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ORIGINAL PAPER

Genetic and physical mapping of a high recombination region on chromosome 7H(1) in barley

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Abstract Approaches utilizing microlinearity between related species allow for the identification of syntenous regions and orthologous genes. Within the barley Chromosome 7H(1) is a region of high recombination flanked by molecular markers cMWG703 and MWG836. We present the constructed physical contigs linked to molecular markers across this region using bacterial artificial chromosomes (BAC) from the cultivar Morex. Barley expressed sequence tags (EST), identified by homology to rice chromosome 6 between the rice molecular markers C425A and S1434, corresponded to the barley syntenous region of Chromosome 7H(1) Bins 2–5 between molecular markers cMWG703-MWG836. Two hundred and thirteen ESTs were genetically mapped yielding 267 loci of which 101 were within the target high recombination region while 166 loci mapped elsewhere. The 101 loci were joined by 43 other genetic markers resulting in a highly saturated genetic map. In order to develop a physical map of the region, ESTs and all other molecular markers were used to identify Morex BAC clones. Seventy-four BAC contigs were formed containing 2–102 clones each with an average of 19 and a median of 13 BAC clones per contig. Comparison of the BAC contigs, generated here, with the Barley Physical Mapping Database contigs, resulted in additional overlaps and a reduction of the contig number to 56. Within cMWG703-MWG836 are 24 agriculturally important traits including the seedling spot blotch resistance locus, *Rcs5*. Genetic and physical analysis of this region and comparison to rice indicated an inversion distal of the *Rcs5* locus. Three BAC clone contigs spanning the *Rcs5* locus were identified.

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Introduction

Cultivated barley has evolved, with other cultivated cereals, from common ancestry over millions of years (Devos 2005). Rice, wheat, maize, rye, and barley all share various degrees of microlinearity at the genetic level (Moore et al. 1995; Devos and Gale 1997). By using related genomes as templates, the genomes of other cereals can be recreated (Moore et al. 1995). In particular, barley chromosome 7H(1) can be reconstructed by using Rice Chromosome 6 (Saghai Maroof et al. 1996) as well as wheat chromosome 7A (Dubcovsky et al. 1996) as templates. Since many of these species co-evolved with the predecessors of today's plant pathogens and were also once targets of pathogen attack, it is likely that related grass species share defense related genes for common pathogens. Although barley and rice do not share an abundance of diseases, pathogens that

once infected a common ancestor of barley and rice have co-evolved with current crop species and so may share similar Avirulence (*Avr*) or virulence effectors as well as the corresponding crop hosts sharing similar Resistance (*R*) genes.

Barley Chromosome 7H(1)S between markers cMWG703 and MWG836 was previously identified as a very High Recombination Region (Kunzel et al. 2000) and represents a region that is likely gene rich and contains agronomically important traits. A very High Recombination Region in barley was defined by Kunzel et al. (2000) as a region of <1.0 Mb/cM and a High Recombination Region as a region of 1.0–4.4 Mb/cM. We will use the abbreviation HRR to refer to these regions. We attempted to construct a physical map across the region using markers derived from wheat and the sequenced rice genome.

The syntenous rice and wheat regions are represented by rice chromosome 6 markers C425A-S1434 (Saghai Maroof et al. 1996) and wheat chromosome 7A markers XWG834-XABC58 (Dubcovsky et al. 1996). Using the recently sequenced rice genome as a template we identified homologous Expressed Sequence Tags (EST) from barley and wheat using rice P1 derived Artificial Chromosome and Bacterial Artificial Chromosome (BAC) clone sequences between markers C425A and S1434 available from the Rice Genome Research Program database (http://rgp.dna.affrc.go.jp/giot/INE.html). The clone sequences were used to identify Triticeae ESTs that correspond to the short arm of Barley Chromosome 7H(1). Although barley, rice, and wheat do not share chromosome number and each has evolved to contain unique genetic material, all share regions of synteny that can be exploited to identify homologous genes controlling important functions (Dunford et al. 1995; Kilian et al. 1995; Kilian et al. 1997; Han et al. 1998, 1999; Smilde et al. 2001; Collins et al. 2003; Yan et al. 2003; Stein et al. 2005; Turner et al. 2005). Within Bins 2–5 of barley chromosome 7H(1) (Kleinhofs and Graner 2001) there are 17 agronomic, 5 disease resistance, 1 crossability factor, and a malting quality trait (Table 1). The syntenous region in rice contains 121 agronomic, 11 disease, and 11 abiotic stress QTLs (Supplementary Material 3). By comparing the related genomes we can identify genes that control conserved traits or identify genes controlling unique traits. Of interest to our study is the seedling spot blotch resistance gene (*Rcs5*) found within this region in barley.

