Dissection of barley chromosome 5H in common wheat

Taizo Ashida¹, Shuhei Nasuda¹, Kazuhiro Sato²
and Takashi R. Endo^{1*}

¹Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University,
Kyoto 606-8502, Japan

²Research Institute for Bioresources, Okayama University,
Okayama 710-0046, Japan

(Received 9 January 2007, accepted 30 January 2007)

We dissected barley chromosome 5H added to common wheat by a genetic method or the gametocidal system. Firstly, we induced chromosomal breaks in the offspring of a 5H addition line of common wheat carrying a gametocidal chromosome and cytologically screened for plants with structural chromosomal changes involving 5H, such as deletions and translocations. Secondly, we screened the progeny of such plants to establish common wheat lines carrying structurally changed chromosomes containing single segments of the dissected 5H. Using 23 representative 5H dissection lines, we physically mapped 97 barley EST markers assigned to 5H. The ESTs fell into 20 regions of 5H between the breakpoints of the 23 dissected segments, distributing rather evenly along the chromosome, with significantly higher frequency in the distal region of the long arm. The ESTs, in turn, allowed us to distinguish the breakpoints of dissected 5H segments. We demonstrated by PCR (polymerase chain reaction), as well as by *in situ* hybridization, that these dissected 5H segments were stably transmitted in the dissection lines. We discuss the usefulness of the 5H dissection lines for physical mapping of DNA markers. These 5H dissection lines are available from National BioResource Projects-Wheat, Japan.

Key words: barley, chromosome 5H, common wheat, physical mapping, structural changes

INTRODUCTION

Six of the possible seven wheat-barley disomic chromosome addition lines of common wheat were produced by Islam et al. (1981) (1H addition line is not available), and they can be primary materials useful for transferring barley genes into wheat. Attempting to transfer genes from barley to wheat, some researchers isolated wheat-barley recombinant chromosomes that were induced by homeologous pairing (Islam and Shepherd 1992; Sherman et al. 2001; Murai et al. 1997; Taketa et al. 2005).

Besides its agronomic importance, barley, which is diploid and self-fertile, has been one of the model plants for genetic and genomic studies. However, its large genome (5439 Mbp, Bennett and Leitch (2005)) containing a high percentage of repetitive sequences (over 80%, Schulman et al. (2004)) has been a major obstacle to sequencing the entire genome. In barley, therefore, the

construction of chromosome maps is still the first step in attempts to clone agronomically important genes. Genetic maps have been constructed in barley by using various molecular markers, including expressed sequence tag (EST)-derived SSR markers (Varshney et al., 2006). ESTs and sequenced cDNA fragments, which are rapidly being developed in barley genomics, would enable us to access genes of interest in the genome with ease. Recently, more than 130,000 barley ESTs have been sequenced and analyzed in the Research Institute for Bioresources at Okayama University, Japan, and National Institute of Genetics, Japan (<http://www.shigen.nig.ac.jp/barley/>).

More than 10,000 primer sets have been developed and a high-density barley genetic map was constructed by using these barley EST markers by Sato et al. (2004). These barley EST markers can be applied widely to the genomics of other species; many of them show polymorphism between wheat and other Triticeae species (Hagras et al. 2005). These barley ESTs are useful not only as markers for chromosome mapping but also for the

Edited by Minoru Murata

* Corresponding author. E-mail: trendo@kais.kyoto-u.ac.jp

arrangement of BAC contigs (Yu et al., 2000; Saisho et al., 2007) and microarrays (Close et al., 2004).

In general, genetic maps do not necessarily represent actual physical locations of genes or molecular markers on chromosomes (DeScenzo and Wise, 1996). Cytological maps, in which genes and molecular markers are physically located on specific chromosomal regions, can rectify such shortcomings. In barley, physical RFLP maps have been constructed by two different methods. One utilizes microdissected translocation chromosomes as templates for PCR (polymerase chain reaction) with primers designed from sequenced RFLP probes (Sorokin et al., 1994; Künzel et al., 2000). The other is deletion mapping using deletions and translocations involving a dissected barley chromosome in common wheat (Serizawa et al., 2001). In the latter case, the structural changes of the barley chromosome were induced in a wheat-barley addition line using the gametocidal system (Shi and Endo, 1997, 1999, 2000). The same gametocidal system was applied successfully to the induction of terminal deletions in common wheat chromosomes (Endo, 1988, 1990; Endo and Gill, 1996). The deletion stocks of common wheat were used to map wheat RFLPs (Werner et al., 1992), AFLPs (Zhang et al., 2000) and ESTs (Gustafson et al., 2004; Qi et al., 2004). Nasuda et al. (2005) allocated barley EST markers to each barley chromosome-arm by using a set of wheat-barley chromosome addition lines, and they also demonstrated the effectiveness of physical mapping of ESTs assigned to 7H using dissected 7H chromosomes induced using the gametocidal system in common wheat. There is no doubt about the usefulness of physically mapped ESTs, e.g. as landmarks in arranging barley BAC clones into contigs. For the physical mapping of barley ESTs, it is needed to establish an array of stable common wheat lines carrying dissected fragments of each barley chromosome.

In this study, we focused on barley chromosome 5H, formerly chromosome 7 (Linde-Laursen et al. 1997), which has agronomically important genes related to early heading, vernalization requirement and photoperiodic sensitivity (Koba et al., 1997). We report on the induction of structural changes involving 5H using the gametocidal system, on the physical mapping of barley EST markers, and on the establishment of common wheat lines carrying structurally changed 5H chromosomes.

