Variation and diversity of the breakpoint sequences on 4AL for 4AL/5AL translocation in Triticum

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1 Variation and diversity of the breakpoint sequences on 4AL for
2 4AL/5AL translocation in *Triticum*

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Abstract: The translocation of 4AL/5AL in *Triticum*, occurred before the differentiation of *T. urartu* and einkorn, is an important chromosomal rearrangement incident. Recently the first identification of breakpoint sequence on 4AL for this translocation provides the chance to analyze the variation and diversity of this breakpoint in *Triticum*. In this study, the breakpoint regions of 52 accessions from 21 species were isolated and further characterized. The sequences were divided into 12 types based on length differences ranging from 2009 to 2552 bp. Cluster analysis showed that they were further divided into three groups. Interesting evolutionary relationships among a few of the species were observed and discussed. Multiple sequence alignment of the 52 sequences detected 13 insertion and deletion length polymorphism (InDel) and 101 single nucleotide polymorphism (SNP). Besides, several species or accession specific SNP or InDel were also identified. Based on BLAST analysis of the conserved sequences, the breakpoint was narrowed down to a 125 bp fragment. Taken together, the results obtained in this study enrich our understanding of the breakpoints and will be helpful for identification of other breakpoints in wheat.

Key words: 4AL/5AL translocation, *Triticum*, breakpoint, diversity

44 Introduction

45 Chromosomal rearrangements play an important role in the evolution of various
 46 species (Morrow and Cooper 2012; Sankoff and Nadeau 2003). Previous studies have
 47 identified quite a few of chromosomal rearrangements in wheat (Berkman et al. 2012;
 48 Devos et al. 1995; Ma et al. 2015a; Ma et al. 2013; Ma et al. 2014; Ma et al. 2015b;
 49 Nelson et al. 1995). The most well-known of such rearrangements are the
 50 non-homoeologous translocations (4A-5A-7B) occurred between chromosomes 4A,
 51 5A and 7B in the hexaploid wheat (*Triticum aestivum* L., $2n=6x=42$, genomes
 52 AABBDD). It is widely believed that the 4AL/5AL translocation occurred at the
 53 diploid level and the 4A/7B translocation must have occurred at the tetraploid level as
 54 it is also present in *T. durum* ($2n=4x=28$, genomes AABB) (Devos et al. 1995). Most
 55 recently, the breakpoints of the 4AL/5AL translocation were identified and the 4AL
 56 junction was narrowed down to ~1000 bp in *T. monococcum*, *T. urartu* and *T.*
 57 *aestivum* (Li et al. 2016).

58 It was documented that more than ten species or subspecies were classified into
 59 *Triticum* (Briggle and Reitz 1963; Dong 1979). Scientists have long been focused on
 60 studying the origin of the species. To date, geneticists still hold different opinions on
 61 the origins of some species. For example, Yang et al. (1992b) thought that Tibetan
 62 semi-wild wheat (*T. aestivum* ssp. *tibetanum*) is the progeny of tetraploid wheat and
 63 Chinese *Aegilops tauschii* Cosson, while Tsunewaki et al. (1990) suggested it is the
 64 offspring of Tibetan common wheat. Chen et al. (1985) thought Xinjiang rice wheat (*T.*
 65 *petropavlovskyi*) was evolved from natural hybridization of *T. polonicum* with
 66 common wheat and then derived from the backcross with common wheat, yet Yang et
 67 al. (1992a) and Chen (1999) inferred it came from the distant hybridization
 68 descendant of *T. polonicum* and *A. tauschii*. In sunflower, chromosomal
 69 rearrangements play a substantial role in adaptive divergences, species integrity and
 70 genetic identity in extensive hybridization and gene flow (Strasburg et al. 2009). Thus,
 71 analysis of the breakpoints in *Triticum* could benefit our understanding of the origin

72 and evolution of some species.

73 Genetic variation is directly involved into the environmental adaptability of plant
74 (Nevo et al. 1982; Prentis et al. 2008). In *Triticum* species, the evolutionary processes
75 might cause genetic variations of a given gene or a sequence (Gautier et al. 2000;
76 Huang et al. 2002b; Ma et al. 2017). Therefore, analysis of different genes could help
77 to understand the origin and evolution of the *Triticum* species. For example, Xu et al.
78 (2009) detected 37 high molecular weight glutenin subunits alleles in diploid,
79 tetraploid and hexaploid wheat, and they presumed that *T. carthlicum* might
80 experience a different evolutionary way with other tetraploid wheat given their owned
81 special *1Ay* alleles. Huang et al. (2002a) analyzed the divergence and evolution of
82 wheat and other grasses based on the genes encoding acetyl-CoA carboxylase and
83 plastid 3-phosphoglycerate kinase.

