensis translocations for wheat genetic improvement.

**MATERIALS AND METHODS**

**Plant Materials**

*Triticum aestivum* cv. Jinan17 (JN17) and Jimai22 (JM22) were maintained at the Crop Research Institute, Shandong Academy of Agricultural Sciences in Jinan. *T. aestivum* cv. Chinese Spring (CS) was provided by Prof. Z. J. Yang, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu. The diploid A. sharonensis accession (TA1995) was provided by Mr. J. Raupp, Wheat Genetic and Genomic Resources Center, Kansas State University, United States. The CS-A. sharonensis amphiploid (JIC-31) and six unidentified CS-A. sharonensis chromosome lines (JIC-32, JIC-33, JIC-34, JIC-35, JIC-36, and JIC-37) were kindly provided by Prof. S. M. Reader, John Innes Centre, United Kingdom.

**Fluorescence in situ Hybridization Analysis**

Root tip treatments, chromosome slide preparations, and chromosome counting were according to Liu et al. (2011). Fifteen seeds of each of the materials were germinated for collection of root tips for FISH analysis and fifteen cells of each of the materials were studied. Probes Oligo-pTa535-1, Oligo-pSc119.2-1, and Oligo-(GAA)<sub>8</sub> were synthesized by Chengdu Ruixin Biological Technology Co., Ltd. Probe sequences, the fluorochromes for probe labeling, FISH protocols and labeled DNA signal detection methods were according to Danilova et al. (2012) and Tang et al. (2014) after comparison with the CS standard FISH map. FISH using Oligo-(GAA)<sub>8</sub> as a probe could be used to identify wheat chromosomes except 1A, 3D, 4D, 5D, and 6D, as described by Danilova et al. (2012). FISH using Oligo-pSc119.2-1 and Oligo-pTa535-1 probes could identify all 42 wheat chromosomes simultaneously as described by Tang et al. (2014). pTa71 (45S rDNA) contains a 9-kb EcoRI fragment isolated from bread wheat (Gerlach and Bedbrook, 1979), which could be used to identify homoeologous groups 1 and 6 of *Triticum* and *Aegilops*. Photomicrographs of FISH chromosomes were taken using an Olympus BX-51 microscope.

**DNA Isolation and PLUG-PCR**

Total genomic DNA isolation was according to the protocol of Liu et al. (2006). A total of 526 PLUG primer pairs were synthesized according to Ishikawa et al. (2009). All primer pairs were synthesized by Chengdu Ruixin Biological Technology Co., Ltd., and PCR protocol was according to Ishikawa et al. (2009). In order to obtain high levels of polymorphism, the PCR products were digested with the four-base cutter enzymes *Hae*<sub>III</sub> or *TaqI* according to Ishikawa et al. (2009) and were separated on 2% agarose gels.

**Disease Resistance Testing**

The resistance reactions to stripe rust, leaf rust, stem rust, and powdery mildew of the six suspected CS-A. sharonensis derivatives were tested. We investigated the disease resistance data for two consecutive years in 2015 and 2016, and 20 individual plants of each line were investigated each year. CS is highly susceptible to all four pathogens, hence the disease response scoring did not begin until CS was fully infected. According to Wang et al. (2014), the disease responses were scored on a 0–4 rating scale, 0 means immune; 0; indicates nearly immune but showing a small fleck on the leaf, 1 means highly resistant, 2 indicates moderately resistant, 3 means moderately susceptible, and 4 indicates highly susceptible. Scores of 0–2 were classified as resistant and 3–4 as susceptible. The pathogenic race selection and disease response rating scale of the four diseases were all according to Gong et al. (2017). The pathogen inoculation methods for stripe rust, leaf rust and powdery mildew were according to Liu et al. (2013), while stem rust inoculation was according to Han et al. (2018). Stripe rust
resistance was determined on adult plants using mixed isolates of races CY32, CY33, and Su-4 in the experimental farmland of School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, Sichuan Province. Stem rust resistance was determined on seedlings using mixed isolates of pathotypes 34MKGQM and 21C3THSM in the greenhouse of College of Plant Protection, Shenyang Agricultural University, Shenyang, Liaoning Province. Leaf rust resistance was determined on seedlings using mixed isolates of THTT, PHTT, THKS, THTS, and THKT (these isolates are prevalent and highly damaging on wheat crops throughout China) in the greenhouse of College of Plant Protection, Hebei Agricultural University, Baoding, Hebei Province. Powdery mildew resistance was determined on seedlings (in the greenhouse) and also on adult plants (in the field) following inoculation with mixed powdery mildew races collected from four different cities including Jinan, Linyi, Dezhou, and Heze of Shandong Province.