MATERIALS AND METHODS

Plant materials We used a wheat-barley addition line of common wheat (*Triticum aestivum* cv. Chinese Spring (abbreviated CS), $2n = 6x = 42$, genome formula AABBDD) that has a pair of 5H chromosomes of barley (*Hordeum vulgare* cv. Betzes, $2n = 2x = 14$, HH) (Islam et al., 1981). This line was crossed with a CS line carrying a pair of gametocidal $3C^{SAT}$ chromosomes and the F_1 was back-

crossed to the 5H addition line to screen for 45-chromosome plants carrying a pair of 5Hs and one $3C^{SAT}$ chromosome. Structurally aberrant 5H chromosomes were expected to occur in the progeny of the 45-chromosome plants (Endo, 2007). We also used 5HS and 5HL ('S' and 'L' stand for the short and long arm of 5H, respectively) addition lines of CS (Islam, 1983), and euploid CS and Betzes barley as controls. These genetic stocks were obtained from National BioResource Project-Wheat (<http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp>).

Cytological screening and characterization of 5H fragments

We screened the progeny of the 5H addition line with one $3C^{SAT}$ chromosome by simultaneous FISH (fluorescence *in situ* hybridization) with the barley-specific subtelomeric sequence HvT01 and GISH (genomic *in situ* hybridization) with the total barley genomic DNA. HvT01 is a 118-bp satellite DNA repeat that is present in the chromosomes in tandem arrays (Belostotsky and Ananiev, 1990) and is localized in the terminal regions of all barley chromosomes (Schubert et al., 1998; Shi and Endo, 1999). Chromosome preparation, probe labeling, and detection of FISH/GISH probes were conducted as described (Schubert et al. 1998). The breakpoints of the deletions and translocations were analyzed by C-banding, followed by FISH/GISH on the same metaphase cells, as described by Masoudi-Nejad et al. (2002).

Barley 5H EST primers We used 100 pairs of EST primers that are specific to 5H. These EST primers amplify 5H-specific PCR products that have already been assigned to either 5HS (23) or 5HL (77) (Nasuda et al., 2005). The primer sequences are in the APPENDIX.

DNA extraction and PCR We extracted genomic DNAs from fresh or frozen young leaves by the modified CTAB (cetyltrimethylammonium bromide) method (Saghai-Marooif et al. 1984) and used 2.5 μ l of the DNA solution (about 20 ng/ μ l) as a template for PCR. The PCR mixture consisted of 1.25 μ l of $10 \times$ PCR buffer, 1 μ l of dNTP (2.5 mM each), 0.75 μ l of $MgCl_2$ of (25 mM), 0.25 μ l of primers (10 pmol/ μ l), 0.1 μ l of Ex.Taq polymerase (Takara), and 6.65 μ l of dH_2O . The thermal cycling was carried out in an iCycler (BioRad) using the following conditions: 94°C for 2 min, 5 cycles of 94°C for 30 sec, 60°C for 30 sec (with the temperature subsequently decreased 1°C per cycle), and 72°C for 1 min, 35 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min, and 72°C for 7 min. We checked the PCR products by agarose (Agarose S, Nippon gene) gel electrophoresis at 100 V for 80 min.

RESULTS AND DISCUSSION

Screening for 5H structural changes We cytologically examined the self-pollinated progeny (1333 plants)

Table 1. Breakpoints of the 123 aberrant 5H chromosomes

Type of aberration	Breakpoint in 5H				
	5HS	5HL	Both arms	Centromere ^a	Unknown
Terminal deletion	8	12	2	6 (5HS), 2 (5HL)	0
Whole-arm translocation	–	–	–	14 + 1 ^b (5HS), 7 (5HL)	0
Translocation with the 5H centromere	6	13	1	–	0
Translocation with the wheat centromere	14	14	–	–	11
Intercalary translocation	0	0	1	–	1
Isochromosome	–	–	–	5 (5HS), 5 (5HL)	0

^a 5HS and 5HL in parentheses represent the 5H chromosome arm retained in the aberrations.

^b One chromosome lacked the terminal region of 5HS.

of the 45-chromosome ($21'' + 1''5H + 1'3C^{SAT}$) plants and obtained 132 plants with structurally changed 5H chromosomes, which were either self-pollinated or backcrossed to CS. Forty-eight out of the 132 plants had two or more structural changes involving 5H, presumably originated from reciprocal translocations or fertilization of male and female gametes containing different aberrant chromosomes carrying dissected 5H segments. We again screened the progeny of 78 plants randomly chosen from the 132 plants. By examining ten or fewer plants for each progeny, we recovered the dissected 5H segments in the progenies of 70 plants; we found no 5H segments in the remaining eight progenies (two, four and ten plants were examined for one, five and two progenies, respectively). In total, we found 135 chromosomes carrying dissected 5H segments, for 123 of which we determined the type of structural changes (Table 1). There were 30 terminal deletions, 83 translocations between 5H and wheat chromosomes, and 10 isochromosomes. These rearrangements are characterized by breakpoints in 5HS (28), in 5HL (39), in both arms (4), in the centromere (40), and in unknown positions (12). It is noteworthy that the $3C^{SAT}$ chromosome generated many more translocations than simple terminal deletions and induced the most severe breakage in the centromeric region. The satellite sequences and retrotransposons localized at the barley centromere might be vulnerable to the gametocidal action as reported for barley chromosome 7H (Nasuda et al. 2005). After the second-round screening, we established 68 common wheat lines that carry single segments of the dissected 5H chromosome in either homozygous or hemizygous condition (we call these lines '5H dissection lines'). From the 5H dissection lines we chose 23 lines such that the breakpoints of the dissected 5H chromosomes cover the entire 5H (Fig. 1), for the physical mapping of the barley ESTs. Eight lines had deleted 5H chromosomes with breakpoints in 5HS (5H-7 and 5H-9) or in 5HL (5H-10, 5H-11, 5H-13, 5H-15, 5H-19 and 5H-20); 15 lines had translocations between wheat and 5H chromosomes with breakpoints in 5HS (5H-1, 5H-2, 5H-3, 5H-4, 5H-5, 5H-6 and 5H-8), in 5HL (5H-12, 5H-14, 5H-16, 5H-17, 5H-18,

