Genomic differentiation of *Hordeum chilense* from *H. vulgare* as revealed by repetitive and EST sequences

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Hordeum vulgare, cultivated barley, and its wild relative, *H. chilense*, have several important traits that might be useful for wheat improvement. Here, *in situ* hybridization and barley expressed sequence tag (EST) markers were used to characterize and compare the chromosomes of *H. chilense* with those of *H. vulgare*. FISH with four repetitive DNA sequences, AG, AAG, 5S rDNA and 45S rDNA, was applied to the mitotic chromosomes of *H. vulgare*, *H. chilense* and available wheat-*H. chilense* addition and substitution lines. FISH with the AAG repeat differentiated the individual chromosomes of *H. chilense* and *H. vulgare*. The patterns of FISH signals in the two species differed greatly. The 45S rDNA signals were observed on two pairs of chromosomes in both species, while the 5S rDNA signals were observed on four pairs of chromosomes in *H. vulgare* and on one pair in *H. chilense*. The AG repeat showed FISH signals at the centromeric regions of all chromosomes of *H. vulgare* but none of the chromosomes of *H. chilense*. These results indicate that the chromosomes of the two species are highly differentiated. To study the homoeology between the two species, 209 EST markers of *H. vulgare* were allocated to individual chromosomes of *H. chilense*. One hundred and forty of the EST markers were allocated to respective chromosomes of *H. chilense* using the wheat-*H. chilense* addition and substitution lines. Twenty-six EST markers on average were allocated to each chromosome except to the chromosome 2H^{ch}S, to which only 10 markers were allocated. Ninety percent of the allocated EST markers in *H. chilense* were placed on *H. vulgare* chromosomes of the same homoeologous group, indicating that the expressed sequences of the two species were highly conserved. These EST markers would be useful for detecting chromatin introgressed from these species into the wheat genome.

Key words: *H. chilense*, *H. vulgare*, alien chromosome addition lines, FISH, EST, homoeology

INTRODUCTION

Related, wild and progenitor species represent a rich reservoir of useful genetic variation that can be exploited for crop improvement. Interspecific and intergeneric hybridization in the tribe Triticeae have been successfully used to facilitate the transfer of genes from wild relatives to cultivated species. Many amphiploids between wheat

and other species, and alien chromosome addition, substitution or translocation lines have been produced and maintained for this purpose. Agronomically important traits, including resistance to diseases, pests and abiotic stresses, have been transferred from these genetic stocks into wheat (Knott and Dvorak, 1976; Sharma and Gill, 1983; Gale and Miller, 1987; Jiang et al., 1994; Friebe et al., 1996).

Cytological and molecular characterization of alien chromosomes in a wheat genetic background would accelerate production of such genetic stocks and introgression of alien chromosome segments including useful genes into

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wheat. Fluorescence *in situ* hybridization (FISH) is well established as a highly efficient method for cytological characterization. It has been used for physical mapping of specific sequences in wheat and its relatives (Mukai et al., 1990 and 1992; Leitch et al., 1991; Jiang and Gill, 1994) to locate the breakpoints of chromosome translocations (Friebe et al., 1991; Mukai et al., 1993; Jiang et al., 1994; Molnar-Lang et al., 2000) and in karyotyping (Cabrera et al., 1995; Tsujimoto et al., 1997).

H. chilense is a wild species of *Hordeum*. However, its genome (H^{ch}) and geographical distribution (South America) are quite different from those of cultivated barley, *H. vulgare*. The use of *H. chilense* in breeding programs has focused on the development of tritordeums (amphiploids between wheat and *H. chilense*) to be used as new cereals (Martín, 1998) and for the introgression of novel traits into wheat. The amphiploids could be used as a bridge for the genetic transfer of useful genes from *H. chilense* to wheat or barley (Martín et al., 1998). Many *H. chilense* novel traits have been allocated to specific chromosomes: resistance to root-rot nematode on chromosome $1H^{ch}$ S, tolerance to salt on chromosomes $1H^{ch}$, $4H^{ch}$ and $5H^{ch}$, resistance to *Septoria* on chromosome $4H^{ch}$, and high carotenoid pigment content and resistance to common bunt both on chromosome $7H^{ch}$ (Person-Dedryver et al., 1990; Martín et al., 2000). *H. chilense* chromosome addition lines of hexaploid wheat have been obtained (Miller et al., 1982) and characterized by C-banding and *in situ* hybridization (Cabrera et al., 1995). The addition lines could be very useful tools for the introgression of *H. chilense* chromatin in wheat if such introgressions could be easily and reliably characterized using genetic markers.

Recently, a total of 120,000 clones of barley expressed sequence tags (ESTs) have been sequenced and analyzed, and 10,336 primer sets developed by Sato et al. (2003). Of these primers, 5,133 sets were tested for polymorphism between barley (cv. Betzes) and bread wheat (cv. Chinese Spring). One-third of the tested primers showed a presence/absence type of polymorphism between

wheat and barley. Previously, we tested this group of primers for polymorphisms between bread wheat and ten alien Triticeae species, including *H. chilense* (Hagras et al., 2005). A large number of such barley EST markers were effective in *H. chilense* and many of them were polymorphic between wheat and *H. chilense*. These markers can be used to identify DNA of *H. chilense* in a wheat genetic background. To examine the similarity of the chromosomes of *H. chilense* and *H. vulgare*, in this study we allocated most of these barley EST markers to specific chromosomes of *H. chilense* and compared the chromosome morphology between *H. chilense* and *H. vulgare* by FISH with four repetitive sequences.

MATERIALS AND METHODS

Plant materials *Hordeum vulgare* cv. Betzes ($2n = 2x = 14$; genome HH), *H. chilense* ($2n = 2x = 14$; genome $H^{ch}H^{ch}$), common wheat cv. Chinese Spring ($2n = 6x = 42$; genome AABBDD) and six *H. chilense* addition and substitution lines of Chinese Spring wheat were used in this study (Table 1). The chromosome addition line of $2H^{ch}$ that was used was addition of the short arm of $2H^{ch}$. The *H. chilense* chromosome addition and substitution lines, produced by Miller et al. (1982), were introduced from Wheat Genetics Resource Center, Kansas State University. The plant materials used in this study have been maintained in Tottori Alien Chromosome Bank of Wheat (TACBOW), managed by the National Bioresources Project-Wheat, Japan.

