1   **Genome-wide homology analysis reveals new insights into the origin of the wheat B genome**

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Abstract

Wheat is a typical allopolyploid with three homoeologous subgenomes (A, B, and D). The ancestors of the subgenomes A and D had been identified, but not for the subgenome B. The goatgrass *Aegilops speltoides* (genome SS) has been controversially considered a candidate for the ancestor of the wheat B genome. However, the relationship of the *Ae. speltoides* S genome with the wheat B genome remains largely obscure, which has puzzled the wheat research community for nearly a century. In the present study, the genome-wide homology analysis identified perceptible homology between wheat chromosome 1B and *Ae. speltoides* chromosome 1S, but not between other chromosomes in the B and S genomes. An *Ae. speltoides*-originated segment spanning a genomic region of approximately 10.46 Mb was identified on the long arm of wheat chromosome 1B (1BL). The *Ae. speltoides*-originated segment on 1BL was found to co-evolve with the rest of the B genome in wheat species. Thereby, we conclude that *Ae. speltoides* had been involved in the origin of the wheat B genome, but should not be considered an exclusive ancestor of this genome. The wheat B genome might have a polyphyletic origin with multiple ancestors involved, including *Ae. speltoides*. These novel findings provide significant insights into the origin and evolution of the wheat B genome, and will facilitate polyploid genome studies in wheat and other plants as well.

Keywords: *Aegilops speltoides*; genome homology; genomic *in situ* hybridization (GISH); meiotic pairing; single nucleotide polymorphisms (SNPs); wheat B genome

62   **Introduction**

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64   Wheat (*Triticum aestivum*, 2n=6x=42, genome AABBDD), a major food grain source for

65   humans, has been considered a typical allohexaploid originated from the interspecific

66   hybridization involving three diploid ancestors (Sakamura 1918; Kihara 1919, 1954; Kihara et al.

67   1959; Sax 1922). This evolutionary theory of allopolyploid has led to successful identification of

68   the ancestors for the wheat subgenomes A and D, but not yet for the subgenome B. The wild

69   grasses *T. urartu* (2n=2x=14, genome AA) and *Aegilops tauschii* (2n=2x=14, genome DD)

70   contributed A and D subgenomes to wheat, respectively (Kihara 1944; McFadden and Sears

71   1946; Dvorak et al. 1993). The ancestor of the subgenome B, however, remains controversial

72   even though tremendous research efforts have been made to tackle this evolutionary puzzle of

73   wheat for nearly a century.

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75   Early studies on meiotic pairing, karyotyping, plant morphology, and geographic distribution of

76   wheat-related wild species and their hybrids with wheat species (*Triticum* L.) identified the

77   goatgrass *Ae. speltoides* (2n=2x=14, genome SS) as the closest ancestor of the wheat B genome

78   (Jenkins 1929; Pathak 1940; Sarkar and Stebbins 1956; Riley et al. 1958). Meanwhile, questions

79   had been raised about the meiotic pairing-based assessment of genome homology in these early

80   studies because *Ae. speltoides* was suspected to contain genetic factors with epistatic effect on

81   the wheat diploidization system, now designated *Ph* (pairing homoeologous) gene (Jenkins 1929;

82   Sarkar and Stebbins 1956; Riley et al. 1958). The *Ph* gene limits meiotic pairing to homologous

83   chromosomes in wheat and wheat hybrids with its relatives. Also, it had been assumed that the

84   ancestral form of the B genome might have undergone a series of changes since its incorporation

85   into wheat (Jenkins 1929; Sarkar and Stebbins 1956).

86

87   In a later study, Riley et al. (1961) confirmed the presence of the gene(s) in *Ae. speltoides* that

88   suppresses the effect of the *Ph1* gene located on wheat chromosome 5B. Three genotypes with

89   high, intermediate, and low ability to suppress *Ph1* activity were identified in *Ae. speltoides*

90   (Dvorak 1972). According to these findings, Kimber and Athwal (1972) reassessed meiotic

91   pairing in the hybrids and amphiploids involving wheat and *Ae. speltoides* accessions with

92   different levels of suppression for the *Ph1* activity. They determined that the variation of meiotic

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93   pairing in the hybrids resulted from the presence of different *Ph1* suppressors in the *Ae.*

94   *speltoides* accessions. Also, they found that chromosomes predominantly paired as bivalents in

95   the amphiploid involving polyploid wheat and a low pairing *Ae. speltoides* accession, which was

96   very similar to a normal diploidized allopolyploid. As a result, they concluded that *Ae. speltoides*

97   could not be considered as the ancestor of the wheat B genome. This was also supported by the

98   evidence of chromosome banding patterns (Gill and Kimber 1974) and protein electrophoretic

99   profiles (Johnson 1972). More recently, three *Ph1* suppressor gene loci were identified and

100   mapped to chromosome 3S, 5S, and 7S of *Ae. speltoides*, respectively (Dvorak et al. 2006).

101

102   In contrast, molecular analyses of both nuclear and extranuclear genomic DNAs suggested that

103   *Ae. speltoides* or a species in the evolutionary lineage of *Ae. speltoides* could be the most likely

104   ancestor of the wheat B genome as well as wheat plasmon (Ogihar and Tsunewaki 1988; Dvorak

105   and Zhang 1990; Sasanuma et al. 1996; Wang et al. 1997; Kilian et al. 2007). However,

106   comparative analysis of several gene loci and nearby genomic regions across the *Triticum* and

107   *Aegilops* species did not reveal clear evidence supporting that conclusion (Hang et al. 2002;

108   Salse et al. 2008). The *Ae. speltoides* S genome was considered to be evolutionarily closer to the

109   wheat B genome than to the A and D genomes, but its candidacy as the ancestor of B genome

110   remained undetermined in both studies.

111

112   The wheat B genome has significantly higher genetic variability than A and D genomes (Chao et

113   al. 1989; Felsenburg et al. 1991; Siedler et al. 1994; Petersen et al. 2006). These findings support

114   the hypothesis that the wheat B genome had diverged from its ancestor through various genomic

115   modifications (Jenkins 1929; Sarkar and Stebbins 1956; Blake et al. 1999). In addition, *Ae.*

116   *speltoides* has significantly higher intraspecific genetic variability than any of the other four

117   *Aegilops* species in the Sitopsis section, which is even comparable to the interspecific variability

118   among the other four *Aegilops* species in the section (Sasanuma et al. 1996). This appears to

119   support the hypothesis that *Ae. speltoides* might contribute to the origin of the wheat B genome,

120   but the current version of *Ae. speltoides* had diverged from the original ancestor of the B genome

121   (Salse 2008). Another hypothesis, proposed by Zohary and Feldman (Zohary and Feldman

122   1962), states that the wheat B genome is a reconstructed genome resulted from meiotic

123   homoeologous recombination between multiple ancestral genomes of the *Aegilops* species. This

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124   evolutionary recombination process was assumed to occur in the hybrids of the tetraploid

125   amphiploids that combined the different ancestral *Aegilops* genomes and a common ancestral A

126   genome of *T. urartu*. In other words, the wheat B genome might have a polyphyletic origin.