5H-21 and 5H-22), or in both arms (5H-23). These 5H fragment lines provided 10 breakpoints in the short arm (two of which were in the satellite) and 14 in the long arm.

Physical mapping of barley ESTs We excluded three ESTs out of the 100 specific EST markers from the physical mapping analysis because they did not give clear PCR amplification products. Using template DNA from the 23 5H dissection lines, and 5H, 5HS and 5HL addition lines, we examined the presence or absence of the 97 ESTs in these lines (Fig. 2A, Table 2). This PCR analysis allowed us to identify the chromosomal positions of the 97 ESTs between the breakpoints of two dissected 5H segments, one of which retained the EST and the other of which did not. We located the positions of the ESTs on either 5HS (23) or 5HL (74). The result was consistent with that reported by Nasuda et al. (2005). Thus, the 97 ESTs were assigned to 9 regions of 5HS and to 11 regions of 5HL (Fig. 3). At least one EST marker (and up to 20) was allocated to each region. In fact, the EST mapping distinguished the breakpoint positions of the dissected 5H segments that were indistinguishable by FISH/GISH. For example, PCR analysis revealed that the breakpoint of 5H-19 was proximal to that of 5H-20 (Fig. 1, Table 2). However, the ESTs did not specify the breakpoint positions of 5H-8 and 5H-9 within 5HS nor those of 5H-11, 5H-12 and 5H-23 within 5HL.

Using the arm ratio of the intact 5H chromosome and the arm ratio of 5H-16, we calculated that 5H-16 retained about 56% of the 5HL length. The fact that 5H-16 contained 17 out of the 74 ESTs of 5HL indicates that 77.0% of the EST markers are located in the 5HL distal region that accounts for about 44% of its length. Eight (34.8%) out of the 23 ESTs of 5HS belong to the satellite, which occupies about 26.2% of the short arm excluding the nucleolus organizing region. Similar observations, i.e. more ESTs in the distal than in the proximal chromosome regions, were made in barley physical mapping (Künzel et al., 2000; Nasuda et al., 2005) and linkage mapping (Varshney et al., 2006), and in wheat deletion mapping

Fig. 1. C-banding (left) and GISH/FISH (right) images of normal chromosome 5H, telocentric chromosome 5HS, telocentric chromosome 5HL, and aberrant chromosomes carrying dissected 5H segments (5H-1 to 5H-23). 5H-6-2 is a whole-arm translocation between the 5H-6 short arm and an unknown wheat chromosome arm and 5H-13-2 is a whole-arm translocation between the 5H-13 deficient long arm and an unknown wheat chromosome arm. The FISH probe was the barley subtelomeric repeat sequence HvT01 (green) and the GISH probe was barley total genomic DNA (red). The HvT01 signal on the long arm was too weak to be observed always. Chromosomes were counterstained with DAPI (blue).

Fig. 2. A: An example of PCR analysis of the 23 5H dissection lines using EST marker k04947. B: The PCR screening of the progeny of the 5H-6 dissection line using three EST markers (for each bracketed group, left: k02019, middle: k00675, right: k02569), which are located on the deleted 5HS region, the remaining 5HS region, and the intact 5HL of 5H-6, respectively. One plant (5H-6-1) had a whole 5H-6, a second plant (5H-6-2) retained only the short arm of 5H-6 translocated to a wheat chromosome (see Fig. 1, 5H-6-2), a third plant (5H-6-3) had only the 5HL telocentric chromosome, and the remaining seven plants (5H-6-4-10) had no 5H chromatin. "M" stands for 1-kb DNA ladder marker.

(Erayman et al., 2004).

Stability and use of 5H dissection lines We checked the transmission of the dissected 5H chromosomes in ten plants of the selfed progeny for each 5H dissection line, by FISH/GISH and PCR using primer sets for the ESTs flanking the breakpoints of the dissected 5H segments and for an EST on the opposite intact arm when it existed. The screening results of the FISH/GISH and PCR analyses were always consistent. We never observed secondary breakage in the dissected 5H chromosomes except in the centromere. We recovered the critical dissected 5H chromosomes from all lines, and obtained homozygous plants for 5H-12, 5H-15, 5H-16 and 5H-17, as well as for 5H-7, 5H-13 and 5H-22 that had already been homozygous in the parental lines. We found that the dissected 5H chromosomes were vulnerable to centromere misdivision, even in disomic condition, generating telocentric chromosomes and new whole-arm translocations with wheat chromosome arms. For example, one of the ten selfed progeny of the 5H-6 line had an intact 5H-6 chromosome, but a second plant had a whole-arm translocation between the 5H-6 short arm and an unknown wheat chromosome arm (Fig. 1, 5H-6-2; Fig. 2B) and a third plant had only the 5H-6 long-arm telosome; the other seven plants contained no 5H-6 chromosome. Eight of the ten selfed progeny of the 5H-13 disomic plant were

disomic for 5H-13, but one plant had a whole-arm translocation between the 5H-13 deficient long arm and an unknown wheat chromosome arm (Fig. 1, 5H-13-2) and another plant was devoid of 5H-13. This suggests that aberrant chromosomes containing dissected 5H segments do not pair well and often suffer from centromere misdivision during meiosis. Therefore, it is desirable to check the chromosome constitutions of the 5H dissection lines before planting, even when parents were homozygous.

