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Dissection and cytological mapping of barley chromosome 2H in the genetic background of common wheat

Giri Prasad Joshi, Shuhei Nasuda and Takashi R. Endo*

Laboratory of plant genetics, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

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We used gametocidal (Gc) chromosomes 2C and $3C^{SAT}$ to dissect barley 2H added to common wheat. The Gc chromosome induces chromosomal breakage resulting in chromosomal aberrations in the progeny of the 2H addition line of common wheat carrying the monosomic Gc chromosome. We conducted in situ hybridization to select plants carrying structurally rearranged aberrant 2H chromosomes and characterized them by sequential C-banding and in situ hybridization. We established 66 dissection lines of common wheat carrying single aberrant 2H chromosomes. The aberrant 2H chromosomes were of either deletion or translocation or complicated structural change. Their breakpoints were distributed in the short arm (2HS), centromere (2HC) and the long arm (2HL) at a rough 2HS/2HC/2HL ratio of 2:1:2. We conducted PCR analysis of the 66 dissection lines using 115 EST markers specific to chromosome 2H. Based on the PCR result, we constructed a physical or cytological map of chromosome 2H that were divided into 34 regions separated by the breakpoints of the aberrant 2H chromosomes. Forty-seven markers were present in 2HS and 68 in 2HL. We compared the 2H cytological map with a previously reported 2H genetic map using 44 markers that were used in common to construct both maps. The order of markers in the distal region was the same on both maps but that in the proximal region was somewhat contradictory between the two maps. We found that the markers distributed rather evenly in the genetic map were actually concentrated in the distal regions of both arms as revealed by the cytological map. We also recognized an EST-marker or gene-rich region in the 2HL interstitial region slightly to the telomere.

Key words: barley, chromosome 2H, common wheat, gametocidal chromosome, cytological map, EST

INTRODUCTION

Barley (*Hordeum vulgare*; 2n = 2x = 14, genome formula HH), one of the members of Triticeae, is widely cultivated in the temperate zone. Like wheat, barley belongs to the oldest and most important crops of the Fertile Crescent (Badr et al., 2000). The species is more drought tolerant and much more salt tolerant than wheat. It is used for animal feed, is the main cereal for malt production, and is also an important human food. Besides its agronomic importance, because of its diploid and self-fertile nature, barley serves as a suitable material for genetic and genomic studies. The main advantage of barley is its relatively low DNA content (5000 Mbp DNA per haploid genome), which is one third of hexaploid wheat (common wheat or bread wheat; *Triticum aestivum*, 2n = 6x = 42, genome formula AABBDD, ca. 17000 Mbp per haploid genome) (Bennett and Leitch, 1995). In addition, the barley genome has homology to the wheat genomes (Linde-Laursen et al., 1997), and therefore barley has become a model plant of Triticeae that includes agronomically important macaroni wheat (*T. durum*, 2n = 4x = 28, genome formula AABB) and bread wheat.

Improvement of crops by introducing valuable genes from related alien species has been practiced for a long time (Jiang et al., 1994). In this regard the first wheat-barley hybrid was developed by Kruse (1973). Furthermore, Islam et al. (1981) produced six of the seven possible wheat-barley ('Chinese Spring'- 'Betzes') disomic addition lines for barley chromosomes 2H to 7H, and Islam and Shepherd (2000) produced an addition line for

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* Corresponding author. E-mail: endo.takashi.2e@kyoto-u.ac.jp

chromosome 1H that carries one intact 1H chromosome, one 1H short arm and a pair of 6H chromosomes. In alien chromosome or chromosome-arm addition lines, the whole genome can be said to be dissected into its component chromosomes or chromosome arms (Doležel et al., 2007). For genome analysis, alien addition lines carrying single alien chromosomes or chromosome arms are useful and those carrying sub-arm segments would be more valuable.

Chromosome maps provide primary information in cloning genes of interest. Genetic chromosome maps have been constructed in barley with various molecular markers: restriction fragment length polymorphisms (RFLPs) (Kleinhofs et al., 1993), amplified fragment length polymorphisms (AFLPs) (Qi and Lindhout, 1997), random amplified microsatellite polymorphisms (RAMPs) (Becker and Heun, 1995), simple sequence repeat (SSR) (Ramsay et al., 2000), retrotransposon markers (Manninen et al., 2000), and EST markers (Sato et al., 2009). High-density genetic maps can be efficiently constructed and applied to QTL analysis in barley (Hori et al., 2003). However, genetic maps, which are based on genetic recombination values, do not necessarily represent actual physical locations of genes and molecular markers because recombination does not occur evenly along a chromosome (DeScenzo and Wise, 1996). Distortion of gene loci determined by genetic mapping can be remedied by physical or cytological mapping.

In cytological mapping, a target chromosome needs to be divided into segments by some means. The gametocidal (Gc) system acts as a biological mutagen in common wheat. Certain alien chromosomes, called Gc chromosomes, introduced from *Aegilops* species into common wheat cause rearrangements in the host wheat chromosomes (Endo, 1990) and also induce chromosome mutations in alien chromosomes added to common wheat, such as those of barley and rye (Endo, 2007). Using the Gc chromosome as a tool of chromosome manipulation, Endo and Gill (1996) produced an array of deletion stocks of common wheat, Shi and Endo (1999, 2000) proved the possibility of producing deletion stocks of barley chromosomes in the genetic background of common wheat, and Endo et al. (1994) and Friebe et al. (2000) demonstrated that the Gc system induce rearrangements in rye chromosomes added to common wheat. Using the Gc system different researchers so far produced arrays of barley dissection lines of common wheat carrying rearranged chromosomes containing barley chromatin derived from single barley chromosomes and constructed cytological maps for barley chromosomes: 3H with ESTs (Sakai et al., 2009), 4H with ESTs (Sakata et al., 2010), 5H with ESTs (Ashida et al., 2007), 7H with AFLPs and STSs (Serizawa et al., 2001), 7H with AFLPs and SSRs (Masoudi-Nejad et al., 2005), and 7H with ESTs (Nasuda et al., 2005).

In this study we focused on chromosome 2H, which contains genes controlling a variety of traits of agronomic and commercial importance, such as cleistogamy (Turuspekov et al., 2004), reproductive frost tolerance (Reinheimer et al., 2004; Li et al., 2005), photoperiod response (Laurie et al., 1994), head architecture (Pourkheirandish et al., 2007), and the production of hordatines A and B, strong antifungal components in shoots of barley seedlings (Nomura et al., 1999, 2007). In the cytological map reported herein, the 2H chromosome was dissected into 34 regions and became the most finely dissected chromosome among the barley chromosomes so far dissected by the Gc system.

MATERIALS AND METHODS

Plant material We used three alien addition lines of common wheat cultivar Chinese Spring (CS) that were disomic respectively for a barley (cv. Betzes) chromosome 2H (Islam et al., 1981), a gametocidal (Gc) chromosomes 2C (Endo, 1988) and a Gc chromosome $3C^{SAT}$ (Endo and Gill, 1996). We also used, as controls, euploid CS and CS-Betzes disomic addition lines for the short arm of chromosome 2H (2HS) and for the long arm of chromosome 2H (2HL), which had been developed by Islam et al. (1981). These lines were obtained from National BioResource Project-Wheat (NBRP-Wheat) (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp). The F_1 hybrids (2n = 44, 21" + 1' 2H + 1' 2C or $3C^{SAT}$) between the 2H and Gc addition lines were backcrossed to the 2H addition line to produce their BC_1 progeny, from which we selected plants disomic for the 2H chromosome and monosomic for the Gc chromosome (2n = 45, 21" + 1"2H + 1'2C or $3C^{SAT}$). The BC_1 plants were then self-pollinated or cross-pollinated with euploid CS pollen to generate structural changes involving the 2H chromosome in their progeny. We also used 23 prescreened CS lines carrying different 2H aberrations induced by the gametocidal system as described above (unpublished data).

