

Fine mapping of *Hch1*, the causal D-genome gene for hybrid chlorosis in interspecific crosses between tetraploid wheat and *Aegilops tauschii*

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Hybrid chlorosis, one of the reproductive barriers between tetraploid wheat and its D-genome progenitor, *Aegilops tauschii*, inhibits normal growth of synthetic wheat hexaploids. Hybrid chlorosis appears to be due to an epistatic interaction of two loci from the AB and D wheat genomes. Our previous study assigned the causal D-genome gene for hybrid chlorosis, *Hch1*, to the short arm of chromosome 7D. Here, we constructed a fine map of 7DS near *Hch1* using 280 F₂ individuals from a cross of two wheat synthetic lines, one showing normal growth and the other showing hybrid chlorosis. The hybrid chlorosis phenotype was controlled by a single dominant allele of the *Hch1* locus in the synthetic hexaploids. *Hch1* was closely linked to four new markers within 0.2 cM, and may be localized near or within the two *Ae. tauschii* scaffolds containing the linked markers on 7DS. Comparative analysis of the *Hch1* chromosomal region for *Ae. tauschii*, barley and *Brachypodium* showed that a local inversion occurred in the region proximal to *Hch1* during the divergence between barley and *Ae. tauschii*, and that the *Hch1* region on wheat 7DS is syntenic to *Brachypodium* chromosome 1. These observations provide useful information for further studies toward map-based cloning of *Hch1*.

Key words: allopolyploidy, inversion, molecular marker, reproductive barrier, synteny

INTRODUCTION

Many hybrid growth abnormalities such as hybrid necrosis and hybrid chlorosis are controlled by the interaction of at least two loci or alleles, which is the basis of the Dobzhansky-Muller model (Bombliès and Weigel, 2007). Hybrid incompatibility generally promotes intraspecific differentiation and establishes reproductive isolation between two intraspecific lineages (Rieseberg and Willis, 2007). On the other hand, hybrid incompatibility in interspecific crosses between related species inhibits allopolyploid speciation, which is a major evolutionary process in higher plants such as wheat and its relatives. Common wheat (*Triticum aestivum* L., genome constitution AABBDD) is an allohexaploid species derived through interspecific crossing between cultivated tetraploid wheat (*T. turgidum* L., AABB genome) and the wild diploid progenitor *Aegilops tauschii* Coss. (DD genome) (Matsuoka, 2011). This evolutionary process can be arti-

ficially reproduced (Kihara and Lilienfeld, 1949), and a number of synthetic hexaploid wheat plants have been produced from ABD hybrids of tetraploid wheat and *Ae. tauschii* (Mujeeb-Kazi et al., 1996; Matsuoka and Nasuda, 2004; Kajimura et al., 2011; Jones et al., 2013). From ABD triploids obtained from the tetraploid wheat cultivar Langdon (Ldn) and various *Ae. tauschii* accessions, several hybrid growth abnormalities have been observed in many cross combinations (Mizuno et al., 2010). The incompatibilities in the ABD triploids include hybrid chlorosis, severe growth abortion, and two types of hybrid necrosis (type II and type III), all of which act as postzygotic reproductive barriers inhibiting the production of synthetic hexaploid wheat lines (Mizuno et al., 2010).

Four *Ae. tauschii* accessions induce hybrid chlorosis in ABD triploids crossed with Ldn, and leaves of the hybrid plants exhibiting chlorosis gradually turn yellowish with age (Mizuno et al., 2010). Hybrid chlorosis with mild or severe abnormalities has been observed in wheat triploids, and abnormal chloroplasts form in the mesophyll cells before the leaves yellow, suggesting that senescence is accelerated in the hybrid chlorosis lines (Nakano et al.,

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2015). Hybrid chlorosis lines with mild chlorosis show increased resistance to wheat blast and powdery mildew fungi (Nakano et al., 2015). The hybrid chlorosis in ABD triploids occurs due to epistatic interaction between the AB and D genomes, which is postulated to depend on a phenomenon described by the Dobzhansky-Muller model. The causal D-genome gene, *Hybrid chlorosis1* (*Hch1*), for hybrid chlorosis in ABD triploids and wheat synthetics was assigned to the short arm of chromosome 7D (Nakano et al., 2015). Another *Hch* gene, *Hch2*, complementary to *Hch1* is proposed to be present in the AB genome of tetraploid wheat, but no information on it or its chromosome location has been reported. In hybrid necrosis of higher plants, most causal genes that have been isolated to date are related to disease resistance (Bomblies et al., 2007; Alcázar et al., 2009, 2010; Yamamoto et al., 2010). In our previous study, comparative transcriptome analyses showed that up-regulation of defense- and carbohydrate metabolism-related genes is common in hybrid chlorosis and type III necrosis, suggesting the existence of common mechanisms that induce their symptoms (Nakano et al., 2015). Therefore, *Hch1* may be any of the disease resistance genes located in the 7DS region.