Physically mapped barley ESTs are useful for the barley genome project because it is not easy to sequence the gigantic barley genome consisting mostly of repetitive DNA sequences. For example, the ESTs can be used to localize the positions of barley BAC (bacterial artificial chromosome) clones on chromosomes. Barley dissection lines would be useful for mapping specific genes and DNA markers, including ESTs, and also in transcriptome analyses of barley genes as done by Cho et al. (2006) using wheat-barley chromosome addition lines. The 5H dissection lines established in this study are available from National BioResources Project-Wheat (<http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp>).

This article is a contribution (No. 592) from the Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University.

Table 2. Presence or absence of the 97 ESTs in the 23 5H dissection lines¹⁾[illegible]

EST on 5HL		5H	S	5HL	-1	-2	-3	-4	-5	-6	-7	-23	-8	-9	-10	-11	-12	-23	-13	-14	-15	-16	-17	-18	-19	-20	-21	-22	Region
k04305	*														*	*	*	*	*	*	*	*	*	*	*	*	*	*	X
k04508	*														*	*	*	*	*	*	*	*	*	*	*	*	*	*	X
k04717	*															*	*	*	*	*	*	*	*	*	*	*	*	*	XI
k01962	*															*	*	*	*	*	*	*	*	*	*	*	*	*	XI
k03400	*																	*	*	*	*	*	*	*	*	*	*	*	XII
k03449	*																		*	*	*	*	*	*	*	*	*	*	XIII
k01322	*																		*	*	*	*	*	*	*	*	*	*	XIII
k02998	*																		*	*	*	*	*	*	*	*	*	*	XIII
k03232	*																		*	*	*	*	*	*	*	*	*	*	XIII
k03364	*																		*	*	*	*	*	*	*	*	*	*	XIII
k04166	*																			*	*	*	*	*	*	*	*	*	XIV
k04921	*																			*	*	*	*	*	*	*	*	*	XIV
k05150	*																			*	*	*	*	*	*	*	*	*	XIV
k01302	*																				*	*	*	*	*	*	*	*	XV
k03357	*																				*	*	*	*	*	*	*	*	XV
k04996	*																				*	*	*	*	*	*	*	*	XV
k01041	*																				*	*	*	*	*	*	*	*	XV
k00669	*																					*	*	*	*	*	*	*	XVI
k02336	*																					*	*	*	*	*	*	*	XVI
k02464	*																					*	*	*	*	*	*	*	XVI
k02556	*																					*	*	*	*	*	*	*	XVI
k02723	*																					*	*	*	*	*	*	*	XVI
k03435	*																					*	*	*	*	*	*	*	XVI
k00970	*																					*	*	*	*	*	*	*	XVI
k00101	*																					*	*	*	*	*	*	*	XVI
k00192	*																					*	*	*	*	*	*	*	XVI

continued

	5HS	5HL	-1	-2	-3	-4	-5	-6	-7	-23	-8	-9	-10	-11	-12	-23	-13	-14	-15	-16	-17	-18	-19	-20	-21	-22	Region
k03075	*																				*	*	*	*	*		XVI
k00950	*																				*	*	*	*	*		XVI
k01353	*																				*	*	*	*	*		XVI
k01426	*																				*	*	*	*	*		XVI
k00038	*																				*	*	*	*	*		XVI
k00102	*																				*	*	*	*	*		XVI
k00446	*																				*	*	*	*	*		XVI
k00741	*																				*	*	*	*	*		XVI
k00263	*																				*		*	*	*		XVII
k03278	*																				*		*	*	*		XVIII
k04727	*																				*		*	*	*		XVIII
k01017	*																				*		*	*	*		XVIII
k00046	*																				*		*	*	*		XVIII
k02647	*																				*		*	*	*		XVIII
k00760	*																				*		*	*	*		XVIII
k00839	*																				*		*	*	*		XVIII
k00642	*																				*		*	*	*		XVIII
k01087	*																				*		*	*	*		XIX
k04025	*																				*		*	*	*		XIX
k04030	*																				*		*	*	*		XIX
k04207	*																				*		*	*	*		XIX
k02593	*																				*		*	*	*		XIX
k00162	*																				*		*	*	*		XIX
k00572	*																				*		*	*	*		XIX
k01139	*																				*		*	*	*		XIX
k03356	*																				*		*	*	*		XIX
k04732	*																				*		*	*	*		XIX
k03038	*																				*		*	*	*		XIX
k01319	*																				*		*	*	*		XX
k01359	*																				*		*	*	*		XX
k01445	*																				*		*	*	*		XX
k03359	*																				*		*	*	*		XX
k03513	*																				*		*	*	*		XX
k04765	*																				*		*	*	*		XX
k04947	*																				*		*	*	*		XX
k05087	*																				*		*	*	*		XX
k00006	*																				*		*	*	*		XX
k00213	*																				*		*	*	*		XX
k02569	*																				*		*	*	*		XX
k03320	*																				*		*	*	*		XX
k01110	*																				*		*	*	*		XX
k01230	*																				*		*	*	*		XX
k01516	*																				*		*	*	*		XX
k03445	*																				*		*	*	*		XX
k04973	*																				*		*	*	*		XX
k00578	*																				*		*	*	*		XX
k01987	*																				*		*	*	*		XX
k04490	*																				*		*	*	*		XX

¹⁾ “*” and “blank” represent the occurrence and absence of PCR amplification, respectively.