The spot blotch pathogen, an Ascomycete fungus, *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur (Anamorph *Bipolaris sorokiniana*) is a globally important disease on barley causing marked reduction in yield and quality of grain (Kiesling 1985; Nutter et al. 1985; Mathre 1997; Kumar et al. 2002). The spot blotch disease is characterized by a central necrotic lesion that can be round or oblong and may also be accompanied by a chlorotic halo, which is likely a response to toxins produced by the fungus. A nine-point resistance scale created by Fetch and Steffenson (1999) identifies the following phenotypes: resistant class (ratings 1–3), susceptible class (ratings 6–9), and intermediate class (ratings of 4 or 5). Seedling resistance to the pathotype 1 of *C. sativus* was mapped to the short arm of Chromosome 7H(1) within the HRR (Steffenson et al. 1996).

Cochliobolus sativus is a hemibiotrophic pathogen, which means that it has two life stages. The first stage is a biotrophic phase where it initiates infection by invading a single epidermal cell of the leaf facilitated by appressoria-like structures in order to gain nourishment from living cells (Kumar et al. 2002). The second stage is a necrotrophic phase that results in plant cell death. The dead plant cells can then be used by the fungus for nourishment. Resistance to spot blotch was identified in the North Dakota barley breeding line NDB112 which is the presumed source of resistance for the barley malting variety Morex used in this study (Rasmusson and Wilcoxson 1979; Wilcoxson et al. 1990). This resistance has remained effective for over 40 years and is considered durable (Steffenson et al. 1996).

Similar hemibiotrophic pathogens like the rice blast fungus, *Magnaporthe grisea*, or the oat leaf blight fungus, *Cochliobolus victoriae* are avirulent on hosts that harbor the corresponding resistance genes. The rice blast resistance genes, *Pib* (Wang et al. 1999; Bryan et al. 2000), *Pi-Ta* (Bryan et al. 2000), and *Pi9* (Qu et al. 2006) encode NBS-LRR-like proteins while *Pi-d2* (Chen et al. 2006) encodes a serine-threonine protein kinase (S/TPK)-like protein. The *Puccinia coronata* oat crown rust resistance gene, *Pc-2* confers resistance to crown rust and sensitivity to the victorin toxin (Lorang et al. 2007). The null or non-functional allele is attributed to victorin insensitivity presumably by the inability of the *Pc-2* NBS-LRR gene to activate a hypersensitive response. Collectively, NBS-LRR and S/TPK genes represent two important classes of resistance genes cloned and characterized to date (Martin et al. 2003).

Seedling resistance to spot blotch was mapped in the Steptoe × Morex doubled haploid (DH) population to the short arm of chromosome 7H(1) and designated *Rcs5* (Steffenson et al. 1996). *Rcs5* is flanked by the Bin markers ABC151A and ABG380, a region that corresponds to a syntenous region of the sequenced rice genome on rice Chromosome 6 (Kleinhofs et al. 1993; Saghai Maroof et al. 1996).

Previous efforts to clone genes by utilizing synteny between grasses have met with limited success. The rice orthologs of the wheat *Vrn1* (Yan et al. 2003), barley *ror2* (Collins et al. 2003), *rym4/5* (Kanyuka et al. 2005; Stein et al. 2005), and *Ppd-H1* (Turner et al. 2005) are all present in the syntenous positions of the rice genome. However, the orthologs of maize *Adh1* (Tarchini et al. 2000), *Triticeae*