Here, we report fine mapping of *Hch1* as a step toward map-based cloning of the hybrid chlorosis-causative gene in future studies. Recently, a draft genome sequence of *Ae. tauschii* from a whole-genome shotgun strategy was made available, which anchored 1.72 Gb of the 4.36-Gb genome to physical chromosomes (Jia et al., 2013). A physical map of the *Ae. tauschii* genome that covers 4 Gb is also available (Luo et al., 2013). In addition, barley genome information efficiently helps to assign wheat genomic and transcript sequences *in silico* to the barley physical map (Iehisa et al., 2012, 2014a, b). Thus, the scaffold sequence data of the *Ae. tauschii* genome are useful for fine mapping of loci assigned to the D genome, such as *Iw2* on 2DS (Nishijima et al., 2014). To make a fine map of the *Hch1* region on 7DS, we developed *Hch1*-linked markers using *Ae. tauschii* and barley genome information, and compared the chromosomal structure of the *Hch1* region with the barley orthologous region based on marker order.

MATERIALS AND METHODS

Plant materials Two synthetic hexaploid wheat lines generated through interspecific crosses of tetraploid wheat accession Ldn with two *Ae. tauschii* accessions, IG47202 and CGN10768 (Mizuno et al., 2010), were used in this study. One synthetic hexaploid, Ldn/IG47202, revealed hybrid chlorosis with mild abnormalities (Nakano et al., 2015), and the other, Ldn/CGN10768, showed normal growth (wild type; WT). An F₂ mapping population was produced from crosses between Ldn/IG47202 and Ldn/CGN10768. Seeds of the Ldn/

IG47202/Ldn/CGN10768 population were sown in November 2013, resulting in 280 F₂ individuals, which were grown in the experimental field of Kobe University.

Phenotype evaluation as a quantitative trait The degree of chlorosis symptom development was determined based on the position of the uppermost leaf with yellowish sectors on the leaf blade at the heading stage in May 2014. In plants with the strongest symptoms, designated position 1, the flag leaf showed yellowish sectors. The position value was determined for the main tiller of each F₂ individual.

Marker development and genotyping Information on SSR markers and their annealing temperature was obtained from the National BioResource Project KOMUGI website (<http://www.shigen.nig.ac.jp/wheat/komugi/strains/aboutNbrpMarker.jsp>) and the GrainGenes website (<http://wheat.pw.usda.gov/GG2/maps.shtml>); the SSR markers are listed in Supplementary Table S1. Some SSR (*kupg*) markers were designed based on the survey sequence data for the short arm of chromosome 7D (Berkman et al., 2011) by searching for SSR motifs using SciRoKo version 3.4 software (Kofler et al., 2007). For mapping of leaf rust-resistance locus *Lr34* (Krattinger et al., 2009), the primer pair 5'-TGCGGCGATTCTATACTACT-3' and 5'-CCGACATCAAGAACCTCC-3' was designed. PCR-amplified products were digested by the 4-bp-cutting restriction enzyme TaqI.

In our previous studies, we performed deep-sequencing analyses of the leaf and spike transcriptomes of two *Ae. tauschii* accessions that represent two major lineages, and more than 16,000 high-confidence single nucleotide polymorphisms (SNPs) were found in 5,808 contigs (Iehisa et al., 2012, 2014a). Contig sequences with the SNPs were used with the BlastN algorithm to search the *Ae. tauschii* and barley genomes (The International Barley Genome Sequencing Consortium, 2012; Jia et al., 2013). These genome sequences included high-confidence genes with an *E*-value threshold of 10⁻⁵ and hit length ≥ 50 bp, fingerprinted contigs, and whole-genome shotgun assemblies.

To choose scaffolds for *Ae. tauschii* sequences throughout the *Hch1* chromosomal region, all the genes contained in each scaffold were used with BlastN to search the barley genomic sequence using the parameters described above. Scaffolds containing at least one gene aligning to the distal region of chromosome 7HS (between 3.66 Mb and 5.51 Mb) were considered candidates for marker development. Scaffolds without genes were anchored based on respective results from BlastN searches against the barley genome. First, high-confidence SNPs (Iehisa et al., 2012, 2014a) plotted on barley 7HS were used for marker development to refine the *Hch1* region. Next, SciRoKo software was used with the search mode setting

“mismatched; fixed penalty” to identify additional SSR markers in sequence data of candidate scaffolds. Additional SNPs were identified in the *Hch1* regions by sequencing approximately 700 bp of amplified DNA of *Ae. tauschii* accessions IG47202 and CGN10768. The nucleotide sequences were determined using an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and SNPs were found through sequence alignment using GENETYX-MAC version 12.00 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). The identified SNPs were converted to cleaved amplified polymorphic sequence (CAPS) markers. The primer sequences for newly developed markers and any relevant restriction enzymes are listed in Supplementary Table S1.

