Sequence elimination in hybrid offspring of wheat-*Agropyron cristatum* (L.) Gaertn introgression line Pubing3504 × common wheat cultivar Jing4839

Wu Xiaoyang*, Chen Dan*,†,‡,§,**, Lu Yuqing*, Zhang Jinpeng*, Liu Weihua*, Yang Xinming*, Li Xiuquan*, Du Juan* and Li Lihui*

*The National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. Beijing 100081, P. R. China

†Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650223, P. R. China

‡Yunnan Provincial Key Lab of Agricultural Biotechnology, Kunming 650223, P. R. China

§Key Lab of Southwestern Crop Gene Resource and Germplasm Innovation, Ministry of Agriculture, Kunming 650223, P. R. China

**Scientific Observation for Rice Germplasm Resources of Yunnan, Ministry of Agriculture, Kunming 650223, P. R. China

18 **Sequence elimination in hybrid offspring of Pubing3504 × Jing4839**

19 **KEYWORDS** Common wheat; *Agropyron cristatum*; Molecular marker; Sequence

20 elimination; Chromosome differentiation

21 Corresponding author: Li Lihui, Tel: +86-10-62186670, Fax: +86-10-62189650,

22 E-mail: lilihui@caas.cn.

23

ABSTRACT

Sequence elimination is one of main reasons for homologous chromosome differentiation in common wheat. Sequence elimination can occur in genome-specific sequences, chromosome-specific sequences, and repeat sequences in the wheat genome. Genetic polymorphism loci in chromosome-specific sequences can be used to develop molecular markers including simple sequence repeats (SSRs), insertions and deletions, and single nucleotide polymorphisms (SNPs). Pubing3504 is a wheat-*Agropyron cristatum* (L.) Gaertn introgression line, and Jing4839 is a common wheat cultivar. Assessment of their recombinant inbred line (RIL) population using 120 pairs of SSR markers covering all wheat chromosomes indicated that sequence elimination occurred at the short arm of chromosome 1A (1AS). We developed 13 pairs of new co-dominant SSR markers and constructed a genetic linkage map of 1AS; we found that the segment with sequence elimination is from *SSR110* to the end of 1AS. We further developed 10 pairs of dominant SNP markers of Pubing3504, 10 pairs of dominant SNP markers of Jing4839, and 10 pairs of primers designed in SNP flanking sequences to assess RILs. We found that all chromosome segments with sequence elimination came from Jing4839. The sequence elimination occurred in SSR loci, SNP loci, and coding sequences. There was no homologous recombination in the chromosome segment with sequence elimination. We suggest that sequence elimination causes the differentiation of chromosomes and the chromosome differentiation affects the homologous pairing at the chromosome segment in meiosis, which further affects the occurrence of homologous recombination at the chromosome

46 segment.

47

INTRODUCTION

Allopolyploidization is one of the main forces driving the evolution of plant genomes, inducing structure–function and modification differentiation in plant genomes (Wendel 2000; Feldman and Levy 2005; Otto 2007; Leitch and Leitch 2008). This differentiation is known as "genome shock" (McClintock 1993). "Genome shock" widely exists in natural and artificial interspecific hybridization. Allopolyploidization brings rapid changes in the genome of hybrid offspring (Comai 2000; Chen 2007). These genome changes include chromosomal rearrangements, chromatin remodeling, gene expression alteration, DNA methylation, histone modification, and reactivation of transposable elements (Kashkush et al. 2003; Pontes et al. 2003; Pontes et al. 2004; Adams and Wendel 2005; Gaeta et al. 2007; Ha et al. 2009).

Common wheat (*Triticum aestivum*) is an allohexaploid with genome composition AABBDD, which has experienced at least two interspecific hybridizations in the process of its evolution. The genetic polymorphism of the wheat genome not only comes from the accumulation of random mutations, but the changes also largely come from allopolyploidization (Feldman and Levy 2005; Feldman and Levy 2012). During the evolution of common wheat, the low-copy sequences (Liu et al. 1998a) and the repeated sequences (Salina et al. 2004; Han et al. 2005) in the genome have been eliminated in large scale. Sequence elimination is not a random event, and the elimination of coding and non-coding sequences results in the generation of the chromosome differentiation and new genetic diversity in the wheat

genome. Furthermore, these eliminated sequences are within the recognition site of homologous chromosome pairing in meiosis (Feldman et al. 1997). In the process of allopolyploidization, sequence elimination is usually accompanied by wide changes in epigenetic modification and gene expression (Shaked et al. 2001; Kashkush et al. 2002; Kashkush et al. 2003; Levy and Feldman 2004; Feldman and Levy 2005; Feldman and Levy 2009).

Genomic and transcriptional changes after allopolyploidization exhibit genome preference (Ozkan et al. 2001; Ma et al. 2004). In hybrid progeny between *Aegilops sharonensis* (S^lS^l) and *Aegilops umbellulata* (S^uS^u), 14% of the *Ae. sharonensis* genome sequence was eliminated, but only 0.5% of the *Ae. umbellulata* genome sequence was eliminated. In hybrid progeny between *Ae. sharonensis* and *Triticum monococcum*, the eliminated sequence in the *T. monococcum* genome was twice as large as that of *Ae. Sharonensis* (Shaked et al. 2001). Analysis of genome-wide transcription of the hybridization progeny and the parents revealed that the patterns of gene expression in synthetic hexaploid wheat are homoeolog specific, non-additive, and parentally dominant (Akhunov et al. 2010; Chagué et al. 2010; Qi et al. 2012). In allohexaploid wheat, morphology and environmental adaptability are usually controlled by only one genome among the A, B, and D genomes (Peng et al. 2003; Nalam et al. 2006; Feldman and Levy 2012). However, the mechanism of its establishment and maintenance is still not clear.

Agropyron cristatum L. Gaertn is one of the main plant species of *Triticeae*. Its chromosome composition is seven as the base number, and the chromosomes are

92 diploid and tetraploid (Dong 1993). Our laboratory successfully completed the
93 hybridization between tetraploid *A. cristatum* and common wheat, and subsequently, a
94 large number of introgression lines were created (Wang et al. 2011; Dan et al. 2012;
95 Lu et al. 2015). The chromosome number of these materials is consistent with
96 common wheat, but their alien genetic component cannot be effectively detected by
97 cytology or the molecular marker method. For this reason, we know little about the
98 changes in the genomes of these introgression lines. However, it is clear that these
99 introgression lines have undergone more allopolyploidization than has common wheat.
100 In addition, many of these introgression lines show excellent breeding traits including
101 multiple grains and extensive resistance to wheat diseases and insect pests. Some
102 materials have been used in breeding and gene mapping.

103 Pubing3504 is one of the wheat-*Agropyron cristatum* (L.) Gaertn introgression
104 lines, and a recombinant inbred line (RIL) population was established by the
105 hybridization of Jing4839 (a common wheat) and Pubing3504. The sequence
106 elimination at the short arm of chromosome 1A (1AS) was found from the RIL
107 population. In this study, we developed a series of molecular markers for the
108 elimination of chromosome segments used chromosome-specific sequences to
109 determine the range and the origin of the chromosome segment with sequence
110 elimination. In addition, we further studied the effect of sequence elimination on the
111 coding sequence and homologous recombination.