Cytological screening We detected aberrant 2H chromosomes by fluorescence in situ hybridization (FISH) using the probe of the HvT01 sequences, specific to subtelomeric regions of all barley chromosomes (Belostotsky and Ananiev, 1990), and genomic in situ hybridization (GISH) using the probe of the total barley genomic DNA. The procedures of chromosome preparation and simultaneous FISH/GISH were as described by Sakai et al. (2009). We estimated approximate fractions of the remaining segments in the deleted or translocated 2H arms by comparing the arm ratios of the intact and dissected 2H chromosome (two to six chromosomes were measured for each chromosome), as calculated in Endo and Gill (1996). We also conducted sequential C-banding and FISH/GISH, as described by Masoudi-Nejad et al. (2002), to identify the breakpoints of rearranged 2H chro-

mosomes relative to the C-bands.

PCR analysis We used DNA extracted from leaves by the CTAB method (Saghai-Maroof et al., 1984) and 127 EST markers that had been assigned to chromosome 2H (Nasuda et al., 2005). We added 1 μl of DNA solution (ca. 30 ng/μl) as a template to a PCR mixture consisting of 4 μl of 5 x PCR buffer, 0.4 μl of dNTP (10 mM each), 1.5 μl of $MgCl_2$ (25 mM), 1 μl of primers (10 pmol/ μl), 0.1 μl of KAPATaq Extra DNA Polymerase (5 U/μl KAPABIOSYSTEMS), and 12 μl of dH_2O. We conducted thermal cycling with an iCycler (BioRad) using the following conditions: 94°C for 2 min, 5 cycles of 94°C for 30 sec, 65°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 PCR products by agarose (1.5% Agarose S, Nippon gene) gel electrophoresis (200 V, 40 min).

RESULTS AND DISCUSSION

Cytological screening Chromosome 2H had a strong HvT01 FISH signal at the 2HS distal end and minor HvT01 FISH signals in the 2HL distal region. We screened most of the structural rearrangements involving the 2H chromosome based on the HvT01 signals. We also applied sequential C-banding and FISH/GISH to the screened aberrant 2H chromosomes to confirm complicated rearrangements and to determine the breakpoint positions of the aberrant 2H chromosomes relative to the C-bands. Chromosome 2H was a metacentric chromosome with an arm ratio 1.2 (average of 10 chromosome measurements), and both of the 2H arms had two prominent C-bands in the proximal region and two minor bands, which were often invisible, in the distal region (Fig. 1).

We cytologically screened the backcrossed progeny of the 45-chromosome plants (21" + 1" 2H + 1' 2C or 21" + 1" 2H + 1' $3C^{SAT}$) and found that the 2C and $3C^{SAT}$ Gc chromosomes induced chromosome mutations of the 2H chromosome in 4.4% (39 out of 871 plants) and 2.1% (21 out of 982 plants), respectively. A total of 47 plants carrying single or multiple 2H aberrations survived and set seeds. We further screened their progeny and the progeny of the 23 prescreened lines to establish 2H dissection lines with single rearranged 2H chromosomes. In the end we established 66 dissection lines carrying single aberrant 2H chromosomes: 21 of them had 2H deletions with single breakage, 4 had 2H deletions with multiple breakpoints, 36 had 2H-wheat chromosome translocations with single translocation points, 1 had a 2H-wheat chromosome translocation with double translocation points, and 3 had aberrant 2H chromosomes including both a deletion and a translocation (Fig. 1, Table 2). Out of the 66 lines, 22 lines were homozygous and 44 lines were hemizygous for aberrant 2H chromosomes, and 26 lines originated from the progeny of the line with $3C^{SAT}$ and 40 lines from the progeny of the line with 2C (Table 2).

The dissection lines with the same numeral followed by "a", "b" or "c" had different aberrant 2H chromosomes that were originated in the same plants. Two aberrant 2H chromosomes in each of the six pairs, 2H-4a/2H-4b, 2H-8a/2H-8b, 2H-13a/2H-13b, 2H-32a/2H-32b, 2H-37a/2H-37b and 2H-50a/2H-50b, were generated independently in the same plants, and then isolated from each other by backcrossing into separate lines. Aberrant 2H chromosomes in each of the seven sets, 2H-2a/2H-2b, 2H-9a/2H-9b, 2H-30a/2H-30b, 2H-31a/2H-31b, 2H-40a/2H-40b, 2H-42a/2H-42b/2H-42c and 2H-46a/2H-46b, were derived from one aberrant 2H chromosome by secondary rearrangement, centromeric breakage or fusion.

PCR analysis of the dissection lines and characterization of aberrant 2H chromosomes We checked the PCR amplification of the 127 EST markers in the control lines (CS, 2H, 2HS and 2HL) to confirm their specificity to chromosome 2H and successfully assigned 115 out of the 127 markers to the 2H chromosome arms: 47 of them were located on 2HS and 68 were on 2HL. The remaining 12 EST markers showed ambiguous PCR amplification in the control lines and therefore we excluded them from this study. Using these 115 markers (Table 1), we conducted PCR analysis with the 66 2H dissection and four control lines (Table 3). The PCR analysis confirmed the chromosomal locations of the breakpoints of all aberrant 2H chromosomes, either in the 2HS arm, in the 2HL arm or in the centromere (Table 2).

Three aberrant chromosomes 2H-9b, 2H-15, 2H-42c had breakpoints in the 2HS arm and centromere. Chromosome 2H-37a had breakpoints in both arms. Chromosome 2H-37b was a wheat chromosome containing a 2HS segment and chromosomes 2H-32b and 2H-39 were wheat chromosomes containing 2HL interstitial segments. FISH/GISH analysis revealed that chromosome 2H-47 had been a dicentric translocation chromosome between the 2H chromosome and a wheat chromosome, became again a dicentric chromosome in subsequent generations with a shorter truncated 2HS segment joined to the same or a different wheat chromosome. PCR analysis revealed that chromosome 2H-49 had an interstitial deletion in the 2HS arm induced by two breakages occurred close to the centromere. Chromosome 2H-50b was a translocation chromosome with a large 2HL interstitial segment into which a small wheat chromosomal fragment was inserted, which generated four breakpoints in the 2HL arm. Three pairs of chromosomes, 2H-8a/2H-8b, 2H-13a/2H-13b and 2H-32a/2H-32b, showed FISH/GISH images implying that each pair had originated from reciprocal

translocations, and this was proved so by PCR analysis because the rearranged chromosomes of each pair had breakpoints flanked by two common markers, k04763 and k03341 for 2H-8a/2H-8b, k03201 and k05033 for 2H-13a/2H-13b, and k01321 and k05173 for 2H-32a/2H-32b. In summary, out of the 77 breakpoints in the 66 aberrant 2H chromosomes, 31 were in the 2HS arm, 19 in the centromere, and 27 in the 2HL arm (Table 2).