Total DNA was extracted from leaves of the parental strains and F_2 individuals. For SSR genotyping, 40 cycles of PCR were performed using 2x Quick Taq HS DyeMix (TOYOBO, Osaka, Japan) and the following conditions: 10 s at 94 °C, 30 s at the annealing temperature, and 30 s at 68 °C. For CAPS markers, the PCR annealing temperature was 58 °C. PCR products and their digests were resolved in 2% agarose or 13% non-denaturing polyacrylamide gels and visualized under UV light after staining with ethidium bromide.

Construction of the 7DS linkage map and QTL analysis For the initial mapping and quantitative trait locus (QTL) analysis of *Hch1*, 90 F_2 plants were used in the Ldn/IG47202//Ldn/CGN10768 population. Then, 190 F_2 plants from the Ldn/IG47202//Ldn/CGN10768 population were added for fine mapping of *Hch1* with tightly linked molecular markers.

The MAPMAKER/EXP version 3.0b package was used for genetic mapping (Lander et al., 1987). The threshold for log-likelihood (LOD) scores was set at 3.0, and genetic distances were calculated with the Kosambi function (Kosambi, 1943). QTL analyses were carried out by composite interval mapping with Windows QTL Cartographer version 2.5 software (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>; Wang et al., 2011) using the forward and backward method. An LOD score threshold for each trait was determined by computing a 1000 permutation test. The percentage of phenotypic variation explained by a QTL for a trait and any additive effects were also estimated.

Blast analysis of the *Ae. tauschii* genes relative to the *Brachypodium* genome Nucleotide sequences of the selected *Ae. tauschii* scaffolds and annotation information were analyzed with reference to the *Ae. tauschii* draft genome data, which were published by Jia et al. (2013). Reference sequences from *Brachypodium* (The International Brachypodium Initiative, 2010) were used to search the National Center for Biotechnology Informa-

tion NR protein database using the BlastX algorithm with an *E*-value cut-off of 10^{-3} .

RESULTS AND DISCUSSION

QTL analysis of hybrid chlorosis induction In the Ldn/IG47202 hexaploid line, yellowish sectors appeared gradually on the leaf surface, starting with older tissues. F_1 plants from crosses between the Ldn/IG47202 and Ldn/CGN10768 lines also showed the hybrid chlorosis phenotype. F_2 plants exhibiting yellowish sectors on leaves were found among the Ldn/IG47202//Ldn/CGN10768 mapping population. The chlorosis-displaying F_2 plants generated more yellowish sector-containing leaves than normal plants such as Ldn/CGN10768 hexaploids. To evaluate the vigor of each F_2 individual, the position of leaves having yellowish sectors at the heading stage was scored. Heading times of the parental lines, Ldn/IG47202 and Ldn/CGN10768, were 175 and 169 days in 2014, respectively. Mean values for the parental lines, Ldn/IG47202 and Ldn/CGN10768, were 1 and 5, respectively (Fig. 1). Many F_2 individuals showed flag leaves with yellowish sectors (score 1) at the heading stage. F_2 plants with higher scores between 4 and 6 ($N = 69$) showed normal growth and had fewer yellowish sectors than those with lower scores between 1 and 3 ($N = 211$) in the Ldn/IG47202//Ldn/CGN10768 population. Based on this observation, the 280 F_2 individuals were segregated into 211 plants with the chlorosis phenotype and 69 WT plants, significantly fitting a 3:1 ratio ($\chi^2 = 0.02$, $P = 0.89$).

Ninety plants among the 280 F_2 individuals were used to construct the primary linkage map on 7DS. A total of 19 loci including *Lr34*, 13 SSR and 4 CAPS markers, and *Hch1* were used to determine the chromosomal positions

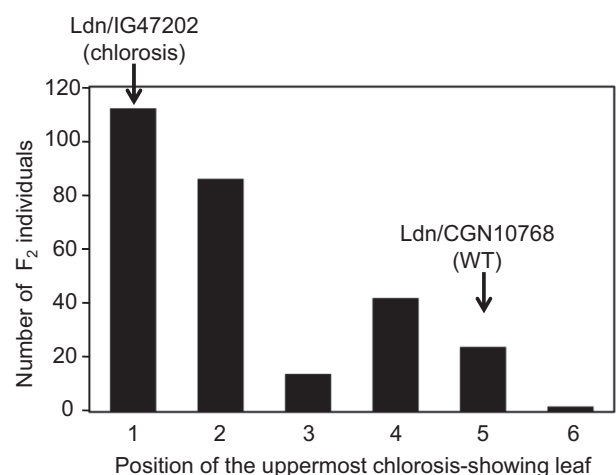


Fig. 1. Frequency distribution in F_2 individuals of the position of the uppermost leaf with yellowish sectors at the heading stage in the Ldn/IG47202//Ldn/CGN10768 population. Parental values are indicated by arrows.