112 ## MATERIALS AND METHODS

113 ### Plant materials

The wheat-*Agropyron cristatum* (L.) Gaertn introgression line Pubing3504, common wheat cultivar Jing4839, and their RIL population (F8) including 336 lines were used for this study.

Development of molecular markers

We developed new simple sequence repeat (SSR) markers in 1AS. The diploid wild einkorn wheat (*Triticum urartu*) is the progenitor species of the A genome. Its scaffold sequence bin mapping in 1AS was used in this study to develop SSR markers (Ling et al. 2013). A high-density genetic linkage map was constructed using the wheat 90K chip (Wang et al. 2014). We further obtained a scaffold sequence using the chip-probe sequence in the 1AS blast scaffold sequence of *T. urartu*. The screening standard was identities higher than 95%. The Perl script of S. Cartinhour (Temnykh et al. 2000) was used to search for SSRs in the scaffold sequence, and SSR sites with repeats of >10 were used to design SSR markers.

We developed new single nucleotide polymorphism (SNP) markers in 1AS using the allele-specific PCR (AS-PCR) technique (Wangkumhang et al. 2007). Two methods were used to obtain SNP sites between the parents; the first method was wheat 90K chip detection, and the second was RNA sequencing described in *transcriptome analysis*. The chromosomal locus information of the chip-SNP was derived from the reference genetic map of the 90K chip. The flanking sequence information of the chip-SNP was obtained using the probe sequence of the 90K chip blast scaffold sequence of *T. urartu*. The screening standard was identity values higher than 95%. The chromosomal loci and sequence information of

RNA-sequencing SNP were derived from transcriptome analysis. Primer3 was used for primer design (Koressaar and Remm 2007).

Whole-genome sequencing was completed for China Spring (CS), a common wheat cultivar (Mayer et al. 2014). The cDNA sequence, scaffold sequence, and ensemble sequence (http://plants.ensembl.org/Triticum_aestivum/Info/Index) of CS were used in this study. The positions of markers in ensemble chromosome 1A were obtained by marker sequence as query to blast ensemble chromosome 1A sequence.

Detection of molecular markers and construction of the genetic map

Genomic DNA was extracted from young leaves of all plant materials (Allen et al. 2006). We used 120 polymorphic SSR markers published in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml) to genotype the RIL population. PCR amplification was performed in reactions (10 μL final volume) containing 60 ng template DNA, 1 U Taq polymerase, 1× PCR buffer, 200 μmol/L dNTP, and 0.25 μmol/L of primers. DNA was amplified for 5 min at 94°, then 35 cycles of 60 s at 94°, 60 s at 50–65°, and 60 s at 72°; and 10 min at 72° for a final extension. The PCR products were separated on 8% polyacrylamide denaturing gels (Acr:Bis = 19:1) and visualized with silver staining. All marker data were scored by visual inspection, and ambiguous bands were not scored. The genetic map was constructed using QTL IciMapping software (Meng et al. 2015).

Transcriptome analysis

The scaffold sequence of 1AS was used as the reference in this study. Young leaves of two parents and a sequence elimination line from the RILs were used for

158 transcriptome sequencing. Reads of the two parents were mapped to the reference
159 sequence using bwa (Li and Durbin 2009). SAMtools and BCFtools were used for
160 SNP calling (Li et al. 2009; Li 2011). We further obtained SNPs between Pubing3504
161 and Jing4839 through the comparison of the parental SNP calling results. Flanking
162 sequences (60 bp) of these SNPs were used to blat (Kent 2002) reads of the sequence
163 elimination line. The SNPs that did not appear in reads of the sequence elimination
164 line were used to develop SNP markers.

165 **RESULTS**

166 **Detection of sequence elimination using co-dominant SSR markers**

167 To map the breeding traits of Pubing3504, we assessed 336 RIL lines
168 constructed from the hybrid combination between Pubing3504 and Jing4839. We
169 obtained 120 pairs of polymorphic markers covering the 21 pairs of wheat
170 chromosomes using parental genomic DNA to screen SSR markers published at the
171 GrainGenes website. All 120 pairs of polymorphic markers were used to genotype the
172 RIL population. In the RIL population, the normal segregation ratio of the parental
173 genotypes is 1:1. In the genotype result, however, some markers exhibited abnormal
174 separation; the number of lines that had the Pubing3504 genotype was significantly
175 higher than that of lines that had the Jing4839 genotype, and the number of lines that
176 had missing genotype was higher than normal. These abnormally separated markers
177 were *Xwmc24*, *Xpsp2999*, *Xbarc263*, *Xcwm75*, and *Xcfa2153*, all located in 1AS
178 (Table 1).

179 We carried out further research on 1AS. We first developed 13 pairs of new SSR

markers located on 1AS (Table 2). Of these, nine pairs of markers (*SSR11*, *SSR26*, *SSR110*, *SSR113*, *SSR114*, *SSR115*, *SSR122*, *SSR144*, and *SSR365*) could be used to detect the sequence elimination. SSR markers are co-dominant, and polyacrylamide gel electrophoresis (PAGE) can be used to distinguish different genotypes due to molecular weight differences. In this study, SSR markers were able to detect the two parental genotypes and sequence elimination. The results with *SSR11* are shown in Figure 1A as an example. A 1AS genetic linkage map was constructed using the RIL population (Figure 2). The genetic linkage map indicated that the SSR markers that could detect the sequence elimination are concentrated at the end of 1AS. We also found that the junction of the sequence elimination chromosome and the normal chromosome is located between *SSR110* and *SSR283*; the genetic distance between these two markers is 0.98 cM.

The origin of the chromosome segment with sequence elimination

We carried out further studies to determine the origin of the chromosome segment with sequence elimination. Theoretically, it may originate from Pubing3504, from Jing4839, or both. First, we developed dominant SNP markers for Pubing3504 using AS-PCR technology. Ten pairs of SNP markers (*SNP5*, *SNP11*, *SNP26*, *SNP32*, *SNP35*, *SNP45*, *SNP48*, *SNP51*, *SNP54*, and *SNP61*) were assessed and added to the chromosome segment of sequence elimination in the genetic linkage map (Figure 2, Table 3). Sequence elimination did not affect the genetic mapping of these SNP markers. The Jing4839 genotype and sequence elimination both presented as a missing genotype when using these SNP markers to detect RILs. The results with

SNP5 are shown in Figure 1B as an example.

We then developed ten pairs of dominant SNP markers for Jing4839 using AS-PCR technology. They are *SNP_174, SNP_035, SNP_054, SNP_009, SNP_095, SNP_097, SNP_102, SNP_120, SNP_069,* and *SNP_024* (Table 4). These SNP loci were derived from the coding sequences on 1AS. The Pubing3504 genotype and sequence elimination both presented as a missing genotype when using these SNP markers to assess the RIL population. The results with *SNP_174* are shown in Figure 1C as an example. However, because so many of the RILs presented as missing genotype, these markers could not be mapped to the genetic linkage map. Therefore, it is reasonable to consider Jing4839 and sequence elimination as the same genotype to construct the genetic linkage map. Judging from these results, the chromosome segment with sequence elimination originated from Jing4839.