²⁾ Region I-XX are depicted Fig. 3.

Fig. 3. A schematic representation of the 5H-segment regions (vertical solid bars) of the aberrant chromosomes in the 23 dissected 5H lines relative to normal chromosome 5H in the center (solid horizontal bars: C-bands, reticulate circles: HvT01 FISH signals). Numbers in parentheses indicate the numbers of ESTs allocated to each of the 20 regions, I to XX.

REFERENCES

- Belostotsky, D. A., and Ananiev, E. V. (1990) Characterization of relic DNA from barley genome. *Theor. Appl. Genet.* **80**, 374–380.
- Bennett, M. D., and Leitch, I. J. (2005) Angiosperm DNA C-values database (release 6.0).
- Cho, S., Garvin, D. F., and Muehlbauer, G. J. (2006) Transcriptome analysis and physical mapping of barley genes in wheat-barley chromosome addition lines. *Genetics* **172**, 1277–1285.
- Close, T. J., Wanamaker, S., Caldo, R. A., Turner, S. M., Ashlock, D. A., Dickerson, J. A., Wing, R. A., Muehlbauer, G. J., Kleinhofs, A., and Wise, R. P. (2004) A new resource for cereal genomics: 22K barley GeneChip comes of age. *Plant Physiol.* **134**, 960–968.
- DeScenzo, R. A., and Wise, R. P. (1996) Variation in the ratio of physical to genetic distance in intervals adjacent to the *Mla* locus on barley chromosome 1H. *Mol. Gen. Genet.* **251**, 472–482.
- Endo, T. R. (1988) Induction of chromosomal structural changes by a chromosome of *Aegilops cylindrica* L. in common wheat. *J. Heredity* **79**, 366–370.
- Endo, T. R. (1990) Gametocidal chromosomes and their induction of chromosome mutations in wheat. *Jpn. J. Genet.* **65**, 135–152.