(Fig. 2A). The four *Xctg* loci of CAPS markers represented high-confidence SNPs derived from the *Ae. tauschii* RNA-seq-derived SNP information (Iehisa et al., 2012, 2014a). The map length of 7DS was 50.0 cM with an average spacing of 2.78 cM between loci. The *Hch1* locus was located 0.7 and 0.8 cM from two *Ae. tauschii* scaffold-derived SSR markers, *S25709_1* and *S59343_1*, respectively. The chromosomal position of *Hch1* seemed to correspond to the one previously reported using another F_2 mapping population between a WT line, Ldn/PI476874, and a severe chlorosis line, Ldn/KU-2111 (Nakano et al., 2015).

To confirm the *Hch1* position on 7DS, QTL mapping for the position of leaves with yellowish sectors was performed using the 90 F_2 individuals in the Ldn/IG47202//Ldn/CGN10768 population. The LOD threshold for the QTL was calculated as 2.2, and a single QTL, named *qHch.kpg-7D.1*, was found in the *Lr34* to *S46843_1* region on 7DS (Fig. 2A). The highest LOD score was 26.87 in the QTL region, and the peak position corresponded to

Hch1. *qHch.kpg-7D.1* explained 83.89% of the variation for the position of leaves with yellowish sectors in the F_2 population. The IG47202-derived allele at *qHch.kpg-7D.1* negatively affected the position number of yellowish sector-containing leaves (Fig. 2B), and the additive effect was -1.78 .

These results indicate that the hybrid chlorosis phenotype is controlled by a single dominant allele of the *Hch1* locus in the F_2 population of the present study. Therefore, *Hch1* was used as a qualitative trait marker for construction of a linkage map.

Fine mapping of *Hch1* on 7DS Next, three *Xctg* loci were assigned to the *Hch1* region on 7DS in the Ldn/IG47202//Ldn/CGN10768 population. Physical map information for *Ae. tauschii* and 16,876 scaffolds that constitute 1.49 Gb from the draft *Ae. tauschii* genome sequence have been anchored to the *Ae. tauschii* linkage map (Jia et al., 2013; Luo et al., 2013). The nucleotide sequences of the seven cDNAs corresponding to these