Fluorescence *in situ* hybridization (FISH) FISH was carried out as described by Kishii et al. (1999). Collected root tips were pretreated with ice water for 24 h and fixed in acetic acid: ethanol (1:3) for more than 3 days at room temperature. Mitotic slides were prepared by the acetocarmine squash method. Then the slides were denatured in 0.15 N NaOH (in 70% ethanol) for 5 min at room temperature, and dehydrated sequentially in cold 70%, 90%, and 99.5% ethanol. Five probes: *H. chilense*

Table 1. Plant materials and genomic constitutions used in this study

Line	Genomic constitution	Accession number
<i>Triticum aestivum</i> cv. 'Chinese Spring'	AABBDD	HT23S1
<i>Hordeum vulgare</i> cv. 'Betzes'	HH	TACBOW0116
<i>H. chilense</i>	$H^{ch}H^{ch}$	TACBOW0115
$1H^{ch}$ disomic substitution line	AABBDD - $1^{ra} + 1H^{ch''}$	TACBOW0051
$2H^{ch}$ S ditelosomic addition line	AABBDD + $2H^{ch}S''$	TACBOW0052
$4H^{ch}$ disomic substitution line	AABBDD - $1^{ra} + 4H^{ch''}$	TACBOW0053
$5H^{ch}$ disomic addition line	AABBDD + $5H^{ch''}$	TACBOW0054
$6H^{ch}$ disomic addition line	AABBDD + $6H^{ch''}$	TACBOW0055
$7H^{ch}$ disomic addition line	AABBDD + $7H^{ch''}$	TACBOW0056

^a The substituted chromosomes of wheat are unknown.

Table 2. Primer sequences and chromosomal locations of the EST markers on *H. chilense* chromosomes and on *H. vulgare* chromosomes

EST marker	Primer (L)	Primer (R)	Chromosomal location	
			<i>H. chilense</i>	<i>H. vulgare</i> ^a
BAWU12	TCCAGACTCTCTGGCGCTAT	GGCGGGCTCTATGAACACTA	1H ^{ch}	1H (P, G)
BAWU136	CAACCCAGCTACAGGGTGAT	CGTCATGTGCGTTCACCTAC	1H ^{ch}	1H (P, G)
BAWU162	CCCCATATCATCCTCCCTTT	GCAGTAATGACCTTTCCCCA	1H ^{ch}	1H (P, G)
BAWU211	AGCCTGTAGCAGCATGGATT	CACATCGACATGTTCCCTTGG	1H ^{ch}	1H (P, G)
BAWU243	ATCAAAGGAATGCAGGGTTG	TCAGTTTCATCGCACTCTGG	1H ^{ch}	1H (P, G)
BAWU316	TAGCTGGTCACCTCGCTCTT	TGCTGCTTGACTCGTTCATC	1H ^{ch}	1H (P, G)
BAWU541	CCGGTATTGACAAGCATCAA	ATTCATGGCACTCCGTCTTC	1H ^{ch}	1H (P, G)
BAWU585	AAGGGGCCGAATTATTTT	ACGGACATAGGTCAAATCCG	1H ^{ch}	1H (P, G)
BAWU616	CAAGCAAATCCTCACTGCAA	GACCTCAAGTCGTACCTGGC	1H ^{ch}	1H (P, G)
BAWU17	GAAGTGTGCCTTGCTCATCA	ACCTCGCTGCTAGAGCACAT	1H ^{ch}	1H (P)
BAWU25	TGTGTTTTATTTCAGCTGGCG	TGCTCTTTCCCTACCAACCT	1H ^{ch}	1H (P)
BAWU299	ATCTGGTTTCCGTTTCATTCG	TCACGCAACATTCCCTCTCTG	1H ^{ch}	1H (P)
BAWU366	TGGGCTTAATTTTCATCAGCC	GACCCCTTTGAAGATCCGTGA	1H ^{ch}	1H (P)
BAWU404	GACGCTCTGAACGACAAACA	ACTTCATCAACTGCCTTCGC	1H ^{ch}	1H (P)
BAWU498	ACCCACTTTTGAAGCTCTCA	CCTGTGGCGAAAAAGAAGAG	1H ^{ch}	1H (P)
BAWU574	AAAACAGAACCGTGGAGTGG	AATGTGGTTGGCAGAAAAGG	1H ^{ch}	1H (P)
BAWU671	CTGTTCGGACCAGTTGGTTT	ACAAAGAATGTCTGCGGGAC	1H ^{ch}	1H (P)
BAWU735	CTTGCTCAAAGTCGTTTCCA	CAGGCTTTACCGGAACATTG	1H ^{ch}	1H (P)
BAWU756	AGTTCCCTCCTTTGGTGGGT	ATCAGGACCCCTTCCTTCAT	1H ^{ch}	1H (P)
BAWU760	ACACGTTGGTGGCAGATACA	ACGAACATCTACGGAATGGC	1H ^{ch}	1H (P)
BAWU532	CCCAAACGTCCTTGTTCCTA	CGCAGGTAGCCAAAAATAGC	1H ^{ch}	2H (P, G)
BAWU785	TCAGCTCCATCAAACTTCG	AGCTGGTGATAGGGCATTG	1H ^{ch}	7H (P, G)
BAWU524	GTTGCACGCAAACGTTTCTA	AGCTTGGCTATCTTGCTGGA	1H ^{ch}	4H, 5H, 6H (P)
BAWU216	CGGAACCACACACAAAACT	CCTTCCCTGGTGATTGAAAC	1H ^{ch}	—
BAWU235	TCAAGGCACAAGGCAGTATG	TCAATGCAAGTGAGGAACCA	1H ^{ch}	—
BAWU343	TCGTACCATAACAATGCTAACCG	TGCAGAGGTTTGAAGAAGCC	1H ^{ch}	—
BAWU719	GACAGCCTTGCGAGCAATACA	GTCAGCCATCTCACCCTCAT	1H ^{ch}	—
BAWU842	ATGTAGAACCCAATCGCGTC	CAAAATGAGCAAGCCTGTGA	1H ^{ch}	—
BAWU917	GCGCCAGCTTTGTGTATGTA	ATGCTTCTGGGTAACGTTGG	1H ^{ch}	—
BAWU288	TAGGGTTTCGTCGCTGCTAGT	ACCAAAGAAGGAGGTGGCTT	2H ^{ch}	2H (P, G)
BAWU32	GTTTCGATCAGACATTCCGGT	TTTTCTCTAAACCCCTCGC	2H ^{ch}	2H (P, G)
BAWU40	CGGAGCTTCTGGTTGATTGT	AACTGCCAGTCTTCCAATG	2H ^{ch}	2H (P, G)
BAWU759	TCGACATCTCTCCATTTC	AACCAGATATGGATGCCAGG	2H ^{ch}	2H (P, G)
BAWU153	ATGGGCCAATATCAATTCCA	TGCTGCGTTTCAGCTTTAAGA	2H ^{ch}	2H (P)
BAWU181	TTTCGTGCCTTTGCTTCTTT	ATGGTTGCTGAAGTCCGAAG	2H ^{ch}	2H (P)
BAWU555	AAGAAATTGCGAGACGGAAG	TCCGTGTGAGTTTAGCATCG	2H ^{ch}	2H (P)
BAWU660	TGCAGCACACACTTCTTTCC	GATCGCTTCGGAGCATAGAC	2H ^{ch}	2H (P)
BAWU387	AGTTTTGTTGCCAAACGTCC	GCTTCATACCTTCGCCCTCTG	2H ^{ch}	4H (P, G)
BAWU835	GAAAAAGAAGCACGAGTCGG	CTCGCTGGAAACAGATCCAT	2H ^{ch}	—
BAWU112	GCACAACTGTCCCATTTGTTG	GCCAAAGGAGGGAAAAACTC	4H ^{ch}	4H (P, G)
BAWU303	AATGTGCCTCCACAGGGTAG	GATACTGAGTGGAAGCGGC	4H ^{ch}	4H (P, G)
BAWU618	AGATCGACACAAGGCCATTTC	GAGCTTGAGCTAATGACCG	4H ^{ch}	4H (P, G)
BAWU670	CAACCTAGCATCGCTCATCA	ATTCGTCAAGAAATGGGCAC	4H ^{ch}	4H (P, G)
BAWU755	TTCCGGACTATGAAACCTG	TTGTTCGCTGCTTACAGTGG	4H ^{ch}	4H (P, G)
BAWU766	AAACGGGAAATGTTTTGCAG	TCAAGAAGGCCATCGAGAAG	4H ^{ch}	4H (P, G)
BAWU915	AGGCGTCCTATGGACACAAC	CCCTCGACACGACCATCTAT	4H ^{ch}	4H (P, G)
BAWU118	CCAGTTCTTCTTGAGCAGGG	GTCTGGATTCCATGGACGTT	4H ^{ch}	4H (P)
BAWU152	GATGGTTGTGCTGCTGCTTA	AGCTCCTAAACATGGACCGA	4H ^{ch}	4H (P)
BAWU171	ATAACACGGGCAGAAATTCG	GGTACTGCCGTGTTTGGTT	4H ^{ch}	4H (P)