Table 1 Barley traits and QTL mapping within the Chr 7H(1)S Bins 2–5 region

Phenotype	Trait	BIN[a]	Marker[b]	Marker[c] Interval	Population[d]	Citation[e]
Agronomic						
Yield and yield components	Plant grain weight	3	ABC151A	WG789-WG178	Blenheim/Kym	Bezant et al. 1997
Phenology	Head date	3	ABC151A	CDO475	Gobern./CMB	Zhu et al. 1999
Yield and yield components	Grain yield	1–3	ABG320	MWG530-MWG635	Igri/Danilo	Backes et al. 1995
Phenology	Head date	2–4	ABC151A	MWG635-MWG527	Igri/Danilo	Backes et al. 1995
Yield and yield components	Grain yield	3–4	ABC151A	WG789A-ABG380	Steptoe/Morex	Hayes et al. 1993
Phenology	Head date	3–4	ABG380	dRcs5-ABC158	Harrington/Morex	Marquez-Cedillo et al. 2001
Seed characters	Kernel length	4–5	ABG380	MWG527-MWG104	Igri/Danilo	Backes et al. 1995
Seed characters	Kernel shape	4–5	ABG380	MWG527-MWG104	Igri/Danilo	Backes et al. 1995
Yield and yield components	Grain yield	4–7	ABC255	ABG380-VAtp57A	Harrington/TR306	Tinker et al. 1996
Phenology	Head date	4–7	ABG701	ABG380-VAtp57A	Harrington/TR306	Tinker et al. 1996
Plant architecture and morphology	Height	1–2	ABG320	ABA301-WG789A	Steptoe/Morex	Hayes et al. 1993
Phenology	Head date	1–4	ABC151A	dRpg1-ABG380	Harrington/TR306	Tinker et al. 1996
Plant architecture and morphology	Height	1–4	ABG320	dRpg1-ABG380	Harrington/TR306	Tinker et al. 1996
Phenology	Head date	2–3	ABG151A	MWG089	Igri/Triumph	Laurie et al. 1995
Phenology	Head date	2–4	KsuA1A	Glx-KsuA1A	Steptoe/Morex	Hayes et al. 1993
Phenology	Head date	4–5	KsuA1A	ABC158-Brz	Dicktoo/Morex	Pan et al. 1994
Phenology	Head date 8h v	4–5	ksuA1	ABC158-Brz	Dicktoo/Morex	Pan et al. 1994
Biotic						
Disease resistance	Fusarium HB	2	ABG320	Wx	Gobern./CMB	Zhu et al. 1999
Disease resistance	Scald	2–4	ABC151A	MWG635-MWG527	Igri/Danilo	Backes et al. 1995
Disease resistance	Fusarium HB	1–2	ABC151A	MWG530-MWG564	Chevron/M69	de la Pena et al. 1999
Disease resistance	Net blotch	1–2	ABG320	BCD129/Glx	Steptoe/Morex	Steffenson et al. 1996
Disease resistance	Spot blotch (*Rcs5*)	3–4	ABG380	WG789A-ABC158	Steptoe/Morex	Steffenson et al. 1996
Others						
	Crossability w/wheat	3	ABC151A	WG789A-MWG089	Steptoe/Morex	Taketa et al. 1998
QTL						
Malt quality	Water sensitivity	2–5	NA	E37m39a	Blenheim/E224/3	Powell et al. 1997

[a] Bin location with reference to the Steptoe × Morex Bin map

[b] Closest mapped marker

[c] Marker interval

[d] Population used to map

[e] References to the original publication. See also (http://barleyworld.org/northamericanbarley/QTLsum9150x.htm)

Sh2 (Li and Gill 2002), and barley *Vrs1* (Pourkheirandish et al. 2007) lie in non-syntenous rice genomic regions and may represent translocation events. Also, the barley *Rpg1* (Brueggeman et al. 2002), *Rpg5* (Brueggeman et al. 2008), and wheat *Ph1* (Griffiths et al. 2006) genes have no rice ortholog in their syntenous position and so represent novel regions containing unique genetic material. Regardless of which scenario *Rcs5* will fall into, the rice genome represents a rich source of molecular markers that can facilitate construction of high-resolution genetic and physical maps across the region of interest essential for positional cloning.

The objective of this study was to develop a saturated genetic map of barley Chromosome 7H(1) bins 2 to 5 with special emphasis on bins 3 and 4. A saturated genetic map would facilitate development of a physical map to aid in the isolation of *Rcs5* and confirm or refute the cMWG703 to MWG836 region as a very High Recombination Region as identified by Kunzel et al. (2000).

Materials and methods

EST identification

Rice genomic clone sequences representing chromosome 6 from the Rice Annotation Project (RAP) were searched using the BLASTn function from the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/) for "EST_others" with limits within Triticeae. Blast hits above a score of 80 were identified and sorted into unigenes to minimize repeated clones. The identified ESTs were then analyzed using the BLASTx function from the NCBI website to assign putative functions. If the EST had homology to repetitive sequence (retrovirus, gag-pol polyprotein) it was removed. The EST probe was analyzed for Restriction Fragment Length Polymorphism (RFLP) between the parents Steptoe and Morex with the enzymes *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, and *Xba*I using Southern Blot analysis.

Southern analysis

Barley genomic DNA isolation, Southern blotting and hybridization were carried out as previously described (Kleinhofs et al. 1993). Approximately 25 ng of cDNA insert was labeled with ^{32}P dCTP (New England Nuclear, Waltham, MA, USA) using the RTS RadPrime DNA labeling system (Gibco BRL, Carlsbad, CA, USA) according to manufacturers instructions. Membranes were exposed to X-ray film for 1–7 days depending on hybridization intensity using NEN Reflection QF intensifying screens (New England Nuclear, Waltham, MA, USA).