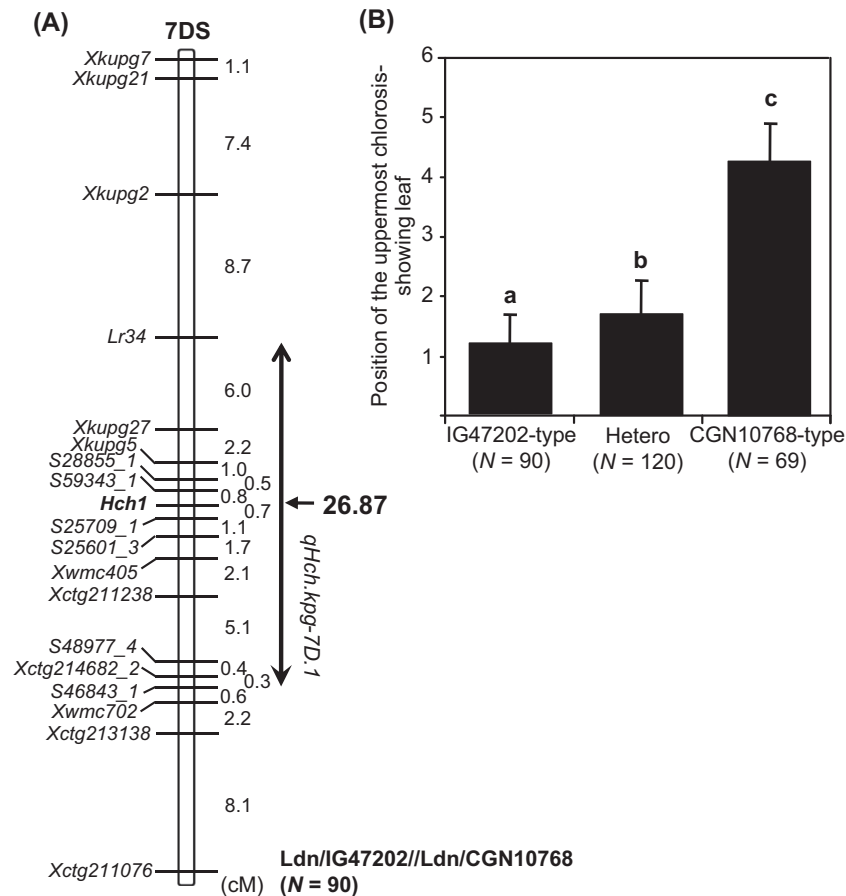


Fig. 2. Detection of QTL for chlorotic-leaf position. (A) Linkage map and position of QTL for hybrid chlorosis on 7DS. The double-headed arrow marks the position and boundaries of the QTL, and an arrow indicates the position showing the highest LOD score of the QTL. Genetic distances are represented in centimorgans on the right of the chromosome. (B) Comparison of the position of the uppermost leaf showing chlorosis between *S40898_1* and *S40898_3* genotypes. Means $\pm$ SD with different letters are significantly different ($P < 0.05$, Tukey-Kramer HSD test).

Xctg loci were used as queries to select carrier scaffolds from the *Ae. tauschii* sequences. We selected the *Ae. tauschii* scaffolds that mapped near the *Xctg*-carrying *Ae. tauschii* scaffolds based on synteny between the wheat and barley genomes and the barley physical map (Mayer et al., 2011). In all, 23 *Ae. tauschii* scaffolds were

assigned *in silico* to an area of the *Ae. tauschii* genome that corresponded to the *Hch1* region in the physical map of barley chromosome 7H (Fig. 3). Using a physical map of the *Ae. tauschii* 7DS chromosome (Luo et al., 2013), we mapped eight *Ae. tauschii* scaffolds *in silico* to the corresponding region in the 7DS physical map. Nucleotide