Region selectivity of sequence elimination

This study found that the sequence elimination frequently occurred at an SSR locus. To study the sequence elimination of SNP loci, we designed ten pairs of primers to amplify the sequence flanking these SNP sites (Table 5). Three pairs of these primers (*SNP_SE1*, *SNP_SE4*, and *SNP_SE7*) could be used to detect sequence elimination. These primers effectively amplified genomic DNA of the parental and offspring lines with no sequence elimination, and offspring lines with sequence elimination were indicated as genotype missing. The results with *SNP_SE4* are shown in Figure 1D as an example. These diagnostic primers indicated that sequence elimination occurred in 76 lines of the RIL population. These 76 lines were used in

the following studies.

We further studied the sequence elimination on gene loci. RNA-sequencing was used in the two parents and a sequence elimination line from RILs**.** The results showed that some genes in 1AS were expressed in both parental transcriptomes, but were not expressed in the sequence elimination line. Ten gene loci with sequence elimination are shown in Table 4. These gene sequences have SNPs in common between the parents. We developed Jing4839 dominant SNP markers using these gene sequences. These markers were used to detect gene loci in the 76 RIL lines with sequence elimination, and we found that some gene sequences were eliminated from 1AS. Thus, sequence elimination can affect gene coding sequences in the genome and hence may alter gene expression.

Genetic characteristics of the chromosome segment with sequence elimination

We examined the reorganization in the 336 lines of the RIL population using markers in the chromosome segment with sequence elimination. This chromosome segment in the genetic linkage map contains 14 pairs of SSR markers and 10 pairs of SNP markers (Figure 2). Recombination in the chromosome segment occurred in 60 lines, and recombination did not occur in 276 lines. We found that 184 of the 276 chromosome segments were from Pubing3504, 16 were from Jing4839, and 76 were from sequence elimination. Recombination only occurred between the homologous chromosomes of Pubing3504 and Jing4839 in the 60 RILs.

Finally, we established a genetic model to illustrate the sequence elimination that occurred in hybrid offspring of Pubing3504 and Jing4839 (Figure 3). F1 was obtained

246 by Pubing3504 and Jing4839 hybridization. After F1 selfing, a new chromosome
247 segment with sequence elimination was generated. The three chromosome segments
248 could form six possible chromosome combinations. The combination of Pubing3504
249 and Jing4839 could undergo normal homologous recombination; however, the
250 chromosome with sequence elimination could not undergo homologous
251 recombination with either of the parentally derived chromosome segments.

252 **DISCUSSION**

253 **Characteristics of sequence elimination**

254 Allopolyploidization causes rapid changes in the wheat genome, which improves
255 the speed of evolution. These rapid changes include non-random sequence elimination
256 in coding and non-coding DNA sequences, epigenetic changes in coding and
257 non-coding DNA sequences, and changes in the activity of transposable elements
258 affecting the expression of adjacent genes (Feldman and Levy 2005; Feldman and
259 Levy 2009; Feldman and Levy 2012).

260 Sequence elimination is a major genomic change resulting from
261 allopolyploidization. Eliminated sequences include both repeat and low-copy DNA
262 sequences (Han et al. 2005; Guo and Han 2014). In the wheat genome, more than 80%
263 of the DNA sequence is repeat sequence (Gustafson et al. 2007; Wicker et al. 2011).
264 Because of this, sequence elimination can cause a reduction in the amount of genomic
265 DNA. Some studies have shown that the amount of genomic DNA in common wheat
266 and synthetic hexaploid wheat is less than the total amount of the parents (Ozkan et al.
267 2003; Gustafson et al. 2008; Eilam et al. 2010). Low-copy sequences in the wheat

genome can be divided into genome-specific sequences found only in the A, B, or D genome and chromosome-specific sequences, for example, only in the 1A, 1B, and 1D chromosomes. Some studies found that some chromosome-specific sequences are eliminated from a genome or two genomes in common wheat and synthetic hexaploid wheat compared with their diploid progenitors (Feldman et al. 1997; Liu et al. 1998B; Ozkan et al. 2001).

In this study, we found that sequence elimination occurred in the hybrid offspring of Pubing3504 and Jing4839. We observed sequence elimination, all occurring at the end of 1AS. The eliminated sequences we found are low-copy sequences in the genome. These sequences are both A genome–specific sequences and 1A chromosome–specific sequences, which are commonly used for marker development to construct the genetic linkage map. We observed sequence elimination in both SSR loci and SNP loci. At these loci, there are base differences between Pubing3504 and Jing4839. Thus, base differences between the parents may trigger the sequence elimination. We also found that sequence elimination occurred in the gene coding sequence. Some gene coding sequences were eliminated from the genome through sequence elimination. These genes could be expressed in the transcriptome of Pubing3504 and Jing4839, but were not expressed in the transcriptome of the line with sequence elimination. Therefore, sequence elimination can affect gene expression.

Sequence elimination is asymmetric or preferential to different genomes. In the octoploid triticale genome (AABBDDRR), the wheat genome (AABBDD) is

290 conservative, and the rye genome (RR) has undergone great changes (Ma et al. 2004).
291 Some studies have indicated that sequence elimination tends to be in the larger
292 genome (Schwarzacher et al. 2011). The preference of sequence elimination results in
293 gene expression as a diploid pattern (Le Comber et al. 2010; Qi et al. 2012). In this
294 study, we found that the sequence elimination was also asymmetric.

295 Sequence elimination usually occurs in the early generations after hybridization.
296 Previous studies showed that the sequence elimination may take place in the F1
297 generation after hybridization (Salina et al. 2004). We previously demonstrated
298 sequence elimination in F2 after hybridization between Pubing3504 and Jing4839
299 (Chen 2012). In the F1 generation, the chromosome doubling is not yet complete
300 (Ozkan et al. 2001; Han et al. 2003; Ma and Gustafson 2006). Therefore, the
301 elimination of sequences is not caused by chromosome doubling. Some studies
302 suggest that sequence elimination may be caused by DNA methylation (Han et al.
303 2003; Guo and Han 2014). The reason for the sequence elimination is not yet
304 definitively known.

305 **Sequence elimination causes chromosome differentiation**

306 The genome of common wheat is composed of seven parts of homologous
307 groups. For example, the first homologous group includes the 1A, 1B, and 1D
308 chromosomes. All chromosomes in a homologous group have a common ancestor, so
309 they have similar gene order and DNA sequences. Although they are homologous
310 chromosomes, they do not pair during meiosis. One reason for this is that a major
311 gene *Ph1*, which is mapped in 5BL, allows homologous chromosome pairing from the

312 same genome (for example between 1A and 1A) and inhibits non-homologous
313 chromosome pairing and homologous chromosome pairing from different genomes
314 (for example between 1A and 1B, between 1A and 1D, or between 1B and 1D) that
315 have evolved in the common wheat genome. So far, the mechanism by which *Ph1*
316 controls homologous chromosome pairing is not clear (Sears 1976).