Linkage map construction

The 150 DH Steptoe × Morex mapping population (Kleinhofs et al. 1993) was used to select 61 lines with at least one recombination on chromosome 7H(1). The resulting 61 DH lines were used to map markers within the HRR between markers ABG320 and ABC255, previously defined as bins 2 through 5 (Kleinhofs and Graner 2001), and genetic distances were calculated based on the 150 DH line population. The previously mapped markers from the 150 DH line Steptoe × Morex map were transferred to the HRR map and used as a framework to map the polymorphic EST's identified as described above. For linkage analyses, the MapManager software was used with the Kosambi function (Manly et al. 2001). Markers were placed on the framework map by minimizing the number of recombinants within the progeny and to limit the length of the map.

BAC clone library hybridization

The cv. Morex BAC clone library (Yu et al. 2000) was searched for positive clones using a probe of 20–30 bulked EST's for hybridization. Hybridization and visualization on X-ray film was as described above. To identify individual BAC clones with specific probes, BAC clones were grown in 96 or 384-well microtiter plates overnight in 400 µl Terrific Broth (Tartof and Hobbs 1987) at 37°C. One-half volume of 50% glycerol was added for a final glycerol concentration of 16% and frozen at −80°C for storage. Plates were replicated onto 8 by 10 cm charged Genescreen Plus-nylon membranes (New England Nuclear, Waltham, MA, USA), which were placed onto 150 by 15 mm Petri plates containing LB agar and grown overnight at 37°C. The bacterial colonies were lysed by floating the membranes on a solution containing 0.4 M NaOH and 0.6 M NaCl for 5 min. The treated membranes were dried at 65°C for 30 min and hybridized with individual clones as previously described. Hybridizations were performed in an Innova 4080 benchtop shaker/incubator at 65°C and three washes at 65°C of decreasing 1× SSC-0.5× SSC-0.25× SSC and 0.4%-0.2%-0.1% SDS, respectively, with a final wash at 65°C with 0.25× SSC 0.1% SDS. Confirmed BAC clones were analyzed for overlaps between co-segregating and closely linked markers and BAC clone contigs constructed.

BAC clone contig construction

Expressed sequence tags probes hybridizing with common overlapping BAC clones were placed into contigs. Assembled contigs were oriented on the genetic map based on mapped EST markers and arranged by collinear position on the rice chromosome. Contigs assembled in this study were extended using the barley BAC clone physical database from May 2006 (http://phymap.plantsciences.ucdavis.edu:8080/barley/) (Close et al. 2006). The barley BAC physical database is a collection of barley cultivar Morex BAC clones assembled into physical contigs based on a preponderance of shared restriction enzyme sites between BAC clones. Physical database BAC clone contigs were searched for clones identified in our study and contigs with clones in common were merged.

Results

Linkage map

Based on synteny with rice, we identified 242 non-redundant EST's with potential to map within the HRR region flanked by markers cMWG703 and MWG836. Of these 213 were polymorphic and were genetically mapped yielding 267 loci. Of the 267 loci, 101 mapped within the target interval (corresponding to 91 EST's) as well as 43 previously mapped markers, while 166 loci mapped outside of the region (Supplementary Material 1). Of the 29 ESTs that