127

128   A species with a genome more closely related to the wheat B genome than the S genome of *Ae.*

129   *speltoides* has not been discovered even though intensive search for the ancestor of B genome

130   has been performed over nearly a century. It seems inevitable to reason that *Ae. speltoides* might

131   contribute to the origin and evolution of the wheat B genome to some extent according to the

132   previous studies. The present study aimed to assess the homology of individual wheat B-genome

133   chromosomes with their homoeologous counterparts in the S genome of *Ae. speltoides* and to

134   detect the *Ae. speltoides* genomic components in the wheat B genome if there are any. A novel

135   integrative cytogenetic and genomic approach was taken to accomplish this research, which

136   could not be done in the previous studies due to the lack of the genomics/cytogenetics tools and

137   resources. This work shed new light on the origin and evolution of the wheat B genome, and will

138   facilitate further studies of the complex polyploid genomes in wheat and other plants.

139

140   **Materials and Methods**

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142   **Plant Materials**

143   Six "Chinese Spring" (CS) wheat B genome-*Ae. speltoides* disomic substitution lines [DS

144   1S(1B), DS 2S(2B), DS 4S(4B), DS 5S(5B), DS 6S(6B), and DS 7S(7B)] (Friebe et al. 2011),

145   one substitution line involving chromosome 3S and 3A [DS 3S(3A)], and the CS *ph1b* mutant

146   were the initial genetic stocks used in this research. They were provided by the Wheat Genetics

147   Resource Center at Kansas State University, USA. DS 3S(3A) was included in this study because

148   DS 3S(3B) is not available (please see results in this study). In addition, six CS wheat B genome-

149   *Thinopyrum elongatum* (2n=2x=14, genome EE) disomic substitution lines [DS 1E(1B), DS

150   2E(2B), DS 3E(3B), DS 5E(5B), DS 6E(6B), and DS 7E(7B)] and one substitution line involving

151   chromosome 4E and 4D [DS 4E(4D)] were used as controls to assess B-S genome homology in

152   this study. DS 4E(4B) was not available. They were kindly supplied by J. Dvorak at UC Davis.

153   Each of these disomic substitution lines has a pair of wheat chromosomes replaced by their

154   homoeologous counterparts of *Ae. speltoides* or *Th. elongatum*. The common and durum wheat

155   (*T. turgidum* ssp. *durum*, 2n=4x=28, genome AABB) accessions used in this study were selected

156   from the worldwide diversity panel of the Triticeae Coordinated Agricultural Project (T-CAP).

157   The other wheat species/accessions that contain the B genome were obtained from the U.S.

158   National Plant Germplasm System. A total of 179 accessions under 13 wheat species (*Triticum*

159   L.) were chosen based on their geographic origin and distribution, representing a diverse

160   worldwide collection of the tetraploid and hexaploid wheat species (File S1). A subset of

161   representative wheat species/accessions (n=88) were selected for single nucleotide

162   polymorphism (SNP) genotyping from the 179 accessions (File S2).

163

164   **Construction of the special genotypes for meiotic pairing analysis**

165   The CS-*Ae. speltoides* and CS-*Th. elongatum* disomic substitution lines were crossed and

166   backcrossed with the CS *ph1b* mutant to construct the special genotypes monosomic for the

167   individual B/A-S or B/D-E homoeologous pairs in the presence and absence of *Ph1*, respectively

168   (Fig. S1). The chromosome-specific DNA markers (File S3) were employed to assist selection of

169   the double monosomics for each of the homoeologous pairs. The selected individuals were

170   verified for the monosomic condition by genomic *in situ* hybridization (GISH). The *Ph1*-specific

171   DNA markers (Roberts *et al.*, 1999) were used to select the double monosomics with *Ph1* as well

172   as those without *Ph1* (i.e. homozygous for *ph1b* deletion mutant) (Fig. S1).

173

174   Anthers with meiocytes [pollen mother cells (PMCs)] at metaphase I (MI) were collected for

175   meiotic pairing analysis from the heterozygotes with and without *Ph1* following the procedure of

176   Cai and Jones (1997). A total of over 100 meiocytes at MI from 1-6 plants were observed and

177   analyzed for each of the special genotypes. GISH was used to differentially paint chromosomes

178   of *Ae. speltoides*, *Th. elongatum*, and wheat for meiotic pairing analysis.

179

180   **DNA Marker Analysis**

181   DNA samples were prepared as described by Niu et al. (2011). Chromosome-specific DNA

182   markers, including SSRs (simple sequence repeats) and STSs (sequence-tagged sites), were

183   developed and used for the identification of individual B-, S-, and E-genome chromosomes as

184   described by Chen et al. (2007). Two STS markers (*PSR128* and *PSR574*) that tag the *Ph1* allele

185   were used to identify individuals homozygous for the *ph1b* deletion mutant (Roberts et al.,

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186   1999). The wheat 90K iSelect SNP arrays were used to perform SNP genotyping assay for CS

187   wheat, the disomic substitution lines, and the 88 representative wheat species/accessions (45

188   hexaploids and 43 tetraploids) (File S2) using the Illumina iScan instrument. SNP allele

189   clustering and genotype calling were conducted using the GenomeStudio v2011.1 software

190   (Illumina, Inc.) (Wang et al. 2014). The polymorphisms for each of the homoeologous pairs at

191   the SNP loci were calculated as the percentages of the polymorphic loci out of the total loci

192   genotyped. The graphical view of the genotypes for the 88 wheat species/accessions at the 68

193   SNP loci within the distal end of 1BL/1SL was constructed using the Flapjack software (Milne et

194   al. 2010). Genetic diversity was calculated based on the SNP genotyping data of the 88

195   representative wheat species/accessions (Nei 1973) and plotted against the SNP consensus

196   linkage map of wheat chromosome 1B (Wang et al., 2014). The SNP genotype-based cluster

197   dendrogram was developed using R package "ape" (https://cran.r-project.org/).

198

199   **Fluorescent *in situ* hybridization (FISH)**

200   Fluorescent genomic *in situ* hybridization (FGISH) was performed to differentiate wheat B-

201   genome and S/E-genome chromatin from each other as described by Cai et al. (1998). Total

202   genomic DNAs of *Ae. speltoides* and *Th. elongatum* were labeled with biotin-16-dUTP by nick

203   translation as probe DNA for detecting *Ae. speltoides* and *Th. elongatum* chromatin, respectively.

