Mapping barley genes to chromosome arms by transcript profiling of wheat–barley ditelosomic chromosome addition lines

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Abstract: Wheat–barley disomic and ditelosomic chromosome addition lines have been used as genetic tools for a range of applications since their development in the 1980s. In the present study, we used the Affymetrix Barley1 GeneChip for comparative transcript analysis of the barley cultivar Betzes, the wheat cultivar Chinese Spring, and Chinese Spring – Betzes ditelosomic chromosome addition lines to physically map barley genes to their respective chromosome arm locations. We mapped 1257 barley genes to chromosome arms 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL based on their transcript levels in the ditelosomic addition lines. The number of genes assigned to individual chromosome arms ranged from 24 to 197. We validated the physical locations of the genes through comparison with our previous chromosome-based physical mapping, comparative *in silico* mapping with rice and wheat, and single feature polymorphism (SFP) analysis. We found our physical mapping of barley genes to chromosome arms to be consistent with our previous physical mapping to whole chromosomes. *In silico* comparative mapping of barley genes assigned to chromosome arms revealed that the average genomic synteny to wheat and rice chromosome arms was 63.2% and 65.5%, respectively. In the 1257 mapped genes, we identified SFPs in 924 genes between the appropriate ditelosomic line and Chinese Spring that supported physical map placements. We also identified a single small rearrangement event between rice chromosome 9 and barley chromosome 4H that accounts for the loss of synteny for several genes.

Key words: wheat–barley ditelosomic addition line, Barley1 GeneChip, transcriptome, physical mapping, single feature polymorphism, synteny, chromosomal rearrangement.

Résumé : Les lignées d'addition chromosomique disomiques et ditélosomiques entre le blé et l'orge ont été utilisées comme outils d'analyse génétique pour diverses fins depuis leur développement dans les années 80. Dans le présent travail, les auteurs ont utilisé la puce Affymetrix Barley1 GeneChip pour des analyses transcriptomiques comparées de l'orge Betzes, du blé Chinese Spring et des lignées d'addition ditélosomiques Chinese Spring – Betzes pour effectuer la cartographie physique des gènes de l'orge sur leurs bras chromosomiques respectifs. Les auteurs ont placé un total de 1257 gènes de l'orge sur les bras chromosomiques 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS et 7HL sur la base de l'abondance du transcrit au sein des lignées d'addition ditélosomiques. Le nombre de gènes par bras chromosomique variait entre 24 et 197. Les auteurs ont confirmé la position de ces gènes en comparant les résultats avec les résultats de leurs travaux antérieurs de cartographie physique, en pratiquant la cartographie physique *in silico* avec le riz et le blé et par analyse du polymorphisme d'hybridation sur sonde unique (SFP ou « single feature polymorphism »). La cartographie des gènes sur des bras chromosomiques a montré une bonne concordance avec les résultats de la cartographie physique. L'approche *in silico* pour cartographier les gènes de l'orge a révélé que la synténie génomique moyenne avec les bras chromosomiques du blé et du riz était respectivement de 63,2 % et 65,5 %. Au sein des 1257 gènes cartographiés, les auteurs ont identifié 924 SFP entre les lignées ditélosomiques appropriées et Chinese Spring, ce qui confirmait la position de ces gènes. Les auteurs ont également identifié un seul petit réarrangement entre le chromosome 9 du riz et le chromosome 4H de l'orge qui explique l'absence de synténie pour plusieurs gènes.

Mots-clés : lignée d'addition ditélosomique blé–orge, puce Barley1 GeneChip, transcriptome, cartographie physique, polymorphisme d'hybridation sur sonde unique (« SFP »), synténie, réarrangement chromosomique.

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Introduction

Aneuploid addition lines have alien donor chromosomes or chromosome arms introduced into a recipient genetic background. Such lines partition the donor genome into its constituent chromosomes or chromosome arms and serve as a genetic tool for analysis of alien chromosomes. Disomic and ditelosomic chromosome addition lines have been instrumental in a variety of plant species for gene mapping (e.g., Dunn et al. 1993; Garvin et al. 1998; Spielmeyer et al. 2004), analysis of alien gene expression (e.g., Hart et al. 1980; Islam and Shepherd 1990; Muehlbauer et al. 2000), analysis of meiotic pairing behavior and chromosome structure (Qi et al. 2005), and isolation of chromosome arms (Gill et al. 1999).