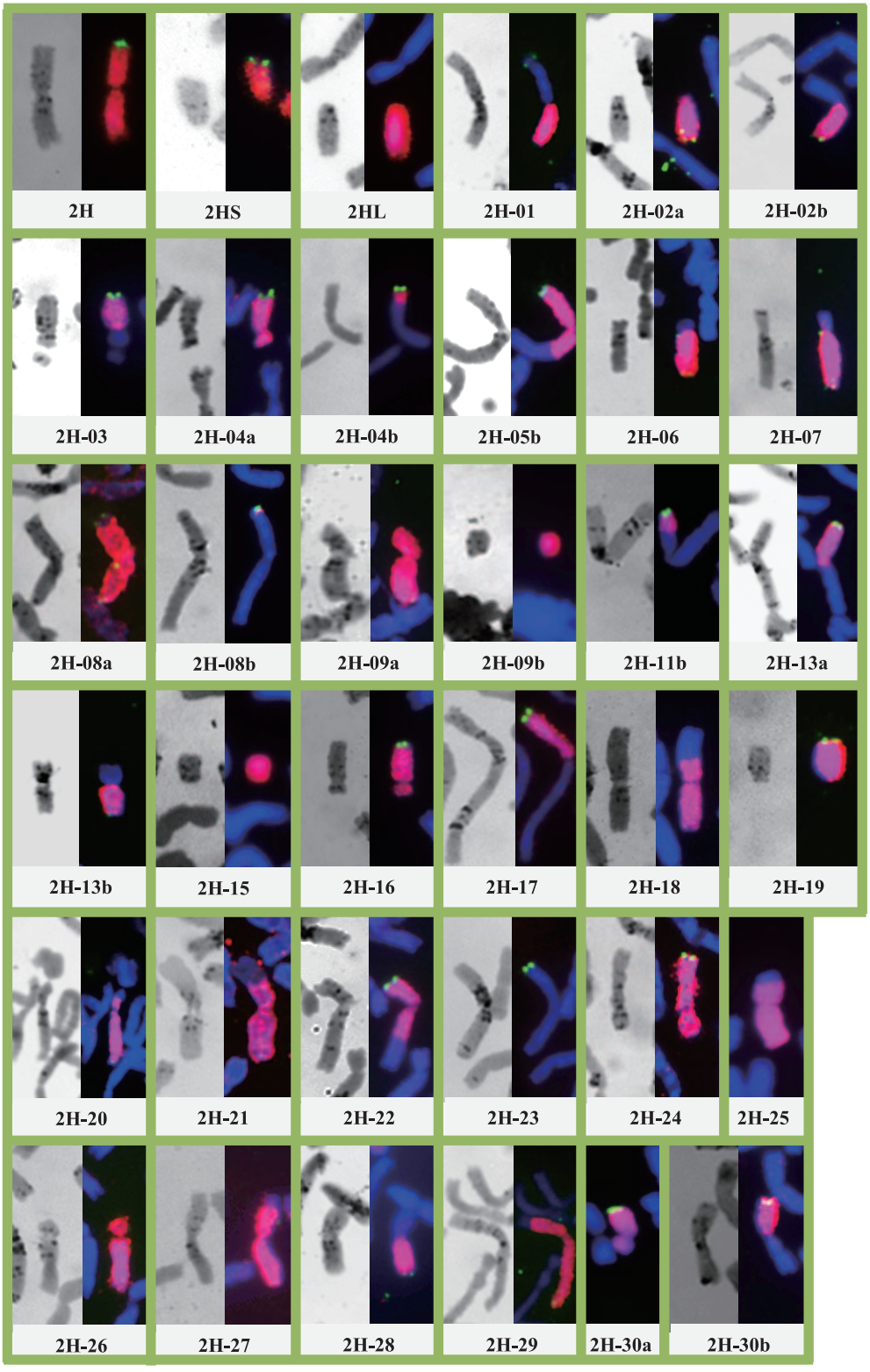

Fig. 1. Continued

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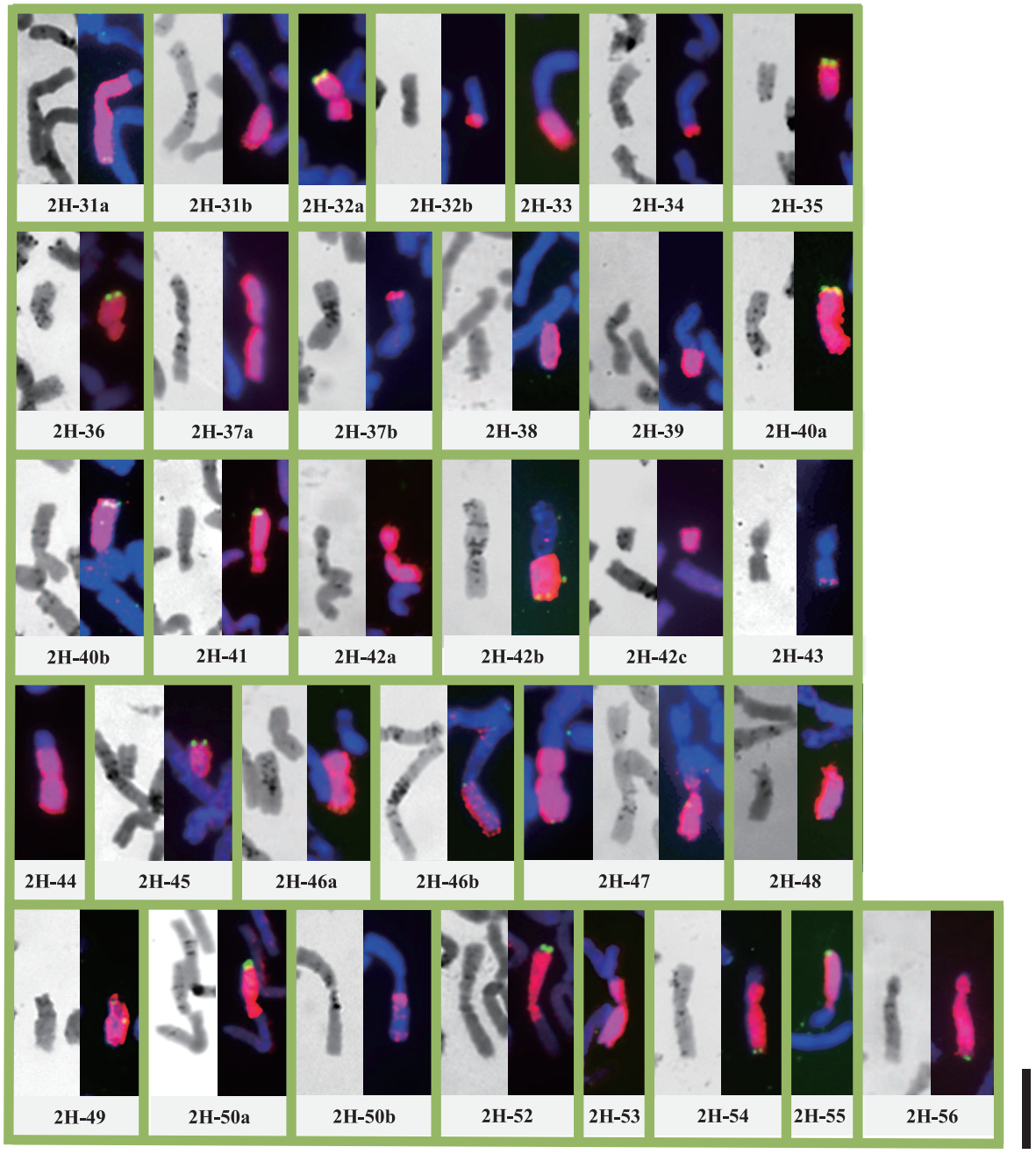

Fig. 1. Sequential C-banding and FISH/GISH images of a normal chromosome 2H, a telosome of the 2HS arm, a telosome of the 2HL arm, and 66 aberrant chromosomes carrying dissected 2H segments; no C-banding photos were available for seven aberrant chromosomes. Chromosome 2H-47; left (FISH/GISH) image of previous year and right in subsequent generation. The aberrant 2H chromosomes were numbered in the order of initial identification; therefore some numbers are missing. Those with the same numerals followed by "a", "b" and "c" originated from the same plant. The FISH probe was the barley subtelomeric repeat sequence HvT01 (green) and the GISH probe was barley total genomic DNA (red). Chromosomes were counterstained with DAPI (blue). Bar = 10 μm.

We examined the transmission of the aberrant 2H chromosomes in the progeny of the 2H dissection lines (five lines were excluded). We used two seeds for each of the homozygous lines (10 lines) and 10 seeds for each of the hemizygous lines (42 lines) and found 100% transmission in the homozygous lines and about 30% transmission in the progeny of the hemizygous lines except for two lines for which we found no plant carrying the aberrant 2H chromosomes among the 10 progeny. Although we did not examine the two lines any further, we can safely say that the rearranged 2H chromosomes induced by the gametocidal system are stable in mitosis and meiosis.