Spike and Grain Characteristics
Chinese Spring and the six suspected CS-A. sharonensis chromosome lines were planted in the field at Jinan in Shandong Province on October 25, 2015 and harvested on June 5, 2016. The spikes were collected for photographs on May 10, 2016 then threshed and the grain extracted when fully mature. The spike and grain characters of these materials were investigated and described according to Li et al. (2006).

RESULTS

Cytogenetic Identification of Wheat-A. sharonensis Chromosome Lines
Sequential FISH with probes Oligo-pSc119.2-1, Oligo-pTa535-1, and Oligo-(GAA)$_3$ was used to detect the chromosome constitution of wheat-A. sharonensis amphiploid JIC-31 (Figure 1). The karyotype of the seven pairs of A. sharonensis chromosomes in JIC-31 is shown in Figure 1C. Cytological studies revealed that the chromosome numbers of JIC-32 to JIC-37 were 44, 44, 42 + monotelosomic, 42, 44, and 44, respectively. FISH on mitotic metaphase chromosomes of these lines showed that the lines JIC-32 and JIC-33 had 42 wheat chromosomes, while the 4D chromosomes in JIC-34 to JIC-37 were missing. A pair of A. sharonensis chromosomes with distinct FISH signals different from wheat chromosomes was detected in JIC-32, JIC-33, and JIC-35. Two different pairs of A. sharonensis chromosomes were found in JIC-36 and JIC-37, while disomic and monotelosomic additions of A. sharonensis chromosomes were detected in JIC-34. Therefore, JIC-32, JIC-33, and JIC-35 are CS-A. sharonensis disomic addition lines, JIC-36 and JIC-37 are CS-A. sharonensis disomic substitution + disomic addition lines. JIC-34 is CS-A. sharonensis disomic substitution + monotelosomic addition line. FISH patterns of JIC-32 and JIC-33 are shown in Figure 2 (FISH patterns of JIC-34 to JIC-37 are shown in Supplement Figure 1).

Molecular Identification of Wheat-A. sharonensis Chromosomes
In order to identify the homoeologous groups of each of the A. sharonensis chromosomes in JIC-32 to JIC-37, a total of 526 PLUG primer pairs were used to develop A. sharonensis chromosome-specific markers. As a result, fifty-one primer pairs could generate polymorphisms in A. sharonensis, the CS-A. sharonensis amphiploid, CS, JM22, and JN17. Among them, four, eight, nine, six, two, five, and seventeen belonged to chromosome homoeologous groups 1–7, respectively (Table 1, 2). The percentage of primers showing polymorphisms across the seven types of A. sharonensis chromosomes ranged from 2.6 to 15.6% (Table 1). The PCR patterns of primer pairs TNAC1137, TNAC1197, TNAC1398, TNAC1740, TNAC1867, and TNAC1924 are shown in Figure 4. TNAC1102 and another seven primer pairs specific to chromosome homoeologous group 2, could amplify polymorphisms in the A. sharonensis chromosomes of JIC-32, indicating that the pair of A. sharonensis chromosomes in that line was 25$^s$h. The chromosome number of JIC-32 was 44, including the 42 complete wheat chromosomes, therefore, JIC-32 was a CS-A. sharonensis 2$^s$h disomic addition (Figures 2A, B). Based on the results of molecular markers and cytological identification, the same analysis method was performed on JIC-33 to JIC-37, indicating that JIC-33 was a CS-A. sharonensis 4$^s$h disomic addition (Figures 2C, D), while JIC-34 was a CS-A. sharonensis 4$^s$h (4D) substitution + 5$^s$hL monotelosomic addition (Supplementary Figures 1A, B), JIC-35 was a CS-A. sharonensis 6$^s$h disomic addition (Supplementary Figures 1C, D), JIC-36 was a CS-A. sharonensis 4$^s$h (4D) substitution + 6$^s$h disomic addition (Supplementary Figures 1E, F) and JIC-37 was a 4$^s$h (4D) substitution + 7$^s$h disomic addition line (Supplementary Figures 1G, H).