317 Sequence elimination is another mechanism to inhibit homologous chromosome
318 pairing from different genomes in common wheat, which causes differentiation of
319 homologous chromosomes from different genomes to inhibit chromosome pairing
320 between them (Feldman et al. 1997; Ozkan et al. 2001; Feldman and Levy 2012). In
321 previous studies, 18 newly formed allopolyploids were used to study chromosome
322 pairing in meiosis and seed fertility in S1, S2, and S3 generations; the results show
323 that the number of bivalents in meiosis and the seed fertility increase with the increase
324 of the generation of the allopolyploid. The cytological behavior of the allopolyploid
325 was considered to begin as the diploid in early generations after chromosome
326 differentiation caused by sequence elimination (Gustafson et al. 2009). In our research,
327 we used high-density molecular markers to detect the chromosome segment with
328 sequence elimination in RILs, and we found that there was no homologous
329 recombination in this chromosome segment. We think that the sequence elimination
330 caused the differentiation of chromosomes, and the chromosome differentiation
331 affected the homologous pairing at this chromosome segment in meiosis, which
332 further affected the occurrence of homologous recombination at this chromosome
333 segment.

334 **Acknowledgments**

335 This work was supported by the Agricultural Science and Technology Innovation
336 Program of CAAS and the National Key Technologies R&D Program of China during
337 the 12th Five-Year Plan period (grant No. 2013BAD01B02).

338

339 **Table 1. The detection result of the RIL population using 120 pairs of SSR markers in GrainGenes 2.0**

Marker name	Chromosome	Number of Pubing3504 genotypes	Number of J4839 genotypes	Number of missing genotypes	X^2 value
Xwmc24	1A	227	33	76	144.94
Xbarc263	1A	223	37	76	131.22
Xpsp2999	1A	225	35	76	136.99
Xcwm75	1A	224	36	76	132.14
Xcfa2153	1A	226	34	76	141.78
Xbarc105	7D	176	159	1	0.76
Xbarc117	5A	175	161	0	0.58
Xbarc12	3A	161	175	0	0.58
Xbarc121	7A\7D	171	163	2	0.30
Xbarc142	5B	184	152	0	3.05
Xbarc147	3B	177	159	0	0.96
Xbarc176	7B	184	151	1	3.44
Xbarc177	5D	171	165	0	0.11
Xbarc186	5A	167	169	0	0.01
Xbarc21	6D	189	147	0	5.25
Xbarc232	5B	175	159	2	0.96
Xbarc252	7D	185	151	0	3.44
Xbarc292	7A	175	161	0	0.58
Xbarc310	3A	172	164	0	0.19
Xbarc322	5D	168	168	0	0.00
Xbarc3220	NA	164	170	2	0.19
Xbarc324	3A	174	162	0	0.43
Xbarc49	7A	181	155	0	2.01
Xbarc50	7B	180	156	0	1.71
Xbarc59	5B	171	162	3	0.43
Xbarc67	3A	180	156	0	1.71
Xbarc68	6B	175	161	0	0.58
Xbarc70	7A	190	146	0	5.76
Xbarc72	7B	175	161	0	0.58
Xbarc75	3B	166	170	0	0.05
Xbarc90	7B	171	165	0	0.11
Xcfa161	NA	172	162	2	0.43
Xcfa2028	7A\7B	162	174	0	0.43
Xcfa2147	1A\1B\1D	169	167	0	0.01
Xcfa2219	1A	170	166	0	0.05
Xcfa2256	4A	180	151	5	3.44
Xcfa2263	2A	157	179	0	1.44
Xcfd49	6D	161	174	1	0.58
Xcfd54	4B\4D	173	163	0	0.30
Xcfd55	3D	168	165	3	0.00

Xcfd8	5D	166	170	0	0.05
Xcfd84	4D	172	164	0	0.19
Xgwm122	2A	165	169	2	0.11
Xgwm126	5A	182	154	0	2.33
Xgwm132	6B\6D	177	159	0	0.96
Xgwm140	1B	173	163	0	0.30
Xgwm149	4B\4D	162	174	0	0.43
Xgwm155	3A	163	173	0	0.30
Xgwm182	5D	179	157	0	1.44
Xgwm213	5B	183	153	0	2.68
Xgwm219	6B	148	188	0	4.76
Xgwm232	1D	157	179	0	1.44
Xgwm251	4B\4D	152	184	0	3.05
Xgwm294	2A	157	175	4	1.44
Xgwm297	7B	174	162	0	0.43
Xgwm3	3D	162	174	0	0.43
Xgwm304	5A	175	155	6	2.01
Xgwm310	NA	171	165	0	0.11
Xgwm312	2A	149	187	0	4.30
Xgwm333	7B	175	158	3	1.19
Xgwm334	6A	159	177	0	0.96
Xgwm337	1B\1D	153	183	0	2.68
Xgwm350	4A	188	148	0	4.76
Xgwm376	3B	163	173	0	0.30
Xgwm389	3B	163	173	0	0.30
Xgwm397	4A	185	147	4	5.25
Xmag3810	7A	173	163	0	0.30
Xpsp3000	1B\1D	164	172	0	0.19
Xwmc1	6A	163	173	0	0.30
Xwmc170	2A	162	174	0	0.43
Xwmc177	2A	184	152	0	3.05
Xwmc198	2A	160	176	0	0.76
Xwmc222	1B\1D	168	168	0	0.00
Xwmc238	4B	156	180	0	1.71
Xwmc256	6A	160	176	0	0.76
Xwmc283	7A	188	148	0	4.76
Xwmc2830	NA	171	165	0	0.11
Xwmc296	2A	166	170	0	0.05
Xwmc326	3B	154	180	2	2.33
Xwmc335	7B	172	164	0	0.19
Xwmc338	7B	185	149	2	4.30
Xwmc364	7B	174	162	0	0.43
Xwmc382	2A\2B	181	152	3	3.05
Xwmc407	2A	173	163	0	0.30