Table 1. Primer sequences of the 115 EST markers used for PCR analysis

EST- ID	F	R	Chromosomal location
k00908	ACGCCATTAGGGAAGTGTTG	ACCTGCAGGGTTATCTGACG	long arm
k00932	GATGCAACGAACGAGCACTA	AAGACGAGGACACGGAGAGA	long arm
k00998	AACAGTGTTCGTTCCGTTCC	AGATCCGTGGTGTAGGGTTG	long arm
k01019	CCAATCACACACGTGGTCTC	TATTCGGGTTCGTGACCTTC	long arm
k01073	TCAATTGCACGACGATCATT	GGCGAAAGAAACACGAAGAG	long arm
k01086	CTTCGTGGTGAACCTTGGAT	GGGTTTCCTTACAGGCATGA	long arm
k01170	AAACCCTGACGACAAGATGG	TCGACCTCATTTGAGCTGTG	long arm
k01216	GAGGGAATCGACCAATTCAA	GGCGTACCAAACGACAGTCT	short arm
k01238	CACATCGTTTGGTGCATAGC	CTGTGTCATGGCATCTTTGG	long arm
k01219	TGGACGTGGTGAGACTACGA	TACCGTATGGGCTCTTCCTG	long arm
k01224	CATGTCTGGTGTCTGGCTTG	GTGCAAGAGGTCAAGGCTTC	long arm
k01253	CCAGTTCCGCAACTACAACA	CTCGAGGACGTGAACACTGA	short arm
k01312	AAAGTTTGTATTGCCCGCTG	AGCCTGTCAAACTTCCCTGA	short arm
k01321	CACCATGTTACCACTGTCGC	TACGAGGTTTGTTCTGCACG	long arm
k01360	ATGGGCCAATATCAATTCCA	TGCTGCGTTCAGCTTTAAGA	short arm
k01362	CCGACATGAATGCAGGATTA	CTGCTCAGGGACATGAAGGT	long arm
k01407	CGCTGACAAGGCTTTCAAAT	AGAGAGGCCACAGGTGAAGA	long arm
k01408	TCTAGCGGACAGCTAAACAGC	CCATCCTCATCACCCTCACT	long arm
k01418	TAGCTCCGGCTGTTCTTGAT	ACCAAGCCATGGCCATATAA	long arm
k01423	TATCCCTTGCTCTTGCTCGT	GGAGCTTTTGTCGTCCTCTG	long arm
k01457	CGGGCAAAACATCGAATACT	CTCAGGATTCTTCAACCGGA	short arm
k01467	ACATAAGTGGGCTCAATGCC	CTCGCTTGATGAAGTGTCCA	long arm
k01579	AAACAGTACGCTCATTGGGG	ACTGCAACAACATCGTGGAG	long arm
k01603	TCCAAATTGCAGCACTTGTC	GGCGTACCACCAGTACATCC	short arm
k02630	CCTCGCTGACCCTTCAAGTA	CACAAACGACGTCTGCAGTT	short arm
k03044	GATGGGAGCACACCAGCTAT	TGCTATTCGCAGTGAGGATG	long arm
k03085	TCAAACTGCATGACAGCCTC	CTACGAGAAGCTGACCCTCG	long arm
k03160	TTAAATGGCATTGCAGTGGA	GATTGGTAGGTGCTGGAAGC	long arm
k03186	CTGAATCCTGCAACGACAGA	GATCGCAGGCAAATCAGTTT	long arm
k03195	AGCGAAATAAAAAGGACCCC	GACCGAGGAGTTGAAGCAGT	long arm
k03231	GCTAGACACAACCGTCCCAT	GGGTTGCAGTTGACAAGGAT	long arm
k03187	AGGATCAACTGCGACCTGAC	GCATCCTCTTGCTGTTGTGA	long arm
k03201	TACAGCGCTGAAAAATGCAC	CAGAACAAGCGAGCACAGAG	long arm
k03341	GCGACCTCCATGAATCCTAA	TTGGAGAGGTAAATGGCGAG	short arm
k03300	AACATTCGGTAGATGGCAGC	GTGGCAAGTATGGCCTTGTT	short arm
k03332	GGATTTGGCGGATCTAACAC	TGGTCTCCAATGCACAAAGA	short arm
k03376	ATGAGGCGATCAAAATCAGG	CAAGATCCCTGTTGGGAAGA	long arm
k03409	ACAAAATCTGGGTGGCTGAC	GAGAAGACCGCGACCACTAA	long arm
k03436	GAGGCTGTGACATTCCAACA	ACTGCGACAAGCAAGGATTT	short arm
k03370	AAAGGGAAAAGGCGACTCAT	ATTCTTAGTGCGGCAATGCT	long arm
k03404	CAACACGGTGGAACATTCAG	CCAGAGGATCTTTTGCAAGC	long arm
k03443	ATTTTGAGTGGAGCACCCAG	GCATAGATGCAAGGGGGTTA	long arm
k03454	GAGCATCAGGAGTCCCAAAC	GACCACTCAGCTGCTGTGAA	long arm
k03467	AAAACCATACCGCAAATCCA	CAAGCTCACCGTCACCTACA	short arm
k03501	CGTGGCAACACACAGCTATT	AGAATGCCACACCAAAGACC	long arm
k03538	AGGAACCACAAAGGCTCAGA	CAACATCGTTGTGGATGGAG	short arm
k03601	CTACCTGCAAACACGCAGAC	ACGAGAGCCCAAACCCTACT	short arm
k03626	CAAACAAATTCGGCAGGTG	TCAGTTGAGAAAGAAGCGCA	long arm
k03622	GAACCGACCGAAGATTCAAA	ACAAGGACATGAGGCAATCC	long arm
k03744	CTCGCTAGCTCAGTTGAGGG	CAGGGTCGTTCCCAGTGTAT	short arm
k04721	TCGACATCTCTCCCATTTCC	AACCAGATATGGATGCCAGG	short arm
k04782	CCGGGTCGTAGTCTTGTTGT	GAACGGAACGAGCTCAACAT	short arm
k04759	GGTTAAATCCTCCATGCCAA	CTACGTGGAGAGGATCCAGC	short arm
k04763	ATGCCTCCAGTGGACCTATG	AGTCTGCTGGTTTGGGACAG	short arm
k04771	TATACCAGCGCTGCACTTTG	ACCCAAACGCAAACAGACTC	long arm
k04773	CGTTCAAGGACCAACCAGAT	TATTACCGCTGCACATCGTC	short arm
k04784	TATGTTTCGGCACCGTACAA	CCCATAGTCAAAGCCAGGAA	long arm