84 In the present study, the breakpoint regions of 4AL/5AL chromosomal translocation
85 on 4AL were sequenced and analyzed in diploid, tetraploid and hexaploid species of
86 *Triticum*. Quite a few of insertion and deletion length polymorphism (InDel) and
87 single nucleotide polymorphism (SNP) were detected among species. The
88 relationships among different species were also analyzed based on the breakpoint
89 sequences. The breakpoint interval was narrowed to 125 bp based on the chromosome
90 arm location pattern of the flanking sequences.

91 **Materials and Methods**

92 **Plant materials and DNA isolation**

93 A total of 52 *Triticum* accessions from 21 species were used in this study (Table 1).
94 All of the accessions were planted at the experimental farm of the Triticeae Research
95 Institute, Sichuan Agricultural University, China in 2015-2016. The fresh leaves of the
96 plants at seedling stage were harvested and ground into powder with liquid nitrogen,
97 and then the genomic DNA was extracted from it by using the
98 cetyltrimethylammonium bromide method (Li et al. 2012) and stored at -20 °C for the

99 following procedure.

100 Sequencing of the breakpoint

101 Two pairs of universal primers (P1 and P2) were designed to amplify the breakpoint
102 region of 4AL based on the sequences of *T. urartu*, *T. monococcum* and *T. aestivum*
103 (Li et al. 2016), and the reference sequences were shown in Table S1. The forward
104 primer F1 of primer P1, 5'-CAGAGCATTTGAAATATCA-3', was closed to the 3'
105 part of *WD3L*, and the reverse primer R1, 5'-GCACCATTTTCATTCTCCATTGC-3',
106 was connected with 3' part of *PMEIL* (Fig. 1A). The forward primer F2 of primer P2,
107 5'-CATGGACGGCATAACAGAG-3', and the reverse primer R2,
108 5'-GCTGGTGGTTGATGTGATTG-3', were connected with 3' part of *WD3L* and
109 *PMEIL* (Fig. 1A), respectively. The primers were synthesized by Tsingke Biological
110 Technology (Chengdu, China; <http://www.tsingke.net/>).

111 The PCR was carried out in a 50 µl mixture containing 25 µl 2× TSINGKE Master
112 Mix (blue) (Tsingke Biological Technology), 5 µl DNA template (~ 200 ng/µl), 2.5 µl
113 of each primer (10 µM) and 15 µl ddH₂O. At least five independent PCR mixtures for
114 each accession were collected and sequenced in both directions by Tsingke Biological
115 Technology (Chengdu, China). The sequences amplified by P1 and P2 from a given
116 accession were assembled by DNAMAN 6.0 (Lynnon Biosoft, USA).

117 Sequence analysis

118 The analysis of breakpoint sequence and multiple sequence alignment were carried
119 out by DNAMAN 6.0. The construction of bootstrap consensus tree and analysis of
120 nucleotide polymorphism (including InDel and SNP) were performed by MEGA 6.0
121 (Tamura et al. 2013).

122 Results

123 Isolation of breakpoint sequences

124 The expected fragments of breakpoint sequences for all the accessions in the present
 125 study were successfully isolated using two sets of primers (Fig. 1B and 1C). Multiple
 126 sequence alignments showed that all the obtained sequences belong to the breakpoint
 127 on 4AL for 4AL/5AL translocation based on the result of Li et al. (2016). Sequencing
 128 and assembly identified 12 types of sequences with different length termed from Type
 129 I to Type XII (Table 2). The sequence length of these groups ranged from 2009 bp
 130 (XII) to 2552 bp (I) (Table 2). The obtained breakpoint sequences of seven species
 131 belong to Type II, while only one species belong to each of Type I, V, VII, IX, X and
 132 XII (Table 2).