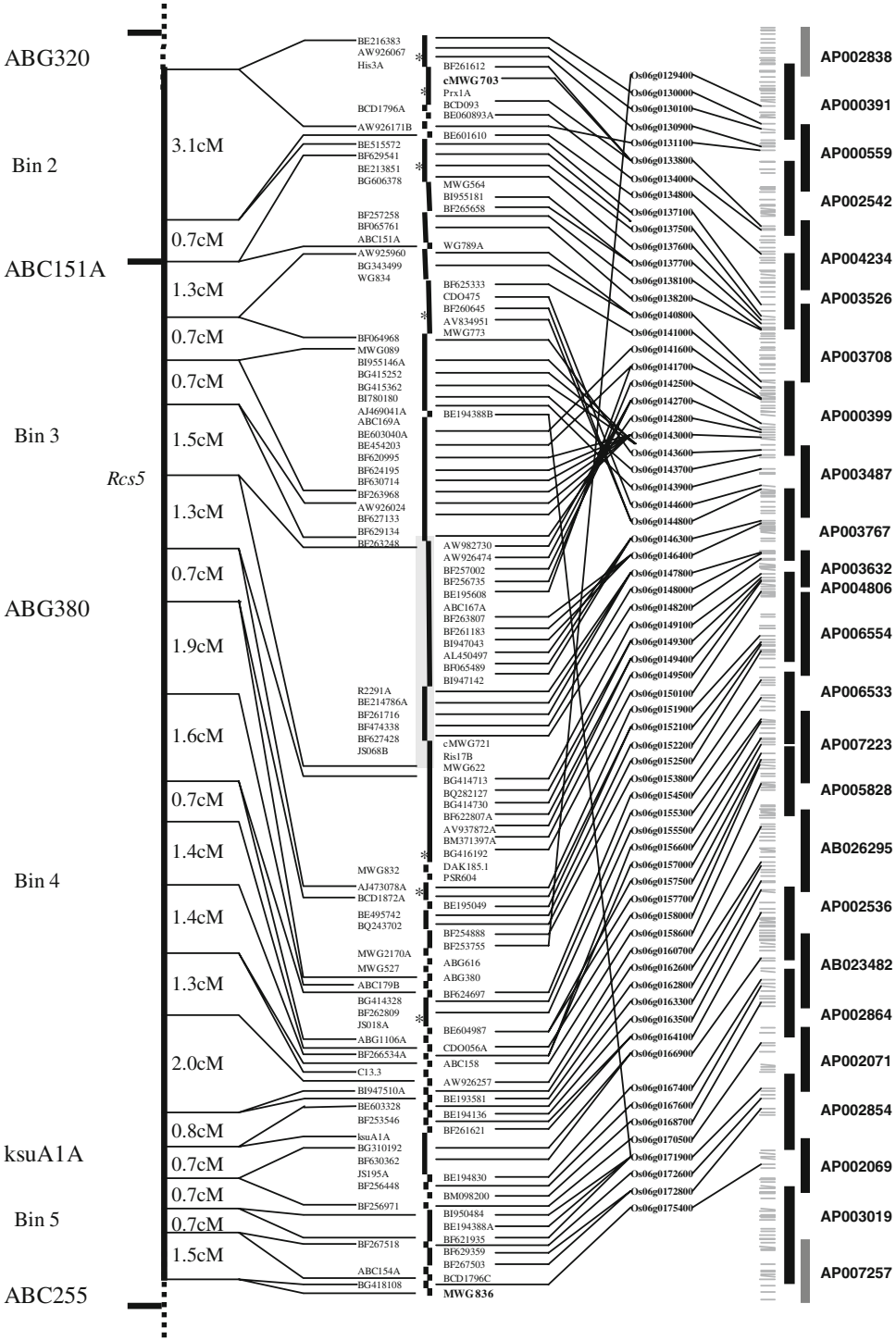

Fig. 1 Barley genetic and physical map of the High Recombination Region between markers cMWG703 and MWG836 in chromosome 7H(1). Barley genetic and physical maps of the short arm of chromosome 7H(1) are shown compared to the syntenous rice chromosome 6 annotated sequence from the Rice Annotation Project (NC_008399 between 1,392–4,088 kb). Rice BAC/PAC clones are shown on the right as vertical bars with predicted genes indicated to the *left* as *light gray horizontal lines*. Rice genes used to identify barley homologues are identified (Os06g…) and connected to the barley ESTs by *thin lines*. The barley ESTs are identified and aligned on both sides of the barley BAC contigs shown as vertical *black bars* in the *middle*. Many of the barley ESTs identifying different BAC contigs co-segregated. These are indicated between the physical map flanking markers, which are connected to the barley genetic map position by thin lines. Linkage distances (cM) between groups of co-segregating markers are denoted on the left and have been adjusted in reference to the Steptoe × Morex map. Bin markers are in *large font* on the *left*. *Asterisk* denotes BAC contigs that were merged using the barley physical mapping database contigs at http://phymap.plantsciences.ucdavis.edu:8080/barley/ (See Supplementary Material 1)

were not mapped, 22 were not polymorphic in the population with the restriction enzymes used and 7 were high copy on Southern Blots and could not be mapped. The constructed linkage map resulted in 21 groups of non identical co-segregating markers (Fig. 1).