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EST- ID	F	R	Chromosomal location
k04909	TCGAAGCGACAAACTTCAAA	GACCCAGAGAAATCCGATGA	short arm
k04935	TCCAAAGTTGGACCGTTCTC	ACATGAGCAGCATTAGCACG	short arm
k04929	TGCTTCAGTACCCTGCTCCT	GGACAATACTGAGCCTGGGA	long arm
k04939	CCCACCCTTACCACTAGGCT	GACCGTGGAGTAGAGAAGCG	long arm
k04988	AGGTGGTGTTTGGTTTGAGG	CGACCGTAGAACGTGGATTT	long arm
k05033	GCGAAAGCCAAAACTTGAAC	TCACAGATGTCTCAGCAGGG	long arm
k05056	GAAAGCTCGGCGACACTAAC	TGCAGTCCATCTACGAGCAG	short arm
k05037	GGTTTTGTCGTTGACCTCGT	CCCACTGAACCACGAGATTT	long arm
k00074	ACCTTGGTGGCTCGTATTTG	GGTGTTTACGGAGGAGTCCA	short arm
k00091	CAACATGCACGAGCAAAACT	ACATTTGTTCAAGGCGTTCC	short arm
k00186	GTTCGATCAGACATTCCGGT	TTTTCTCTAAACCCCCTCGC	short arm
k00132	TCTTCCACCACTCTCCCAAC	CGCCTGACCGAGAAATCTAC	long arm
k00144	CCTTCCCTTCACACATTCGT	CTGCTCAAGCTCGTCAGTTG	short arm
k00168	CGGAGCTTCTGGTTGATTGT	AACTGCCAGTCCTTCCAATG	short arm
k00265	CTACCAAATCTTGGCCCTCC	CGCACAAACACAGACACACA	short arm
k00246	ACAGCTCTCGCCTTTTCTTG	CCGGGGGTGCTATAGTTCTT	long arm
k00289	CAGCTTCCTCTTGTTTTGCC	ACGGTGTCCTTGCTGGTTAC	long arm
k00313	AAGGGCCTTTGGAAGAATTG	CCTGCGAATGTCACTGCTAA	short arm
k00314	AGAACTCGGCTCCATGATTG	GGGGGTGTAGCCGTATATCA	long arm
k00363	AACACAAGGAGAGCACACGA	GGAAGTAGATGCCGTTTTCG	long arm
k00376	CCGTCGATTGACCATTTCTT	GTCCGTCTTCAGGGAGACAA	short arm
k00323	ATTCCAAGCACAACACACCA	CGGTGAAATGGTGCCTAACT	long arm
k00434	CCACGAAAATGCATGAAACA	AAGTTCATCGCGAGTCCTGT	short arm
k00491	TTAGCACCGCAAACACACTC	ACTCCAGTACCGACGACCAC	long arm
k00579	ATCCTCGGCCATTCTACCTC	CGTCATCTTCCTCAAGCACA	long arm
k00757	AAGACTCACAACCGGAGTGG	GGCTATAGGTGGCGCAAGTA	short arm
k00748	ACCATGTCCTCGGAAACAAG	TGTGGAGGACAATGTGGAGA	long arm
k00677	GCTTTGTAAACCCCGTCAAA	ACACGCTGAAGAAGATTGCC	short arm
k00679	CAAATTGGCATCCTTGTCCT	GCAGTAGAGCGAGCGAAGAC	long arm
k00730	TTGATCTCACGATCTCACGG	GAGATCGACGAACTTGGAGC	long arm
k00776	CCAAACAAAACAGCACATGG	GAGGGAACATCCTACAGCCA	short arm
k00777	GCGTCGAGCACATGAAAGTA	GAGGTGGTGTATTGCTCGGT	long arm
k00838	TAGCTGCTTCCGTTCTTCGT	CATCATGCCTAAGCCAGACA	short arm
k00852	TGGCTCAATTCACGTTTCTG	CGCCTCAAACACGATCTACA	short arm
k01975	GGATCCCTGCATCGACTTTA	TCCAGATTTAAGGCCACCAC	long arm
k01932	ATGGACAACTACAAAGCGGG	TAGGTTCCACATGGACGACA	short arm
k02202	TTTCGTGCCTTTGCTTCTTT	ATGGTTGCTGAAGTCCGAAG	short arm
k02121	TCCTTGCAGGACTCGAAGTT	CTACAACTGGCCTGATGGGT	short arm
k02245	ACTCCTTGAACACCAATCCG	TAAGTTGGTTTGGGGCACTC	long arm
k02482	GCAGTTTCTTTTCTCCGCAC	AAAACCCTCAGGCTGCCTAT	long arm
k02521	TAGGGTTCGTCGCTGCTAGT	ACCAAAGAAGGAGGTGGCTT	short arm
k02551	TCAGCAAGCAAACATTCAGG	GGTTGGTCGCTGTTGGTACT	long arm
k02590	TGCATAAAACACCTCACCCC	CACACTAGCGGGTTCCATTT	long arm
k02580	TATGGCCACCAAATCTGTCA	TTCCCTATGCAGAAGGGTTG	long arm
k03899	TCCAACACCATCCACTACGA	ATGACCCGGTCGATACAAGA	long arm
k04003	TTTTACCAACGGCAGTCCTC	TCTCCAAAGGAAGCAGAAGG	long arm
k04017	CCCAAACGTCCTTGTTCCTA	CGCAGGTAGCCAAAAATAGC	long arm
k04039	GGCCCAAAACGAGTCTACAA	GACGCTACTACGTCGCTCCT	short arm
k04102	TCTTTGCCTGGAAGAAGGAA	ACTCCCCACAATCAAGCAAG	short arm
k04142	ACGCACAAATGACTGGTCTG	TTATCGACATCACCCCCAAT	long arm
k04286	CACTCACTGGAGCTTGGACA	ATGCTTGCTTGCTCACACAC	long arm
k04267	CATGTCGTGCGAATCAAATC	CGATGTTTACGCATTTGGTG	short arm
k04365	TGACGACAATGAACGAGGAG	TTGCTAAAGAGGCGACCAGT	long arm
k04377	TGCGGATCAACACCAGATTA	AGCAGCACACAACAGACCAC	short arm
k04415	CAAGTGTTTTCTGTGGGCAA	ACACAGGTCTCGCCAAGAGT	short arm
k04446	ACCAAGCATGTACCCCAAAG	TCACTGAAGGCATAACTGCG	long arm
k04629	ATGCTAAGCAGAGAGCCGAG	CTGTACGGGAACCTCGACAT	short arm
k05173	GCCAAGCTCATATAGGGCAG	TATCTGTGATGCCACATCCG	long arm

Table 2. Dissection lines of chromosome 2H

Line	Rearranged 2H chromosome				
	Gc*	State	Type of rearrangement	Breakpoint position**	FL***
2H-01	2C	homozygous	translocation	centromere (l)	0.00
2H-02a	2C	hemizygous	deletion	short arm	0.26
2H-02b	2C	hemizygous	translocation	centromere (l)	0.00
2H-03	$3C^{SAT}$	homozygous	translocation	centromere (s)	0.00
2H-04a	$3C^{SAT}$	homozygous	deletion	long arm	0.30
2H-04b	$3C^{SAT}$	hemizygous	translocation	short arm	0.66
2H-05b	$3C^{SAT}$	hemizygous	translocation	long arm	0.57
2H-06	$3C^{SAT}$	homozygous	translocation	centromere (l)	0.00
2H-07	$3C^{SAT}$	hemizygous	translocation	centromere (l)	0.00
2H-08a	$3C^{SAT}$	homozygous	translocation	short arm	0.77
2H-08b	$3C^{SAT}$	hemizygous	translocation	short arm	0.77
2H-09a	$3C^{SAT}$	hemizygous	deletion	short arm	0.66
2H-09b	$3C^{SAT}$	hemizygous	deletion/deletion	centromere (s)/short arm	0.00/0.66
2H-11b	$3C^{SAT}$	homozygous	translocation	short arm	0.59
2H-13a	$3C^{SAT}$	homozygous	translocation	long arm	0.17
2H-13b	$3C^{SAT}$	homozygous	translocation	long arm	0.17
2H-15	$3C^{SAT}$	hemizygous	deletion/deletion	centromere (s)/short arm	0.00/0.66
2H-16	$3C^{SAT}$	homozygous	deletion	long arm	0.30
2H-17	$3C^{SAT}$	hemizygous	translocation	centromere (s)	0.00
2H-18	$3C^{SAT}$	homozygous	translocation	short arm	0.60
2H-19	$3C^{SAT}$	hemizygous	deletion	centromere (s)	0.00
2H-20	$3C^{SAT}$	homozygous	translocation	short arm	0.26
2H-21	$3C^{SAT}$	homozygous	translocation	short arm	0.58
2H-22	$3C^{SAT}$	homozygous	translocation	long arm	0.60
2H-23	2C	hemizygous	translocation	short arm	0.85
2H-24	2C	hemizygous	deletion	long arm	0.45
2H-25	2C	hemizygous	translocation	short arm	0.64
2H-26	2C	hemizygous	deletion	short arm	0.40
2H-27	2C	homozygous	translocation	short arm	0.64
2H-28	2C	homozygous	translocation	centromere (l)	0.00
2H-29	2C	hemizygous	translocation	short arm	0.64
2H-30a	2C	hemizygous	deletion	long arm	0.47
2H-30b	2C	hemizygous	translocation	centromere (s)	0.00
2H-31a	2C	homozygous	translocation	short arm	0.64
2H-31b	2C	hemizygous	translocation	cenromere (l)	0.00
2H-32a	2C	hemizygous	deletion	long arm	0.60
2H-32b	2C	hemizygous	deletion/transl	long arm/long arm	0.60
2H-33	2C	hemizygous	translocation	long arm	NC
2H-34	2C	hemizygous	translocation	long arm	NC
2H-35	2C	homozygous	deletion	centromere (s)	0.00
2H-36	2C	hemizygous	deletion	long arm	0.38
2H-37a	2C	hemizygous	deletion/deletion	short arm/long arm	0.64
2H 37b	2C	hemizygous	deletion/transl	short arm/short arm	0.00/0.26