Wheat (*Triticum aestivum* L.) – barley (*Hordeum vulgare* L.) disomic chromosome addition lines and ditelosomic chromosome arm addition lines have been developed through hybridization between the hexaploid ($2n = 6x = 42$) wheat cultivar Chinese Spring (CS) and the diploid ($2n = 2x = 14$) barley cultivar Betzes (BZ) (Islam et al. 1981). These lines include 6 of the 7 possible disomic barley chromosome addition lines for chromosomes 2(2H), 3(3H), 4(4H), 7(5H), 6(6H), and 1(7H) (Islam et al. 1981) and 13 of 14 ditelosomic addition lines (Islam et al. 1981; Islam 1983; Islam and Shepherd 1990).

Wheat–barley addition lines have been used to physically map isozymes and DNA markers to chromosomes and chromosome arms (e.g., Islam and Shepherd 1990; Garvin et al. 1998). Recently, Cho et al. (2006) extended the use of the wheat–barley chromosome addition lines by coupling these genetic stocks with the Affymetrix Barley1 GeneChip to physically map 1787 barley genes to 6 different barley chromosomes. The Barley1 GeneChip probe array (Close et al. 2004) provides the opportunity to examine approximately 23 000 transcripts in BZ, CS, and the disomic addition lines in parallel. This study employed the rationale that a transcript detected in BZ and a given CS–BZ addition line, but not in CS, is a signature for a barley gene located on the barley chromosome present in the addition line.

GeneChip technology also enables detection of single feature polymorphisms (SFPs) between 2 genotypes. SFPs are differences between genotypes in hybridization to a single probe within a probe set, and they can result from single nucleotide polymorphisms (SNPs), insertion/deletions, or differences in mRNA processing between the genotypes evaluated. Both Rostoks et al. (2005) and Cui et al. (2005) detected SFPs between 2 barley genotypes based on RNA hybridizations to the Barley1 GeneChip. Since each Barley1 GeneChip is composed of approximately 23 000 probe sets, each with 11 perfect-match 25-mer oligonucleotide probes, SFP detection in RNA populations from different genotypes is a powerful approach for identifying potentially thousands of polymorphic markers for mapping in barley.

In this study, we used wheat–barley ditelosomic addition lines in combination with the Barley1 GeneChip to extend large-scale physical mapping of barley genes to chromosome arms. Our specific objectives were to (*i*) physically map genes to barley chromosome arms using transcript signatures, (*ii*) validate the physical mapping results based on the analysis of chromosome arms, and (*iii*) use barley–rice synteny data as a tool to identify discrete genomic rearrangements between rice and barley.

Materials and methods

Genetic stocks

The wheat cultivar Chinese Spring (CS, barley chromosome arm recipient), the barley cultivar Betzes (BZ, barley chromosome arm donor), and 11 different CS–BZ ditelosomic addition lines carrying the short or long chromosome arms from barley chromosomes 1(7H), 2(2H), 3(3H), 4(4H), and 7(5H) and the short arm from chromosome 5(1H) were used (Islam 1983; Islam and Shepherd 1990). The ditelosomic addition line for the long arm of barley chromosome 5(1H) was not included because it is sterile. The long and short arms of Betzes chromosome 6(6H) were used previously to validate transcript-based mapping of genes to chromosome arms (Cho et al. 2006). The identity of the genetic stocks was validated using at least 2 barley simple sequence repeat markers for each chromosome arm. For simplicity, barley chromosome arms are referred to as 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL in the remainder of this paper.

Plant growth and experimental design

Seeds of both parents and the ditelosomic addition lines were cold-treated at 4 °C in the dark for 96 h in sterile Petri dishes on 3 layers of filter paper wetted with 8.0 mL of distilled water. Following the cold treatment, the Petri dishes were placed in a growth chamber in a randomized complete block design with 3 replications, where they were kept at 22 °C with a 16 h light : 8 h dark regime at a light intensity of 360 $\mu E \cdot m^{-1} \cdot s^{-1}$ (1 μE = 1 μmol photons). After both 24 and 48 h, 1.0 mL of sterile water was added to each Petri dish to maintain moisture levels. After 72 h, 11 to 13 seedlings per replication were sampled for each genotype by detaching the seedlings from the seeds. The seedling tissue was immediately frozen in liquid nitrogen. The plants for the 2HL addition lines were grown in a separate experiment with their own CS and BZ controls and analyzed independently. The experimental conditions applied to the 2HL addition lines were the same as those applied to the rest of the lines.

