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ORIGINAL PAPER

# Whole genome approaches to identify early meiotic gene candidates in cereals

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**Abstract** Early events during meiotic prophase I underpin not only viability but the variation of a species from generation to generation. Understanding and manipulating processes such as chromosome pairing and recombination are integral for improving plant breeding. This study uses comparative genetics, quantitative trait locus (QTL) analysis and a transcriptomics-based approach to identify genes that might have a role in genome-wide recombination control. Comparative genetics and the analysis of the yeast and Arabidopsis sequenced genomes has allowed the identification of early meiotic candidates that are conserved in wheat, rice and barley. Secondly, scoring recombination frequency as a phenotype for QTL analysis across wheat, rice and barley mapping populations has enabled us to identify genomic regions and candidate genes that could be involved in genome-wide recombination. Transcriptome data for candidate genes indicate that they are expressed in meiotic tissues. Candidates identified included a non-annotated expressed protein, a DNA topoisomerase 2-like candidate, *RecG*, *RuvB* and *RAD54* homologues.

**Keywords** Genome-wide recombination · QTL · Comparative genetics · Transcriptomics · Meiosis

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## Introduction

Meiosis is a process integral to all sexually reproducing organisms. It results in a reduction of chromosome number by half before haploid gamete formation. During meiosis, a single round of chromosome replication is followed by two rounds of chromosome segregation (meiosis I and meiosis II). In meiosis I, pairs of replicated maternal and paternal chromosomes (referred to as homologous chromosomes or homologues) segregate (a reductional division); in meiosis II, the sister chromatids segregate (an equational division). In many organisms, effective meioses are reliant on pairing of homologous chromosomes, synapsis (the formation of the intimate proteinaceous synaptonemal complex between homologous chromosomes) and recombination (exchange of chromosome segments). These events take place during prophase I of meiosis I and are (generally) required to ensure proper homologue segregation (for comprehensive reviews, see Roeder 1997; Zickler and Kleckner 1999; Krogh and Symington 2004).

The ability to effectively utilise the wild relative gene pool in small grain cereals centres on promoting not only pairing between homoeologous or non-homologous chromosomes but also increasing the frequency of recombination events. Bread wheat (*Triticum aestivum* L.) contains three closely related genomes, and the *Ph1* locus on the long arm of chromosome 5B suppresses homoeologous pairing, such

that only homologous chromosomes pair and recombine (Riley and Chapman 1958). Lines in which the *Ph1* phenotype has been suppressed (such as nullisomic/tetrasomic lines) have been used to produce inter-specific hybrids (Sears 1976), but this approach has had limited success, with large linkage blocks which display undesirable linkage drag often introgressed (Brevis et al. 2008). By increasing the amount of recombination, problems associated with linkage drag may be overcome. The first step in assessing the effectiveness of this approach lies in identifying wheat, rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) homologues of meiotic genes that have been identified in yeast (*Saccharomyces cerevisiae*) and other model organisms.

Traditionally, identification of meiotic genes has been achieved through reverse genetics by observing genes that contribute to a phenotype, as was done with the radiation-sensitive (*rad*) mutants in yeast (Cox and Parry 1968). However, with the advent of large-scale transcriptomics approaches such as microarrays, the meiome is beginning to be explored at a whole-genome level. Chu and colleagues (1998) identified more than 1,000 genes that were differentially expressed during sporulation in budding yeast, while Primig et al. (2002) identified 1,600 genes differentially regulated during sporulation and estimated that approximately 150 of them have meiosis-specific functions. In a higher eukaryote, Crismani and colleagues (2006) identified 1,350 transcripts that were temporally regulated during meiosis in wheat. Another more recent strategy to identify candidate meiotic genes was reported by Esch and colleagues (2007), who used a quantitative trait loci (QTL) mapping approach to find genomic regions which contribute to global recombination frequency in individual maize (*Zea mays*), wheat and mouse (*Mus musculus*) mapping populations, and five Arabidopsis (*Arabidopsis thaliana*) mapping populations. Encouragingly, QTL were identified in each of the species examined, with the same genomic region being detected in two of the five Arabidopsis populations.

In the current study, we use a combination of comparative genetics, QTL mapping and transcriptomics to identify candidates in wheat, rice and barley. We have identified several candidates that are conserved across these three cereal genomes through comparison with key meiotic genes from yeast and Arabidopsis. By extending the findings of Esch et al. (2007), we have also identified recombination QTL that show conservation between these three cereals. Two regions were identified, with one on chromosome group 6 of wheat and barley and the other on group 2 of wheat and barley and group 7 of rice (which shares synteny to wheat and barley group 2). Meiotic genes that underlie the QTL identified are considered strong candidates for increasing the natural recombination rate in each of these important crop species.

## Materials and methods

### Database compilation and comparative analysis

To identify wheat, rice and barley putative orthologues of known meiotic genes, an in-house database of key meiotic genes was compiled in Microsoft Office Access 2003. The genes chosen for inclusion in the database were predominantly from yeast (*S. cerevisiae*) and Arabidopsis (*A. thaliana*) and were chosen for their roles in double strand break (DSB) formation, recombination, synaptonemal complex assembly, chromosome pairing and DNA mismatch repair. Coding strand sequence data from selected genes were downloaded from Genbank (http://www.ncbi.nlm.nih.gov/; release 164, February 2008) and used as queries for BLASTN and TBLASTX analysis against all green plants at The Institute for Genomic Research's (TIGR) Plant Transcript Assembly (TA) database (see Childs et al. 2007). The plant TA database contains expressed sequence tags (ESTs) from all green plants for which there are at least 1,000 sequences available (Childs et al. 2007) and constructs these into contiguous sequences based upon overlapping sequence conservation. Searching against this database therefore reveals genes in wheat, rice and barley for which there is evidence of expression. A significance value of $>E^{-20}$ from the TBLASTX analysis was used to identify putative orthologues in wheat, rice and barley.