Continued

Continued

Line	Rearranged 2H chromosome				
	Gc*	State	Type of rearrangement	Breakpoint position**	FL***
2H-38	2C	homozygous	deletion	centromere (l)	0.00
2H-39	2C	hemizygous	deletion/transl	long arm/long arm	0.60
2H-40a	2C	hemizygous	deletion	long arm	0.60
2H-40b	2C	hemizygous	translocation	centromere (s)	0.00
2H-41	2C	homozygous	deletion	long arm	0.30
2H-42a	2C	hemizygous	deletion	short arm	0.63
2H-42b	2C	hemizygous	translocation	centromere (l)	0.00
2H-42c	2C	hemizygous	deletion/deletion	centromere (s)/short arm	0.00/0.63
2H-43	2C	hemizygous	translocation	long arm	NC
2H-44	2C	homozygous	translocation	short arm	0.79
2H-45	2C	homozygous	deletion	centromere (s)	0.00
2H-46a	2C	hemizygous	deletion	short arm	0.59
2H-46b	2C	hemizygous	translocation	centromere (l)	0.00
2H-47	2C	hemizygous	translocation	short arm	0.25
2H-48	2C	homozygous	deletion	short arm	0.26
2H-49	2C	hemizygous	deletion	short arm/short arm	NC
2H-50a	2C	hemizygous	deletion	long arm	0.30
2H-50b	2C	hemizygous	translocation	long arm/long arm/long arm/long arm	0.60
2H-52	$3C^{SAT}$	hemizygous	translocation	long arm	0.30
2H-53	$3C^{SAT}$	hemizygous	deletion	short arm	0.59
2H-54	$3C^{SAT}$	hemizygous	translocation	short arm	0.52
2H-55	$3C^{SAT}$	hemizygous	translocation	long arm	0.11
2H-56	$3C^{SAT}$	hemizygous	deletion	short arm	0.40

* 2C and $3C^{SAT}$ are the gametocidal (Gc) chromosomes used for the induction of rearrangements in chromosome 2H.

** (s) and (l) represent the whole or part of the chromosomes arm retained by the rearranged 2H chromosomes, and '/' indicates the occurrence of separate breakages.

*** FL stands for 'fraction length' and 'NC' indicates that FL was not calculable.

Cytological mapping of barley EST markers

Based on the result of PCR analysis, we assigned the 47 2HS-specific markers to 16 regions and 68 2HL-specific markers to 18 regions. These regions were divided by the breakpoints of the 66 rearranged 2H chromosomes; in other words, these breakpoints were grouped into 34 clusters flanked by the EST markers and cytological landmarks, the centromere and telomeres (Fig. 2). Each of the regions contained 1 to 15 markers. We measured the distances between the C-bands and the centromere for the 2HS and 2HL arms to draw an ideogram of chromosome 2H. We also measured fraction lengths of the rearranged 2H chromosomes, as conducted for wheat deletion chromosomes by Endo and Gill (1996), and placed the 115 EST markers on the ideogram in relation to the C-bands and fractional length.

The breakpoints of chromosomes 2H-47 (FL = 0.25), 2H-26 (FL = 0.40), 2H-18 (FL = 0.60) and 2H-44 (FL = 0.79) physically divided the 2HS arm into five parts at intervals of ca. 20% length of the arm. The five parts from the centromere to the telomere respectively contained 0.0%, 10.6%, 27.6%, 34.0% and 27.6% of the EST markers assigned to the 2HS arm. Chromosome 2H-47 retained ca. 25% of the 2HS arm but had no EST marker, and 61.7% of the EST markers were distributed in the distal region accounting for ca. 40% of the 2HS arm, and 10.6% of the EST markers were found in the proximal region accounting for ca. 40% of the 2HS arm. The 2HL arm was divided into six parts by the breakpoints of chromosomes 2H-13a (FL = 0.17), 2H-4a (FL = 0.30), 2H-24 (FL = 0.45), 2H-22 (FL = 0.60) and 2H-37a (FL = 0.64). The six parts from the centromere to the telomere respectively contained 5.9%, 4.4%, 5.9%, 13.2%, 48.5%, and 22.0% of the EST markers assigned to the 2HL arm. In other words, almost half of the EST markers (33/68) were found in the ca. 4.0% region between the breakpoints of

Table 3. PCR analysis of the 66 dissection and four control lines using 115 EST markers ("+" and "–" represent PCR-

EST	CS	2H	2HS	2HL	23	8b	4b	11b	44	8a	9a	9b	15	25	27	29	31a	42a	42c	18	46a	53	21	54	49	26	56	37b	2a	20	48	1	2b	6
k04102	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04782	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04909	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04935	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00074	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00144	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00434	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04629	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k01216	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k01312	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k01603	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04267	–	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k01253	–	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04763	–	+	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k03341	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k05056	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k02521	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00677	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k02630	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04377	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k03538	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k03601	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04721	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k04773	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k03436	–	+	+	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00313	–	+	+	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k03467	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00852	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00757	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00186	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
k00265	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
k00376	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
k00168	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
k00776	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
k01932	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
k01360	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
k03332	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
k01457	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
k03300	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
k03744	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
k04759	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
k04039	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
k00091	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
k00838	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
k02202	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–	–	–	–
k04415	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	–	–	–	–
k02121	–	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	–	–	–	–
k03501	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01407	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00363	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03201	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k05033	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04365	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01238	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03186	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04142	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00679	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+

Cotinued

–amplification and no amplification, respectively).

7	28	31b	38	42b	46b	47	3	17	19	30b	35	40b	45	55	13a	41	4a	16	50a	52	36	24	30a	5b	22	32a	40a	33	13b	34	43	32b	39	50b	37a
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
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−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
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−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
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−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
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+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	+

Continued

Cotinued

EST	CS	2H	2HS	2HL	23	8b	4b	11b	44	8a	9a	9b	15	25	27	29	31a	42a	42c	18	46a	53	21	54	49	26	56	37b	2a	20	48	1	2b	6
k03195	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00132	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00491	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k02551	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03409	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03404	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03231	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03187	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00246	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01321	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k05173	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01975	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k02245	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00289	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04771	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04784	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04929	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04939	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04988	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03443	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01579	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00908	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01073	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04003	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03160	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01362	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01219	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00998	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01019	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01086	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k02590	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00748	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k02482	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00777	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03454	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00314	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03622	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01170	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04286	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k02580	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03376	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k05037	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04017	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00932	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03044	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03085	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00323	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00579	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k00730	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03899	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k04446	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03370	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01467	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01418	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01408	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k03626	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01224	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
k01423	–	+	–	+	–	–	–	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+

Continued

7	28	31b	38	42b	46b	47	3	17	19	30b	35	40b	45	55	13a	41	4a	16	50a	52	36	24	30a	5b	22	32a	40a	33	13b	34	43	32b	39	50b	37a
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	+	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	+	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	+	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	+	+
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	+
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chromosomes 2H-22 and 2H-37a, and 70.5% of the EST markers were present in the distal region accounting for ca. 40% of the 2HL arm. These facts suggested that the EST markers or genes on chromosomes 2H were more abundant in the distal region than in the proximal region in either arm, as observed for the other barley chromosomes (Künzel et al., 2000; Nasuda et al., 2005; Ashida et al., 2007; Sakai et al., 2009; Sakata et al., 2010). This tendency was obvious in the subtelomeric regions. Chromosomes 2H-23 and 2H-43 retaining small subtelomeric regions including the HvT01 sequences contained 11 (23.4%) of the 2HS-specific EST markers and 15 (22.0%) of the 2HL-specific EST markers, respectively. Marker k03501 on the 2HL arm was found to be the closest EST marker to the centromere.