Spike and Grain Characters of Wheat-A. sharonensis Chromosome Lines
Compared to spike morphologies of CS, the spikes of the six CS-A. sharonensis chromosome derivatives were all varied (Figure 5). Spikes of the CS-A. sharonensis 2$^s$h disomic
FIGURE 1 | FISH identification of the chromosome constitution of wheat-Aegilops sharonensis amphiploid JIC-31. Panel (A) shows the probes were Oligo-pSc119.2-1 (green) and Oligo-pTa535-1 (red). Panel (B) shows the probe was (GAA)$_8$ (red). (C) shows the karyotype of the seven pairs of A. sharonensis chromosomes in JIC-31; Panel (A,B) indicates unidentified chromosome. Bar indicates 10 µm.

FIGURE 2 | FISH using Oligo-nucleotides as probes on the CS-A. sharonensis 2S$_{sh}$ disomic addition (A,B) and the 4S$_{sh}$ disomic addition (C,D). Panels (A,C) are double-color FISH patterns using Oligo-pTa535-1 (red) and Oligo-pSc119.2-1 (green) as probes; (B,D) are double-color FISH patterns using pTa71 (red) and (GAA)$_8$ (green) as probes. Bar indicates 10 µm.
addition had short awns and narrow spikes. The lower inter-
spikelet segments of the heads of CS-\(A. \text{sharonensis}\) 4\(S\text{sh}\) (4D) substitution + 5\(S\text{sh}\)L monotelosomic addition, 6\(S\text{sh}\) disomic addition, 4\(S\text{sh}\) (4D) substitution + 6\(S\text{sh}\) disomic addition and 4\(S\text{sh}\) (4D) substitution + 7\(S\text{sh}\) disomic addition lines were more
elongated than that of CS. The CS-\(A. \text{sharonensis}\) 4\(S\text{sh}\) disomic addition line (JIC-33) showed slightly elongated spikelets and
overall longer spikes than that of CS. The CS-\(A. \text{sharonensis}\) 4\(S\text{sh}\) (4D) substitution + 6\(S\text{sh}\) disomic addition (JIC-36) showed
shorter spikes and fewer spikelets per head than that of
CS (Figure 5).

Grain morphologies of the six CS-\(A. \text{sharonensis}\) chromosome
derivatives identified above were similar to that of CS, while the
CS-\(A. \text{sharonensis}\) 2\(S\text{sh}\) disomic addition (JIC-32) showed slender
grains and darker pericarp color than that of CS (Figure 5) and the 4\(S\text{sh}\) disomic addition (JIC-33) showed smaller grains
than those of CS.

**Disease Resistance Tests of Wheat-\(A. \text{sharonensis}\) Chromosome Lines**

Stripe rust, leaf rust, stem rust, and powdery mildew resistance
tests showed that all the materials were moderately to highly
susceptible to stripe rust, leaf rust, and stem rust (Table 3) except
that the infection reaction for leaf rust on the CS-\(A. \text{sharonensis}\)
4\(S\text{sh}\) (4D) substitution + 7\(S\text{sh}\) disomic addition (JIC-37) was not
obtained. The CS-\(A. \text{sharonensis}\) 4\(S\text{sh}\) (4D) substitution + 7\(S\text{sh}\)
disomic addition (JIC-37) was nearly immune to powdery
mildew, while CS and other CS-\(A. \text{sharonensis}\) chromosome lines
tested were highly susceptible to powdery mildew (Table 3),
suggesting that chromosome 7\(S\text{sh}\) of \(A. \text{sharonensis}\) might carry
powdery mildew resistant gene(s).