Xwmc415	5B	155	181	0	2.01
Xwmc44	1B	153	183	0	2.68
Xwmc47	4B	175	161	0	0.58
Xwmc476	7B	173	163	0	0.30
Xwmc487	6B	160	176	0	0.76
Xwmc494	6B	161	175	0	0.58
Xwmc503	2D	184	152	0	3.05
Xwmc506	7D	180	153	3	2.68
Xwmc532	3A	161	175	0	0.58
Xwmc533	3B\3D	182	151	3	3.44
Xwmc631	3D	158	178	0	1.19
Xwmc634	7D	171	165	0	0.11
Xwmc651	2A\3A	167	169	0	0.01
Xwmc696	7B	170	166	0	0.05
Xwmc710	4B	150	186	0	3.86
Xwmc720	4D	172	164	0	0.19
Xwmc728	1B	170	166	0	0.05
Xwmc752	5A	185	146	5	5.76
Xwmc758	7B	177	159	0	0.96
Xwmc765	5D	175	161	0	0.58
Xwmc766	1B	180	156	0	1.71
Xwmc773	6D	172	164	0	0.19
Xwmc783	5B	163	173	0	0.30
Xwmc93	1A\1D	180	152	4	3.05
XgwmA413	1B	173	163	0	0.30
XgwmA425	2A	167	169	0	0.01
XgwmA437	7D	165	171	0	0.11
XgwmA459	6A	162	174	0	0.43
XgwmA484	2D	174	162	0	0.43
XgwmA493	3B	166	170	0	0.05
XgwmA499	5B	168	168	0	0.00
Xgwm52	3D	181	150	5	3.86
Xgwm566	3B	173	163	0	0.30
Xgwm583	5D	168	168	0	0.00
Xgwm617	5A\6A	153	183	0	2.68
Xwmc710	4B	151	185	0	3.44

340

341 **Table 2. The co-dominant SSR markers in 1AS developed in this research[a]**

Marker name	Left primer sequence (5′-3′)	Right primer sequence (5′-3′)	Scaffold number	KD number
SSR11	AATGCGTTTGCAGTGTTTTG	GCCCGACTTCATATCAAAGG	scaffold35243	KD115173
SSR12	ATTTATGATGTGCTTGGAGCAGT	CTTTGTTTTTCACGAAAATGGTC	scaffold59118	KD212170
SSR26	CTTACTTGCATGCCCTACCTTC	TGCATGCTAAGCTAGAGAGACG	scaffold3016	KD255423
SSR110	CACCCGTTAATTCCGATAAAGA	AATGTCCAGCCTTCTCCTACAG	scaffold132282	KD052721
SSR113	CATACCCGGGAATTCCTTCT	TTCTTCAAGCATGCAAGGTG	scaffold19707	KD100855
SSR114	AAAAATTCTTTCGCCCGACT	ATTCTGCACCCTGGGTTATG	scaffold5139	KD103520
SSR115	AACCCATTTCCAGGAAGACA	CGGCCTTGATCCTTGTATCT	scaffold91171	KD106287
SSR122	GCTTCCTAGTAGACGAGCCTGAG	GAGATTTCCTGAGGCTAACGATT	scaffold51260	KD149568
SSR144	CTCTTCCCCGCTCTTGTGT	AGGTGCGTCTTGTGGATCG	scaffold4334	KD211995
SSR156	CTGCGTCTCTCCTCCTATTGTC	CGAGAACAAGATGTGGGTAGTG	scaffold13889	KD028551
SSR263	CTGTTCTCGCTTTTGCTTTACAT	TTTTGAAGAGTGTTTTTGTGTGTG	scaffold36257	KD024925
SSR283	GGGTTGCAATAGTTGCTTCAA	CACGCACACTCTCTCCCTTAC	scaffold86180	KD152767
SSR365	CGGGGTCCTATTTTGGAGA	CTCCTTCCTGCCCTTAGCA	scaffold69185	KD033102

342 [a]These markers were developed using the scaffold sequences of *Triticum urartu*

343

344 **Table 3. The dominant SNP markers of Pubing3504 in 1AS developed in this research[a]**

Marker name	Left primer sequence (5′-3′)	Right primer sequence (5′-3′)	SNP name in 90K chip	Scaffold number	KD number
SNP5	TCAGGCATGCATAAGTACCGA	AGTTCAACCGGTGCAGCTAT	*Excalibur_c11258_1700*	scaffold14562	KD046732
SNP11	TTCGTCTATGATGCAGGATGC	TTGACCCAAACAAAGCACAA	*BobWhite_c46501_92*	scaffold152900	KD142770
SNP26	AGGTTTTTGCATGGTCCAGTT	GGCATTATAGGCCGTCTTGA	*BS00022270_51*	scaffold35848	KD274627
SNP32	GTCACATTCATATGGCAAACC	CTGCCTTTCAACCTCTTTGG	*Excalibur_c20777_315*	scaffold52299	KD115293
SNP35	GCAGTGTTGACAACTAGGGCA	GCCAATTCCTGTAGGTCCAA	*BS00074511_51*	scaffold227092	KD126126
SNP45	GTAGCCAATGGAATTTGGAGC	TAAGGCCCAACAGTTTCCTG	*wsnp_RFL_Contig4735_5673999*	scaffold10430	KD211942
SNP48	CCCTCATACAGGTTCTCCAAG	GAAGGTTGGGGTGAACTTGA	*tplb0043h23_1346*	C167212184	KD498128
SNP51	TGTTGCCGCTAACAAACTAAC	TGTCAACAGTGTGCCATGTG	*wsnp_Ex_c2868_5293485*	scaffold69185	KD033102
SNP54	GCAAACTGATCTGTTCTTGGA	ACTGCCTCCTTCTGGTTCTG	*BS00110709_51*	scaffold35848	KD274627
SNP61	GAGTTTGCTGGAGAGAATGCC	GCCTCACCCTCACTTTCAAC	*BobWhite_c1361_1187*	scaffold8519	KD250690

345 [a]These markers were developed using the scaffold sequences of *Triticum urartu*

346

347 **Table 4. The dominant SNP markers of Jing4839 in 1AS developed in this research[a]**

Marker name	Left primer sequence (5′-3′)	Right primer sequence (5′-3′)	Scaffold number	Gene number[b]	Position[c]
SNP_174	TCAAGCATCAAGCGTTCCTT	GTGCTGGATGAGGGTGAAGT	1AS_3311520	Traes_1AS_972AA6CB4	169086
SNP_035	AGTTTGCTGGAGAGAATGCC	CACTACCAGAAGCGTCCTCG	1AS_3313778	Traes_1AS_49D585BA6	410323
SNP_054	CAGAACCACTGTCTGCTGGA	CCTTCTGCAGTAAACGCCTC	1AS_3271165	Traes_1AS_87B7A8114	1470541
SNP_009	AACTATGAAATGCACCGGCA	AACTTGTCGATGTCGATCCC	1AS_3274088	Traes_1AS_097B121E3	1527571
SNP_095	CTGGAGCAGCTGCCATACAA	AAGAGGAGAGGTTTGGCTCC	1AS_3287754	Traes_1AS_8BAC45B9A1	3222564
SNP_097	CTGGTCACAAACTTCTCCCG	TCCACTGGTATTTGAACCGA	1AS_3307156	Traes_1AS_429D67C42	3607350
SNP_102	GAAATGCATGAGCTTCGACA	TGCAGAATTAATTGGCAGGA	1AS_2146315	Traes_1AS_4369A7326	4049964
SNP_120	GCATTCTGTCAGCAGCGATT	AGCCACAAAGAACCGAGCTA	1AS_3266548	Traes_1AS_20FF2F450	4691663
SNP_069	CCGCACCTTTATCTTCTTTG	TGTCTTTCTCTCCACTCCGC	1AS_3283727	Traes_1AS_E700FE9D9	5200276
SNP_024	ACTAAACTGTAGATGCGCCG	TGATGGATAATTCCGATATGCT	1AS_3314952	Traes_1AS_7BFBBDCE6	8325073