204   Total genomic DNA of CS wheat was used as blocking DNA. *Ae. speltoides*/*Th. elongatum*

205   chromatin was painted with fluorescein isothiocyanate-conjugated avidin (FITC-avidin) as

206   yellow-green and wheat chromatin was counter-stained with propidium iodide (PI) as red.

207   Multicolor FISH was conducted following the procedure of Liu et al. (2006). The clone *pTa71*, a

208   wheat 9 kb rDNA repeating unit that contains the 18S, 5.8S, and 26S rRNA genes and intergenic

209   spacer (Gerlach and Bedbrook, 1979), was supplied by Peng Zhang at The University of Sydney,

210   Australia. It was labeled with dig-11-dUTP and detected by anti-dig-rhodamine as red. This

211   rDNA probe was used to tag the nucleolar organizer region on wheat chromosomes 1B and 6B.

212   Total genomic DNA of *Ae. speltoides* was labeled with biotin-16-dUTP and detected by FITC-

213   avidin as yellow-green. This genomic probe was used to identify *Ae. speltoides* chromatin in the

214   wheat genome. Wheat chromatin was counter-stained with 4',6-diamidino-2-phenylindole

215   (DAPI) as blue. The fluorescence microscopy system BX51 (Olympus, Japan) was used to

216   visualize GISH/FISH-painted chromosomes.

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217

218 **DNA Sequence Analysis**

219 The DNA sequences of wheat chromosome 1B was extracted from the IWGSC RefSeq v1.0

220 (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies). The contextual sequences of

221 the SNP loci at the distal ends of both CS wheat 1BL and *Ae. speltoides* 1SL were aligned to the

222 DNA sequences of chromosome 1B using the Splign software (Kapustin et al. 2008). The

223 physical order of the SNP loci was determined based on the DNA sequence alignment.

224

225 **Results**

226

227 **Homology analysis of the individual B/A-S homoeologous chromosome pairs**

228 Meiotic pairing has been considered direct cytological evidence for genome homology. It can,

229 however, be influenced by the genetic factors in addition to homology, such as *Ph1* gene in

230 wheat and *Ph1* suppressors in *Ae. speltoides*. To take account of the non-homology factors in the

231 B-S genome homology analysis, we investigated meiotic pairing of individual B-S

232 homoeologous pairs under the same genetic background of CS wheat in the presence and

233 absence of *Ph1*. The CS wheat B genome-*Ae. speltoides* S genome disomic substitution lines

234 dissect the S genome of *Ae. speltoides* into individual chromosomes in the CS wheat

235 background. They were used to construct double monosomics for the individual B- and S-

236 genome chromosomes. Meanwhile, the *ph1b* deletion mutant of *Ph1* was introduced into the

237 double monosoimcs with assistance of the *Ph1*-specific DNA markers. Thus, we were able to

238 investigate meiotic pairing of individual B-S homoeologous chromosome pairs in the presence as

239 well as absence of *Ph1*. In addition, we investigated meiotic pairing of the individual B-E

240 homoeologous chromosome pairs as controls for B-S genome homology analysis.

241

242 Chinese Spring wheat chromosome 1B was found to pair with *Ae. speltoides* chromosome 1S in

243 67 of the 134 PMCs analyzed (50.00%), while other B/A-S homoeologous pairs had a relatively

244 low meiotic pairing frequency ranging from 0.00 to 8.63% in the presence of *Ph1* (Fig. 1). In

245 addition, we noticed that 1B-1S meiotic pairing predominantly involved the long arms of

246 chromosome 1B (1BL) and 1S (1SL). Surprisingly, wheat 1BL was found to contain a small *Ae.*

247 *speltoides* S genome-derived chromosomal segment at its distal end, where meiotic pairing

8

248   initiated (Fig. 2a). Also, the same *Ae. speltoides*-derived segment was observed on the unpaired

249   1BL (univalent) (Fig. 2b). The *Ph1* suppressor genes mapped to *Ae. speltoides* chromosomes 3S,

250   5S, and 7S (Dvorak et al. 2006). We found that meiotic pairing involving chromosome 5S was

251   noticeably higher than that involving 2S, 3S, 4S, 6S, and 7S in the presence of *Ph1* (Fig. 1).

252   Thus, there might be a *Ph1* suppressor on this particular *Ae. speltoides* chromosome 5S, but not

253   on chromosomes 3S and 7S involved in this study. In the absence of *Ph1* (i.e. *ph1bph1b*),

254   chromosomes 1B and 1S paired at a frequency of 60%, which was higher than the 1B-1S pairing

255   frequency (50%) in the presence of *Ph1*. Meiotic pairing of other homoeologous pairs (2B-2S,

256   3A-3S, 4B-4S, 5B-5S, 6B-6S, and 7B-7S) was dramatically enhanced by *ph1b* mutant (Fig. 1).

257   5B^{ph1b}-5S also exhibited a high pairing frequency (42.16%), suggesting absence of *Ph1* on *Ae.*

258   *speltoides* chromosome 5S (Griffiths et al. 2006).

259

260   Meiotic pairing was not observed between CS wheat chromosome 1B and *Th. elongatum*

261   chromosome 1E in the 105 PMCs analyzed under the presence of *Ph1*. The other B/D-E

262   homoeologous chromosome pairs also showed a low meiotic pairing frequency when *Ph1* was

263   present (Fig. 1). Meiotic pairing of all B/D-E homoeologous chromosome pairs was enhanced by

264   the *ph1b* mutant, but not as extensively as that with the B/A-S homoeologous chromosome pairs

265   except 4D-4E and 5B-5E. Apparently, CS wheat chromosome 5B^{ph1b} had a high meiotic pairing

266   affinity with *Ae. speltoides* chromosome 5S as well as *Th. elongatum* chromosome 5E (Fig. 1).