Although this study encompassed Bins 2 through 5, of special interest was the region covered by Bins 3 and 4. Within Bin 3 there are eight groups of co-segregating markers with an average of 1.03 cM and a range of 0.7–1.5 cM between the groups. Bin 3 contains *Rcs5*; a locus controlling seedling spot blotch resistance. We mapped the approximate position of *Rcs5* 1.5 cM proximal of the marker BF263248 and 1.3 cM distal of BG414713 (Fig. 1). The 15 Barley EST's that mapped within this interval and their homologies are given in Table 2. Within Bin 4 there are nine groups of co-segregating markers with

Table 2 Barley Expressed Sequence Tags mapping within the *Rcs5* interval and their predicted protein homologies

EST accession	Protein homology
BF257002, AW982730, AW926474	Cytochrome c oxidase subunit Vb precursor (Oryza)
BF256735	Wall associated kinase (Oryza)
BE195608	Early nodulin protein (Oryza)
BF263807	No known homology
BF261183	No known homology
BI947043	hesB-like domain-containing protein-like (Oryza)
AL450497	No known homology
BF065489	No known homology
BI947142	Hypothetical protein (Oryza)
BE214786	MAP Kinase Kinase (Arabidopsis)
BF261716	MAP Kinase Kinase (Arabidopsis)
BF474338	No known homology
BF627428	Proline-rich APG-isolog (Arabidopsis)

an average of 1.39 cM and a range of 0.7–2.0 cM between the groups.

A comparison of collinearity between barley and rice within this region indicated that the interval from CDO475 to BE195608, representing approximately 200 kb in rice, was inverted between the two species. This region is flanked by the markers BF625333 and AL450497, both of which have homologous genes in rice. Twenty one barley ESTs were mapped within this interval (Supplementary Material 2) which spans five BAC clone contigs.

Previous studies indicated a genetic distance of 40 cM between cMWG703 and MWG836, however with the addition of the molecular markers used in this study the genetic distance was estimated to be 25 cM.

BAC clone contig construction

All molecular markers that mapped within the HRR were used to identify BAC clones from the Morex BAC library resulting in 1,270 BAC clones. Co-segregating markers identified overlapping BAC clones resulting in 72 BAC clone contigs across the region. Using the Barley BAC clone physical database from 2006 and searching for overlaps from co-segregating contigs, extended the contigs and reduced the number of contigs to 56. These contigs ranged from 2 to 102 BAC clones each with an average of 19 and a median of 13. For Bin 2 there were 10 contigs ranging from 4 to 44 BAC clones per contig and a total of 223. For Bin 3 there were 19 contigs ranging from 4 to 58 BAC clones per contig and a total number of 568. For Bin 4 there were 15 contigs ranging from 2 to 64 BAC clones per contig and a total number of 267. For Bin 5 there were 12 contigs ranging from 4 to 102 BAC clones per contig and a total number of 212.

Chromosome walk

The seedling spot blotch resistance locus, *Rcs5*, is within the interval between BF263248 and BG414713 (Fig. 1). This region is represented by 3 BAC clone contigs (Fig. 2) with an approximate size of 1908.4 kb. In order to complete the physical map across this region we linked the contigs together. We could not orient the direction of the BAC clone contig to the genetic map since all markers co-segregated so we began chromosome walking from the central contig represented by the BAC clones 053N03, 612G14, 452P09, and 808M17. A subclone from 053N03, KAJ076 was non-polymorphic in the enzymes used, however when hybridized to the BAC clone library it identified the distal contig containing BAC clone 222O19 linking the two contigs together. Two subclones from BAC clone 808M17, KAJ185, and KAJ189 identified the proximal contig containing BAC clone 794L20 linking the next two contigs together. Additional walks to link the other contigs are in progress (Fig. 2).

Discussion

Using microlinearity between rice and barley, we saturated Bins 2 through 5 of the barley chromosome 7H(1) short arm with 101 loci from 91 polymorphic barley EST's. The relative percentage of EST's that mapped within the syntenous interval was 91 out of 213 or 43%. Previous reports have noted that within barley Chromosome 3H(3) approximately 57% of rice cDNA's were collinear (Smilde et al. 2001) and on Chromosome 2H(2)S around the *rph16* leaf rust locus, 97% of polymorphic EST's mapped within the collinear interval (Perovic et al. 2004). It is clear that the expected value of collinearity within any interval is dependent on the conservation of genetic material between related species as well as the ability to correctly identify the orthologous gene between species. Also, EST probes producing multiple bands on RFLP blots could result in identifying polymorphism and mapping of a paralogous copy. The variability observed between the chromosome 3H(3) collinearity and the chromosome 2H(2)S *rph16* collinearity, combined with this study for chromosome 7H(1)S HRR demonstrates this dependence.

The 91 polymorphic barley EST's mapping within Bins 2–5 generated 21 groups of co-segregating markers that correspond to 56 BAC clone contigs. The number of BAC clones identified by markers within the HRR (cMWG703 to MWG836) was 1,270 with an average insert size of 106 kb.