Continued

EST marker	Primer (L)	Primer (R)	Chromosomal location	
			<i>H. chilense</i>	<i>H. vulgare</i> ^a
BAWU217	CGCCCAATAAACCATTAAGC	CATCATCCAGGTGGTCTTCC	4H ^{ch}	4H (P)
BAWU265	TACGACGCGCATACTTCATC	ACATCATAGACCCTGCCCTG	4H ^{ch}	4H (P)
BAWU312	ACATTGGAGGATTCGCAAAG	ATCTCAGGCAACCAAACCTGC	4H ^{ch}	4H (P)
BAWU473	CACCTCATCCATCCTTGCTT	TTGTCATGAACGCATGATCC	4H ^{ch}	4H (P)
BAWU497	TGTGCATCCCTCAAAACAAA	TTGCTCACAAAGTGCTCCTG	4H ^{ch}	4H (P)
BAWU505	ATGGAGGGGATAAATACGGC	TCTGTAATGCTTGTGGCCTG	4H ^{ch}	4H (P)
BAWU507	CACACCTTTTCCGTCCAAGT	TTATGGTAGCATGGCGAGTG	4H ^{ch}	4H (P)
BAWU552	CCCCTGACAGCCTACAGCTA	AGGAGATGGTGTCCAGATCG	4H ^{ch}	4H (P)
BAWU673	AATGGGATCCATTCCCGTAT	AACGTCTCCGTTTCATCAC	4H ^{ch}	4H (P)
BAWU686	GAAGAGACCGTGCCAAAGTGT	CACGGAGGCAACAATCTGTA	4H ^{ch}	4H (P)
BAWU720	GCAACCCCTAACATTGCCTA	TCCTGGCATGCAGTCAATTA	4H ^{ch}	4H (P)
BAWU808	TGCCCCCAAACCTTTATATGC	GAGGGTCTTCTGTGTGGA	4H ^{ch}	4H (P)
BAWU852	ATCTCCACGCTAGCAGAACC	GCTCTGATGGCAATGATCTG	4H ^{ch}	–
BAWU884	GGACACGAGCTTCGTTTTTC	TAGCTGCATTGCTCAACTGG	4H ^{ch}	–
BAWU886	CGTACCTCGACAAAAGCACA	GTGTAAATGGCCAGATGCT	4H ^{ch}	–
BAWU898	ACATATTCTCCCGTGGTTGG	GCATTGCCAACGGTAAAGAT	4H ^{ch}	–
BAWU15	TTGCAGGTACTCCGGGTAAT	TTGTGCGATCGGTTCTTACT	5H ^{ch}	5H (P, G)
BAWU160	GCAACCGTAGTCCTTGTGGT	GTGTGGCCTTTTCAGGTGATT	5H ^{ch}	5H (P, G)
BAWU227	CCAGACTGACGATTTGGGAT	GTGAAGCAATTCATGCAAGC	5H ^{ch}	5H (P, G)
BAWU240	AGGTCATGCAGCTCTGTTCC	TGCGGGAGACCTACTTCAAC	5H ^{ch}	5H (P, G)
BAWU271	TGCACAAGAACAGTGCATCA	TTGAGGAGCTTCTGGCATT	5H ^{ch}	5H (P, G)
BAWU315	GTACCATTAGCTGCCCCAAA	GGCATGCTCTTCGAGTTTTTC	5H ^{ch}	5H (P, G)
BAWU321	CAGATGGCAGGCAATACAGA	ATAGCTTAACTGCCGCCGTA	5H ^{ch}	5H (P, G)
BAWU749	CTTCATCGCAACTTGTGTGG	GAAGTACACGACCGCATTGA	5H ^{ch}	5H (P, G)
BAWU834	AACCACAGGCACACATTCAA	AGGATACAACCCAAGCATCG	5H ^{ch}	5H (P, G)
BAWU891	ACTTGCACACCCAGCTAAT	TGCACAGCTACTGATTTGGC	5H ^{ch}	5H (P, G)
BAWU116	CGATGAGTGTATACGTGCGG	GAACACATCTTCACCGAGGG	5H ^{ch}	5H (P)
BAWU131	GAACGCCAGCCAAATTGTAT	ACCATTTTGATCCTTCTGCG	5H ^{ch}	5H (P)
BAWU30	AACTGAAAAGTTGCCATCCG	GACCACAAAGCCGATGTTTT	5H ^{ch}	5H (P)
BAWU362	TACATTAGGACCGCTGGGAC	AGGGCAAGTCATTTACCACG	5H ^{ch}	5H (P)
BAWU372	GTGCATAGGGCAGATTCCAT	CCAGCTCAAGTTCCAGAGGA	5H ^{ch}	5H (P)
BAWU423	AGGGTGCTGCCATGTTTTAC	CGCCTGTGAGACAAGAATGA	5H ^{ch}	5H (P)
BAWU494	CCGGGGATGAAATAACAGAA	GCAGCATACAAGGCTGTTGA	5H ^{ch}	5H (P)
BAWU495	CGACGCCTAAGGTACATTTCA	CAGATGAAGGGAAGGCTCAA	5H ^{ch}	5H (P)
BAWU651	TTAAACAGCAAAAACGTGCG	AGTGGAAAGGATTTTGTGCG	5H ^{ch}	5H (P)
BAWU780	ACTCGCATGATACATCCACG	TAGATGGGGTCTCTCATGGC	5H ^{ch}	5H (P)
BAWU782	CAACTTGACAAACAACGC	CTTGTGCATGCGCAGAGTAT	5H ^{ch}	5H (P)
BAWU827	GCCAAGCTCATATAGGGCAG	TATCTGTGATGCCACATCCG	5H ^{ch}	2H (P)
BAWU20	ACCCCGGAAGCTAAGATGAT	AGTCGGAACATGCGGTACAC	5H ^{ch}	3H (P, G)
BAWU713	TGATCGTCGTAGAAAGCCCT	AGGTGCCACATTTTCCAGAC	5H ^{ch}	–
BAWU873	ATCACAATTGGGTGTCCTTC	CACCAATCGTTTGGAGAGGT	5H ^{ch}	–
BAWU878	GGGAACATACCAGAGTCGGA	AGAGCTGTTCTCAAGCTGCC	5H ^{ch}	–
BAWU155	TCGACCACATGATCCACAGT	AGAACAACCACTACGACGGC	6H ^{ch}	6H (P, G)
BAWU29	CTCGGGCAACAAAGTAGCTC	AGAAGGTGATGGTACCGCTG	6H ^{ch}	6H (P, G)
BAWU409	ACCTCAAGCAAACCTGCAACC	GAAAGTGCGTGAGGAGAAGC	6H ^{ch}	6H (P, G)
BAWU894	CCTGCAGCTCTTCATGGTTT	ATTTTGCCACTACGGCTGTC	6H ^{ch}	6H (P, G)
BAWU107	CGCCTATTCTGAGCTCCTG	CGAGTATGGGAGTGGCAGTT	6H ^{ch}	6H (P)
BAWU139	TGATCTTTGGCAGAGCACAC	GGTTGGTGGGGAGTTGTATG	6H ^{ch}	6H (P)
BAWU280	TCTAAGCTAAAGCGACCCCA	CGAGAAAACTTCGGAGCATC	6H ^{ch}	6H (P)
BAWU349	AGCTAAGCTGCTTCCACTGC	TGAAGAAGTGAACGTGCTGG	6H ^{ch}	6H (P)