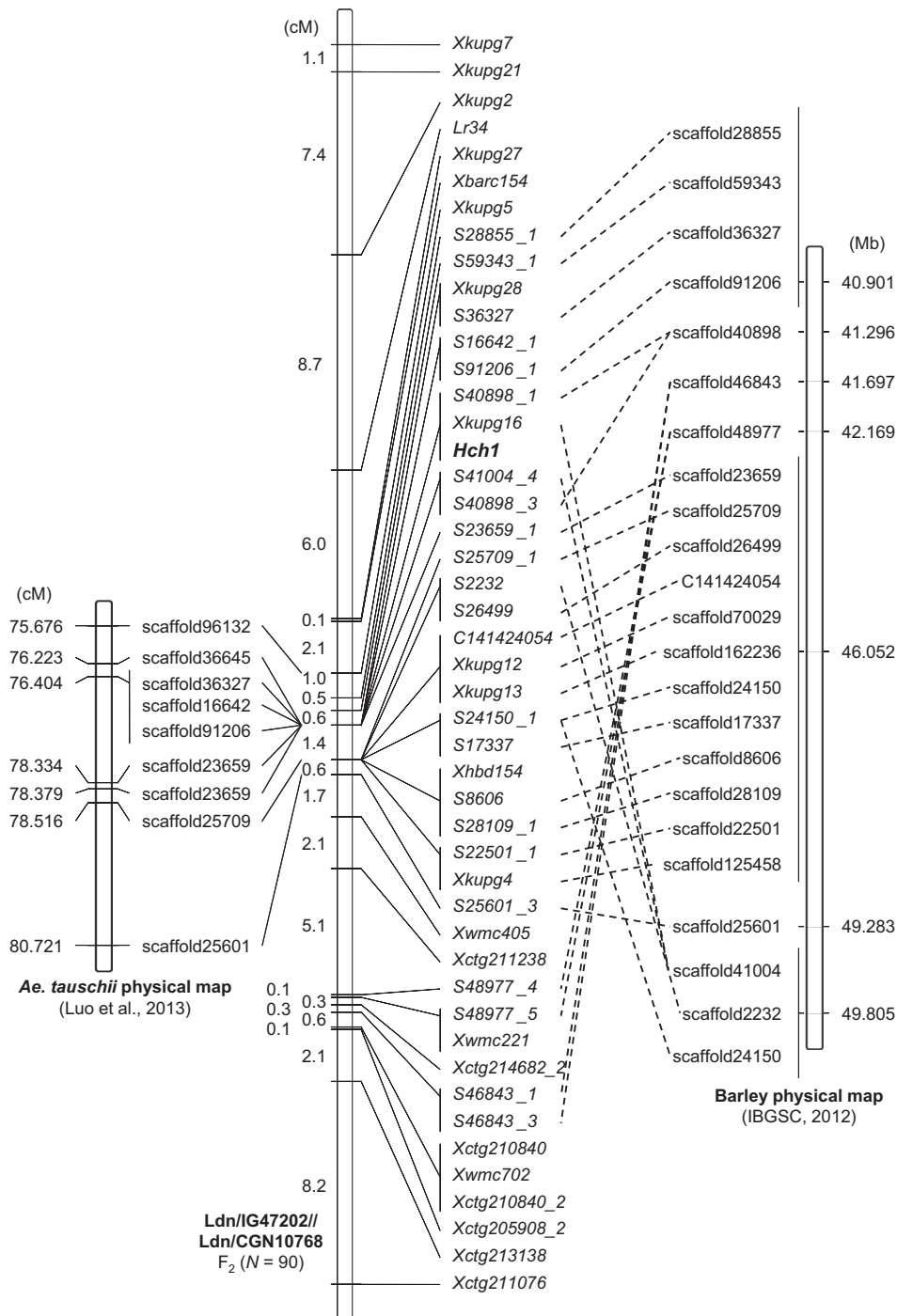


Fig. 3. Comparison of the *Hch1* linkage map, which contains the *Ae. tauschii* scaffolds, with the physical maps of barley and *Ae. tauschii*. The *Ae. tauschii* scaffolds were assigned to regions of the barley physical map of chromosome 7H (The International Barley Genome Sequencing Consortium, 2012). An *Ae. tauschii* physical map with the mapped scaffolds (Luo et al., 2013) is represented.

sequences of the selected scaffolds were used to design CAPS or SSR markers for each scaffold, and the markers that were polymorphic between the parental synthetic hexaploids Ldn/IG47202 and Ldn/CGN10768 were then mapped in the F_2 population (Fig. 3).

All selected scaffolds were mapped to the *Hch1* chromosomal region on 7DS. In the Ldn/IG47202/Ldn/CGN10768 population with 90 F_2 individuals, the *Hch1* locus co-segregated with nine markers derived from seven scaffolds of *Ae. tauschii* (Fig. 3). Next, 280 F_2 individuals of the Ldn/IG47202/Ldn/CGN10768 population and 13 markers including the nine co-segregating markers were used to construct a fine map of *Hch1* (Fig. 4A). On the fine map, *Hch1* was located within the 0.2 cM region between *kupg16* derived from scaffold 41004 and *S40893_3*/*S23659_1* from scaffolds 40898/23659, and co-localized with *S41004_4* from scaffold 41004. These observations suggest that *Hch1* is localized near scaffolds 40898 and 41004 or within one of them on 7DS. The gap length between scaffolds is unknown, so a bacterial artificial chromosome contig covering the *Hch1* region should be

constructed in further studies toward map-based cloning of *Hch1*.

Chromosomal synteny in the *Hch1* region among related species The nine markers co-segregating with *Hch1* in the 90 F_2 individuals of the Ldn/IG47202/Ldn/CGN10768 population covered the 76.223–78.379 cM chromosomal region on 7DS in the *Ae. tauschii* genome (Fig. 3). This *Hch1* chromosomal region corresponded to the 40.901–49.805 Mb region on 7HS of the barley genome (Fig. 3). Scaffold 40898 was more closely linked to *Hch1*, whereas the two scaffolds 46843 and 48977, which existed near scaffold 40898 in the barley 7HS physical map, were more distinct from *Hch1* in the *Ae. tauschii* 7DS linkage map. Instead, scaffold 2232, which was located about 8.5 Mb proximal to scaffold 40898 in the barley physical map, was more closely linked to *Hch1*. These observations indicate that a local inversion occurred in the region proximal to *Hch1* during the divergence between barley and *Ae. tauschii*.