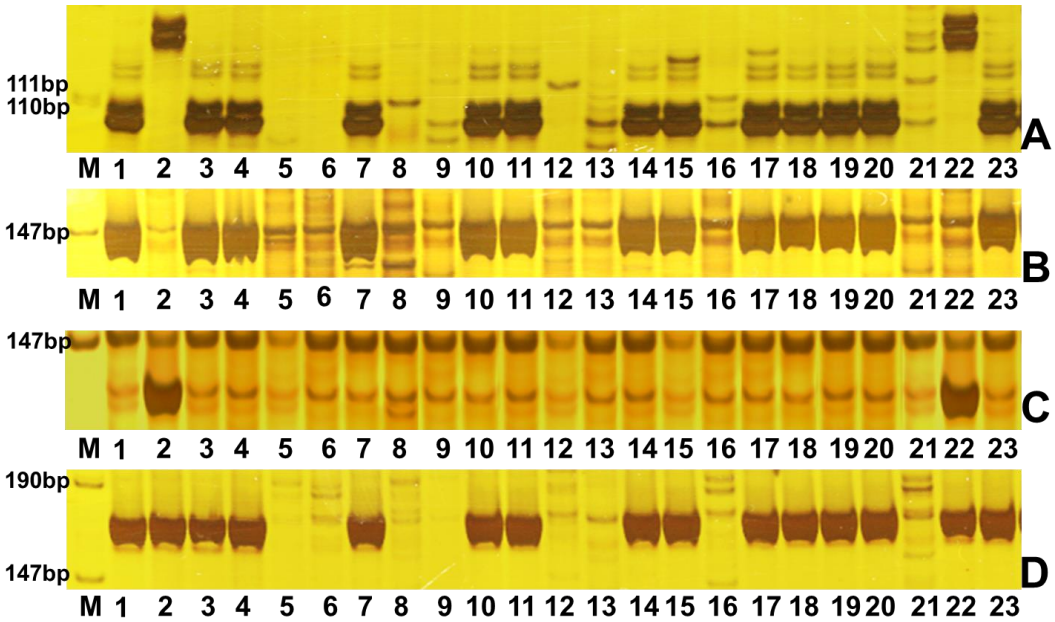
348 [a]These markers were developed using the scaffold sequences of CS. [b]Gene number is the cDNA
349 number of CS. [c]Position is the position in the assembled genome of CS.
350

351 **Table 5. Primers used to detect sequence elimination of SNP loci** [a]

Primer name	Left primer sequence (5′-3′)	Right primer sequence (5′-3′)	SNP loci
SNP_SE1	TTCATAGTGCCCCGTACCTC	CTGAGGCATCCAAAGGACAT	*SNP11*
SNP_SE2	ACTGCCTCCTTCTGGTTCTG	GATGCCTTCGAAGCTACCAT	*SNP26*
SNP_SE3	CGCTTGTGGTCGGAGATAAG	TGCAAAATAGCAGCCATGAG	*SNP35*
SNP_SE4	CCGTCATACCCAGGGATACA	TTGGACGGGTACCTATGGTG	*SNP5*
SNP_SE5	GTGCGAAATACGGAGCTAGG	GCTCATGATCAGAAACCCAAA	*SNP45*
SNP_SE6	ACCTCCCCTCTCCGTTAGAA	ACATCAGATGCTGGGACCAT	*SNP48*
SNP_SE7	AACAGTGTGCCATGTGTATGC	ACAGTGTTGCCGCTAACAAAC	*SNP51*
SNP_SE8	ACTGCCTCCTTCTGGTTCTG	GATGCCTTCGAAGCTACCAT	*SNP54*
SNP_SE9	TTCACTACCAGAAGCGTCCTC	GCTTTGAAGCCGAAAGAAGTT	*SNP61*
SNP_SE10	TTGCTAGGTCAAATGCAACAA	TCGTTAGCATCACCTCCATTC	*SNP32*

352 [a]These markers were developed using the scaffold sequences of *Triticum urartu*

353



354

355 **Figure 1. Detection of different markers in parents and RILs.**

356 A, PAGE result of the co-dominant SSR marker *SSR11*. B, PAGE result of the Pubing3504 dominant

357 SNP marker *SNP5*. C, PAGE result of the Jing4839 dominant SNP marker *SNP_174*. D, PAGE result

358 of primer *SNP_SE4*. M is marker lane; lane 1 is Pubing3504; lane 2 is Jing4839. Lanes 3 to 23

359 are RILs. Lanes 5, 6, 8, 9, 12, 13, 16, and 21 are sequence elimination lines.

360

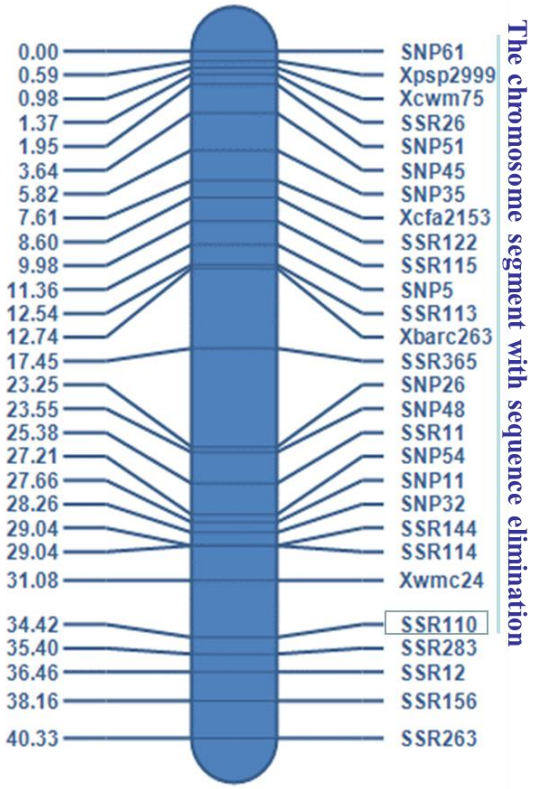


Figure 2. Genetic linkage map of 1AS constructed using the RIL population.

Sequence elimination occurred in the chromosome segment from *SSR110* (boxed) to the end of 1AS. This chromosome segment includes 14 pairs of co-dominant SSR markers and 10 pairs of dominant SNP markers of Pubing3504.