- Endo, T. R., and Gill, B. S. (1996) The deletion stocks of common wheat. *J. Heredity* **87**, 295–307.
- Endo, T. R. (2007) The gametocidal chromosome as a tool for chromosome manipulation in wheat. *Chromosome. Res.* **15**, 67–75.
- Erayman, M., Sandhu, D., Sidhu, D., Dilbirligi, M., Baenziger, P. S., and Gill, K. S. (2004) Demarcating the gene-rich regions of the wheat genome. *Nucleic. Acids. Res.* **32**, 3546–3565.
- Gustafson, J. P., McGuire, P. E., and Qualset, C. O. (2004) Moving wheat into the mainstream in plant genomics. *Genetics* **168**, 583–584.
- Hagras, A. A., Kishii, M., Sato, K., Tanaka, H., and Tsujimoto, H. (2005) Extended application of barley EST markers for the analysis of alien chromosomes added to wheat genetic background. *Breed. Sci.* **55**, 335–341.
- Islam, A. K. M. R., Shepherd, K. W., and Sparrow, D. H. B. (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. *Heredity* **46**, 161–174.
- Islam, A. K. M. R. (1983) Ditelosomic additions of barley chromosomes to wheat. In: *Proc. 6th Intl. Wheat Genet. Symp.* (ed.: Sakamoto S.), pp. 233–238. Plant Germplasm Institute, Kyoto, Japan.
- Islam, A. K. M. R., and Shepherd, K. W. (1992) Production of wheat-barley recombinant chromosomes through induced homoeologous pairing. 1. Isolation of recombinants involving barley arms 3HL and 6HL. *Theor. Appl. Genet.* **83**, 489–494.
- Koba, T., Takumi, S., and Shimada, T. (1997) Isolation, identification and characterization of disomic and translocated barley chromosome addition lines of common wheat. *Euphytica* **96**, 289–296.
- Künzel, G., Korzun, L., and Meister, A. (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* **154**, 397–412.
- Linde-Laursen, I., Heslop-Harrison, J. S., Shepherd, K. W., and Taketa, S. (1997) The barley genome and its relationship with the wheat genomes. A survey with an internationally agreed recommendation for barley chromosome nomenclature. *Hereditas* **126**, 1–16.
- Masoudi-Nejad, A., Nasuda, S., McIntosh, R. A., and Endo, T. R. (2002) Transfer of rye chromosome segments to wheat by a gametocidal system. *Chromosome. Res.* **10**, 349–357.
- Murai, K., Koba, T., and Shimada, T. (1997) Effects of barley chromosome on heading characters in wheat-barley chromosome addition lines. *Euphytica* **96**, 281–287.
- Nasuda, S., Kikkawa, Y., Ashida, T., Islam, A. K. M. R., Sato, K., and Endo, T. R. (2005) Chromosomal assignment and deletion mapping of barley EST markers. *Genes Genet. Syst.* **80**, 357–366.
- Qi, L. L., Echaliier, B., Chao, S., Lazo, G. R., Butler, G. E., Anderson, O. D., Akhunov, E. D., Dvorak, J., Linkiewicz, A. M., Ratnasiri, A., Dubcovsky, J., Bermudez-Kandianis, C. E., Greene, R. A., Kantety, R., La Rota, C. M., Munkvold, J. D., Sorrells, S. F., Sorrells, M. E., Dilbirligi, M., Sidhu, D., Erayman, M., Randhawa, H. S., Sandhu, D., Bondareva, S. N., Gill, K. S., Mahmoud, A. A., Ma, X. F., Miftahudin, Gustafson, J. P., Conley, E. J., Nduati, V., Gonzalez-Hernandez, J. L., Anderson, J. A., Peng, J. H., Lapitan, N. L., Hossain, K. G., Kalavacharla, V., Kianian, S. F., Pathan, M. S., Zhang, D. S., Nguyen, H. T., Choi, D. W., Fenton, R. D., Close, T. J., McGuire, P. E., Qualset, C. O., Gill, B. S. (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics* **168**, 701–12.
- Saghai-Marooif, M. A., Soliman, K. M., Jorgensen, R. A., and Allard, R. W. (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* **81**, 8014–8018.
- Saisho, D., Myoraku, E., Kawasaki, S., Sato, K., and Takeda, K. (2007) Construction and characterization of a bacterial artificial chromosome (BAC) library from the Japanese malting barley variety 'Haruna Nijo'. *Breed. Sci.* (in press)
- Sato, K., Nankaku, N., Motoi, Y., and Takeda, K. (2004) Large scale mapping of ESTs on barley genome. In: *Proc. 9th Intl. Barley Genet. Symp., Vol.1* (eds.: Spunar J. and Janikova J.), pp. 79–85. Brno, Czech Republic.
- Schubert, I., Shi, F., Fuchs, J., and Endo, T. R. (1998) An efficient screening for terminal deletions and translocation of barley chromosomes added to common wheat. *Plant J.* **14**, 489–495.
- Schulman, A. H., Gupta, P. K., and Varshney, R. K. (2004) Organization of retrotransposons and microsatellites in cereal genomes. In: *Gupta PK, Varshney RK (eds) Cereal genomics*. Kluwer, Dordrecht, pp 83–118.
- Serizawa, N., Nasuda, S., Shi, F., Endo, T. R., Prodanovic, S., Schubert, I., and Künzel, G. (2001) Deletion-based physical mapping of barley chromosome 7H. *Theor. Appl. Genet.* **103**, 827–834.
- Sherman, J. D., Smith, L. Y., Blake, T. K., and Talbert, L. E. (2001) Identification of barley genome segment introgressed into wheat using PCR markers. *Genome* **44**, 38–44.
- Shi, F., and Endo, T. R. (1997) Production of wheat-barley disomic addition lines possessing an *Aegilops cylindrica* gametocidal chromosome. *Genes Genet. Syst.* **72**, 243–248.
- Shi, F., and Endo, T. R. (1999) Genetic induction of structural changes in barley chromosomes added to common wheat by a gametocidal chromosome derived from *Aegilops cylindrica*. *Genes Genet. Syst.* **74**, 49–54.
- Shi, F., and Endo, T. R. (2000) Genetic induction of chromosomal rearrangements in barley chromosome 7H added to common wheat. *Chromosoma* **109**, 358–363.
- Sorokin, A., Marthe, F., Houben, A., Pich, U., Graner, A., and Künzel, G. (1994) Polymerase chain reaction mediated localization of RFLP clones to microisolated translocation chromosomes of barley. *Genome* **37**, 550–555.
- Taketa, S., Awayama, T., Ichii, M., Sunakawa, M., Kawahara, T., and Murai, K. (2005) Molecular cytogenetic identification of nullisomy 5B induced homoeologous recombination between wheat chromosome 5D and barley chromosome 5H. *Genome* **48**, 115–124.
- Varshney, R. K., Grosse, I., Hähnel, U., Siefken, R., Prasad, M., Stein, N., Langridge, P., Altschmied, L., and Graner, A. (2006) Genetic mapping and BAC assignment of EST-derived SSR markers shows non-uniform distribution of genes in the barley genome. *Theor. Appl. Genet.* **113**, 239–250.
- Werner, J. E., Endo, T. R., and Gill, B. S. (1992) Toward a cytogenetically based physical map of the wheat genome. *Proc. Natl. Acad. Sci. USA* **89**, 11307–11311.
- Yu, Y., Tomkins, J. P., Waugh, R., Frisch, D. A., Kudrna, D., Kleinbols, A., Brueggeman, R. S., Muehlbauer, G. J., Wise, R. P., and Wing, R. A. (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. *Theor. Appl. Genet.* **101**, 1093–1099.
- Zhang, H., Nasuda, S., and Endo T. R. (2000) Identification of AFLP markers on the satellite region of chromosome 1BS in wheat. *Genome* **43**, 729–735.