267

268   **GISH/FISH analysis of wheat B-genome chromosomes**

269   Meiotic pairing analysis demonstrated a notable homology between CS wheat chromosome 1B

270   and *Ae. speltoides* chromosome 1S (Fig. 1). Apparently, the *Ae. speltoides*-derived chromosomal

271   segment at the distal end of 1BL contributed to the high 1B-1S pairing. To confirm the *Ae.*

272   *speltoides* segment on 1BL and determine whether any additional *Ae. speltoides* segments are

273   present on the CS B-genome chromosomes, we performed multicolor FISH/GISH to the mitotic

274   chromosomes of CS wheat. The CS wheat chromosomes 1B and 6B were tagged by FISH using

275   the rDNA probe *pTa71*; and *Ae. speltoides* chromatin was simultaneously painted by *Ae.*

276   *speltoides* genomic DNA-probed GISH. An *Ae. speltoides* chromosomal segment was clearly

277   detected at the distal end of CS 1BL, but not in other regions of chromosome 1B and other B-

278   genome chromosomes (Fig. 2c). To further verify the origin of the distal segment on 1BL, we

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279 performed *Th. elongatum* genomic DNA-probed GISH to all three chromosome sets of the CS

280 wheat genome. No *Th. elongatum*-derived GISH signals were observed on any of the CS wheat

281 chromosomes, including 1BL (Fig. 3A). Thus, the distal segment on CS chromosomal arm 1BL

282 is *Ae. speltoides* S genome-specific, not a common chromosomal region shared by CS wheat and

283 its relatives. It was derived from the S genome of *Ae. speltoides*.

284

285 We surveyed the B genome of 179 representative accessions under 13 wheat species (*Triticum*

286 L.) for the presence of *Ae. speltoides* chromatin by GISH. They were collected from the different

287 geographic regions around the world and represented a diverse collection of the hexaploid and

288 tetraploid wheat species/accessions that contain the B genome. All of these wheat

289 species/accessions were found to contain an *Ae. speltoides* chromosomal segment at the distal

290 end of 1BL as what we observed on 1BL of CS wheat, but not in the other regions of

291 chromosome 1B and other B-genome chromosomes (Table 1; Fig. 2c). Therefore, the *Ae.*

292 *speltoides*-derived chromosomal segment is universally present at the distal end of 1BL in both

293 tetraploid and hexaploid wheat. It has been part of chromosome 1B probably since the

294 incorporation of the B genome into tetraploid and hexaploid wheat.

295

296 **Comparative analysis of the individual B-S homoeologous pairs**

297 Both CS wheat and CS-*Ae. speltoides* disomic substitution lines were genotyped using wheat

298 90K iSelect SNP arrays. The SNP genotyping results indicated that the substitution line

299 originally designated DS 3S(3B) (Friebe et al. 2011) should be DS 3S(3A), which was further

300 confirmed by SSR markers and chromosome C-banding. Thus, DS 3S(3A), instead of DS

301 3S(3B), was included in the SNP assay in addition to the substitution lines involving other six B-

302 genome chromosomes (1B, 2B, 4B, 5B, 6B, and 7B).

303

304 High-throughput genotyping of CS wheat and the CS B genome-*Ae. speltoides* disomic

305 substitution lines at 17,379 SNP loci identified a total of 6,722 SNPs polymorphic in the seven

306 B/A-S homoeologous pairs. The homoeologous pair 2B-2S showed the lowest polymorphism

307 (33.34%) and 7B-7S the highest (43.37%) at the SNP loci surveyed. The polymorphisms of other

308 five B/A-S homoeologous pairs ranged from 33.89% (3A-3S) to 41.86% (4B-4S) (Fig. 4).

309 Plotting of the SNP polymorphisms between CS wheat chromosome 1B and *Ae. speltoides*

10

310 chromosome 1S against the SNP consensus linkage map of chromosome 1B (Wang et al. 2014)

311 identified a genomic region that shared the same alleles at 65 of the 68 SNP loci within the distal

312 ends of CS wheat 1BL and *Ae. speltoides* 1SL (Fig. 3C). Such a monomorphic linkage block was

313 not detected in other chromosomal regions of the 1B-1S homoeologous pair and on other B/A-S

314 homoeologous pairs (Fig. S2).

315

316 **Genetic and physical characterization of the 1BL distal end**

317 The chromosomal regions at the distal ends of CS wheat 1BL and *Ae. speltoides* 1SL were found

318 to be highly monomorphic at the 68 SNP loci (65 monomorphic/68 SNPs). The three

319 polymorphic SNP loci within the region on 1BL and 1SL were positioned toward the proximal

320 end of the region on the consensus linkage map (Wang et al. 2014). The entire region defined by

321 the 68 SNPs spans a genetic distance of 12.7 cM (Fig. 3B and File S4). The contextual sequences

322 of these 68 SNPs were aligned to the distal region of 1BL according to the IWGSC RefSeq v1.0

323 (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies). Two of the three SNP loci

324 polymorphic between 1BL and 1SL, *Tdurum_contig41999_2908* and *Ex_c1058_1537*, were

325 physically assigned to the proximal end of the region. The other polymorphic SNP within the

326 region, *RFL_Contig785_1156*, was distal to the first two polymorphic SNPs. One monomorphic

327 SNP (*RFL_Contig785_1700*) was physically positioned within the interval between

328 *Tdurum_contig41999_2908* and *RFL_Contig785_1156* according to the sequence alignment

329 (Fig. 3B and File S4).

330

331 The genomic region that spans the 65 SNP loci monomorphic between CS 1BL and *Ae.*

332 *speltoides* 1SL and one polymorphic SNP (*RFL_Contig785_1156*) was estimated to be 9.61 Mb

333 in length according to the DNA sequence assemblies of 1BL (https://wheat-

334 urgi.versailles.inra.fr/Seq-Repository/Assemblies). The two polymorphic SNP loci

335 (*Tdurum_contig41999_2908* and *Ex_c1058_1537*) at the proximal end of that region were not

336 included in the estimate (Fig. 3D). In addition, we identified an extended terminal segment of

337 0.85 Mb distal to the 68 SNP-defined region on 1BL according to the DNA sequence alignment.

338 As a result, the total physical length of the SNP-defined and extended distal genomic region on

339 1BL was estimated to be 10.46 Mb (Fig. 3D). The actual physical size of this distal segment on

340 1BL might be greater than this estimate (10.46 Mb) because the current DNA sequence

11

341 assemblies (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies) we used in this

342 study cover 689.9 Mb out of the total length 849 Mb of chromosome 1B (Šafář et al. 2010).

343

344 Four hexaploid wheat accessions (Cltr8347, PI429624, PI481728, and CI13113), similar to CS

345 wheat, were found to be highly monomorphic with CS DS1S(1B) at the 68 SNP loci within the

346 distal regions of 1BL and 1SL (Fig. S3). They were clustered together with CS wheat and CS

347 DS1S(1B) in the dendrogram (Fig. 5). Cltr8347, PI429624, and PI481728 are the landraces from

348 China, Nepal, and Bhutan, respectively. Both CS and Cltr8347 are the landraces collected

349 probably in southwest China (P.D. Chen, personal communication), which is geographically

350 close to the Himalayan region where Nepal and Bhutan are located. CI13113 is a winter wheat

351 germplasm line with CS involved in the pedigree (http://www.arsgrin.gov/npgs/). Thus, these

352 five hexaploid wheat accessions seem to share a similar origin of this particular *Ae. speltoides*-

353 derived genomic region on 1BL. In addition, we found that 11 tetraploid wheat accessions share

354 the same genotypes at the 68 SNP loci on 1BL (Fig. S3), making them clustered together in the

355 dendrogram (Fig. 5). These tetraploid wheat accessions originated from two primary

356 geographical regions, the Mediterranean Basin and South America (File S2). Overall, the

357 tetraploid wheat accessions showed higher genetic variability than hexaploids in the 68 SNP-

358 defined genomic region on 1BL according to the cluster dendrogram and genetic diversity

359 analysis (Figs. 5 and 6).