RNA extraction, labeling, and hybridization

Seedlings sampled from each replication were ground to a powder in liquid nitrogen. Total RNA was extracted from 1.0 g of powdered tissue of each replicate using the Trizol™ (Invitrogen, Carlsbad, California) protocol. RNA samples were treated with RNase-free DNase and purified on RNeasy™ columns (QIAGEN Sciences, Germantown, Maryland). An Agilent 2100 bioanalyzer (Agilent, Palo Alto, California) was used to check the total RNA quality. Preparation of cRNA probes was conducted with 10.0 μg of total RNA using the GeneChip® One-Cycle Target Labeling and Control Reagents package (Affymetrix, Santa Clara, California) following the manufacturer's recommendations. Probes were hybridized to the Barley1 GeneChip (Affymetrix), which is composed of 22 792 probe sets representing 21 439 barley genes (excluding paralogs or different alleles of genes) (Close et al. 2004).

For each GeneChip hybridization reaction, 20.0 μg of labeled cRNA was used. Barley1 GeneChip hybridizations and acquisition of expression data were conducted at the Biomedical Image Processing Facility at the University of Minnesota following standard Affymetrix procedures (http://www.bipl.ahc.umn.edu/affymetrix.html).

GeneChip data analysis

Expressionist® version EPro1.0.32 (Genedata AG, San Francisco, California) was used for GeneChip data analysis. The Refiner Array module of Expressionist was used for GeneChip quality assessment and normalization of raw data. In Refiner Array, GeneChip and control gene statistics were applied using default settings to check masked and outlier areas (threshold level: 0.2%), corner noise (threshold level: 0.015), and 3′/5′ ratio (threshold level: 2.5). Following these checks, data classified as good or medium quality were condensed using quantile normalization based on perfect-matched values using robust multi-chip array (Irizarry et al. 2003) analysis. The normalized data were stored in CoBi, Expressionist's relational database module, and used for statistical analysis in the Analyst module of Expressionist. The Analyst module was used for statistical analysis and visualization of normalized microarray data.

To identify barley-specific transcripts expressed in the wheat–barley addition lines, we used a 2-step strategy. The first step was to identify those transcripts that exhibited significantly higher abundance in BZ relative to CS. Transcripts showing significantly different expression signals between BZ and CS were identified based on a *t* test ($p \leq 0.001$). *K*-means clustering was then used to select those differentially expressed transcripts that accumulated at higher levels in BZ than in CS. A presence/absence test using Analyst (detection $p \leq 0.001$) was then executed to determine which of these transcripts were present in BZ and absent in CS. A transcript was classified as present if its hybridization signal was detected in at least 2 of 3 GeneChip replications and it was classified as absent if it was detected in one or none of the replications. The second step was to detect barley-specific transcripts in the ditelosomic addition lines. Transcripts classified as present in BZ and absent in CS were examined for their expression pattern in the ditelosomic addition lines. *t* tests ($p \leq 0.001$) were used to compare transcript abundance between the ditelosomic lines and CS. Barley transcripts present at significantly higher levels in a ditelosomic addition line than in CS, as determined by *K*-ordered groups analysis, were assigned to the barley chromosome arm present in the particular ditelosomic line being tested. There are probe sets detecting paralogs or different alleles of genes on the Barley1 GeneChip (Close et al. 2004) that we refer to as "redundant". These redundant probe sets were removed from the chromosome arm gene lists if all copies of the particular redundant probe set had the same unigene sequence and were mapped onto the same chromosome arm or had *E* values equal to those of common rice or wheat BLAST hits. The transcript abundance comparisons between ditelosomic lines and CS were repeated at $p \leq 0.01$ to produce a medium-confidence gene list that provides a larger set of genes for mapping applications in barley.

All data from the Affymetrix Scanner have been deposited in the Plant Gene Expression database (http://www.plexdb.org) as CEL, DAT, CHP, and EXP files. The accession number for the experiment is BB55. The data for 2HL and CS and BZ controls as well as data for disomic chromosome addition lines from Cho et al. (2006) can be retrieved from experiment BB8.

In silico mapping in wheat and rice

Genomic locations of wheat and rice sequences homologous to barley genes assigned to chromosome arms were identified using BLAST searches based on the consensus sequences of probe sets (i.e., unigenes) obtained from HarvEST:Barley version 1.49. Rice synteny was based on BLASTX searches using HarvEST:Barley version 1.49 on 30 June 2006 and rice annotations from the TIGR Rice Genome Annotation Database and Resource version 4. Wheat synteny was based on BLASTN searches against mapped wheat expressed sequence tags (ESTs) using the GrainGenes Web site (http://wheat.pw.usda.gov/GG2/blast.shtml) on 7 July 2006. The best wheat and (or) rice BLAST hits were reported and a similarity was declared significant if the *E* value was equal to or less than e–10.