Continued

EST marker	Primer (L)	Primer (R)	Chromosomal location	
			<i>H. chilense</i>	<i>H. vulgare</i> ^a
BAWU361	GCTGCATGCTGGACTCAATA	GCACTCATTGAACAGCAGGA	6H ^{ch}	6H (P)
BAWU591	AACAAGCAGCCAGATCACAG	CCATTCGGTCTTGACCAGTT	6H ^{ch}	6H (P)
BAWU599	GCAGCTGGGGGATATACAGA	CATCTCCATCATCATCGTCG	6H ^{ch}	6H (P)
BAWU637	AGAAACCAGGTCCAGCCTTT	GGTTACACATGGCGGAAACT	6H ^{ch}	6H (P)
BAWU640	GAAAATACATCGCGGAAAA	TTAGCGTCCAGTCATCATGC	6H ^{ch}	6H (P)
BAWU658	TGTGGACGTGACAAATCCCTA	AACAGAGGAAGAGGAGGGGA	6H ^{ch}	6H (P)
BAWU94	TTTCAAGCAGAGCTGCAAAG	GCTTGCTGAGCGCTTTCTAC	6H ^{ch}	6H (P)
BAWU267	ATCTGATGAACCCGAACCAC	ATCAACAAGCATCCAGGGAG	6H ^{ch}	3H (P), 4H (G)
BAWU233	AAGCATAGCCTTGTCGGTTG	GGCAGATTTGCCATCTTCAT	6H ^{ch}	3H (P, G)
BAWU57	GTGGACTGGGGATACCAATG	ATCAAGGATGGCTGGAGATG	6H ^{ch}	7H (P)
BAWU346	TCTTCCCTTCACTGGATGCT	TCAAGCGTTTCTGTGGTTTG	6H ^{ch}	–
BAWU892	TCCCACGTTGGTCACTGTTA	TTGATATGTGCAAGCGGAAG	6H ^{ch}	–
BAWU104	GGCACCAGGTGAGGAGATTA	TGCACTTTCTGCCTATGTGC	7H ^{ch}	7H (P, G)
BAWU123	GCGGAGGACATACCCCTAA	AAGCTCAAGGGCGAGTACAA	7H ^{ch}	7H (P, G)
BAWU21	CATGCGACACCTACGAGAGA	GAGAGTCACAGATGGCCACA	7H ^{ch}	7H (P, G)
BAWU302	CATCCAATCTCAAATCCGCT	GGCAAAGCAATGCAAAAATC	7H ^{ch}	7H (P, G)
BAWU522	GCGACCACACCCGATATTTA	AAGGACGAGGCACGAAGTTA	7H ^{ch}	7H (P, G)
BAWU576	TGTGGAATGAGTGGAACAA	GGTTGGATCCGCTAGCTACA	7H ^{ch}	7H (P, G)
BAWU631	TACAACAACAATGGGGAGCA	TGAACCTGTGTGAGGGACCA	7H ^{ch}	7H (P, G)
BAWU680	CCCGTCACATGTATCACTGC	CGTGGACATTGAGAGGGAGT	7H ^{ch}	7H (P, G)
BAWU709	CCATAGACCAGCATCCCTTA	CATGTCGAGCAAGAAGACGA	7H ^{ch}	7H (P, G)
BAWU165	ATGAGCTCGGCAAAAATGAG	AGGGATTCCATGTCGTTTAC	7H ^{ch}	7H (P)
BAWU166	TCGCAGTTACAACATCTCGC	CATGACCGCAAAGCTTGATA	7H ^{ch}	7H (P)
BAWU379	TGCTGCACAAAGCATAGGAC	AATGTGAAGGCACATGACGA	7H ^{ch}	7H (P)
BAWU406	GGTTCAGGGACGAAGGTACA	CTACGCCTAGCGGAGATGAG	7H ^{ch}	7H (P)
BAWU517	TTGCGATGATGATGGAGTGT	CTTTTCGTCCTTCTTTGCGG	7H ^{ch}	7H (P)
BAWU550	ATGCCACCATTTACAAAGCC	TTTCTGGGTCCTGATCCTTG	7H ^{ch}	7H (P)
BAWU566	TCATATCAAACCTCCGGGTC	TGCCAAGTTTATCCGAAACC	7H ^{ch}	7H (P)
BAWU763	AGAACCGAGATGAGGAATGTG	AGTCTCTTCGCGGAATCAAG	7H ^{ch}	7H (P)
BAWU764	TGCGGGAAATGGAGAATAAG	GGCTACAAGGACACCCGATA	7H ^{ch}	7H (P)
BAWU79	TTGTACGCCTGTCTTGATC	AACAAGGGTCAACCGATGAG	7H ^{ch}	7H (P)
BAWU829	GAAAAGGTTACATGGGACGC	GGGACAACAGCACCTTTCAT	7H ^{ch}	7H (P)
BAWU381	CACATTAGCAAGCGACAAT	AATTGATCAACCTTGCCCTG	7H ^{ch}	4H (P, G)
BAWU752	TAGTACATGCGTGCCATGCT	GTCATCATCCTCGCCTTAC	7H ^{ch}	5H (P, G)
BAWU559	CCCAAAATTAAGGGCCAGAT	GGGACAAACTTGCTTGGA	7H ^{ch}	5H (P)
BAWU127	CGAATAGTGCCGAAAACAT	TCCATCCCCAGCTAGAAGAA	7H ^{ch}	–
BAWU514	GCTTGAGGGTCTTGTTCTGC	CAAGCTGGAAAAGGAAGCTG	7H ^{ch}	–
BAWU681	GTCACAATTTGCATTGCAGG	GCGTGGAGGAGATATCGTGT	7H ^{ch}	–
BAWU857	AGCTCAAGCCTTTGCAGCTA	TGTTTCAGGTGGTGTTCAT	7H ^{ch}	–
BAWU872	AGTGTCTATTTTGGTGCCGC	CATCTTGCTGGACAGGGATT	7H ^{ch}	–
BAWU879	ACGAAAACCTCGAGTGGAGA	AGCTTGTGGCTGCAAAGAAT	7H ^{ch}	–
BAWU880	TCTTACATCACCAGCGAGGA	AGAAGTTCGCCTACACCGC	1H ^{ch} , 4H ^{ch}	–
BAWU108	AGGGGCTCATGATACATTCG	GATCGCATGATGATGACAGG	1H ^{ch} , 2H ^{ch} , 5H ^{ch}	4H (P)
BAWU114	TGGACGTGGTGAGACTACGA	TACCGTATGGGCTCTTCCTG	1H ^{ch} , 6H ^{ch}	2H (P, G)
BAWU167	ATTTACAGGAAGTGTGGCGG	GTTGCACCTGGTTCCCTAGA	6H ^{ch} , 7H ^{ch}	6H (P)