133 The sequence similarity was 10.32 % for all of the 52 accessions, and that for diploid,
 134 tetraploid and hexaploid wheat were 71.66, 84.02 and 94.58 %, respectively. In
 135 addition, we found that Type VII, VIII and IX were constituted by diploid wheat only;
 136 tetraploid wheat was classified to Type II (only *T. persicum*), III (such as *T.*
 137 *dicoccoides* and *T. persicum*), IV (*T. dicoccoides* and *T. dicoccum*), VI (including *T.*
 138 *durum* and *T. araraticum*), XI (*T. polonicum* and *T. paleo-colchicum*) and XII (only *T.*
 139 *timopheevii*); and hexaploid wheat were categorized to Type I (only *T.*
 140 *petropavlovishyi*), II (i.e. *T. zhukovskyi*, *T. compactum*, *T. macha*, *T. sphaerococum*
 141 and *T. vavilovii*), III (including *T. compactum*, *T. yunnanense* and *T. tibetanum*) and
 142 and X (only *T. zhukovskyi*) (Table 2). Furthermore, both Type II and III were consisted
 143 by tetraploid and hexaploid wheat. These results likely suggested that several types of
 144 breakpoint sequences were present in *Triticum*.

145 **SNPs and InDels in breakpoint region**

146 One hundred and one single nucleotide substitutions and 13 InDels ranging from 1 to
 147 275 bp were detected among all the sequences (Fig. S1). In diploid wheat, 47 single
 148 nucleotide substitutions and 8 InDels were detected. In tetraploid wheat, there were 29
 149 single nucleotide substitutions and 6 InDel regions with length of 1 (two loci), 2, 6, 60
 150 and 105 bp. Furthermore, a total of 62 single nucleotide substitutions and 10 InDel
 151 regions ranging from 1 (6 loci), 6, 101, 105 and 275 bp in length were obtained in

152 hexaploid wheat (Fig. S1).

153 Interestingly several significant diversities were observed: (1) some InDels were
154 detected in several species only, for example, four inserted and one deleted sequences
155 (InDel-A, B, D, F and K) were detected in *T. boeoticum* and *T. monococcum* only; (2)
156 several InDels could be observed in a single species, e.g., there were two InDels in the
157 amplified sequences of 4 accessions of *T. polonicum*; (3) genotype specificity InDel
158 was also detected, for instance, a unique “G” insertion was present in the 495th bp of
159 accession PI393496 for *T. monococcum*) (Fig. S1).

160 **Cluster analysis of breakpoint sequences from diploid, tetraploid and hexaploid** 161 **wheat**

162 To better understand the relationships of the breakpoint sequences for the accessions,
163 a bootstrap consensus tree was generated (Fig. 2). As shown, all the sequences could
164 be divided into three main groups mainly based on the ploidy of the materials. Group
165 I was consisted of einkorn, including *T. boeoticum* and *T. monococcum*. Group II was
166 most complicated and it was mainly composed by tetraploid species. Interestingly, *T.*
167 *urartu* and *T. zhukovskyi* PI355706 belong to Group II. Most of the hexaploid wheat
168 (except *T. zhukovskyi* PI355706), all the *T. persicum* and *T. dicoccoides* AS838 were
169 classified as Group III. It was found that some accessions from the same species or
170 sibling species were categorized together meaning that these accessions may be
171 closely related. For example, *T. boeoticum* and *T. monococcum* were classified as one
172 group with 100 of bootstrap value. However, some accessions from a single species,
173 were fallen into different clades. For example, the three accessions of *T. vavilovii*
174 were in three different clades. In addition, the bootstrap values for many clades were
175 less than 50 reducing the confidence of evolutionary relationships for the species.

176 **Narrowing down the breakpoint interval**

177 In order to identify the accurate breakpoint on 4AL for the 4AL/5AL translocation in
178 wheat, the conserved fragments in the multiple sequence alignment of the *Triticum*

were used for assigning the International Wheat Genome Sequencing Consortium Reference Sequence v1.0 (IWGSC RefSeq v1.0) (<https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies>). We found that fragment A was similar to sequences on 4AL, 4BL and 4DL, while fragments G had similarity to sequences on 4AL, 5BL and 5DL (Fig. 3). No hit was identified for fragment C, E and F, and fragment B and D were similar to sequences on 4AL. There is a significant difference for the sequence relationship between fragments A and G, we thus speculated that the breakpoint is likely located in the 125 bp between fragment A and G (Fig. 3).