Single feature polymorphism detection

Single feature polymorphisms (SFPs) were detected based on hybridization of RNA from BZ, CS, and the ditelosomic addition lines to the same probe on the GeneChip, applying the basic algorithm described in Cui et al. (2005) with the modified probe-level statistical method described in Rostoks et al. (2005). Briefly, perfect-match probe intensity extraction and robust multi-chip array normalization of expression values were performed on individual probes from each probe set using the Bioconductor affy package (http://bioconductor.org) with data extracted from the CEL files. Then, the Bioconductor siggenes package was used to determine the significance of the affinity differences between 2 genotypes. A false discovery rate of 10% was estimated using the significance analysis of microarrays of the siggenes package. SFP detection was conducted using all 3 GeneChip replicates of BZ, CS, and the ditelosomic addition lines. SFPs identified between BZ and CS and not detected between BZ and a given ditelosomic addition line were assigned to genes residing on the chromosome arm of the particular addition line.

Results and discussion

Barley gene expression in wheat–barley ditelosomic addition lines

Transcript accumulation in seedlings of *T. aestivum* 'Chinese Spring' (CS), *H. vulgare* 'Betzes' (BZ), and 11 CS–BZ ditelosomic addition lines was examined using the Barley1 GeneChip. The correlation of expression signals between replications of a single genotype was calculated across all 22 792 probe sets and a high correlation (approximately 0.99) was found for each comparison. We conducted the GeneChip analysis at 2 stringencies, $p \leq 0.001$ and $p \leq 0.01$. Only the high-

Table 1. Number of non-redundant single-copy and multiple-copy barley transcripts assigned to barley chromosome arms at $p \leq 0.001$ and $p \leq 0.01$, and percentage of transcripts showing single feature polymorphisms (SFPs).

		$p \leq 0.001$ level				$p \leq 0.01$ level	
Ditelosomic line	Estimated physical length (μm)*	No. of barley transcripts	No. of single-copy transcripts	No. of multiple-copy transcripts	Percentage of transcripts with SFPs	No. of barley transcripts	Percentage of transcripts with SFPs
1HS	6.0	24	23	1	75.0	100	35.0
2HS	9.9	110	104	6	72.7	228	64.0
2HL	10.8	162	159	3	78.4	397	56.7
3HS	9.3	120	116	4	72.5	196	60.0
3HL	11.7	197	192	5	78.2	408	63.0
4HS	9.5	132	124	8	71.4	240	57.5
4HL	11.6	165	161	4	66.7	286	56.6
5HS	8.3	3	2	1	0.0	53	0.0
5HL	11.6	94	92	2	76.6	327	61.2
7HS (beta)	9.7	119	117	2	64.7	255	49.8
7HL (alpha)	9.8	131	129	2	78.6	230	67.4
Total	—	1257	1219	38	73.5†	2720	57.1†

Note: Single-copy or multiple-copy transcript classification was based on detection of a particular transcript on single or multiple barley chromosome arms added to wheat. Lists of transcripts detected at the $p \leq 0.001$ and $p \leq 0.01$ levels are provided in the supplementary data (Tables S1 and S2, respectively).

*From Brown et al. (1999).

†Average values excluding 5HS.

Table 2. Syntenic relationships between the mapped barley genes and homologous sequences on wheat group chromosome arms based on the best BLAST hits.