Uneven distribution of genes on a chromosome is well documented in wheat and barley (Panstruga et al., 1998; Erayman et al., 2004). Erayman et al. (2004) used deletion stocks of common wheat and identified five major gene rich regions (GRRs) for wheat that contained 26% of the genes but spanned only ca. 3% of the genome. One of the major GRR '2L1.0' lied in 5% region between 95–100% (FL = 0.95–1.00) of the long arms of the group 2 chromosomes. A comparative study of high-density

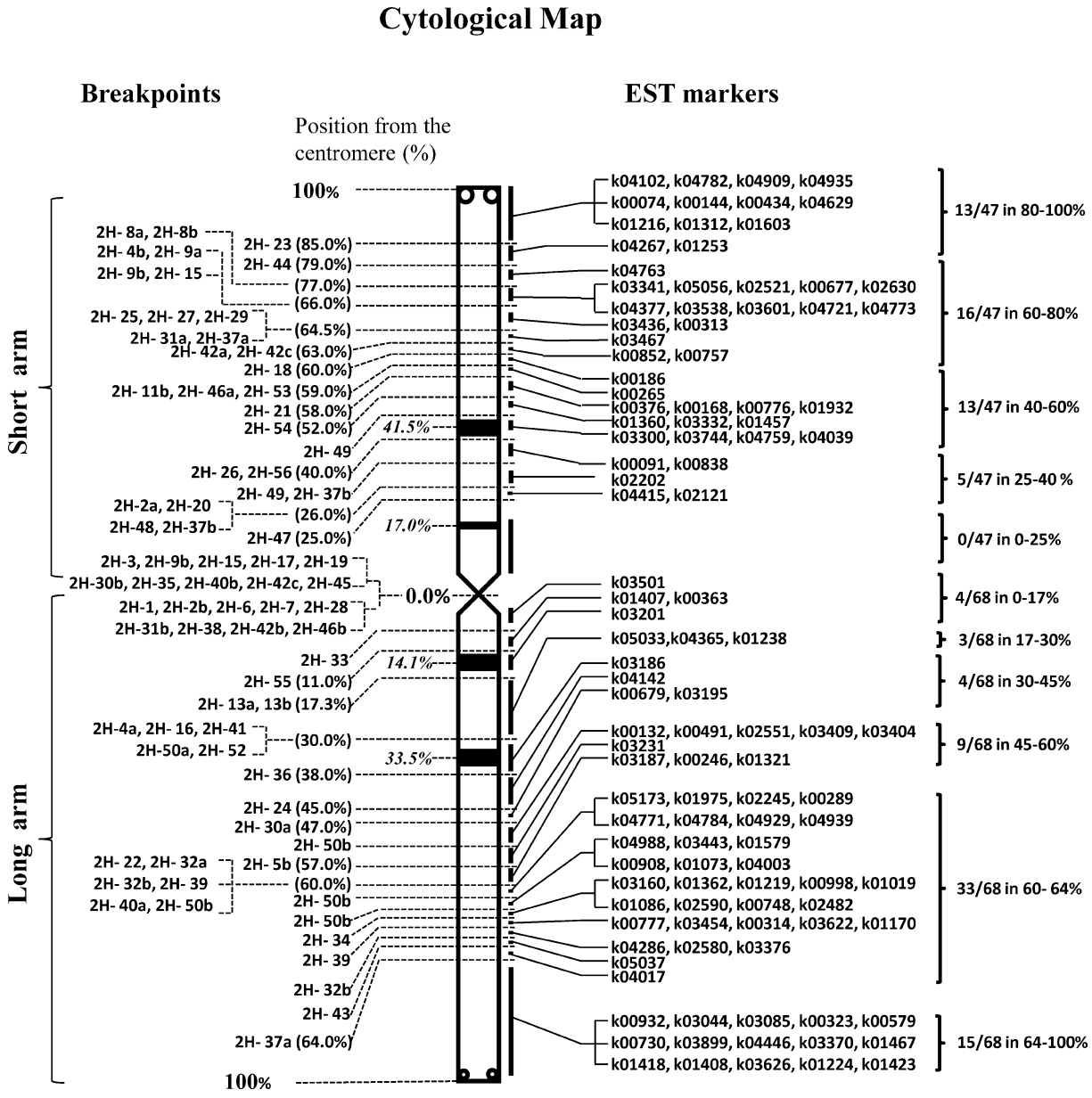

Fig. 2. Cytological map showing the breakpoints of the 66 aberrant 2H chromosomes relative to their fractional length (%). The percentages in italics indicate the positions of the C-bands. The fractions in the right-most column represent the proportions of EST markers mapped at physical intervals (%) in each of the 2H chromosome arms.

detailed linkage and physical maps revealed that wheat and barley shared the same GRRs '2L1.0' lying distal end of the long arm (Dilbirligi et al., 2005). The GRR revealed in the present study seemed to correspond to the GRR '2L1.0', but it was not at the distal end but in an intercalary region.

Comparison of 2H cytological and genetic maps

Sato et al. (2009) constructed a high-density genetic map of chromosome 2H using 492 EST markers and a mapping population of 'Haruna Nijo' (a barley variety) and 'H602' (an accession of *H. vulgare* ssp. *spontaneum*). In this study we employed 44 out of the 492 EST markers. The 44 markers were mapped at 40 genetic distance positions distributed more or less evenly along the 2H chromosome of 360.2 cM. In the cytological map, however, the same markers fell into 19 regions distal to the breakpoint of chromosome 2H-26 (FL = 0.40) in the 2HS arm and distal to that of chromosome 2H-33 (FL = 0.0–0.11) in the 2HL arm (Table 3 and Fig. 3). The order of markers on the genetic map, from k04935 to k03467, covering 121.6 cM of the 2HS arm, was consistent with that on the cytological map stretching distal ca. 40% of the 2HS arm. In this study we separated and repositioned three markers