BAWU310	ATGGTCTGCATGGTGAAACA	TCAAACGCTGCAAACTGAC	2H ^{ch} , 6H ^{ch} , 7H ^{ch}	3 H, 6H (P)
BAWU577	TACATTGGTGGACTTTCGCTG	AGCCAGGCTATTGCTTCAAA	1H ^{ch} , 5H ^{ch}	7H (P, G)
BAWU707	GCTTCTTCTGCCAAGTGAG	CATGAGCAATCCTCTGGGTT	4H ^{ch} , 6H ^{ch}	4H, 5H, 6 H, 7H (P)

^a In *H. vulgare*, chromosomal locations of the ESTs were determined physically (P) by using wheat-*H. vulgare* addition lines (Nasuda et al., 2005) and genetically (G) by linkage analysis (Sato et al., unpublished data).

genomic DNA, and AG, AAG, 5S rDNA and 45S rDNA repeats were used. The microsatellite sequences composed of AG and AAG repeats are organized in long tracts of heterochromatic DNA and are utilized as chromosome markers in some species of Triticeae (Pedersen et al., 1996; Hudakova et al., 2001). Sonicated genomic DNA and 45S rDNA probes were labeled by the random primer labeling method with rhodamine-5-dUTP or FITC-11-dUTP. The AG and AAG repeats were labeled by the 3' end labeling method with rhodamine-5-dUTP or FITC-11-dUTP using oligonucleotide primers (AG)₁₅ and (AAG)₁₀ as templates, respectively. The 5S rDNA was labeled by the PCR method with biotin-16-dUTP. A hybridization solution (50% formamide, 10% dextran sulfate, 1 µg/µl sonicated salmon sperm DNA, 50–500 ng of labeled probe, 2 × SSC) was applied to each slide. For AG, AAG and 45S rDNA probes, after hybridization at 37°C for 12–16 h, the slides were washed in 2 × SSC at room temperature for 5 min, briefly dried, and mounted with Vectashield (Vector) containing 1 µg/µl 4,6-diamidino-2-phenylindole (DAPI) for counterstaining of chromosomes. For the 5S rDNA probe, after hybridization at 37°C for 12–16 h, the slides were washed in 2 × SSC at room temperature for 5 min, 50% formamide in 2 × SSC at 37°C for 15 min, and 4 × SSC at room temperature for 5 min, and then 120 µl of a solution containing 200 µg/µl anti-digoxigenin-rhodamine (Roche) diluted with 1% bovine serum albumin (BSA) in 4 × SSC was applied. After incubation for 30 min at 37°C, the slides were washed in 2 × SSC, 0.1% TritonX-100 (in 2 × SSC) and 2 × SSC at room temperature for 10 min each, briefly dried, and mounted with Vectashield containing 1 µg/µl DAPI. Using a fluorescence microscope (Olympus), photographs were taken with a cooled CCD camera (Photometrics).

DNA isolation and PCR Total genomic DNA was extracted from young plant leaves using the CTAB method (Murray and Thompson, 1980). PCR was performed in a 96-well plate, each well containing a 10-µl reaction mixture of 30 ng of template DNA, 5 pmol of forward and reverse EST primers, 1 µl of 10 × *Ex Taq* buffer (20 mM Mg²⁺), 0.8 µl of dNTP mixture (2.5 mM each), and 0.25 U of TaKaRa *Ex Taq* DNA polymerase (TaKaRa, Japan). After an initial denaturation step at 94°C for 2 min, reactions were subjected to a touch-down amplification: 5 cycles at 94°C for 30 sec, 65°C for 30 sec (decreasing 1°C/cycle), 72°C for 2 min and subsequent 35 cycles at 94°C for 30 sec, 60°C for 30 sec, 72°C for 2 min, with a final extension step at 72°C for 7 min. Electrophoresis of amplification products was performed in 1.5% agarose gels.