	Wheat group (A, B, and D genomes)																	
Barley ditelosomic line	1S	1L	2S	2L	3S	3L	4S	4L	5S	5L	6S	6L	7S	7L	Mapped barley transcripts	Barley transcripts with wheat hits	Barley transcripts with syntenic wheat hits	Percent syntenic wheat hits
1HS	**5**	1	2	1	0	0	0	1	0	1	3	0	1	1	24	13	5	38.5
2HS	1	2	**29**	2	2	6	0	1	5	3	0	2	3	2	110	45	29	64.4
2HL	4	6	10	**55**	1	4	1	8	2	4	10	5	2	2	162	77	55	71.4
3HS	0	3	1	2	**49**	3	0	3	2	3	2	2	5	2	120	62	49	79.0
3HL	1	12	1	1	10	**58**	1	2	3	5	1	0	4	1	197	83	58	69.9
4HS	0	6	3	4	1	5	**27**	30	3	6	1	2	2	4	132	57	27	47.4
4HL	3	11	3	8	1	2	20	**39**	2	13	6	3	5	3	165	70	39	55.7
5HS	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	1	0	0.0
5HL	2	6	2	5	0	2	1	7	4	**23**	3	2	6	2	94	42	23	54.8
7HS	4	3	4	2	3	3	1	11	0	6	5	2	**33**	3	119	55	33	60.0
7HL	1	2	3	5	3	5	1	7	0	4	5	2	7	**37**	131	58	37	63.8
Total															1257*	563*	355*	63.2†

Note: Hits to syntenous wheat chromosome groups are in boldface (short and long arms are designated S and L, respectively). The details of the BLAST hit results (cut-off *E* value ≤ e–10) are shown in the supplementary data (Table S3). The percent syntenic wheat hits was calculated based on the number of barley genes that had wheat BLAST hits.

*Total number of gene transcripts.

†Average percent syntenic wheat hits excluding 5HS.

Table 3. Syntenic relationship between the mapped barley genes and homologous sequences on the rice genome based on the best BLAST

	Rice chromosome arm																		
Barley ditelosomic line	1S	1L	2S	2L	3S	3L	4S	4L	5S	5L	6S	6L	7S	7L	8S	8L	9S	9L	10S
1HS	1	0	1	0	2	1	0	1	**8**	0	0	0	0	2	2	0	0	0	0
2HS	2	2	2	3	4	0	**9**	1	3	0	1	1	5	**55**	1	1	0	2	3
2HL	2	5	1	7	3	2	0	**85**	0	5	2	0	**18**	1	2	3	0	5	0
3HS	**71**	4	2	3	0	3	1	3	1	1	0	3	1	1	1	2	0	2	0
3HL	6	**142**	6	3	0	2	0	2	2	7	1	1	2	3	4	2	0	1	0
4HS	1	3	5	2	6	**38**	0	2	0	2	0	0	0	1	0	2	0	**9**	0
4HL	2	2	1	0	**112**	2	0	3	1	4	1	1	1	2	0	1	1	0	2
5HS	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
5HL	1	1	3	1	3	**27**	0	1	0	2	0	0	5	3	1	3	1	**19**	1
7HS	4	0	2	2	1	3	1	4	0	1	**40**	2	1	2	5	**31**	0	3	1
7HL	5	2	4	3	7	2	1	3	1	0	3	**43**	1	3	**21**	7	1	1	1
Total																			

Note: Hits to syntenous rice chromosome arms are in boldface (short and long arms are designated S and L, respectively) and chromosome arm and on non-syntenic rice chromosomes are underlined. The details of BLAST hit results (cut-off *E* value ≤ e–10) are shown in the supplementary data (Table S3).

*Total number of gene transcripts.

†Average percent syntenic rice hits excluding 5HS.

confidence results obtained at $p \leq 0.001$ are discussed in the text. The moderate-confidence gene lists obtained at $p \leq 0.01$ are presented in Table S2[2].

Of the 22 792 probe sets on the Barley1 GeneChip, 6209 showed significant differences in transcript abundance between BZ and CS ($p \leq 0.001$). Based on a presence/absence test ($p \leq 0.001$), 2600 probe sets were classified as present in BZ and absent in CS. To detect barley-specific transcripts in the ditelosomic addition lines, we compared the transcript abundance of these 2600 probe sets in the ditelosomic addition lines and CS (*t* tests, $p \leq 0.001$). This resulted in identification of 24, 110, 162, 120, 197, 132, 165, 3, 94, 119, and 131 transcripts (1257 in total, not counting 14 redundant probe sets) showing higher detection signals in the addition lines carrying barley chromosome arms 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL, respectively (Table 1).

We then examined how many transcripts were detected in just one ditelosomic addition line. We identified 23, 104, 159, 116, 192, 124, 161, 2, 92, 117, and 129 transcripts (1219 in total) specific to the ditelosomic addition lines carrying barley chromosome arms 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL, respectively. These 1219 transcripts (96.4% of the 1257 mapped genes) were termed "single copy". The remaining 38 transcripts (3.6%) identified by 21 unique probe sets and detected in 2 or more ditelosomic addition lines were termed "multiple copy" (Table 1). These likely represent dispersed members of gene families being detected on different chromosome arms. We observed multiple-copy genes on both arms of the same chromosome in only one case: the same transcript was detected on both the short and long arms of barley chromosome 4H.