APPENDIX Primer sequences of the EST markers allocated to 5H (an Excel file is available at [http://www.soc.nii.ac.jp/gsj3/sup/82\(2\)Ashida/](http://www.soc.nii.ac.jp/gsj3/sup/82(2)Ashida/))

EST-ID	L	R	Arm	Region
k00006	ATAGCTATGGCACGATTGGC	TGACACCGCTGAAGAGATTG	L	XX
k00038	CCTCGGATGTAAACGGAAAA	CAGCCACTCCATGCCTAGAT	L	XVI
k00046	TTGCAGGTACTCCGGGTAAT	TTGTGCGATCGGTTCTACT	L	XVIII
k00101	GCGGTGCTTTGCTCTTTCTA	CTGCAGCTGTTTGCCAAGT	L	XVI
k00102	TTCAGGGTGTGCGATGAATCTC	CACTCTGATGGCAACCTCCT	L	XVI
k00162	AACTGAAAAGTTGCCATCCG	GACCACAAAGCCGATGTTTT	L	XIX
k00192	CTATTGGGCACTACATGCCTC	ATTGGCATCGCACGTCTACT	L	XVI
k00213	CTGCTCGTGTCTTTTCCCAT	ATCAATCCGTGGAAGCATGT	L	XX
k00263	GTACAGCAGTGCACAAGGGA	AGCACTTCTGGCACCATAC	L	XVII
k00446	CTGCCTTTGTCTTCTCAG	GACAGAGGATGTGGCTGGTT	L	XVI
k00572	GAAGTCGGAGATTTTGCTCG	TCTCCTGATTTGGGATTTGC	L	XIX
k00578	ACCGATCTCGCAATTACAGG	TGAAGCACGGGAGGATAAAG	L	XX
k00642	ACCGCGCACTGATTCTAAGT	CGGCAGAACTTCTACCTGC	L	XVIII
k00649	CAACTGTGTTTTCGATTCCA	AGCCTTGCGAGAGTTGGTCAT	S	VI
k00669	AAGTGCTGTTTGAATTGGG	TTCCAGAGAAGGCACCATT	L	XVI
k00675	CATTGCACGTCAAGGACAAC	ACTGCCGTGGGATAATGAAG	S	V
k00741	ATGACCTCCAGATACACCCG	TGTTGGTGTGGACCAGTTA	L	XVI
k00760	GTTTACTTGTGCGCCAGCTCC	TGCCTGAGTTGTGATATGGC	L	XVIII
k00839	TCATCGCACTTGCGAGTTTA	GATCGGGAACATTTCCACTG	L	XVIII
k00914	TTGCTCGGCGAAAATATACA	CATGGACCAGTCTGCTCTGA	S	I
k00950	ACTACAAAAGCCTTCCCCGT	TGCGAAACCATAACCGTAACA	L	XVI
k00970	CTGTGAGTGCGGGAGTATCA	AGTGCAGGCTAACCCTCAGA	L	XVI
k01017	TTTTTCCCTTGACGCCTATG	CGACAGCAGAATGGTTGAGA	L	XVIII
k01041	ACACACGCACACTGAAGAGC	TCTACTACGTTCCGCCCCAC	L	XV
k01087	CTCAACATGGTATTCGCACG	ACAGTGAGTGGGAGTTTGGC	L	XIX
k01110	TTACAGGTGATGGGATGGGT	ACCGTCACAAGAGCAGAGGT	L	XX
k01139	CTTGAACCTTCTCCAGGACGC	GGGTATCGTGAGATGCCAGT	L	XIX
k01183	GAAATTCACATGCTTCCGGT	GTCCATTGATGAGCTGGCTT	S	I
k01230	CCCTTGCAACGATAAGTGAT	CACGCGAAGATGTACGAGAA	L	XX
k01302	CAAACCGACGAACTCCATTT	AAATTTTCGACCGTGGAATG	L	XV
k01319	ATCGCCAGTCAACGATGTTT	TTTAGGGCATTTCTGTTGGC	L	XX
k01320	ACATGGTGTGACAAAAGGCA	CTACACCATGGAACCCATCC	S	IX
k01322	TTGCTGAACTGAGCAAATGG	TCGATGGCTGTTTATTGCAG	L	XIII
k01323	GAACGCCAGCCAAATTGTAT	ACCATTTTGATCCTTCTGCG	S	VI
k01353	GCAACCGTAGTCCTTGTTGGT	GTGTGGCCTTTCAGGTGATT	L	XVI
k01359	GTGCACGGGAGAAACAAAAT	GCTTATCGACAAAGCGGTTT	L	XX
k01422	CCAGCTTCCAGTCTCCTTTG	CTGCTGCAATCATAGTCCCA	S	II
k01426	ATATCATCTATGGCGCGGAA	GATGGGGAACAGTGCATCTC	L	XVI
k01445	CGCTTAGTTAGGGTAGGGCA	TCCAGGACTAGACGGGATATG	L	XX
k01516	CTTCTTATGCGATTCCACCG	TCATCGAAGTTGCACAGGAC	L	XX
k01962	ACCACCTGTAATGGCCTCAG	ACACAGGATTGGGAGGACAG	L	XI
k01987	CCGAAGGCACTTGACATTTT	GCAAAAGATCATCAGCAGCA	L	XX
k02019	GACATGGGCTTCACAACAGA	CAAGATTGGATCCCCAGAA	S	IV
k02336	TCAACATTACTGATCGCGGA	TCAGGTTCAACGCATACAGC	L	XVI
k02464	TGCACAAGAACAGTGCATCA	TTGAGGAGCTTCTGGCATT	L	XVI
k02556	GTACCATTAGCTGCCCCAAA	GGCATGCTCTTCGAGTTTTC	L	XVI