Barley EST primers Two hundred and nine barley EST primer sets were used. The primer names and base

sequences are presented in Table 2. These primers were previously screened for polymorphisms (presence/absence or size polymorphisms) between wheat and *H. chilense* (Hagras et al., 2005). Therefore, they would be expected to amplify markers of *H. chilense* in a wheat genetic background. Moreover, most of these markers have already been allocated to *H. vulgare* chromosomes using wheat-*H. vulgare* addition lines (Nasuda et al., 2005), a linkage map (Sato et al., unpublished data), or both.

RESULTS

Fig. 1 shows root-tip metaphase spreads of *H. chilense* and *H. vulgare* hybridized with AAG, 5S rDNA, AG, and 45S rDNA repeats. AAG showed strong hybridization signals on all of the seven chromosome pairs of *H. vulgare*, while the signals appeared, with varying patterns, on only six pairs of *H. chilense* chromosomes (Figs. 1a and 1b). This suggests that different events of AAG-repeat amplification occurred in the evolution of *H. chilense* and *H. vulgare*. Likewise, hybridization signals for AG were observed only on the centromere of all *H. vulgare* chromosomes. However, no signal appeared on any chromosome of *H. chilense* (Figs. 1e and 1f).

Signals attributable to 5S rDNA appeared on four pairs of chromosomes in *H. vulgare*, but on only one pair in *H. chilense* (Figs. 1c and 1d). On the other hand, the 45S rDNA probe showed the same pattern on the two pairs of satellite chromosomes of both *Hordeum* species (Figs. 1e and 1f).

Allocation of *H. chilense* chromosomes By comparison between the chromosomes in the original plant and those in the addition and substitution lines, the chromosomes of *H. chilense*, showing varied patterns of FISH signals, were allocated to the homoeologous groups (Figs. 2 and 3). AAG signals distinguished individual chromosomes 1H^{ch} to 7H^{ch} by their different patterns. No signals were observed on 6H^{ch}, while the remaining six pairs showed signals of varying distribution and strength. Chromosome 3H^{ch} showed the highest signal number and intensity, while 5H^{ch} showed only one faint interstitial signal (Fig. 3). 5S rDNA signals appeared on the terminus of the short arm in the *H. chilense* chromosome of the 5H^{ch} addition line. 45S rDNA signals appeared on the short arm of addition lines 5H^{ch} and 6H^{ch}. The signal on 6H^{ch} was relatively closer to the centromere and stronger compared to that on 5H^{ch}. No chromosome rearrangements were observed in the chromosomes in the addition or substitution lines.

Comparative FISH analysis of *H. chilense* and *H. vulgare* Pedersen et al. (1996) reported good agreement of the AAG-banding pattern of barley with the C-banding and N-banding patterns. In this study, individual chro-

Fig. 1. FISH with AAG (a, b), 5S rDNA (c, d), and 45S rDNA (green) and AG (red) (e, f) to mitotic chromosomes of *H. chilense* (a, c, e) and *H. vulgare* (b, d, f).

Fig. 2. Cytological characterization of *H. chilense* chromosomes with the AAG probe in wheat-*H. chilense* addition and substitution lines. (a) 1H^{ch} disomic substitution line. (b) 2H^{ch}S telosomic addition line. (c) 4H^{ch} disomic substitution line. (d) 5H^{ch} disomic addition line. (e) 6H^{ch} disomic addition line. (f) 7H^{ch} disomic addition line. Wheat chromosomes appear blue and *H. chilense* chromosomes appear red. All lines except the 6H^{ch} disomic addition line showed AAG repeat signals (green).

Fig. 3. Karyotyping of *H. chilense* chromosomes. The chromosomes of the original *H. chilense* are shown on the left side, and those of addition or substitution lines on the right side of each chromosome pair. (a) AAG repeat, (b) 5S rDNA and (c) 45S rDNA.

Fig. 4. FISH of *H. vulgare* and *H. chilense* with AAG (a), 5S rDNA (b) and 45S rDNA (c) repeats. For each chromosome pair the upper ones are of *H. chilense* and the lower ones are of *H. vulgare*.

mosomes of barley were identified based on the ideogram of the C-banded chromosomes of barley cv. Betzes (Kakeda et al., 1991) and the AAG signal patterns obtained from

this study. Likewise, in *H. chilense* AAG signals partly reflected the C-banding patterns presented by Cabrera et al. (1995). Fig. 4 shows a chromosomal comparison bet-

Fig. 5. Representative PCR amplification patterns. (a) Amplified fragments appeared only in *H. chilense* and one of the *H. chilense* addition/substitution lines (BAWU361). (b) Size-polymorphic bands appeared only in *H. chilense* and one of the *H. chilense* addition/substitution lines (BAWU541). (c) A single band appeared only in *H. chilense* (BAWU414). (d) Amplified fragments appeared in *H. chilense* and two of the *H. chilense* addition/substitution lines (BAWU707).

ween *H. chilense* and *H. vulgare* using FISH with three probes. The signal patterns of the AAG repeat differed between the homoeologous pairs of *H. chilense* and *H. vulgare*. None of the chromosomes of *H. chilense* showed a signal similar to that of the AAG repeats in *H. vulgare*. For instance, while chromosomes 1H and 4H of *H. vulgare* showed two and many signals, respectively, the corresponding homoeologous pairs in *H. chilense* showed one and two signals (Fig. 4).

The locations of 5S rDNA signals differed between the two species. They appeared on chromosomes 2H, 3H, 4H and 7H in *H. vulgare*, but only on the nonhomoeologous chromosome 5H^{ch} in *H. chilense* (Fig. 4). As for 45S rDNA, there was no difference in hybridization pattern between the two species, in both of which hybridization appeared on homoeologous chromosome groups 5 and 6.