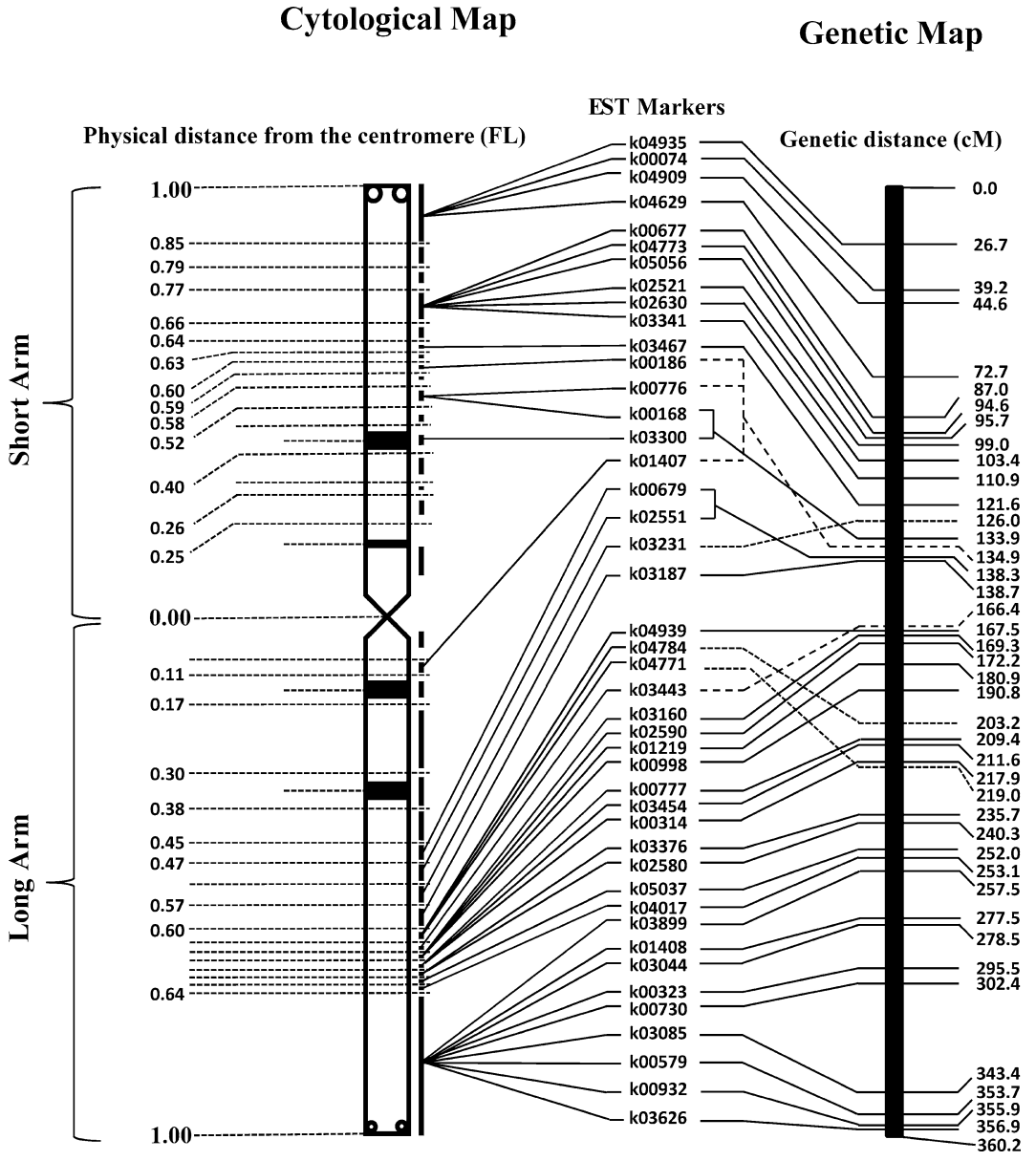

Fig. 3. Cytological (left) and genetic (right) maps of chromosome 2H. The cytological map shows the breakpoints of the 66 aberrant 2H chromosomes relative to the fractional length of the aberrant chromosome. The genetic map shows the genetic distances (cM) of the 44 EST markers that were used in this study.

(k00186, k00776 and k01407) mapped at 134.9 cM and two markers (k00168 and k03300) mapped at 133.9 cM in the genetic map. The breakpoints of chromosomes 2H-11b, 2H-21 and 2H-49 separated these markers, placing them in the order of k00186, k00776, k00168 and k03300, from distal to proximal, on the 2HS arm. The breakpoints of chromosomes 2H-33 and 2H-55 flanked marker k01407 in a proximal region of the 2HL arm. Therefore, the centromere should be flanked by the two markers, k03300 and k01407, covering 1.0 cM; this genetic distance corresponded to a proximal region stretching ca. 40% of the 2HS arm and ca. 11.0% of the 2HL arm. The genetic and cytological maps corresponded closely in terms of the orders of markers on the 2HL arm except for a few markers (Fig. 3), but the two maps were quite different in terms of the distances between markers. For example, three markers k00679, k02551 and k03187 mapped within 0.4 cM between 138.3–138.7 cM (0.4 cM = ca. 0.18% of the total genetic length of ca. 221.9 cM of the 2HL arm) were physically located within at least 10% of the length of the 2HL arm, i.e., between the breakpoints of 2H-5b (FL = 0.57) and 2H-30a (FL = 0.47). Marker k03231 is an example of the discrepancy between the genetic and cytological maps: This marker was genetically mapped at 126.0 cM but cytologically fell between two markers k02551 (138.3 cM) and k03187 (138.7 cM). Out of the 29 2HL-specific markers, 24 markers (k04939 to k03626 covering 189.4 cM) were found in a distal region accounting for ca. 40% of the length of the 2HL arm, and their order on both maps was almost identical, except for three markers k04784, k04771 and k03443 (Fig. 3).

Based on the positions of the 44 common EST markers and on the breakpoint of the aberrant 2H chromosomes used, we analyzed the distribution or density of the 492 EST markers of the 2H genetic map. The breakpoints of chromosomes 2H-23 (FL = 0.85), 2H-18 (FL = 0.60) and 2H-26 (FL = 0.40) divided the 2HS arm into three regions stretching the 15%, 25% and 20% distances from the telomere, and these regions contained 60, 102 and 45 EST markers, respectively (Fig. 4). The 1.0 cM region between two EST markers k03300 (on the 2HS arm) and k01407 (on the 2HL arm) contained 20 markers, but this region physically represented the pericentromeric region covering 40% of the length of the 2HS arm and 11.0% of that of the 2HL arm. The marker density was the highest (20.4 markers per 0.01 FL) in the region between the breakpoints of chromosomes 2H-5b (FL = 0.57) and 2H-37a (FL = 0.64), covering 7% of the length of the 2HL arm (Fig. 4). These facts suggested that the density of EST markers is higher in the distal region than in the proximal region for either of the 2H arms and that crossing over was strongly suppressed in the pericentromeric region. Thus, the genetic map was shown to be precise and accurate in the distal region (ca. 40% of the length of chromosome 2H) of the chromosome where the recombination rate is high. Although not so detailed as the

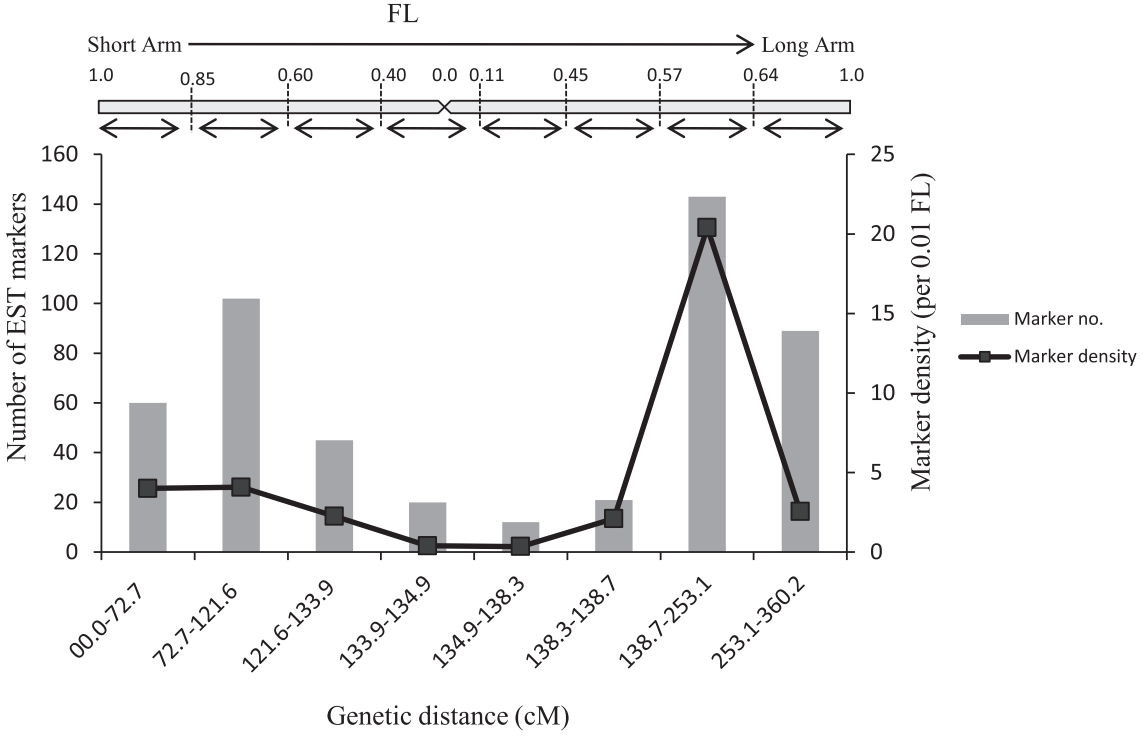

Fig. 4. Distribution of the 492 EST markers on the 2H genetic map in relation to the FL values of the breakpoints. Note that the density of EST markers is much higher in the distal regions than in the pericentromeric region.

genetic map, the cytological map revealed the accurate order of the markers, remedying the order of some of the markers in the genetic map, especially in the proximal region (ca. 60% of the length of chromosome 2H). We conclude that cytological mapping and genetic mapping are complementary strategies to complete the goal of genome sequencing and map based cloning.

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