The expression pattern analysis for ditelosomic line 5HS revealed that only 3 barley gene transcripts were detected (Table 1). This low number of transcripts probably can be attributed to meiotic instability of the barley 5HS ditelosomic line. The meiotic transmission rate of the barley chromosome arm 5HS in wheat was only 11.8%, falling well outside of the meiotic transmission range (76.0%–96.6%) for all the other ditelosomic lines in this study (Islam 1983). The results from this study reveal the impact of meiotic instability and chromosome transmission variability on gene expression levels and suggest that caution should be exercised when using 5HS ditelosomic lines in future mapping projects or studies attempting to quantify levels of alien gene expression.

When the estimated physical size of chromosome arms in barley (Brown et al. 1999) is compared with the number of transcripts detected on each chromosome arm, the data are in broad agreement. It is evident that 1HS is the shortest arm (6.0 μm) and contains the fewest mapped genes (24, Table 1). The other short chromosome arms in barley are similar in size (9.3–9.9 μm, excluding 5HS), and we detected a similar number of transcripts on these arms (110–132, Table 1). A similar observation is valid for the long chromosome arms of barley. The size range of the long chromosome arms is 9.8 to 11.7 μm (Brown et al. 1999) and the corresponding range of gene numbers is 131 to 197 (Table 1), excluding 5HL, which had a comparatively lower number of mapped genes (94). In a previous study (Cho et al. 2006), a similar relationship was found between the size of chromosomes 2H, 3H, 4H, 5H, and 7H and the number of genes they carried. Our results extend these observations to the chromosome-arm level.

Validation of transcript-based physical mapping of barley genes

Comparison with chromosome-based physical mapping

To validate our mapping results using the ditelosomic lines, we first checked whether the expression patterns of

[2] Supplementary data for this article are available on the journal Web site (http://genome.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5197. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

hits.

10L	11S	11L	12S	12L	Mapped barley transcripts	Barley transcripts with rice hits	Barley transcripts with syntenic rice hits	Percent syntenic rice hits	Syntenic rice chromosome arm(s)
1	1	0	1	1	24	22	8	36.4	5S
4	0	4	3	1	110	107	64	59.8	4S, 7L
6	1	2	3	2	162	155	103	66.5	4L, 7S
4	0	8	1	2	120	114	71	62.3	1S
2	2	0	0	2	197	190	142	74.7	1L
1	**26**	**13**	**14**	4	132	128	91	70.3	3L, 11S, 11L, 12S
5	4	1	1	1	165	148	112	75.7	3S
0	0	1	0	0	3	3	0	0.0	—
1	5	1	3	1	94	83	46	55.4	3L, 9L
1	1	2	0	2	119	109	71	65.1	6S, 8L
6	4	1	3	0	131	123	64	58.5	6L, 8S
					1257*	1182*	772*	65.5†	—

centromere locations in rice are based on the information published by the International Rice Genome Sequencing Project (2005). High hits (5 to 9)

genes mapped using ditelosomic lines were consistent with the expression patterns of the same genes mapped by Cho et al. (2006) using wheat–barley disomic lines for chromosomes 2H, 3H, 4H, 5H, 6H, and 7H. Cho et al. (2006) reported the detection of 1787 barley gene transcripts across the 6 lines evaluated. We processed the raw data from CEL data files for the disomic lines from our previous study, following the same normalization and statistical procedures performed in the current study. One hundred and eight, 160, 115, 186, 129, 163, 1, 91, 111, and 128 transcripts (1192 in total) that we mapped to chromosome arms 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL, respectively, showed higher abundance in the corresponding disomic line compared with CS. Levels of all of these transcripts were also higher in BZ compared with CS. Of the 1257 genes mapped in the present study, 1192 (94.8%) were expressed similarly in the disomic lines. Thus, the results from the 2 studies are consistent.