The 13 markers used for the *Hch1* fine mapping were

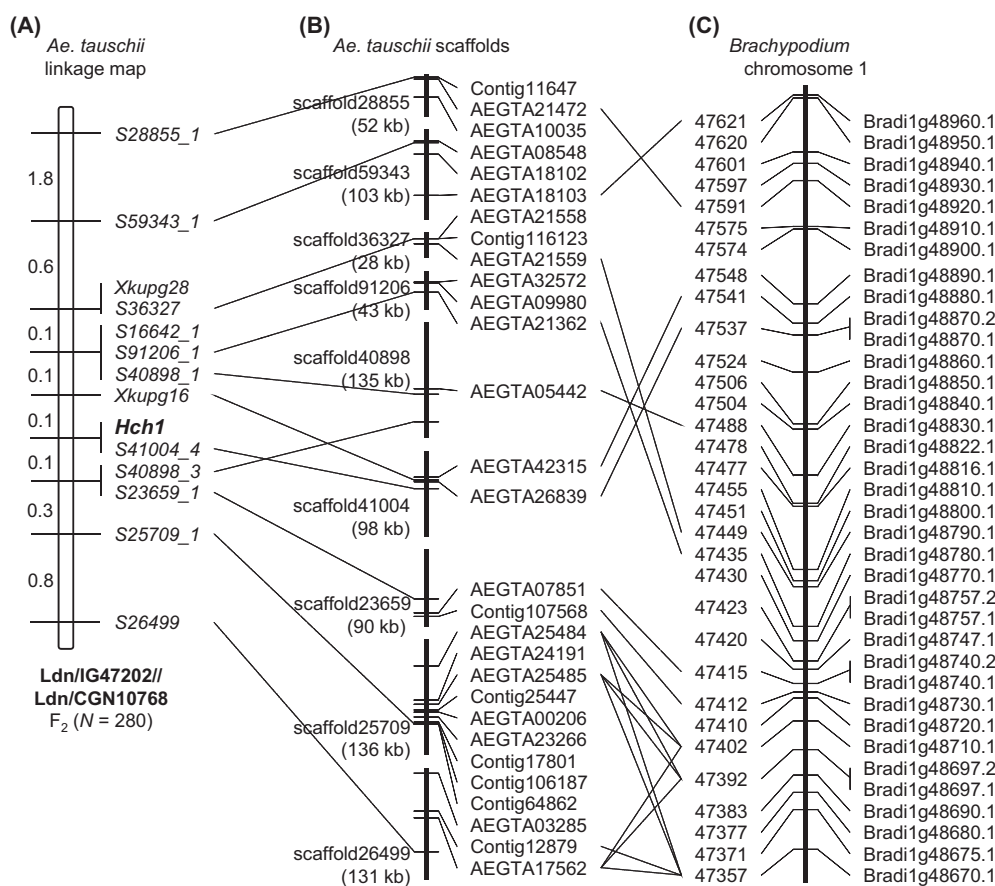


Fig. 4. Comparison of *Ae. tauschii* linkage map of the *Hch1* region, scaffolds, and *Brachypodium* chromosome 1. (A) Linkage map for the region around *Hch1* using 280 F_2 individuals. Genetic distances (in cM) are shown on the left, and markers are shown on the right. (B) *Ae. tauschii* scaffolds putatively anchored to the *Hch1* region. Genes on the scaffolds are shown on the right. (C) The region orthologous to *Hch1* on *Brachypodium* chromosome 1 with gene models on the right and their start position (in kb) on the left.

derived from the nine *Ae. tauschii* scaffolds represented in Fig. 4B, scaffold 16642 for *S16642_1*, and scaffold 36645 for *kupg28*. Each of the 11 scaffolds was 28 to 136 kb and contained one to nine protein-coding genes (Jia et al., 2013; Luo et al., 2013); 33 genes, including 25 with an AEGTA number and 8 contigs, were evident on the 11 scaffolds (Fig. 4B). Scaffolds 16642 and 36645 both con-

tained two AEGTA genes. The *Ae. tauschii* scaffolds that included protein-coding genes were used as queries to search the *Brachypodium* genomic information via a BlastN search. Of the 33 *Ae. tauschii* genes, 27 (20 AEGTAs and 7 contigs) had obvious orthologs in the *Brachypodium* genome (Table 1). Putative orthologs of the *Ae. tauschii* genes from the nine scaffolds were

Table 1. Collinearity between *Ae. tauschii* and *Brachypodium* in the syntenic genomic region of *Hch1*