Allocation of barley EST markers to *H. chilense* chromosomes

Among 209 markers generated in *H. chilense*, 140 markers were allocated to individual chromosomes (Table 2 and Fig. 5). Twenty-nine markers were on 1H^{ch} and 7H^{ch}, 26 were on 4H^{ch} and 5H^{ch}, 21 were on 6H^{ch}, and 10 on the short arm of 2H^{ch}. Seven markers were allocated to two or three chromosomes. For example, marker BAWU707 was allocated to chromosomes 1H^{ch} and 5H^{ch}, and yielded an amplicon of the same size (Fig. 5d). Sixty-one primers amplified no PCR products with any of the used addition or substitution lines and hence these markers could not be allocated to specific chromosomes of *H. chilense* (Fig. 5c). This might be due to three reasons: the unavailability of addition lines of chromosomes 3H^{ch} and 2H^{ch}L, differences between accessions of *H. chilense* used in the production of the addition lines and the one used in this study, and differences in the most appropriate PCR conditions depending on whether the template DNA was from *H. chilense* or the wheat-*H. chilense* addition lines. Comparing the location of these markers on *H. vulgare* (Nasuda et al., 2005; Sato et al., unpublished data) and *H. chilense* chromosomes revealed a high degree of homology between the two species. All the markers allocated to chromosome 4H^{ch} of *H. chilense* were placed onto the homoeologous chromosome of *H. vulgare*. However, some of the markers were allocated to different homoeologous chromosomes. For example, two of the markers allocated to chromosome 5H^{ch} of *H. chilense* were placed on chromosome 2H and 3H of *H. vulgare*, respectively.

DISCUSSION

Marked difference in chromosome morphology between *H. chilense* and *H. vulgare* Characterization of alien chromosomes is useful for their utilization in wheat improvement programs. Differentiation between the genomes of *H. vulgare* ssp. *spontaneum* and *H. chil-*

ense was studied using repetitive sequences (Taketa et al., 2000). In that study, hybridization of the repetitive DNA sequence family pSc119.2 detected signals in *H. chilense* but not in *H. vulgare* ssp. *spontaneum*. Here, four probes of repetitive sequences for FISH and 209 barley EST markers were used to characterize the chromosomes of *H. chilense* and to compare their homoeology with the chromosomes of *H. vulgare*. Three out of the four probes (AAG, 5S rDNA and 45S rDNA) gave diverse signals that enabled us to distinguish individual chromosomes in both *H. chilense* and *H. vulgare*. The hybridization signals in *H. chilense* were conserved in all added or substituted *H. chilense* chromosomes in Chinese Spring wheat. This suggests that no detectable structural changes occurred in *H. chilense* chromosomes during the hybridization with wheat. On the other hand, the hybridization signals revealed differences at the chromosome level between *H. chilense* and *H. vulgare*. The centromeric DNA sequence differed between the two species: AG repeat signals were observed only in *H. vulgare*. In addition, AAG signals were observed on the chromosomes of the two *Hordeum* species but none of the homoeologous pairs showed the same signal pattern. Also, the hybridization signals of the 5S rDNA repeat indicated differences in the numbers of chromosomes containing the repeat between the two species. Conservation of the number and position of signals between *H. chilense* and *H. vulgare* was observed only with the 45S rDNA repeat. The usefulness of both 5S and 45S rDNA as phylogenetic markers in the genus *Hordeum* was previously suggested (de Bustos et al., 1996; Taketa et al., 1999). The physical locations of 5S and 45S rDNA in 15 diploid *Hordeum* species with 'T' genomes were examined (Taketa et al., 2000 and 2005). Those authors concluded that the rDNA sites are useful chromosome markers for investigations of the karyotype evolution and phylogeny of the I-genome species in the genus *Hordeum*.

Conservation of homoeology in *H. chilense* and *H. vulgare*

In a previous study we proposed the usefulness of barley EST markers for the genetic analysis of ten alien Triticeae species related to wheat (Hagras et al., 2005). Out of 1,165 barley EST markers, 610 were transferable to *H. chilense*, and 292 of them were polymorphic between wheat and *H. chilense*. Of the 209 markers tested in this study, 140 were allocated to six pairs of *H. chilense* chromosomes using wheat-*H. chilense* addition and substitution lines. On average, 26 markers were allocated to each chromosome of *H. chilense*. Most of these markers had already been allocated to *H. vulgare* chromosomes (Nasuda et al., 2005; Sato et al., unpublished data). In this study, 90% of the EST markers were allocated to the same homoeologous group of *H. vulgare*, indicating conservation of homoeology between the two species. The remaining markers were allocated to nonhomoeologous or

multiple homoeologous groups. Non-conserved homoeology might be due to undetectable chromosome rearrangements that occurred during the evolution of the species. EST markers might be a good tool for detecting such chromosomal rearrangements.

EST markers have advantages over repetitive sequences in genome comparisons because they originate from expressed sequences and most of them can identify unique regions. Therefore, an EST is more likely to be conserved across species than repetitive sequences, and thus is more likely to be useful for comparative mapping. The degree of homology of the EST markers in *H. chilense* and *H. vulgare* indicates a close relationship between the two species, in spite of the great differences shown by FISH analysis using the four repetitive sequence probes. The EST markers were conserved while the repetitive sequences have rapidly changed during the evolution of the two species. Using ESTs is more reliable than using repetitive sequences to study the homology between these species.

Use of allocated EST markers for genetic studies

The current knowledge about the genomes of wild Triticeae species is limited. Alien chromosome addition and substitution lines in wheat are important genetic resources for genetic studies on individual chromosomes of alien species related to wheat. Moreover, EST markers allocated to individual chromosomes or chromosome arms would make those genetic resources more useful. This study presented 140 EST markers specific to the *H. chilense* chromosomes. This large number of markers should encourage further efforts to construct a fine genetic map for this important resource. The repetitive sequences and EST markers allocated to the specific chromosomes of *H. chilense* in this study will be useful in identifying and tracing introgressed *H. chilense* chromosomes and chromosomal segments in a wheat background.