We also examined the overlap between our physical mapping results from ditelosomic lines and the results for all but 194 of the 1787 genes physically mapped to barley chromosomes by Cho et al. (2006). We used the 1593 gene transcripts physically mapped to chromosomes 2H, 3H, 4H, 5H, and 7H and compared them with the 1234 genes mapped to the same chromosomes in this study. The 23 single-copy gene transcripts assigned to chromosome arm 1HS were excluded from the comparison, since chromosome 1H was not included in the study of Cho et al. (2006). We found that 668 transcripts (54%) overlapped between the 2 studies, indicating that 566 new transcripts were identified and assigned to barley chromosome arms. For chromosomes 2H, 3H, 4H, 5H, and 7H, a larger number of non-redundant transcripts (1593) was identified in the disomic addition line analysis (Cho et al. 2006) than in this study with the ditelosomic lines (1234), and the number of commonly mapped transcripts in the disomic and ditelosomic addition line studies was relatively low (668). Thus, although the expression levels from the disomic and ditelosomic addition lines for the transcripts mapped on chromosome arms were highly comparable, as demonstrated above, the independent results from the disomic and ditelosomic addition line experiments did not completely overlap. This discrepancy highlights the impact of the statistical data analysis and data normalization methodologies on the results. In addition, the lower number of genes detected in this study may be attributed in part to the low number of genes detected on the short arm of chromosome 5H.

Because not all 1593 gene transcripts in the Cho et al. (2006) study were mapped in the present study, we wanted to determine what portion of the 1593 genes determined using whole-chromosome addition lines could be assigned to chromosome arms. We used *K*-means clustering to examine the expression patterns of these 1593 transcripts in the ditelosomic addition lines, and we found that 1538 (97%) were positioned on a chromosome arm consistent with their previous chromosome designation. We partitioned 153, 227, 112, 171, 119, 150, 16, 243, 189, and 172 transcripts to chromosome arms 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL, respectively. The 5HS ditelosomic line had only a few genes mapped, indicating that some of the unmapped genes from the study of Cho et al. (2006) could be on the short arm of chromosome 5H.

Single feature polymorphism (SFP)-based barley gene locations

We examined SFPs between BZ, CS, and the wheat–barley ditelosomic addition lines for all 22 792 probe sets on the Barley1 GeneChip. High false discovery rates are associated with SFP detection (Cui et al. 2005; Rostoks et al. 2005). Thus, we chose to use SFPs only to validate our transcript-based physical mapping. SFPs that were identified as different between BZ and CS but not between BZ and a given addition line were assigned to the chromosome in the particular addition line. In total, 924 (73.5%) of the 1 257 genes that were mapped based on transcript levels exhibited SFPs that permitted assignment to the same chromosome arm determined by transcript-based mapping (Table 1). The number of SFPs detected by an individual probe set varied between 1 and 9, with the majority of probe sets displaying between 1 and 3 SFPs. Even at $p \leq 0.01$, the percentage of

Table 4. Genomic positions of homologous rice loci on chromosome 9 for 8 barley genes mapping to barley chromosome arm 4HS.

Barley probe set	Rice locus	Rice *E* value	Position on rice chromosome 9 (bp)	Rice annotation
Contig2943_s_at	LOC_Os09g39380.1	0	22 644 422 – 22 648 010	Monodehydroascorbate reductase, putatively expressed
Contig7625_at	LOC_Os09g39390.1	2.00e–28	22 648 606 – 22 651 602	Oxidoreductase aldo/keto reductase family protein, expressed
Contig8253_at	LOC_Os09g39400.1	5.00e–52	22 653 383 – 22 656 565	Histidine-containing phosphotransfer protein 1, putatively expressed
Contig7698_at	LOC_Os09g39462.2	6.00e–88	22 682 047 – 22 686 254	Expressed protein
Contig1040_at	LOC_Os09g39500.1	2.00e–69	22 692 186 – 22 694 404	Ubiquitin fusion protein, putatively expressed
Contig4455_at	LOC_Os09g39550.1	1.00e–44	22 702 884 – 22 705 225	Expressed protein
Contig4456_s_at	LOC_Os09g39550.1	1.00e–44	22 702 884 – 22 705 225	Expressed protein
Contig8382_at	LOC_Os09g39780.2	6.00e–84	22 809 423 – 22 810 520	Peptidyl-prolyl *cis-trans* isomerase, putatively expressed

genes showing SFPs was above 50% for most of the chromosome arms (Table 1). These results also provide evidence that our transcript-based mapping is a robust approach to assign genes to chromosome arms.

In silico *comparative mapping of the barley transcriptome*

To further validate the transcript mapping results, we studied the syntenic relationships of the barley gene transcripts detected in the ditelosomic lines with homologous genes in rice and wheat. This approach is possible owing to highly documented synteny between barley, wheat, and rice genomes (e.g., Van Deynze et al. 1995; Gale and Devos 1998; Sorrells et al. 2003; La Rota and Sorrells 2004; Cho et al. 2006).