<i>Ae. tauschii</i> scaffold	<i>Ae. tauschii</i> gene	<i>Brachypodium</i> gene	Annotation
scaffold28855	AEGTA10035		hypothetical protein F775_10035
scaffold28855	AEGTA21472	Bradi1g48920.1	hypothetical protein F775_21472
scaffold28855	Contig11647	Bradi1g48570.1	hypothetical protein F775_21472
scaffold59343	AEGTA08548	Bradi3g17402.1	f-box lrr-repeat protein 9
scaffold59343	AEGTA18102	Bradi1g48560.1	hypothetical protein F775_18102
scaffold59343	AEGTA18103	Bradi1g48960.1	hypothetical protein F775_18103
scaffold36645	AEGTA22321	Bradi4g36350.1	hypothetical protein F775_22321
scaffold36645	AEGTA24215		kelch-like protein 26
scaffold36327	AEGTA21558	Bradi2g40170.1	protoheme ix mitochondrial
scaffold36327	AEGTA21559	Bradi1g48790.1	non-specific lipid-transfer protein 1-like
scaffold36327	Contig116123	Bradi2g40170.1	protoheme ix mitochondrial
scaffold16642	AEGTA15872	Bradi3g18630.1	o-acyltransferase wsd1-like
scaffold16642	AEGTA24437		hypothetical protein F775_24437
scaffold91206	AEGTA09980	Bradi3g19450.1	nuclear nucleic acid-binding protein cld
scaffold91206	AEGTA21362	Bradi1g48780.1	long chain acyl- synthetase 4-like
scaffold91206	AEGTA32572		none
scaffold40898	AEGTA05442	Bradi1g48830.1	flowering locus t protein
scaffold41004	AEGTA26839	Bradi1g48870.1	gdsl esterase lipase at5g45910-like
scaffold41004	AEGTA42315	Bradi1g48880.1	probable beta-galactosyltransferase 14
scaffold23659	AEGTA07851	Bradi1g48740.1	rrna-processing protein fcf1 homolog
scaffold23659	Contig107568	Bradi1g48730.1	glyoxalase bleomycin resistance protein dioxygenase
scaffold25709	AEGTA00206	Bradi5g03790.1	subtilisin-like protease
scaffold25709	AEGTA23266	Bradi5g03790.1	subtilisin-like protease
scaffold25709	AEGTA24191		hypothetical protein F775_24191
scaffold25709	AEGTA25484	Bradi1g48697.2	abc transporter c family member 10-like
		Bradi1g48670.1	abc transporter c family member 10-like
		Bradi1g48697.1	abc transporter c family member 10-like
		Bradi1g48710.1	abc transporter c family member 10-like
scaffold25709	AEGTA25485	Bradi1g48697.2	abc transporter c family member 10-like
		Bradi1g48697.1	abc transporter c family member 10-like
		Bradi1g48670.1	abc transporter c family member 10-like
		Bradi1g48710.1	abc transporter c family member 10-like
scaffold25709	Contig106187	Bradi5g03790.1	subtilisin-like protease
scaffold25709	Contig17801	Bradi3g10037.1	subtilisin-like protease
scaffold25709	Contig25447		none
scaffold25709	Contig64862	Bradi5g03190.1	subtilisin-like protease
scaffold26499	AEGTA03285	Bradi3g35840.1	cytochrome p450 87a3
scaffold26499	AEGTA17562	Bradi1g48670.1	abc transporter c family member 10-like
		Bradi1g48697.2	abc transporter c family member 10-like
		Bradi1g48697.1	abc transporter c family member 10-like
		Bradi1g48710.1	abc transporter c family member 10-like
scaffold26499	Contig12879	Bradi1g48670.1	abc transporter c family member 10

assigned to the 47,357 to 47,621 kb region of *Brachypodium* chromosome 1. Two genes in scaffold 16642 were orthologous to genes, AEGTA15872 and AEGTA24437, assigned to *Brachypodium* chromosome 3 and two genes in scaffold 36645 were orthologous to genes, AEGTA22321 and AEGTA24215, assigned to chromosome 4. With these four exceptions, the *Hch1* region on 7DS therefore showed chromosomal synteny to *Brachypodium* chromosome 1. Although a few local and small inversions and two small translocations were found in the chromosomal region, the gene order and gene content were generally conserved during the divergence between *Brachypodium* and *Ae. tauschii*.

Disease resistance genes encoding NB-LRR-type resistance (R) proteins and R-interacting proteins have been identified as causal genes for hybrid incompatibilities such as hybrid necrosis in higher plants (Bomblies et al., 2007; Alcázar et al., 2009, 2010; Yamamoto et al., 2010; Chen et al., 2013). Among the 33 *Ae. tauschii* genes in the *Hch1* region, no obvious R genes were found (Table 1). Four ABC transporter protein-coding genes were clustered in two scaffolds, 25709 and 26499, proximal to *Hch1*. A putative ABC transporter gene was identified as *Lr34* on 7DS in common wheat (Krattinger et al., 2009), and *Lr34* was mapped about 11 cM distal from *Hch1* (Fig. 3). These observations indicate that several ABC transporter genes are dispersed around the *Hch1* region on 7DS. *Lr34* is involved in resistance against multiple pathogens such as leaf rust, stripe rust and powdery mildew, and stimulates senescence-like processes in the flag leaf tip and edge (Krattinger et al., 2009). A 3-bp deletion in exon 11 and a non-synonymous substitution in exon 12 of the *Lr34* D-genome copy are associated with disease resistance, and two homoeologous copies of *Lr34-D* are assigned to chromosomes 7A (*Lr34-A*) and 4A (*Lr34-B*) in common wheat (Krattinger et al., 2009, 2011). Our previous study showed that senescence was accelerated in leaves with yellowish sectors, and resistance to wheat blast and powdery mildew fungi was significantly enhanced in the wheat synthetics exhibiting hybrid chlorosis (Nakano et al., 2015). Therefore, any of the ABC transporter genes dispersed on 7DS could be candidates for *Hch1*. In our previous studies, two distinct levels of chlorosis severity, severe and mild, were reported (Mizuno et al., 2010; Nakano et al., 2015). Allelic differences in *Hch1* should be analyzed in further studies. In addition, no information on a causative AB-genome gene for hybrid chlorosis, *Hch2*, has been reported. To answer these questions, the molecular nature of *Hch1* should first be elucidated.

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