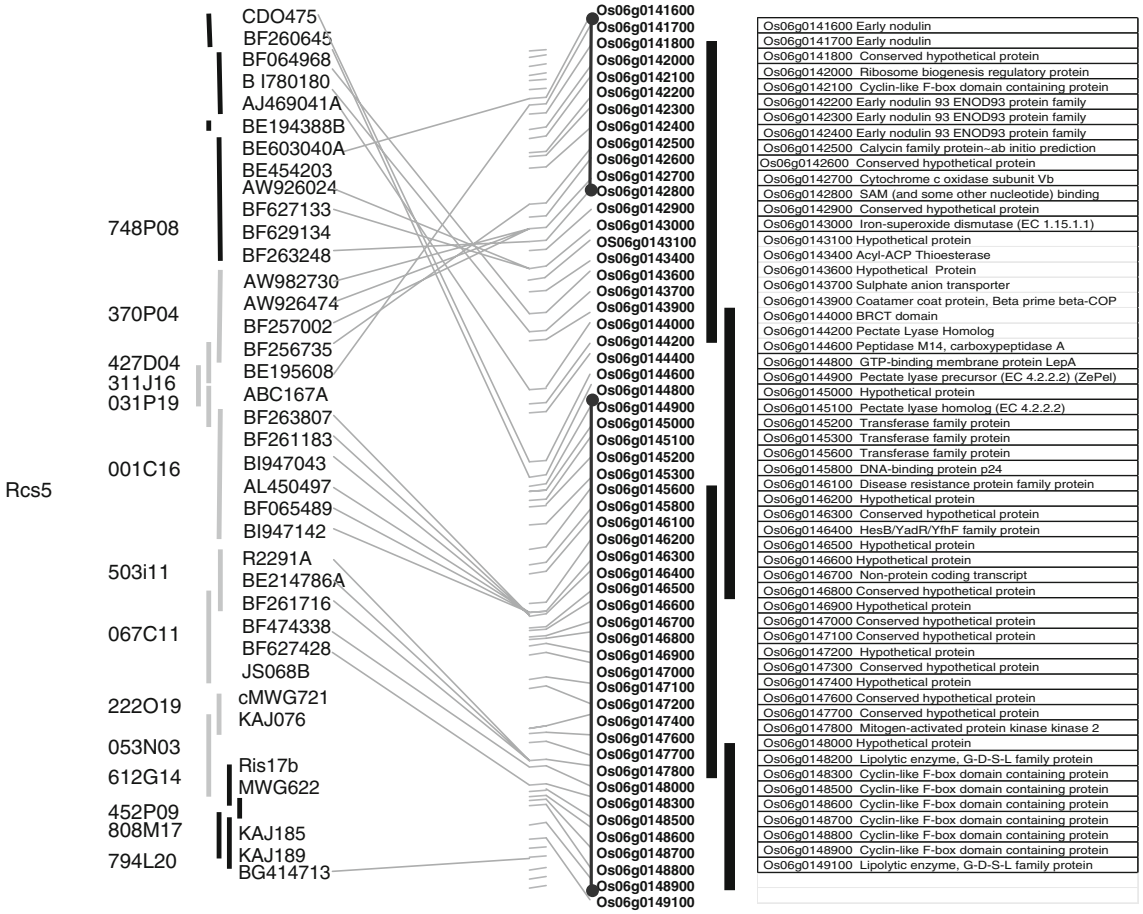

Fig. 2 Comparative barley and rice microlinearity in the *Rcs5* gene interval. The *light gray vertical bars* on the *left* are Morex BAC clones from the *Rcs5* region. The *black vertical bars* represent BAC contigs flanking the *Rcs5* region. The corresponding barley EST's or molecular markers are shown to the *right* of the BAC clones and are connected by *thin lines* to the corresponding rice gene (Os06g…). The syntenous rice chromosome 6 region (NC_008399) between 2,174 and 3,549 kb is shown on the *right* with BAC/PAC clones represented as *vertical bars* and rice genes with predicted protein function. The rice genes mapping outside of the *Rcs5* region are not shown