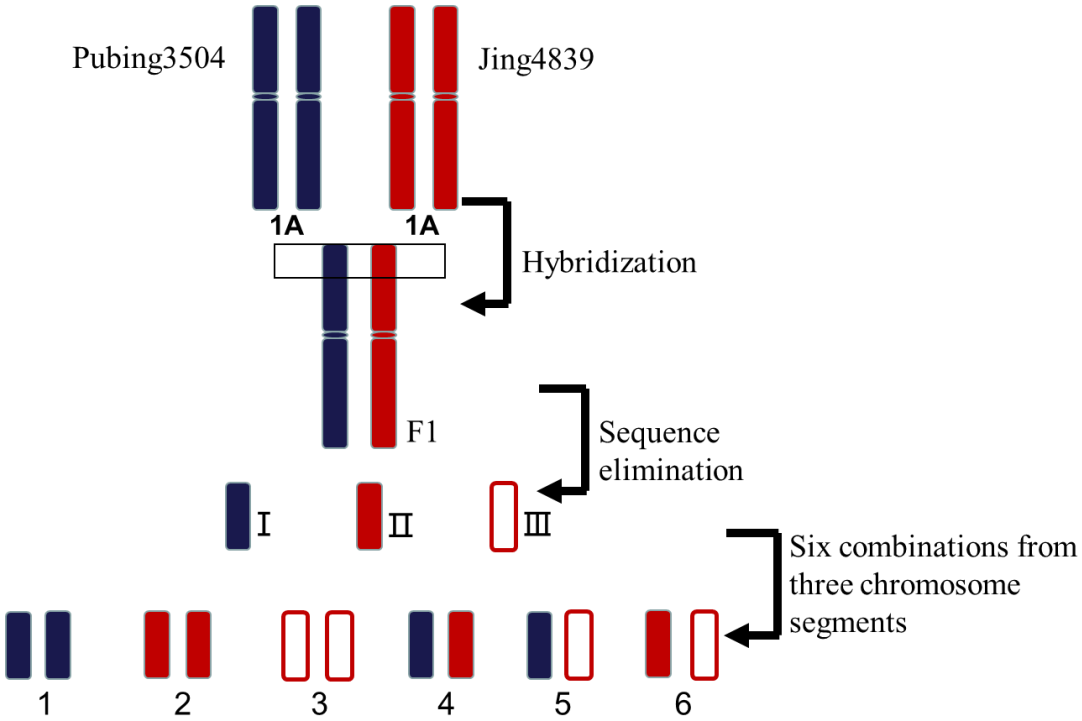


Figure 3. Genetic model for the sequence elimination examined in this research.

F1 was obtained by Pubing3504 and Jing4839 hybridization. After F1 selfing, a new chromosome segment with sequence elimination was generated. The chromosome segments from Pubing3504 (blue) and Jing4839 (red) and the new chromosome segment with sequence elimination (white) are boxed. These chromosome segments could form six combinations; combinations 1–3 are homozygous, combinations 4–6 are heterozygous. Combination 4 (between Pubing3504 and Jing4839) can undergo normal homologous recombination, but combination 5 (between Pubing3504 and sequence elimination) and combination 6 (between Jing4839 and sequence elimination) cannot.

References

Adams, K. L., and J. F. Wendel, 2005 Novel patterns of gene expression in polyploid plants. TRENDS in Genetics 21**:** 539-543.

Akhunov, E. D., A. R. Akhunova, O. D. Anderson, J. A. Anderson, N. Blake *et al.*, 2010 Nucleotide diversity maps reveal variation in diversity among wheat genomes and chromosomes. BMC genomics 11**:** 1.

Allen, G., M. Flores-Vergara, S. Krasynanski, S. Kumar and W. Thompson, 2006 A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nature protocols 1**:** 2320-2325.

Chagué, V., J. Just, I. Mestiri, S. Balzergue, A. M. Tanguy *et al.*, 2010 Genome-wide gene expression changes in genetically stable synthetic and natural wheat allohexaploids. New Phytologist 187**:** 1181-1194.

Chen, D., 2012 Genetics and Utilization of Noval Wheat-*Agropyron cristatum* Germplasm with Multi-Kernel. Doctoral Dissertation in Chinese Academy of Agricultural Sciences.

Chen, D., J. P. Zhang, J. S. Wang, X. M. Yang, W. H. Liu *et al.*, 2012 Inheritance and availability of high grain number per spike in two wheat germplasm lines. Journal of Integrative Agriculture 11**:** 1409-1416.

Chen, Z. J., 2007 Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annual review of plant biology 58**:** 377.

Comai, L., 2000 Genetic and epigenetic interactions in allopolyploid plants. Plant

molecular biology 43**:** 387-399.

Dong, Y. C., and L. H. Li., 1993 Progress in Studies of Agropyron Gaertn. Hereditas (Beijing) 1**:** 014.

Eilam, T., Y. Anikster, E. Millet, J. Manisterski and M. Feldman, 2010 Genome size in diploids, allopolyploids, and autopolyploids of Mediterranean Triticeae. Journal of Botany 2010.

Feldman, M., and A. Levy, 2005 Allopolyploidy-a shaping force in the evolution of wheat genomes. Cytogenetic and genome research 109**:** 250-258.

Feldman, M., and A. A. Levy, 2009 Genome evolution in allopolyploid wheat-a revolutionary reprogramming followed by gradual changes. Journal of Genetics and Genomics 36**:** 511-518.

Feldman, M., and A. A. Levy, 2012 Genome evolution due to allopolyploidization in wheat. Genetics 192**:** 763-774.

Feldman, M., B. Liu, G. Segal, S. Abbo, A. A. Levy *et al.*, 1997 Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147**:** 1381-1387.

Gaeta, R. T., J. C. Pires, F. Iniguez-Luy, E. Leon and T. C. Osborn, 2007 Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. The Plant Cell 19**:** 3403-3417.

Guo, X., and F. Han, 2014 Asymmetric epigenetic modification and elimination of rDNA sequences by polyploidization in wheat. The Plant Cell 26**:** 4311-4327.

Gustafson, P., T. Eilam, Y. Anikster, E. Millet, J. Manisterski *et al.*, 2008 Nuclear

DNA amount and genome downsizing in natural and synthetic allopolyploids of the genera Aegilops and Triticum. Genome 51**:** 616-627.

Gustafson, P., T. Eilam, Y. Anikster, E. Millet, J. Manisterski *et al.*, 2007 Genome size and genome evolution in diploid Triticeae species. Genome 50**:** 1029-1037.

Gustafson, P., H. Ozkan and M. Feldman, 2009 Rapid cytological diploidization in newly formed allopolyploids of the wheat (Aegilops-Triticum) group. Genome 52**:** 926-934.

Ha, M., J. Lu, L. Tian, V. Ramachandran, K. D. Kasschau *et al.*, 2009 Small RNAs serve as a genetic buffer against genomic shock in Arabidopsis interspecific hybrids and allopolyploids. Proceedings of the National Academy of Sciences 106**:** 17835-17840.

Han, F. P, G. Fedak, W. Guo and B. Liu, 2005 Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics 170**:** 1239-1245.

Han, F. P, G. Fedak, T. Ouellet and B. Liu, 2003 Rapid genomic changes in interspecific and intergeneric hybrids and allopolyploids of Triticeae. Genome 46**:** 716-723.

Kashkush, K., M. Feldman and A. A. Levy, 2002 Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics 160**:** 1651-1659.