Discussion

Breakpoint sequences based cluster analysis of the origin and evolution of *Triticum*

Cluster analysis indicated that *T. urartu* and the species with G genome including *T. timopheevii* (AAGG), *T. araraticum* (AAGG) and *T. zhukovskyi* PI355706 (AAA^mA^mGG) were clustered together with a high bootstrap value (77), which means that *T. urartu* and *T. timopheevii* or *T. araraticum* might be involved in the origin of *T. zhukovskyi*. This was consistent with previous studies pointing that *T. timopheevii* was the ancestor of *T. zhukovskyi* (Talbert et al. 1991) and the ancestor of *T. urartu* was the donor of A genome of tetraploid and hexaploid wheat (Allaby et al. 1999).

As *T. zhukovskyi* was suggested to have arisen via hybridization of *T. timopheevii* originated from crossing of *Ae. speltoides* with *T. urartu* (Allaby and Brown 2000; Dvorák et al. 1993; Kilian et al. 2007) and *T. monococcum* with subsequent chromosome doubling (Bowden 1959; Dvorák et al. 1993), two different amplified sequences orthologous to those of *T. urartu* (A) and *T. monococcum* (A^m), respectively, could be obtained. Using a pair of primers spanning an ~2.4 kb fragment, Li et al. (2016) amplified two weak bands in *T. zhukovskyi* corresponding to the A and A^m genome. In this study, a single band similar to that from *T. urartu* was obtained

only in *T. zhukovskyi* by the designed primers. We thus speculate that the sequence on A^m genomes of *T. zhukovskyi* may be obtained by newly designed primers.

T. petropavlovishyi, *T. tibetanum* and *T. yunnanense* are landraces found only in China. They showed similar original characters including brittle rachis and hard-threshability. It was suggested they should have similar evolutionary process (Jiang et al. 2014; Wang et al. 2005; Yang 1992). Multiple sequences alignments in this study showed that a 60 bp fragment (InDel-J) was missing from *T. petropavlovishyi* (Fig. S1). Only *T. tibetanum* and *T. yunnanense* were clustered in one clade (Fig. 2) showing a farther genetic relationship with *T. petropavlovishyi*. This was consistent with the results of taxonomic classifications (Chen et al. 2016; Ward et al. 1998; Yang et al. 1993; Yang et al. 1992b) based on morphological traits, cytogenetics, ecogeographical origins and restriction fragment length polymorphism (RFLP) analysis indicating that *T. tibetanum* and *T. yunnanense* might experience a similar origin and (or) evolution compared to *T. petropavlovishyi*.

It was widely believed that *T. dicoccum* was the A and B donors of *T. spelta* (Peng et al. 2011). Here we found that *T. tibetanum* and *T. yunnanense* were clustered with *T. dicoccoides* (Fig. 2), likely suggesting that *T. tibetanum* and *T. yunnanense* may be derived from *T. dicoccoides*. This possible evolutionary difference between *T. spelta* and *T. tibetanum* and *T. yunnanense* may contrite to their different brittle rachis characters (Wang and Zheng 2004).

Different from previous evolutionary analyses based on phenotype characteristics (Peng et al. 2011), chromosome paring (El Baidouri et al. 2017; Feldman and Levy 2005; Peng et al. 2011), and gene similarity (Novoselskaya-Dragovich et al. 2018; Peng et al. 2011; Yan et al. 2017), we here conducted cluster analysis of the main species in *Triticum* by employment of the breakpoint sequences on 4AL for 4AL/5AL translocation. We indeed observed a few of evolutionary relationships among some species being consistent with those detected in previous studies. For example, in addition to those discussed above, we also found that both einkorn and emmer wheat

234 were expectedly clustered together closely (Fig. 2). However, quite a few of the
235 detected relationships among some species were different from those previously
236 observed by traditional classification. For example, *T. persicum*, *T. spelta* and *T.*
237 *vavilovii* were categorized into more than one clades being different from traditional
238 classification (Fig. 2). The reason is likely that the breakpoint sequences suffered
239 from extensive, intense and unidirectional variations as the environment changed after
240 divergence from the ancestor. These sequence polymorphisms masked the real
241 evolutionary picture of different species in *Triticum*. We thus suggested that the ability
242 of breakpoints based analysis of evolution could be limited. The identification of
243 other breakpoints for 4A-5A-7B translocation is yet to be further employed in
244 evolutionary analysis.