**DISCUSSION**

**Chromosomes Transferred From \(A. \text{sharonensis}\) Into Wheat**

Transferring each pair of \(A. \text{sharonensis}\) chromosomes into
wheat is difficult due to the presence of gametocidal (\(Gc\)) genes
that control the preferential transmission of chromosome
4\(S\text{sh}\) (Endo, 1982; Miller et al., 1982). Therefore, it is not
easy to produce a complete set of wheat-\(A. \text{sharonensis}\)
additions or substitutions (Miller et al., 1982; Olivera and
Steffenson, 2009). Maan found a \(T. \text{urartu}-A. \text{sharonensis}\)
ampeloploid TA3398 in North Dakota in 1972 (unpublished).
## TABLE 2 | Markers specific for A. sharonensis chromosomes developed by the current study.

| No. | Primer | Primer sequence (5′–3′) | Wheat chromosomal location | Location on S<sup>th</sup> chromosome | Enzyme used | Product size (bp) |
|-----|--------|--------------------------|-----------------------------|---------------------------------------|-------------|------------------|
| 1   | TNAC1042 | F:GACAAACCCCAACGACATGC  
R:TATACCGCTGATCGGTGCAGA | 1AL-1BL-1DL | 7S<sup>th</sup> | – | 750 |
| 2   | TNAC1041 | F:TCACCATCCTCTTTCAAGTGCTT  
R:GCATCAAGGATGAGGTGCTG | 1AL.4-0.56-0.61  
1BL.2-0.69-0.85  
1DL.9-0.64-1.00 | – | TaqI | 450 |
| 3   | TNAC1079 | F:CACCTGAAGGACATGGATGC  
R:TCATCGAGGTGTGATCACCCTC  
R:TCATCGAGGGTGAACACCTCC | 1AL  
1BL  
1DL | – | TaqI | 750 |
| 4   | TNAC1089 | F:GATGAGGACAGATGACAGACC  
R:TCGTTCTCCATCATCACACAA | 1AL  
1BL  
1DL | – | TaqI | 350 |
| 5   | TNAC1102 | F:GGAAGGCTGAAGGACCAAACCTC  
R:CTTGCGAGGATGAGGTGCTT | 2AS.5-0.78-1.00  
2BS.8-0.84-1.00  
2DS.8-0.47-1.00 | 2S<sup>th</sup> | – | 1,200/1,000 |
| 6   | TNAC1137 | F:GCTGATGTCATCAACACATCGT  
R:CGATGCCTGAACCGATCG | 2AL.4-0.27-0.77  
2BL.4-0.65-0.89  
2DL.9-0.76-0.94 | 2S<sup>th</sup> | – | 1,400 |
| 7   | TNAC1140 | F:TCGCAAAATTCAAGGCTCA  
R:AGGAACCTTGACGAGTGGAAA | 2AL.3-0.77-1.00  
2BL.6-0.89-1.00  
2DL.9-0.94-1.00 | 2S<sup>th</sup> | – | 700 |
| 8   | TNAC1142 | F:GCCTGAAGGAGTACATGGTCGAG  
R:CAAGATCCCATACACCAGGT | 2AL.3-0.77-1.00  
2BL.6-0.89-1.00  
2DL.9-0.94-1.00 | 2S<sup>th</sup> | – | 1,400 |
| 9   | TNAC1197 | F:CAAGATGTCAGGCTCTCCAC  
R:TCGCGAGTATTGAAGT | 2AL  
2BL  
2DL | – | TaqI | 980 |
| 10  | TNAC1204 | F:GAGAGAGGTTGAGGTAAGTGG  
R:AGACCATCTTCTCGGCTTTGG | 2AL.4-0.27-0.77  
2BL.7-0.50-0.58  
2DL.1-0.49-0.58 | 2S<sup>th</sup> | – | 800/700 |
| 11  | TNAC1206 | F:ACCTCTACACCCAGACGATCG  
R:CGGAAACCTTGGGACCGAC | 2AL  
2BL  
2DL | 2S<sup>th</sup> | – | 950 |
| 12  | TNAC1176 | F:CTTCATGTTGCTACGAACT  
R:CATTGGAAGGGATGTGCTC  
R:CATTGGAATTGCTTCTT | C-2AS.5-0.78  
2BS.1-0.27-0.53  
2DS.1-0.33-0.41 | 2S<sup>th</sup> | TaqI | 1,000 |
| 13  | TNAC1248 | F:ATGATGCAAGGCGAATAACCA  
R:CTGAGAGGAGCTCTCAACTTC  
R:CTGAGAGGAGCTCTCAACTTC | 3AS.4-0.45-1.00  
3BS.8-0.33-0.33  
3DS.3-0.24-0.31 | 3S<sup>th</sup> | – | 800 |
| 14  | TNAC1294 | F:CGGAACCTTTAGCCTCTTCGCT  
R:GTCTGTCAGGATCTGTGCTGT | 3AS.4-0.45-1.00  
3BS.9-0.57-0.78  
3DS.4-0.59-1.00 | 3S<sup>th</sup> | – | 600 |
| 15  | TNAC1254 | F:ATTAGCTTTGAGCCCTGGAGGT  
R:CTACTGCAACCGACCAGAAGGT | 3AL  
3BL  
3DL | – | TaqI | 850 |
| 16  | TNAC1269 | F:AACGGTTTGTGTCCTTCAAGA  
R:CTGAGAAGGACCTGAACAGC | 3AL  
3BL  
3DL | – | TaqI | 850 |
| 17  | TNAC1335 | F:CTCTACAGAGGCTCGATGCTAT  
R:GGGAAGTTTCTCCTCAATGG | C-3AL.2-0.21  
C-3BL.2-0.22  
C-3DL.1-0.23 | 3S<sup>th</sup> | – | 900 |
| 18  | TNAC1337 | F:CTCCTCCATCATGCTTCCTCAAA  
R:CCTCCTCTCAGCTTAACTCC | 3AL  
3BL  
3DL | – | TaqI | 900, 1,000 |
| 19  | TNAC1341 | F:GTGAAGCCCTACGTCGCAAACAC  
R:TAGCATGGGCTCTCAAAATG | 3AL.1-0.26-0.42  
3BS.2-0.22  
3DS.1-0.23 | – | TaqI | 500 |
| 20  | TNAC1356 | F:CCTGCAAGCTCTCTCAACAGC  
R:GGCCGATGCTGCTACCAACAC  
R:GGCCGATGCTGCTACCAACAC | 3AL.3-0.42-0.61  
3BL.10-0.50-0.63  
3DL.1-0.23-0.81 | – | TaqI | 350 |
| 21  | TNAC1365 | F:CTTGGGCAAGGCTTTTCTCCTA  
R:GGAAGCCCTTGGCTTCTCTCTC | 3AL  
3BL  
3DL | – | TaqI | 850 |
| 22  | TNAC1412 | F:CTAGTGCAGACCCATGGAGTA  
R:CGGGACATTGCTGCTCTTG | 4AS.3-0.78-1.00  
4S<sup>th</sup> | – | 1,600 |