Kashkush, K., M. Feldman and A. A. Levy, 2003 Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nature genetics 33**:** 102-106.

444 Kent, W. J., 2002 BLAT-the BLAST-like alignment tool. Genome research 12**:**
445 656-664.
446 Koressaar, T., and M. Remm, 2007 Enhancements and modifications of primer design
447 program Primer3. Bioinformatics 23**:** 1289-1291.
448 Le Comber, S., M. Ainouche, A. Kovarik and A. Leitch, 2010 Making a functional
449 diploid: from polysomic to disomic inheritance. New Phytologist 186**:**
450 113-122.
451 Leitch, A., and I. Leitch, 2008 Genomic plasticity and the diversity of polyploid plants.
452 Science 320**:** 481-483.
453 Levy, A. A., and M. Feldman, 2004 Genetic and epigenetic reprogramming of the
454 wheat genome upon allopolyploidization. Biological Journal of the Linnean
455 Society 82**:** 607-613.
456 Li, H., 2011 A statistical framework for SNP calling, mutation discovery, association
457 mapping and population genetical parameter estimation from sequencing data.
458 Bioinformatics 27**:** 2987-2993.
459 Li, H., and R. Durbin, 2009 Fast and accurate short read alignment with
460 Burrows–Wheeler transform. Bioinformatics 25**:** 1754-1760.
461 Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan *et al.*, 2009 The sequence
462 alignment/map format and SAMtools. Bioinformatics 25**:** 2078-2079.
463 Ling, H.-Q., S. Zhao, D. Liu, J. Wang, H. Sun *et al.*, 2013 Draft genome of the wheat
464 A-genome progenitor Triticum urartu. Nature 496**:** 87-90.
465 Liu, B., J. Vega and M. Feldman, 1998a Rapid genomic changes in newly synthesized

amphiploids of Triticum and Aegilops. II. Changes in low-copy coding DNA sequences. Genome 41**:** 535-542.

Liu, B., J. Vega, G. Segal, S. Abbo, M. Rodova *et al.*, 1998b Rapid genomic changes in newly synthesized amphiploids of Triticum and Aegilops. I. Changes in low-copy noncoding DNA sequences. Genome 41**:** 272-277.

Lu, Y. Q., X. Y. Wu, M. M. Yao, J. P. Zhang, W. H. Liu *et al.*, 2015 Genetic mapping of a putative Agropyron cristatum-derived powdery mildew resistance gene by a combination of bulked segregant analysis and single nucleotide polymorphism array. Molecular Breeding 35**:** 1-13.

Ma, X. F., P. Fang and J. P. Gustafson, 2004 Polyploidization-induced genome variation in triticale. Genome 47**:** 839-848.

Ma, X. F., and J. P. Gustafson, 2006 Timing and rate of genome variation in triticale following allopolyploidization. Genome 49**:** 950-958.

Mayer, K. F., J. Rogers, J. Doležel, C. Pozniak, K. Eversole *et al.*, 2014 A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345**:** 1251788.

McClintock, B., 1993 *The significance of responses of the genome to challenge*. Singapore: World Scientific Pub. Co.

Meng, L., H. Li, L. Zhang and J. Wang, 2015 QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3**:** 269-283.

Nalam, V. J., M. I. Vales, C. J. Watson, S. F. Kianian and O. Riera-Lizarazu, 2006

488 Map-based analysis of genes affecting the brittle rachis character in tetraploid
489 wheat (Triticum turgidum L.). Theoretical and Applied Genetics 112**:** 373-381.
490 Otto, S. P., 2007 The evolutionary consequences of polyploidy. Cell 131**:** 452-462.
491 Ozkan, H., A. A. Levy and M. Feldman, 2001 Allopolyploidy-induced rapid genome
492 evolution in the wheat (Aegilops–Triticum) group. The Plant Cell 13**:**
493 1735-1747.
494 Ozkan, H., M. Tuna and K. Arumuganathan, 2003 Nonadditive changes in genome
495 size during allopolyploidization in the wheat (Aegilops-Triticum) group.
496 Journal of Heredity 94**:** 260-264.
497 Peng, J., Y. Ronin, T. Fahima, M. S. Röder, Y. Li *et al.*, 2003 Domestication
498 quantitative trait loci in Triticum dicoccoides, the progenitor of wheat.
499 Proceedings of the National Academy of Sciences 100**:** 2489-2494.
500 Pontes, O., R. J. Lawrence, N. Neves, M. Silva, J.-H. Lee *et al.*, 2003 Natural
501 variation in nucleolar dominance reveals the relationship between nucleolus
502 organizer chromatin topology and rRNA gene transcription in Arabidopsis.
503 Proceedings of the National Academy of Sciences 100**:** 11418-11423.
504 Pontes, O., N. Neves, M. Silva, M. S. Lewis, A. Madlung *et al.*, 2004 Chromosomal
505 locus rearrangements are a rapid response to formation of the allotetraploid
506 Arabidopsis suecica genome. Proceedings of the National Academy of
507 Sciences 101**:** 18240-18245.
508 Qi, B., W. Huang, B. Zhu, X. Zhong, J. Guo *et al.*, 2012 Global transgenerational
509 gene expression dynamics in two newly synthesized allohexaploid wheat

(Triticum aestivum) lines. BMC biology 10**:** 1.

Salina, E., O. Numerova, H. Ozkan and M. Feldman, 2004 Alterations in subtelomeric tandem repeats during early stages of allopolyploidy in wheat. Genome 47**:** 860-867.

Schwarzacher, T., M. Bento, J. P. Gustafson, W. Viegas and M. Silva, 2011 Size matters in Triticeae polyploids: larger genomes have higher remodeling. Genome 54**:** 175-183.

Sears, E. R., 1976 Genetic control of chromosome pairing in wheat. Annual review of genetics 10**:** 31-51.

Shaked, H., K. Kashkush, H. Ozkan, M. Feldman and A. A. Levy, 2001 Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. The Plant Cell 13**:** 1749-1759.

Temnykh, S., W. D. Park, N. Ayres, S. Cartinhour, N. Hauck et al., 2000 Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 100: 697-712.

Wang, J. S., W. H. Liu, H. Wang, L. H. Li, J. Wu *et al.*, 2011 QTL mapping of yield-related traits in the wheat germplasm 3228. Euphytica 177**:** 277-292.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao *et al.*, 2014 Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12**:** 787-796.

Wangkumhang, P., K. Chaichoompu, C. Ngamphiw, U. Ruangrit, J. Chanprasert *et al.*,

2007 WASP: a Web-based Allele-Specific PCR assay designing tool for detecting SNPs and mutations. BMC genomics 8**:** 1.

Wendel, J. F., 2000 Genome evolution in polyploids, pp. 225-249 in *Plant molecular evolution*. Springer.

Wicker, T., K. F. Mayer, H. Gundlach, M. Martis, B. Steuernagel *et al.*, 2011 Frequent gene movement and pseudogene evolution is common to the large and complex genomes of wheat, barley, and their relatives. The Plant Cell 23**:** 1706-1718.