360

361 Four of the 68 SNP loci (46805, 31066, 50867, and 76928) showed no allelic variation at all in

362 the 88 wheat accessions. The other four SNPs (71971, 65270, 71898, and 78965) had very

363 minimal variation in the wheat accessions (Fig. S3; File S4). Apparently, these SNP loci have

364 been very conservative over the evolutionary process of this chromosomal region.

365

366 **Discussion**

367

368 *Ae. speltoides* has been considered the diploid species with a genome most closely related to the

369 wheat B genome according to the previous studies (Jenkins 1929; Pathak 1940; Sarkar and

370 Stebbins 1956; Riley et al. 1958; Dvorak and Zhang 1990; Sasanuma et al. 1996; Wang et al.

371 1997; Kilian et al. 2007). However, the relationship of the *Ae. speltoides* S genome with the

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372   wheat B genome remains largely obscure. Previous meiotic pairing-based homology analyses

373   had led to inconsistent conclusions about the evolutionary relationship of the B and S genomes

374   due primarily to the influence of the *Ae. speltoides*-derived *Ph1* suppressors on meiotic pairing

375   (Jenkins 1929; Riley et al. 1958; Kimber and Athwal 1972). In the present study, we partitioned

376   the B and S genomes and investigated meiotic pairing of the individual B/A-S homoeologous

377   pairs in the presence as well as absence of *Ph1*. This allowed us to monitor the effect of *Ph1* and

378   *Ph1* suppressors in meiotic homoeologous pairing and to precisely assess homology for the

379   individual B/A-S homoeologous pairs.

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381   Substantial meiotic pairing was observed between CS wheat chromosome 1B and *Ae. speltoides*

382   chromosome 1S in the presence of *Ph1* and absence of other S-genome chromosomes. The 1B-

383   1S pairing frequency (50.00%) was significantly higher than any other B/A-S homoeologous

384   pairs (0.00-8.63%). Chromosome 1S does not contain a *Ph1* suppressor gene (Dvorak et al.

385   2006). Therefore, no *Ph1* suppressor was involved in the 1B-1S meiotic pairing analysis. It was

386   homology that made CS wheat chromosome 1B pair with *Ae. speltoides* chromosome 1S in a

387   relatively high frequency. A small *Ae. speltoides*-derived chromosomal segment was detected at

388   the distal end of CS wheat 1BL by GISH. Also, we found that 1B-1S meiotic pairing occurred

389   mostly on the long arms (i.e. 1BL-1SL) that share the *Ae. speltoides* segment at the distal ends.

390   Therefore, this *Ae. speltoides*-derived segment was the homologous counterpart on chromosomes

391   1B and 1S that initiated high meiotic pairing between them. Extremely low meiotic pairing (0 out

392   of 105 PMCs) was observed between CS wheat chromosome 1B and *Th. elongatum* chromosome

393   1E in the presence of *Ph1*. Also, a *Th. elongatum*-specific segment was not detected on 1B and

394   other B-genome chromosomes of CS wheat by GISH. As a control, these findings further

395   confirmed the *Ae. speltoides* origin of the distal 1BL region in CS wheat.

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397   We detected the *Ae. speltoides*-originated chromosomal segment at the distal end of 1BL in a

398   representative worldwide collection of tetraploid (including wild and cultivated emmer wheat)

399   and hexaploid wheat (n=179) in addition to CS wheat. Apparently, this *Ae. speltoides*-originated

400   segment on 1BL has been part of the B genome in both tetraploid and hexaploid wheat species

401   probably since the initial incorporation of the B genome into tetraploid wheat. Also, we found

402   that tetraploid wheat had higher genetic diversity than hexaploid wheat within the *Ae. speltoides*-

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403   originated genomic region on 1BL, suggesting an evolutionary pattern similar to other genomic

404   regions of polyploid wheat (Doebley et al. 2006). All these new findings consistently support the

405   conclusion that *Ae. speltoides* had been involved in the origin of the wheat B genome. The *Ae.*

406   *speltoides*-originated chromosomal segment on wheat chromosome 1BL has been retained in the

407   B genome probably throughout the entire evolutionary and domestication process of polyploid

408   wheat.

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410   High-throughput SNP genotyping of individual B/A-S homoeologous pairs and the 88

411   representative wheat species/accessions identified a sizable highly monomorphic linkage block

412   (12.7 cM) at the distal ends of wheat 1BL and *Ae. speltoides* 1SL. This was surprisingly

413   consistent with the meiotic pairing and GISH results, supporting the *Ae. speltoides* origin of the

414   distal segment on 1BL. The SNP-defined monomorphic genomic region and extended distal end

415   on 1BL was estimated to be 10.46 Mb in physical size based on the genomic DNA sequence

416   assemblies of chromosome 1B currently available from IWGSC RefSeq v1.0 (https://wheat-

417   urgi.versailles.inra.fr/Seq-Repository/Assemblies). This estimate is about 1.2% of the total length

418   of chromosome 1B (849 Mb) (Šafář et al. 2010). The GISH-detected *Ae. speltoides* segment on

419   1BL spans approximately 2.0% of the cytogenetic length of chromosome 1B. In addition, the

420   DNA sequence assemblies of the IWGSC RefSeq v1.0 cover approximately 81.3% of the entire

421   chromosome 1B (689.9 Mb out of 849 Mb) (Šafář et al. 2010). Thus, the monomorphic linkage

422   block is probably a major portion of the *Ae. speltoides*-originated chromosomal segment on 1BL.

423   The actual physical size of the *Ae. speltoides*-originated distal segment on 1BL might be greater

424   than the estimate of 10.46 Mb.