Using the 1257 gene transcripts assigned to chromosome arms and the barley unigene sequences derived from the Barley1 GeneChip probe sets, we examined the syntenic relationships with both wheat EST maps and the rice genome sequence. Of the 1257 mapped barley genes, 563 exhibited significant homology (E value ≤ e–10) to at least 1 wheat EST, and 355 of these (63.2%) were on a chromosome arm syntenic to the corresponding wheat group (Table 2). For the majority of the ditelosomic lines, most of the wheat BLAST hits were to genes on the corresponding arm of the homoeologous wheat group (Table 2). Similarly, 1182 barley genes exhibited homology to at least 1 rice genomic sequence, and 772 of these (65.5%) had hits on the corresponding rice chromosome arm(s) (Table 3). The percentage of homologous sequences on syntenous chromosome arms ranged from 38.5% (1HS) to 79.0% (3HS) for wheat, and from 36.4% (1HS) to 75.7% (4HL) for rice (Tables 2 and 3). We observed similar degrees of synteny to wheat (63.2%) and rice (65.5%) even though barley is much more closely related to wheat than to rice. This might be due to the use of EST sequences for wheat homology that have limited genomic coverage compared with the coverage available for rice, which was represented by whole genome sequence data. The average genomic synteny levels we observed are comparable to those observed in previous studies of the syntenic and evolutionary relationships between barley, wheat, and rice (Gale and Devos 1998; Sorrells et al. 2003; La Rota and Sorrells 2004; Cho et al. 2006) and validate our mapping results by locating high levels of homology on the syntenic wheat and rice chromosome arms.

A small barley–rice genomic rearrangement disrupts synteny for multiple genes

The barley genes physically mapped to chromosome arms exhibited many BLAST hits to rice genes on non-syntenic rice chromosomes (Table 3). To determine whether small ancestral rearrangements involving multiple tightly linked genes were contributing to this observation, we examined the genome locations of rice genes in those cases where 5 or more barley genes assigned to a given chromosome arm had best BLAST hits to the same non-syntenic rice chromosome. Sixteen such gene sets were examined (Table 3). We identified 1 region on barley chromosome arm 4HS that contained 8 barley genes homologous to 7 different rice loci in the telomeric region of the long arm of rice chromosome 9. Two of these barley probe sets (Contig4455_at and Contig4456_s_at) were homologous to the same rice lo-

cus (LOC_Os09g39550.1) and thus redundant. The physical distance encompassing the 7 different rice loci (from LOC_Os09g39380.1 to LOC_Os09g39780.2) is approximately 166 kb (Table 4). This suggests that the loss of synteny for this group of genes is due to a single rearrangement event and that the 7 barley genes in question on the short arm of barley chromosome 4H are likely to be in close physical proximity and not dispersed across this chromosome arm. This result demonstrates the use of expression profiling of aneuploid alien addition lines as a genome analysis tool to identify distinct chromosome rearrangement events between related species.

Applications of chromosome arm physical mapping

The results from the analyses presented in this study can be utilized for a variety of mapping studies. Since we were able to identify homologous sequences for the majority of the genes mapped to barley chromosome arms in both wheat and rice, syntenic PCR-based markers can be easily developed in each of these crop species or transferred between species to facilitate discovery and mapping of candidate genes across species. Syntenic relationships between barley and rice chromosomes have proven to be a useful resource for chromosome walking in barley. For example, high levels of synteny have been observed between barley chromosome 3H and rice chromosome 1, and this synteny has been exploited in map-based cloning (Kilian et al. 1997; Brueggeman et al. 2002; Mammadov et al. 2005). Our study also provides a large number of markers that can be exploited conveniently for gene mapping and cloning purposes in other genomic regions of barley where rice synteny has not been established. We propose that the high-confidence gene lists produced in this study be used as starting points for fine mapping studies and the moderate-confidence gene lists be utilized once the possible markers developed based on the first list are exhausted. Although the moderate-confidence gene lists would naturally include more false positives, the decreasing costs associated with marker technologies still make these markers feasible choices for exploration.

In summary, we have demonstrated that wheat–barley addition lines coupled with the Barley1 GeneChip can be used to answer fundamental genetic questions (e.g., alien gene expression, genomic rearrangements) while producing results for applied molecular crop improvement (PCR-based markers for mapping and map-based cloning).

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