245 **Utilization potential of the detected SNPs and InDels**

246 In the genetic research of wheat, one of the most important jobs is to map genes or
247 major quantitative trait loci (QTL). The development of molecular markers based on
248 SNPs or InDels plays vital function in construction of genetic maps, especially fine
249 mapping of genes or QTL. For example, Lu et al. (2016) developed high-resolution
250 melting (HRM) markers to map powdery mildew resistance gene *PmPB74*; Qureshi et
251 al. (2017) designed kompetitive allele specific PCR (KASP) marker to link rust
252 resistance genes *Yr47* and *Lr52*; The InDel and cleaved amplified polymorphic
253 sequence (CAPS) markers were developed to study the grain weight gene *TGW-7A* in
254 wheat (Hu et al. 2016). Abundant of SNPs and InDels were detected in this study, and
255 some of them were specific to a given genotype or species (Fig. S1). For example,
256 InDel-A and InDel-K were inserted and deleted in both *T. beoeticum* and *T.*
257 *monococcum*, respectively; and a 60 bp fragment, i.e. InDel-J, was only deleted from
258 *T. petropavlovishyi*. Thus, the SNPs and InDels identified here may be useful for
259 developing molecular markers to map genes or QTLs flanking the breakpoint on 4A
260 of wheat.

261 **The constantly improved chromosome sequences could facilitate the**

262 identification of breakpoints

263 By using *Aegilops tauschii*, CS chromosome survey sequences, *T. monococcum* and *T.*
 264 *urartu* genomic sequences deposited in public database, the 4AL junction of the
 265 breakpoint for the 4AL/5AL translocation was recently identified, and the breakpoint
 266 was located in an ~1000 bp of interval (Li et al. 2016). Here, the isolated conserved
 267 sequences from 21 species of *Triticum* flanking the 4AL breakpoint were used as
 268 queries for searching the recent assemblies of IWGSC RefSeq v1.0 and the breakpoint
 269 interval was narrowed down to a 125 bp fragment on 4AL. It is believed that other
 270 breakpoints for 4A-5A-7B translocations could be more easily identified with the
 271 publication of updated CS chromosome assembly sequences.

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281 **Conflict of interest** All authors read and agreed with the final manuscript and have
 282 no conflicts of interest.

283

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438 **Table 1** The plant materials used in the present study

439

Species	Genome	Number	Accession
<i>T. urartu</i> [†]	AA	2	PI428270, PI428276
<i>T. beoeticum</i> [†]	AA	2	AS261, AS262
<i>T. monococcum</i> [†]	AA	5	PI362610, PI377666, PI377671, PI393496, PI427959
<i>T. timopheevii</i> [†]	AAGG	1	AS275
<i>T. araraticum</i> [†]	AAGG	3	PI427300, PI427302, PI427408
<i>T. polonicum</i> [†]	AABB	4	PI254214, PI254215, PI266846, PI272564
<i>T. persicum</i> [†]	AABB	4	PI94752, PI182471, PI251914, PI272521
<i>T. turanicum</i> [†]	AABB	2	PI124494, PI352514
<i>T. paleo-colchicum</i> [†]	AABB	1	PI349050
<i>T. durum</i> [†]	AABB	1	AS2247
<i>T. dicoccoides</i> [†]	AABB	3	AS838, AS847, PI470947
<i>T. dicoccum</i> [†]	AABB	5	PI94614, PI94666, PI94670, PI94675, PI113961
<i>T. zhukovskyi</i> [§]	AAA ^m A ^m GG	2	PI296968, PI355706
<i>T. macha</i> [§]	AABBDD	2	PI355513, PI355514
<i>T. compactum</i> [†]	AABBDD	4	AS352, PI129528, PI157920, PI211701
<i>T. spelta</i> [†]	AABBDD	3	AS328, CItr13967, PI15865
<i>T. vavilovii</i> [§]	AABBDD	3	AS345, AS346, PI326319
<i>T. sphaerococcum</i> [†]	AABBDD	2	AS348, AS349
<i>T. petropavlovishyi</i> [†]	AABBDD	1	AS359
<i>T. yunnanense</i> [†]	AABBDD	1	AS338
<i>T. tibetanum</i>	AABBDD	1	Q1028

440 ^{†, ‡, §} The materials were kindly provided by Dr. Lianquan Zhang, Dr. Zehong Yan, and
 441 Dr. Bihua Wu, respectively, of Triticeae Research Institute, Sichuan Agricultural
 442 University, China