Continued

k02569	GGTACCATCACAGTCGCAGA	GTGGTTGGACAGCAACCTTT	L	XX
k02593	GGAACCAGCCCCCTTACTACC	ACATGTC'TTACCGTCTGGGC	L	XIX
k02647	CAGATGGCAGGCAATACAGA	ATAGCTTAACTGCCGCCGT	L	XVIII
k02723	AACGCTGGCAAAGTTATTGG	CAAACCGTGGAAGGCTTAAA	L	XVI
k02762	CAAGGGGAACGCTACACATT	CCAAGAGGAGCAACTTCTCG	S	I
k02998	GTGCATAGGGCAGATTCCAT	CCAGCTCAAGTTCCAGAGGA	L	XIII
k03038	TACATTAGGACCGCTGGGAC	AGGGCAAGTCATTTACCACG	L	XIX
k03039	CAAAACCTGCAAAAGCAAA	CACCTGCAGAGTCCAACAAA	S	IX
k03041	ATCTCAAATGGGGAGGCTCT	GCCTGAGGGTTGGTCAATTA	S	VI
k03075	TCAGTGTGTCTCCGCTTGTC	GGAAATGGGTCGGTAGGAAT	L	XVI
k03232	TACCGCCTATGATACTCCCG	ACCAGTGGAGCTTCCAGAAA	L	XIII
k03278	CTGAATGTAGGGCGGATTGT	TGATGGAAACGACCAAGACA	L	XVIII
k03320	AGATACGGCGAACCACATTC	AACTGGTCGAAATGAGGTGG	L	XX
k03348	AAATGCAGAAGGGCTCGTTA	CCAGAAAGGAATGTTGGGAA	S	VIII
k03356	AGGGTGCTGCCATGTTTAC	CGCCTGTGAGACAAGAATGA	L	XIX
k03357	ACTTTAGCCTTGCTCCCAT	ATGCATTTGCTCAAGAGGCT	L	XV
k03359	ACAGTTATTTGAGGGCACGG	TACTGAGGGTTAAGCGTGGG	L	XX
k03364	TTCTCATCATCCCATCCCAT	TGTGTTTCGATCGAGAAGTGG	L	XIII
k03390	ACATATTGCTGATAGGGGCG	GATGACGAAGAGGATGGGAA	S	IX
k03400	AAGCAGGTTCCAGCTTTTGA	CTGGTGAGGGCAACTCTAGC	L	XII
k03411	TCGTCGCATGGTATCAAAAA	CTTCGAAACAGCGAGAGAGC	S	I
k03435	CCTGCATCACAAGGTCTCCT	GGGAGGCAAAATGCTAACAAC	L	XVI
k03445	TTTCGCACGAACATGTCAAT	TTGGTGGAAGAAAAGCCTG	L	XX
k03449	GCTGTTGTCCGTGTTGATTG	GCTAACCGAGAAGAGCGACA	L	XIII
k03485	CTCCGCAGCAAGTACAACAA	GGCACGAGAAGACCAAGAAG	S	I
k03513	GCCGACAATGACACCACTAA	GAGGTTTCATCATGGAGGCTG	L	XX
k03578	TGACCAAGTACGACACCCAA	ATGGTTGGTGGTGGACAAGT	S	IX
k03684	CGACGCCTAAGGTACATTTCA	CAGATGAAGGGAAGGCTCAA	S	I
k03949	CCAGATCAACAAACACCACG	TGGAGTCATCTGAAGCGATG	S	I
k04025	CAATGCAACAAACTGATGCC	GGAGGATCAGCAGGCAATAC	L	XIX
k04030	GTCCAACCGAGATCAACCAT	ATGAAAACCTTGAGGATGCG	L	XIX
k04091	GGCAACGGCAGATTTCTCTA	TCAGCAATGTGCCTATCGAG	S	VII
k04166	TTGGTGGTGCCATGATTCTA	GCTAACTGACTGCCTCCCTG	L	XIV
k04207	TTGCAATGTATGCAACAGCA	CGAACTCCCCAGTAGACCAA	L	XIX
k04289	GTCCGTCCGTAACAAAGGAA	TGCGATTGATAAACTGCGAG	S	I
k04305	TCCTTGCTCTTGACCGATCT	TTTGTGGAGCATCCTTTTCC	L	X
k04490	GGAAAAGGCATGACAGAGGA	GTGCTTGACAAAAATGATCG	L	XX
k04508	TTCAATGCAGGCGATGATTA	CTGTGCTCTTCCTCTCGCTC	L	X
k04717	AATCATTTTCGGTAGGGAGGG	CTACCAGGTCTTCTGCCAGC	L	XI
k04727	AATGTAGGGTTCCACAAGCG	GCTGCTGCAGAGAGGAACT	L	XVIII
k04732	GTTTACACACACGGCGCTAA	CAGGCGGTTGGTAATTCAGT	L	XIX
k04765	TAGTACATGCGTGCCATGCT	GTCATCATCCTCGCCTTCAC	L	XX
k04921	TGGTTAGGAAGGCACACACA	GTCGACCCGTAGAGCTGAAG	L	XIV
k04947	CAACTTGGAACAACAACGC	CTTGTGCATGCGCAGAGTAT	L	XX
k04962	TTGCCTCGAACCTCTTCACT	TGCTGTTTCGAGGATGTCAAG	S	V
k04973	CAACAACAATACGCACGACA	ATCAATACCTCCGCATCCAG	L	XX
k04992	GTA AAAAGGACCGCTACGCA	TGGAGGAGACAGTCTTGAGC	S	III
k04996	AAACCCGATATGGTCTTCC	TACAACGTTGCCTCTGTTGC	L	XV
k05024	ACCAACGCACCATCCTCTAC	ATTGTGCAGTCTGGCATCAG	S	VIII
k05087	ACCAAAATGGCCATCAACAT	AAAACAATGGGCAAGTGGAG	L	XX
k05150	GCGAGAAAACCTGGAGAGCAA	TTCAGGTTTCAAGAATGGGC	L	XIV