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426   Relatively low meiotic pairing was observed with each of other B/A-S homoeologous pairs in the

427   presence of *Ph1*. In addition, we did not detect any *Ae. speltoides*-originated chromosomal

428   segments in the other regions of chromosome 1B and on other B-genome chromosomes by

429   GISH. Are there additional *Ae. speltoides*-originated chromosomal segments that are too small to

430   leverage meiotic pairing and undetectable by GISH in the wheat B genome? We observed small

431   monomorphic linkage blocks at multiple locations in other regions of chromosome 1B and on

432   other B-genome chromosomes, but none was comparable in size to the one at the distal end of

433   1BL. Also, a clear association of those monomorphic linkage blocks with meiotic pairing could

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434   not be established in this study. Thus, we were unable to determine whether those B-genome

435   chromosomal regions originated from *Ae. speltoides*. Further studies, such as genome-wide

436   sequence comparative analysis, are needed to uncover the evolutionary relationship of those

437   genomic regions with *Ae. speltoides*.

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439   In summary, we conclude that *Ae. speltoides* had been involved in the origin and evolution of the

440   wheat B genome. The current form of *Ae. speltoides* should not be considered an exclusive donor

441   of the B genome. *Ae. speltoides* is probably one of the diploid ancestors involved in the

442   evolutionary lineage of the B genome as stated in the theory of polyphyletic origin (Zohary and

443   Feldman 1962). The wheat B genome might be a genome reconstructed from the homoeologous

444   meiotic recombination between multiple ancestral genomes of *Aegilops* species, including *Ae.*

445   *speltoides*. Further studies of *Aegilops* species, especially those in the Sitopsis section, may

446   reveal additional insights into the origin and evolution of the wheat B genome.

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465   **Author contributions**

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467   W.Z. and M.Z. equally contributed to this work on crossing, meiotic pairing analysis, SNP

468   assays, GISH/FISH, comparative analysis of the homoeologous genomic regions, and manuscript

469   preparation. X.Z. participated in crossing, chromosome-specific marker analysis, GISH, and

470   manuscript preparation. Y.C. participated in GISH analysis. Q.S. performed computation in

471   DNA sequence and comparative analysis. G.M. was involved in material maintenance and

472   crossing. S.H. ran SNP assays and participated in manuscript preparation. C.Y. participated in

473   DNA sequence and comparative analysis. S.S.X. was involved in data analysis and manuscript

474   preparation. X.C. designed and coordinated this work, made crosses, analyzed and interpreted all

475   experimental data, and prepared the manuscript.

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477   **Acknowledgements**

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485   **Conflict of interest statement**

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487   The authors declare that they have no conflict of interest.

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Table 1 Presence of the *Ae. speltoides*-originated segment on chromosome 1BL in the 179 accessions under 13 wheat species (*Triticum* L.)

Species	Genome	No. accessions	*Ae. speltoides* segment on 1BL*	Origin
T. aestivum	AABBDD	61	+	Afghanistan, Algeria, Armenia, Australia, Belgium, Bhutan, Bosnia, Canada, China, Croatia, Czech Republic, Denmark, Egypt, Finland, France, Georgia, Germany, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Japan, Kazakhstan, Macedonia, Mexico, Montenegro, Nepal, Pakistan, Russia, Serbia, Spain, Sweden, Switzerland, Syria, Tajikistan, Tunisia, Turkey, Ukraine, USA
T. compactum	AABBDD	5	+	Turkey, Syria, Egypt, USA
T. macha	AABBDD	3	+	Former Soviet Union, Iran, USA
T. spelta	AABBDD	10	+	USA, Ethiopia, Iran, Germany, Belgium, Afghanistan, Macedonia, Spain
T. sphaerococcum	AABBDD	5	+	Iraq, China, USA, Germany, Pakistan
T. vavilovii	AABBDD	1	+	Sweden
T. dicoccoides	AABB	3	+	Israel, Hungary
T. polonicum	AABB	2	+	Hungary, Jordan
T. dicoccum	AABB	2	+	Russia, USA
T. carthlicum	AABB	2	+	Iran, Georgia
T. turgidum	AABB	2	+	Ethiopia, China
T. turanicum	AABB	2	+	Morocco
T. durum	AABB	81	+	Argentina, Bulgaria, Mexico, Israel, Italy, Canada, Peru, Uzbekistan, Russia, Algeria, Azerbaijan, South Africa, Chile, Ukraine, Ecuador, Lebanon, United Kingdom, Ethiopia, France, China, India, Jordan, Malta, Oman, Hungary, Portugal, Pakistan, Saudi Arabia, Serbia, Nigeria, Morocco, Tunisia, Yemen, Turkey, Afghanistan, Portugal, Cyprus, Egypt, Eritrea, Iran, Iraq, Macedonia, North Africa, Pakistan