(Continued)
| No. | Primer sequence (5′–3′) | Wheat chromosomal location | Location on S<sup>th</sup> chromosome | Enzyme used | Product size (bp) |
|-----|--------------------------|----------------------------|---------------------------------------|-------------|------------------|
| 23  | TNAC1416                 | R:CTTCACACCATCCAAAGCTTTC   | 4BL1-0.71-0.86 4DL1-0.61-0.71         | –           | 1,800            |
| 24  | TNAC1396                 | F:CGGTTTCTGTTCATTACCA      | 4AS 4BL 4DL                            | TaqI        | 1,200            |
| 25  | TNAC1398                 | F:CAAGGCCAGGTGCTGATATTGT   | 4AS3-0.76-1.00 4BL5-0.86-0.95 4DL12-0.71-0.86 | TaqI        | 1,100            |
| 26  | TNAC1457                 | F:TTTGATCCGTACTGCGCTAG     | 4AL12-0.43-1.00 4BS1-0.84-1.00 4DS2-0.82-1.00 | TaqI        | 650              |
| 27  | TNAC1473                 | F:GAAGCAGCAATATTGTGAGT     | 4BL 4DL                               | TaqI        | 700              |
| 28  | TNAC1455                 | F:AGCAACACTTCCTCCACGTTTAT  | 4AL 5AL 5BS 5DL                        | –           | 750              |
| 29  | TNAC1621                 | F:CCCTCTTGCGATCTTCTTGTG    | 5AL 5BL 5DL                           | –           | 1,050            |
| 30  | TNAC1740                 | F:CGGAGTGCTCGATGTTATCT     | 6AL7-0.88-0.90 6BL5-0.40-0.66 6DL6-0.29-0.47 | –/TaqI     | 1,200/250        |
| 31  | TNAC1748                 | F:TCGTAAGTTGGCTGAGCGTATG   | 6AL7-0.88-0.90 6BL8-0.66-0.70 6DL1-0.47-0.68 | TaqI        | 750              |
| 32  | TNAC1751                 | F:CTCTCTTGTGCTGATCGCTG     | 6AL8-0.90-1.00 6BL1-0.70-1.00 6DL1-0.68-0.74 | TaqI        | 900              |
| 33  | TNAC1756                 | F:CTTCATGGACATTTCTGCTA     | 6AL 6BL 6DL                           | TaqI        | 750              |
| 34  | TNAC1763                 | F:CGATTGGGCTGACATTCTTC     | 6AL8-0.90-1.00 6BL1-0.70-1.00 6DL10-0.80-1.00 | TaqI        | 1,000            |
| 35  | TNAC1867                 | F:GCCCTTCTTTTGATGGCTG      | C-7AL1-0.39 7BL2-0.38-0.63 7DL1-0.14-0.30 | TaqI        | 750              |
| 36  | TNAC1924                 | F:TAGCTTGGAGAAGATGGTGG     | 7AL 7BL 7DL                           | –           | 750              |
| 37  | TNAC1801                 | F:CAAGCACTCAGCTTCTTCGAC    | 7AS 7BS 7DS                           | HaeIII      | 550              |
| 38  | TNAC1920                 | F:CTCAGACGGGACTTAGCTGAG    | 7AS 7BS 7DS                           | HaeIII      | 1,500            |
| 39  | TNAC1843                 | F:TGGGAAATCACTCATTCAGT     | 7AL 7BL 7DL                           | TaqI        | 800              |
| 40  | TNAC1881                 | F:GAAGGGCTATGACCAAGCTTCT   | 7AL 7BL 7DL                           | TaqI        | 400              |
| 41  | TNAC1888                 | F:AGGATGTTGGAGCTGTTTA      | C-7AL1-0.39 7BL2-0.38-0.63 7DL5-0.30-0.61 | TaqI        | 750              |
| 42  | TNAC1902                 | F:AAACCAGGACCTCTCAACTTTT   | 7AL 7BL 7DL                           | TaqI        | 1,500            |
| 43  | TNAC1922                 | F:CAAGCAATAAAGTGGACATG     | 7AS 7BS                               | TaqI        | 350              |