443 **Table 2** Classification of the amplified sequences in this study
444

Type	Num. of sp.	Num. of ac.	Length (bp)	Species	Accession
I	1	1	2009	<i>T. petropavlovishyi</i>	AS359
II	7	16	2069	<i>T. persicum</i>	PI94752, PI251914, PI272521
				<i>T. zhukovskyi</i>	PI296968
				<i>T. compactum</i>	PI129528, PI211701
				<i>T. macha</i>	PI355513, PI355514
				<i>T. spelta</i>	AS328, PI15865, CITR13967
				<i>T. sphaerococum</i>	AS348, AS349
				<i>T. vavilovii</i>	AS345, AS346, PI326319
III	5	6	2070	<i>T. dicoccoides</i>	AS838
				<i>T. persicum</i>	PI182471
				<i>T. compactum</i>	PI157920, AS352
				<i>T. yunnanense</i>	AS338
				<i>T. tibetanum</i>	Q1028
IV	2	7	2071	<i>T. dicoccoides</i>	AS847, PI470947
				<i>T. dicoccum</i>	PI94614, PI94666, PI94670, PI94675, PI113961
V	1	2	2073	<i>T. polonicum</i>	PI254214, PI254215
VI	2	4	2076	<i>T. durum</i>	AS2247
				<i>T. araraticum</i>	PI427300, PI427302, PI427408
VII	1	2	2077	<i>T. urartu</i>	PI428270, PI428276
VIII	2	6	2087	<i>T. beoeticum</i>	AS261, AS262
				<i>T. monococcum</i>	PI427959, PI362610, PI377666, PI377671
IX	1	1	2088	<i>T. monococcum</i>	PI393496
X	1	1	2180	<i>T. zhukovskyi</i>	PI355706
XI	2	5	2449	<i>T. polonicum</i>	PI272564, PI266846, PI124494, PI352514
				<i>T. paleo-colchicum</i>	PI349050
XII	1	1	2552	<i>T. timopheevii</i>	AS275

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Figure legends

Fig. 1 Isolation of breakpoint regions of the 4AL junction for the 4AL/5AL translocation in *Triticum*. **A** Locations of *WD3L*, *PEMIL*, P1 (F1 and R1), P2 (F2 and R2) and breakpoint. **B** PCR bands (1500-1800 bp) amplified by primer P1. M, DNA Marker; 1 *T. dicoccoides* AS838; 2 *T. dicoccoides* AS847; 3 *T. dicoccum* PI94666; 4 *T. dicoccum* PI94670; 5 *T. dicoccum* PI94675; 6 *T. polonicum* PI254214; 7 *T. polonicum* PI254215; 8 *T. araraticum* PI427302; 9 *T. urartu* PI428276; 10 *T. monococcum* PI362610; 11 *T. monococcum* PI393496; 12 *T. polonicum* PI266846. **C** PCR bands (600-900 bp) amplified by primer P1. M, DNA Marker; 1 *T. petropavlovishyi* AS359; 2 *T. dicoccoides* AS838; 3 *T. compactum* PI157920; 4 *T. dicoccoides* AS847; 5 *T. dicoccoides* AS847; 6 *T. dicoccum* PI94666; 7 *T. dicoccum* PI94670; 8 *T. araraticum* PI427302; 9 *T. urartu* PI428270; 10 *T. urartu* PI428276; 11 *T. beoeticum* AS261; 12 *T. beoeticum* AS262; 13 *T. monococcum* PI393496; 14 *T. zhukovskyi* PI355706.

Fig. 2 Bootstrap consensus tree was obtained by 1 000 repeat calculation based on 52 breakpoint sequences of the 4AL junction for the 4AL/5AL translocation in *Triticum*. The branch with bootstrap value of 0 indicates that the sequences vary greatly between the species or accessions.

Fig. 3 Multiple sequence alignments of the breakpoints from the studied 21 species. The blue bar indicates the sequence was similar to sequences on 4AL, 4BL and 4DL. The green bar indicates the sequence was similar to sequences on 4AL, 5BL and 5DL. Gray bar indicates the sequence cannot find hits in BLAST analysis. And red bars indicates the sequence had similarity to sequences on 4AL.

47x38mm (600 x 600 DPI)

159x281mm (300 x 300 DPI)

72x20mm (300 x 300 DPI)