* "+" indicates presence of the *Ae. speltoides* segment on wheat chromosome 1BL.
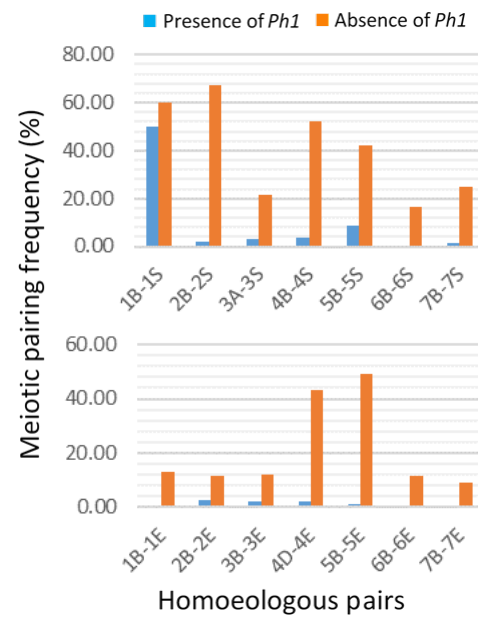
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Figure 1 Meiotic pairing frequency of B/A-S (*top*) and B/D-E (*bottom*) homoeologous pairs in the presence and absence of *Ph1*.
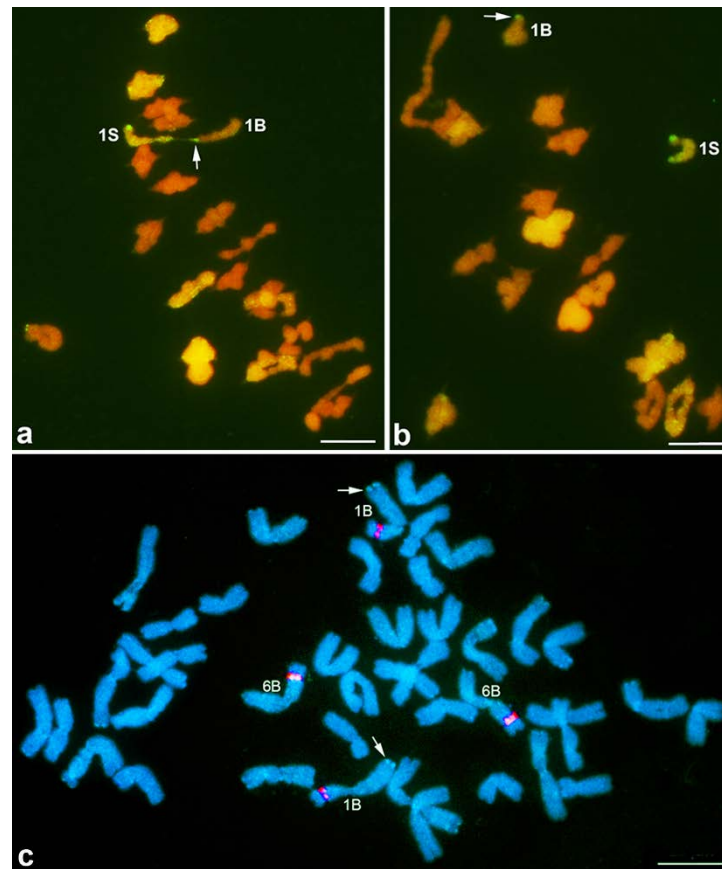
Figure 2 GISH/FISH-painted wheat and *Ae. speltoides* chromosomes. **a)** Showing meiotic pairing of CS wheat chromosome 1B with *Ae. speltoides* chromosome 1S as a rod bivalent; **b)** showing unpaired 1B and 1S chromosomes (univalents); and **c)** showing mitotic chromosomes of CS wheat. Arrows point to the *Ae. speltoides*-originated chromosomal segment on 1BL. Wheat and *Ae. speltoides* chromatin was painted as red and yellow-green by GISH, respectively (**a** & **b**). The *Ae. speltoides*-originated chromosomal segment was painted as light blue-green by GISH and the nucleolar organizer regions on 1BS and 6BS were painted as red by FISH. Wheat chromatin was counter-stained as blue by DAPI (**c**). Scale bar = 10 µm.
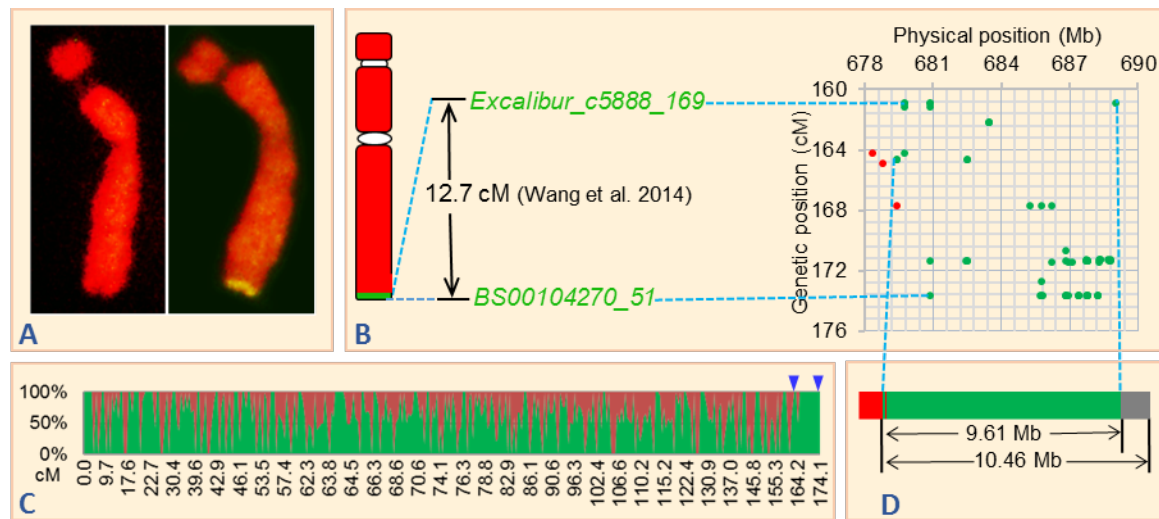
Figure 3 Cytogenetic and molecular mapping of the *Ae. speltoides*-originated chromosomal segment on 1BL. **A)** GISH-painted CS wheat chromosome 1B using *Th. elongatum* genomic DNA as probe (*left*) and *Ae. speltoides* genomic DNA as probe (*right*); wheat chromatin was painted as red and *Ae. speltoides*/*Th. elongatum* chromatin as yellow-green. **B)** Graphical representation of GISH-painted wheat chromosome 1B using *Ae. speltoides* genomic DNA as probe (*left*); genetic size of the highly monomorphic linkage block harboring 