REFERENCES

- Cabrera, A., Friebe, B., Jiang, J., and Gill, B. S. (1995) Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. *Genome* **38**, 435–442.
- De Bustos, A., Cuadrado, A., Soler, C., and Jouve, N. (1996) Physical mapping of repetitive DNA sequences and 5S and 18S–26S rDNA in five wild species of the genus *Hordeum*. *Chromosome Res.* **9**, 491–499.
- Endo, T. R., and Gill, B. S. (1996) The deletion stocks of common wheat. *J. Hered.* **87**, 295–307.
- Friebe, B., Jiang, J., Raupp, W. J., McIntosh, R. A., and Gill, B. S. (1996) Characterization of wheat-alien translocations conferring resistance to disease and pest: current status. *Euphytica* **91**, 59–87.
- Friebe, B., Mukai, Y., Dhaliwal, H. S., Martin, T. J., and Gill, B. S. (1991) Identification of alien chromatin specifying resistance to streak mosaic and greenbug in wheat germplasm by C-banding and in situ hybridization. *Theor. Appl. Genet.* **81**, 381–389.
- Gale, M. D., and Miller, T. E. (1987) The introduction of alien genetic variation into wheat. In: *Wheat breeding: its scientific basis* (Lupton FGH ed). Chapman and Hall. pp. 173–210.
- 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** (in press).
- Hudakova, S., Michalek, W., Presting, G. G., ten Hoopen, R., dos Santos, K., Jasencakova, Z., and Schubert, I. (2001) Sequence organization of barley centromeres. *Nucleic Acids Res.* **29**, 5029–5035.
- Jiang, J., and Gill, B. S. (1994) New 18–26S ribosomal RNA gene loci: chromosomal land markers for the evolution of polyploid wheats. *Chromosoma* **103**, 179–185.
- Jiang, J., Friebe, B., and Gill, B. S. (1994) Recent advances in alien gene transfer in wheat. *Euphytica* **73**, 199–212.
- Kakeda, K., Fukui, K., and Yamagata, H. (1991) Heterochromatic differentiation in barley chromosomes revealed by C- and N-banding techniques. *Theor. Appl. Genet.* **81**, 144–150.
- Kishii, M., Nagaki, K., Tsujimoto, H., and Sasakuma, T. (1999) Exclusive localization of tandem repetitive sequences in subtelomeric heterochromatin regions of *Leymus racemosus* (Poaceae, Triticeae). *Chromosome Res.* **7**, 519–529.
- Knott, D. R., and Dvorak, J. (1976) Alien germplasm as a source of resistance to disease. *Ann. Rev. Phytopathol.* **14**, 211–235.
- Leitch, I. J., Leitch, A. R., and Heslop-Harrison, J. S. (1991) Physical mapping of plant DNA sequences by simultaneous in situ hybridization of two differently labeled fluorescence probes. *Genome* **34**, 329–333.
- Martín, A. (1998) Tritordeum: the first ten years. *Rachis* **7**: 12–15.
- Martín, A., Cabrera, A., Hernandez, P., Ramirez, M. C., Gimenez, M. J., Rubiales, D., and Ballestros, J. (2000) Prospects for the use of *H. chilense* in durum wheat breeding. In: Broertjes C (ed) *proc Int Symp Eucarpia on induced Variability in Plant Breeding* PP. 79–81. Pudoc, Wageningen.
- Martín, A., Martín, L. M., Cabrera, A., Ramirez, M. C., Gimenez, M. J., Rubiales, D., Hernandez, P., and Ballestros, J. (1998) The potential of *Hordeum chilense* in breeding Triticeae species. In: *Triticeae III*. Jaradat, A.A. (ed.) Science publisher, Inc., Enfield, NH, USA, pp. 377–386.
- Miller, T. E., Reader, S. M., and Chapman, V. (1982) The addition of *Hordeum chilense* chromosomes to wheat. In *Induced Variability in Plant Breeding: Proceedings of an International Eucarpia Symposium*, Wageningen, September 1981. Edited by C. Broertjes. Pudoc, Wageningen, The Netherlands, pp. 79–81.
- Molnar-Lang, M., Lince, G., Friebe, B., and Sutka, J. (2000) Detection of wheat-barley translocations by genomic in situ hybridization in derivatives of hybrids multiplied in vitro. *Euphytica* **112**, 117–123.
- Mukai, Y., Endo, T. R., and Gill, B. S. (1990) Physical mapping of the 5S rDNA multigene family in common wheat. *J. Hered.* **81**, 290–295.
- Mukai, Y., Friebe, B., and Gill, B. S. (1992) Comparison of C-banding patterns and in situ hybridization sites using highly repetitive and total genomic rye DNA probes if Imperial rye chromosomes added to Chinese Spring wheat. *J. Genet.* **67**, 71–83.

- Mukai, Y., Friebe, B., Hatchett, J. H., Yamamoto, M., and Gill, B. S. (1993) Molecular cytogenetic analysis of radiation induced wheat-rye terminal and intercalary chromosomal translocations and the detection of rye chromatin specifying resistance to Hessian fly. *Chromosoma* **102**, 88–95.
- Murray, H. G., and Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**, 4321–4325.
- Nasuda, S., Kikkawa, Y., Ashida, T., Rafiqul Islam, A. K. M., Sato, K., and Endo, T. R. (2005) Chromosomal allocation and deletion mapping of barley ESTs. *Genes and Genetic Systems* (in press)
- Pedersen, C., Rasmussen, S. K., and Linde-Laursen, I. (1996) Genome and chromosome identification in cultivated barley and related species of the Triticeae (Poaceae) by in situ hybridization with the GAA-satellite sequence. *Genome* **39**, 93–104.
- Person-Dedryver, F., Jahier, J., and Miller, T. E. (1990) Assessing the resistance to cereal root-knot nematode, *Meloidogyne naasi*. I: a wheat line with the added chromosome arm 1H^{ch}S of *Hordeum chilense*. *J. Genet. Breed.* **44**, 291–296.
- Sato, K., Nankaku, N., Yano, K., and Takeda, K. (2003) Large scale development of barley EST markers. *Ikushugaku Kenkyu*, **5** (suppl. 1): 93.
- Sharma, H. C., and Gill, B. S. (1983) Current status of wide hybridization in wheat. *Euphytica* **32**, 17–31.
- Taketa, S., Ando, H., Takeda, K., Harrison, G. E., Heslop-Harrison, J. S. (2000) The distribution, organization and evolution of two abundant and widespread repetitive DNA sequences in the genus *Hordeum*. *Theor. Appl. Genet.* **100**, 169–176.
- Taketa, S., Ando, H., Takeda, K., Ichii, M., and Von Bothmer, R. (2005) Ancestry of American polyploid *Hordeum* species with the I genome inferred from 5S and 18S–25S rDNA. *Ann. Bot.* (published online on April 13, 2005).
- Taketa, S., Harrison, G. E., Heslop-Harrison, J. S. (1999) Comparative physical mapping of the 5S and 18S–25S rDNA in nine wild *Hordeum* species and cytotypes. *Theor. Appl. Genet.* **98**, 1–9.
- Tsujimoto, H., Yamada, T., and Abe, T. (2001) Chromosome breakage in wheat induced by heavy ion-beam irradiation. *Riken Report* **34**, 172.
- Tsujimoto, H., Mukai, Y., Akagawa, K., Nagaki, K., Fujigaki, J., Yamamoto, M., and Sasakuma, T. (1997) Identification of individual barley chromosomes based on repetitive sequences: Conservative distribution of Afa-family repetitive sequences on the chromosomes of barley and wheat. *Genes and Genetic Systems* **72**, 303–309.