(Continued)
TABLE 2 | Continued

| No. | Primer   | Primer sequence (5′–3′)                                                                 | Wheat chromosomal location | Location on 5th chromosome | Enzyme used | Product size (bp) |
|-----|----------|-----------------------------------------------------------------------------------------|----------------------------|----------------------------|-------------|-------------------|
| 44  | TNAC1774 | F:CAAGTCTGGGATGACCTTCA R:GTGATCATCCGTCTTCACTG                                            | 7DS                        |                             |             | 1,400             |
| 45  | TNAC1781 | F:AACCTGGAATCAAGCAGCAC R:ACACCAGCTCTTCTCTTTATTT                                         | 7AS2-0.73-0.83 7BS2-0.27-1.00 7DS4-0.73-1.00 | 7Sh            |             | 1,700             |
| 46  | TNAC1827 | F:TCCTCATGTCGAAGCAGGA R:TCAATTCAATCTCTGTTCGC                                             | 7AL 7BL 7DL                |                             |             | 750               |
| 47  | TNAC1948 | F:TTGTCTCTAGGCGATCAGG R:GTGTATGATGCGAATGGAAGG                                          | 7AS8-0.45-0.59 7BS1-0.27-0.27 7DS2-0.61-0.73 | 7Sh            |             | 1,100             |
| 48  | TNAC1786 | F:CCCTTCCATATCTCCACCT R:GGAAAGTATCTTCCTGGTTGA                                           | 7AS 7BS 7DS                |                             | TaqI        | 600               |
| 49  | TNAC1788 | F:CTGAGATGATGCGAAGGA R:AGAAATGCCTTCCTCCTGTA                                              | 7AS 7BS 7DS                |                             | TaqI        | 900               |
| 50  | TNAC1806 | F:ATTCTCTGGAATTCTGTGAT R:TCTGCACTGAGGACTGAAA                                             | 7AS8-0.45-0.59 7BS2-0.27-1.00 7DS2-0.61-0.73 | 7Sh            | TaqI        | 800               |
| 51  | TNAC1937 | F:AGGGGATGAGGTGAATCCA A R:GGAGGATGGAAGGACAC                                               | 7AS 7DS                    |                             | TaqI        | 600               |

Information of wheat chromosomal locations is according to Ishikawa et al. (2007).