Given an approximate genome coverage of 6× in the library, the genomic region contained within the very high recombination region would be at least 22 Mb. Kunzel et al. (2000) reported an estimated physical distance of 0.1 Mb/cM between the markers cMWG703 and MWG836 which corresponds to a genetic distance of 39.3 cM on the Igri × Franka genetic map he used for 4.0 Mb estimate. Our estimated 22 Mb is five times larger than expected and may be partially explained by the markers within the HRR having multiple copies that also map elsewhere in the genome. Since these EST probes would identify multiple BAC clones that do not belong to the target region, the number of identified BAC clones as well as the predicted genome space would increase. The 91 EST probes mapping within the HRR also identified an additional 70 loci located in other genomic regions as well as copies with unknown map locations. This would increase the number of BAC clones by at least 420. Correcting for the inclusion of BAC clones from paralogous copies, the number of BAC clones within the HRR decreased to 850, equal to approximately 15 Mb genomic region. This estimate of 15 Mb/25 cM gives a ratio ~0.6 Mb/1 cM which is within Kunzel et al's. (2000) definition for a very high recombination region. However, we probably have not eliminated all of the BAC clones that may be outside the target region. By the same token, it should be noted that this is a minimum estimate since the BAC contig is not complete for the whole region.

The development of a physical map across regions containing important traits will aid in future endeavors for cloning and breeding purposes. As mentioned in the Introduction, there are several important traits contained within chromosome 7H(1) Bins 2 through 5 (See Table 1, Supplementary Material 3). Several yield and malt quality traits as well as heading date and grain characteristics are attributed to this region. Also within this region are agronomically important traits for disease resistance. Two Fusarium head blight resistance traits map within or near Bin 2, a scald resistance locus maps within Bins 2 through 4, net blotch

resistance locus maps within or near Bin 2, and the seedling spot blotch resistance locus, *Rcs5* maps within Bin 3. (Table 1)

A rearrangement distal of the *Rcs5* region (CDO475 to BE195608) resulting in an inversion between rice and barley was observed. A similar inversion was observed in the mapping of the *Lr34/Yr18/Ltn1* locus in wheat (Bossolini et al. 2006). Interestingly, the *Lr34* (Leaf Rust resistance), *Yr18* (Yellow Rust resistance), and *Ltn1* (Leaf tip necrosis), loci have been observed to associate with spot blotch resistance in wheat, but not completely (Joshi et al. 2004). The *Lr34/Yr18/Ltn1* locus was mapped to a syntenous position in rice that is closely linked to the rice syntenous position of *Rcs5*, suggesting that both wheat and barley spot blotch resistance may be attributed to a similar or the same gene. The syntenous position of *Lr34/Yr18/Ltn1* within barley is contained within the marker interval of BF257258 to AV834951, just distal of the inversion and of *Rcs5*. BF257258 to AV834951 is represented by three BAC clone contigs in barley that may potentially contain the barley orthologs of the *Lr34/Yr18/Ltn1* gene(s), however no similar traits have been mapped in barley within this interval.

The inversion distal of *Rcs5* was contained within the flanking markers of BF625333 and AL450497 which represents a portion of the interval that is likely to contain *Rcs5*. Due to the limited number of polymorphic barley EST's within this region it is not possible to determine the entirety of the inversion and so it is described assuming the greatest interval with regards to flanking markers. Within the rice syntenous region of the *Rcs5* locus there are 45 genes, of which ten have homologous barley EST's, eight of which mapped within the *Rcs5* region. Contained within these 45 genes are resistance gene candidates such as; Os06g0146100, an NBS-LRR disease resistance gene and Os06g0147800, a mitogen-activated protein kinase-kinase 2. Both of these represent major classes of resistance genes, but only the mitogen-activated protein kinase-kinase 2 gene identified a homologous barley EST, BF261716, which mapped within the *Rcs5* interval. Interestingly, the Os06g0146100 NBS-LRR identified a barley EST BE216309 with similarity, which was previously mapped in the ThibautRdg2a/Mirco population distal of *Rcs5* (Bulgarelli et al. 2004). The EST BE216309 appears to be identical to a Sequenced Tagged Site, S-9202, that also mapped within the same distal interval in the Steptoe/Morex population (Madsen et al. 2003). Since, NBS-LRR genes tend to be part of gene families with high similarity it is possible that the non-polymorphic band observed in the RFLP Southern analysis of Bulgarelli et al. (2004) may be part of a gene family and may be located within the *Rcs5* region.

In conclusion, using barley–rice microlinearity approach we have confirmed the interval of cMWG703 to MWG836 as a region of high recombination and saturated the seedling spot blotch resistance locus, *Rcs5* with *Triticeae* EST markers. *Rcs5* was confirmed within a genetic interval of 2.8 cM with flanking markers BF263248 and BG414713 which is represented by three contigs of Morex BACs. Future work will include the identification of *Rcs5* as well as the identification of the other traits shared between rice and barley within the HRR.

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