68 SNP loci at the distal ends of 1BL and 1SL (*middle*); and genetic and physical locations of the 68 SNP loci within the region (*right*). Green dots refer to the SNP loci monomorphic between CS wheat 1BL and *Ae. speltoides* 1SL; red dots refer to the polymorphic SNP loci. **C)** SNP-based comparative graph showing the distribution of polymorphisms between CS wheat chromosome 1B and *Ae. speltoides* chromosome 1S. Red areas refer to polymorphisms and green areas to monomorphisms. Arrow heads demarcate the highly monomorphic linkage block. **D)** Estimated physical size of the highly monomorphic linkage block harboring the 66 SNP loci and the extended region at the distal end of 1BL. Red, green, and grey bars refer to the polymorphic, monomorphic, and extended genomic regions, respectively.
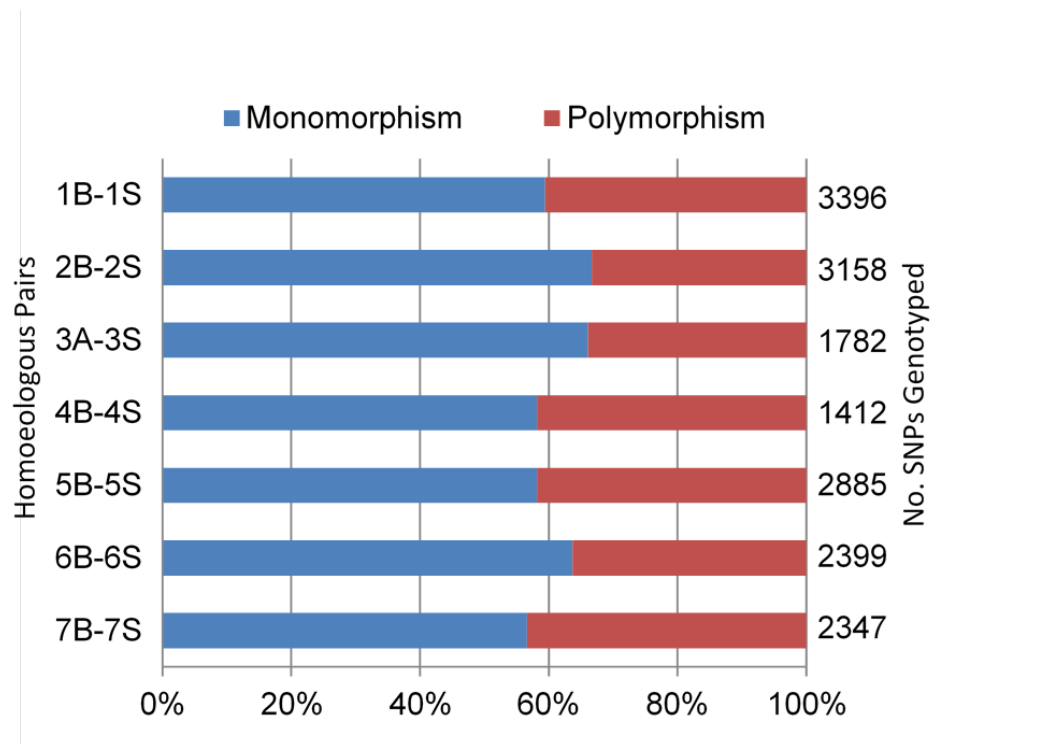
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Figure 4 Polymorphisms of individual B/A-S homoeologous pairs at the SNP loci mapped on wheat B/A-genome chromosomes.
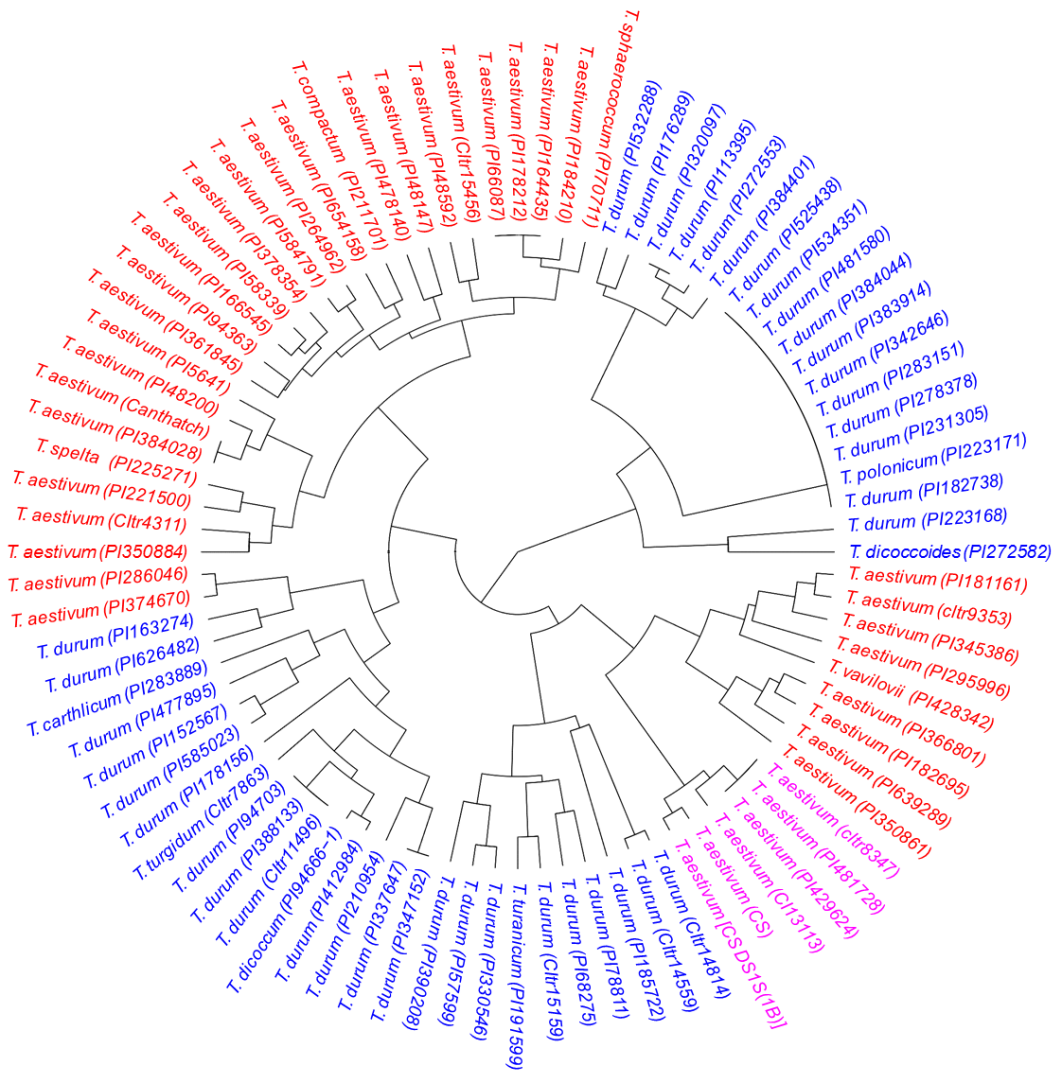
Figure 5 Cluster dendrogram of the 88 representative wheat species/accessions constructed based on the genotypes at the 68 SNP loci within the distal end of 1BL.
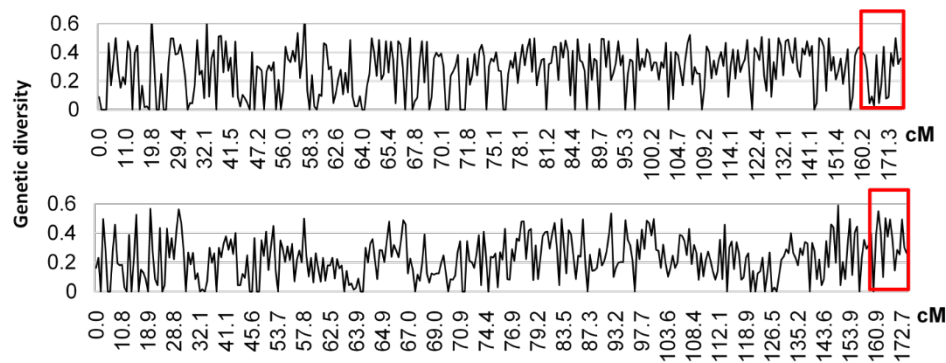
Figure 6 Genetic diversity at the SNP loci on chromosome 1B in 45 hexaploid wheat accessions (*top*) and 43 tetraploid wheat accessions (*bottom*). Red rectangles mark the *Ae. speltoides*-originated chromosomal region spanning the 68 SNP loci at the distal end of 1BL. Y-axis indicates genetic diversity defined as the probability of two different alleles randomly selected from the population.