FIGURE 4 | PCR patterns of primer pair TNAC1137 (A), TNAC1197 (B), TNAC1398 (C), TNAC1740 (D), TNAC1867 (E), and TNAC1924 (F). Lane M indicates Marker DM2000. Lanes 1-8 in panels (A–F) are CS-A. sharonensis amphiploid, CS, CS-A. sharonensis 2Sh disomic addition, 4Sh disomic addition, 4Sh (4D) substitution + 5Ssh monotelosomic addition, 6Ssh disomic addition, 4Sh (4D) substitution + 6Ssh disomic addition and 4Sh (4D) substitution + 7Ssh disomic addition lines, respectively.

King et al. (1991) induced a mutation in the male fertility gene of the preferentially transmitted A. sharonensis chromosome 4S1 (some scientists defined the genome of A. sharonensis as S1S1). Friebe et al. (2003) produced a mutation of the A. sharonensis Gc2 gametocidal gene (Gc2mut), which opened a way for introgression of genes from A. sharonensis into wheat. Zhang et al. (2001) reported the production of additions 1S1, 3S1, 5S1, 6S1, and 7S1 in a 4S1 (4D) background. Antonyuk et al. (2009) studied 26 wheat-A. sharonensis introgression lines which they then separated into six groups based on different substituted chromosomes belonging to definite homoeologous groups and different numbers of translocations. Millet (2007) developed a tetraploid wheat-A. sharonensis amphiploid (genome AABBSS1S1). Li X. Y. et al. (2019); Li et al. (2020) reported 24 HMW-GSs homozygous lines derived from progenies of cross wheat/A. sharonensis, and produced three 1Ssh (1A) substitution lines, two 1Ssh (1B) substitution lines, three 1Ssh (1D) substitution lines and two 1Ssh (5D) substitution lines.

So far, reports regarding the development of wheat-A. sharonensis introgression lines are very rare. Furthermore, none to date has reported the production of wheat-A. sharonensis 2Ssh introgression lines. In this research, six wheat-A. sharonensis introgression lines were identified, including a CS-A. sharonensis 2Ssh disomic addition (JIC-32), a 4Ssh disomic addition...
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FIGURE 5 | Spike and grain morphologies of wheat-A. sharonensis chromosome lines. Grain and spikes from left to right are CS, CS-A. sharonensis 2S disomic addition, 4S (4D) substitution + 5S L monotelosomic addition, 6S (4D) substitution + 6S disomic addition and 4S (4D) substitution + 7S disomic addition lines, respectively.

TABLE 3 | The chromosome composition and Stripe rust, leaf rust, stem rust, and powdery mildew infection types of JIC-32 to JIC-37.

| Line   | 2n  | Chromosome composition          | Stripe rust | Leaf rust | Stem rust | Powdery mildew |
|--------|-----|---------------------------------|-------------|-----------|-----------|----------------|
| JIC-32 | 44  | 42W + 2S<input>2S<sub>sh</sub> | S           | S         | S         | S              |
| JIC-33 | 44  | 42W + 4S<input>4S<sub>sh</sub> | S           | S         | S         | S              |
| JIC-34 | 42  | 40W + 4S<input>4S<sub>sh</sub> + 5S<input>5S<sub>L</sub> | S           | S         | S         | S              |
| JIC-35 | 42  | 40W + 6S<input>6S<sub>sh</sub> | S           | S         | S         | S              |
| JIC-36 | 44  | 40W + 4S<input>4S<sub>sh</sub> + 6S<input>6S<sub>sh</sub> | S           | S         | S         | S              |
| JIC-37 | 44  | 40W + 4S<input>4S<sub>sh</sub> + 7S<input>7S<sub>sh</sub> | S           | –         | S         | R              |

R, resistant; S, susceptible; –, uninvestigated.

Development of New Molecular Markers Specific for A. sharonensis Chromosomes

Previous reports regarding identification of useable molecular markers for A. sharonensis chromosomes indicated that the percentage which were polymorphic was very low, ranging from 1.3 to 11.4% (Zhang et al., 2001; Zhao et al., 2014; Wei Long, 2016;
Li et al., 2020). Zhang et al. (2001) developed 21 RFLP markers to identify CS-A. sharonensis 3S1, 4S1, 5S1, 6S1, and 7S1 addition lines. Antonyuk et al. (2009) used two microsatellite primer pairs to screen wheat-A. sharonensis introgression lines. Zhao et al. (2014) used two pairs of primers from 150 SSR markers to identify the S18 genome of A. sharonensis among F1 hybrids. Wei Long (2016) developed two molecular markers specific to the x- and y-type HMW-GSs genes of A. sharonensis, which were validated in accurately tracing and distinguishing A. sharonensis Glu-1S18 of backcross progenies from Glu-1A, Glu-1B, and Glu-1D of wheat. Li et al. (2020) developed four molecular markers specific to the 15S1 chromosome of A. sharonensis from 35 primer pairs.

In this study, we found that 51 PLUG markers from a total of 526 primer pairs could identify the homoeologous groups of each of the A. sharonensis chromosomes. Among these primer pairs, four, eight, nine, six, two, five, and seventeen belonged to chromosome homoeologous groups 1–7, respectively (Tables 1, 2). The percentage of each homoeologous group primers generated ranged from 2.6 to 15.6%, with an average percentage of 9.7% (Table 1).

**Powdery Mildew Resistance in A. sharonensis**

Wild relatives of wheat are an important gene reservoir for resistance to wheat diseases, and have been exploited extensively around the world for wheat improvement (Olivera and Steffenson, 2009). A. sharonensis, as well as other wild grasses, has co-evolved in association with many cereal pathogens, such as leaf rust, stem rust, stripe rust, and powdery mildew (Wahl et al., 1984). Among them, the highest frequency and level of resistance reported in A. sharonensis was to wheat powdery mildew (Gill et al., 1985; Valkoun et al., 1985; Dhalwal et al., 1993; Olivera et al., 2007). Zhirov and Ternovskaya (1993) first studied a powdery mildew resistance gene in a wheat-A. sharonensis introgression line. Olivera et al. (2008) identified A. sharonensis accessions carrying major resistance genes to powdery mildew, and found different genes from accessions native to the southern and northern coastal plains of Israel.

To date, more than 70 powdery mildew resistance genes have been permanently designated (Li G. Q. et al., 2019). Among them, 19 have originated from wheat's related species (Liu et al., 2019), such as Pm7, Pm8, Pm17, Pm20, and Pm56 from Secale cereale, Pm12, Pm32, and Pm53 from A. speltoides, Pm13 and Pm66 from A. longissima, Pm21 and Pm55 from Dasyphyllum villosum, Pm19, Pm34, Pm35, and Pm58 from A. tauschii, Pm29 from A. ovata, Pm40 and Pm43 from Thinopyrum intermedium, Pm51 from T. ponticum and Pm57 from A. searsii. Among the above 19 genes mentioned, none were derived from A. sharonensis. In our present study, the CS-A. sharonensis 4S18 (4D) substitution + 5S18L monotelosomic addition (JIC-34) and the CS-A. sharonensis 4S18 disomic addition line (JIC-33) were highly susceptible to powdery mildew, indicating that there were no powdery mildew resistance genes on chromosomes 4S18 and 5S18L of A. sharonensis. However, the CS-A. sharonensis 4S18 (4D) substitution + 7S18 disomic addition (JIC-37) was nearly immune to powdery mildew (Table 3), suggesting that the chromosome 7S18 of A. sharonensis might carry new powdery mildew resistant gene(s).

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

CL conceived and designed the experiments. XW, ZHY, HW, JBL, and RH performed the experiments. WX, GL, and JG analyzed the data. XW wrote the manuscript. HL, ZJY, JYL, and CL revised the manuscript. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.708551/full#supplementary-material

**Supplementary Figure 1** | FISH using Oligo-nucleotides as probes on the CS-Aegilops sharonensis 4S18 (4D) substitution + 5S18L monotelosomic addition (A,B), 6S18 disomic addition (C,D), 4S18 (4D) substitution + 6S18 disomic addition (E,F), and 4S18 (4D) substitution + 7S18 disomic addition line (G,H). Panels (A,C,E,G) are double-color FISH patterns using Oligo-pTa535-1 (red) and Oligo-pSc119.2-1 (green) as probes; (B,D,F,H) are FISH patterns using (GAA)6 as probes. Bar indicates 10 μm.
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