### QTL mapping

To identify QTL that contribute to whole genome recombination frequency, we analysed three wheat, five barley and one rice mapping population. Genetic linkage maps of the wheat doubled haploid (DH) populations (2-49/Janz [2/J]; W21MMT70/Mendos [W/M]; and Cranbrook/Halberd [C/H]) were produced as components of the Australian Winter Cereals Molecular Marker Project (see Collard et al. 2005; Lehmensiek et al. 2005; Bovill et al. 2006). Two of the five barley populations (the recombinant inbred line [RIL] population WI2875-1/Alexis [W/A] and the DH population Alexis/Sloop [A/S]) were components of the Australian National Barley Molecular Marker Project (see Barr et al. 2003). Genotypic data from three other RIL populations (Patty/Tallon [P/T]; Foster/CI4196 [F/C]; and Frederickson/Stander [F/S] were previously used to create a barley consensus map by Wenzl et al. (2006). The genotypic data from these populations were provided from the supplementary material provided by Wenzl et al. (2006). Genotypic data for the rice RIL population Bala/Azucena (B/A; Price and Tomos 1997) was obtained from Gramene (http://www.gramene.org/).

REcombination Counting and ORDering (Van Os et al. 2005) was used to order markers into linkage groups in

each of the populations. Linkage groups were assigned to chromosomes based upon consensus maps (see Appels 2003 for wheat; Wenzl et al. 2006 for barley, and Gramene [http://www.gramene.org/] for rice consensus maps). After placing markers into linkage groups, the maps were curated to increase the accuracy of QTL detection as described by Lehmensiek et al. 2005. The total number of genome-wide recombination events in each of the individuals in the respective populations was counted and used as phenotypic data to identify QTL. As we were most interested in identifying QTL that were in the same genomic region within and between species, a low significance threshold of LRS of 5.53 (LOD of 1.2) was used to assign putative QTL. Composite interval mapping (using default parameters) was performed using Windows QTL Cartographer 2.5 (Wang et al. 2007). MapChart (Voorrips 2002) was used for graphical presentation of linkage groups.

### Candidate gene and transcriptomics analysis

To identify candidate genes within the conserved QTL that contributed to genome-wide recombination events, we downloaded brief information of the gene models from The Institute for Genomic Research (http://rice.plantbiology.msu.edu) for these regions and searched for genes whose annotation suggested a possible role in affecting genome-wide recombination. To complement this candidate gene analysis, we interrogated the wheat meiosis microarray dataset generated by Crismani et al. (2006). Crismani et al. (2006) reported 350 genes that showed meiotic regulation in wheat, and by using the Affymetrix wheat GeneChip® probe sequences for each of these, a BLASTN analysis was performed against TIGR's rice database to identify any probes that were located under identified QTL. A BLASTN analysis against Arabidopsis of selected genes at TIGR's Arabidopsis database (http://tigrblast.tigr.org/er-blast/index.cgi?project=ath1) was also performed and the National Centre for Biotechnology's (NCBI; http://www.ncbi.nlm.nih.gov/) EST Profile Viewer used to determine if any of our candidates were up-regulated during meiosis in Arabidopsis.

## Results

### Meiotic gene sequence conservation across eukaryotes

From a total of 53 meiotic gene candidates that are known to have reported roles during early meiosis in yeast and/or Arabidopsis, 33 wheat gene sequences using a stringency of $>E^{-20}$ were identified. Similar numbers were obtained in rice (34) and barley (30) (Table 1). The majority of yeast genes that were not identified in wheat, rice or barley belonged to the DSB class (Electronic Supplementary Material S1). None of these individual yeast candidates have Arabidopsis counterparts either. DSB formation in Arabidopsis is predominantly controlled through two *SPORULATION11* (*SPO11*) candidates, *SPO11–1* and *SPO11–2*, which are represented in the wheat, rice and barley genomes with significant TBLASTX values (Electronic Supplementary Material S1). In addition, a putative orthologue of *RADIATION SENSITIVE52* (*RAD52*), a key gene required for virtually all homologous recombination events in yeast (Paques and Haber 1999), was not identified in any of the cereals.

**Table 1** A summary of the number of yeast and/or Arabidopsis genes associated with meiosis for which putative orthologues were identified in the wheat, barley and rice genomes

| Meiotic functional class | Wheat | Rice | Barley |
|---|---|---|---|
| Cohesins | 4 (5) | 4 (5) | 5 (5) |
| DSB formation | 1 (7) | 1 (7) | 1 (7) |
| RAD52 epistasis group | 9 (13) | 9 (13) | 8 (13) |
| Recombination/synapsis/pairing | 14 (22) | 14 (22) | 11 (22) |
| Mismatch repair machinery | 5 (6) | 6 (6) | 5 (6) |
| Total | 33 (53) | 34 (53) | 30 (53) |

For complete information, including EST or transcript assembly accession numbers and TBLASTX values, see Electronic Supplementary Material, S1. The total number in each class is displayed in parentheses

### Recombination frequency

In each of the populations examined, the histograms display a continuous distribution which is typical of a quantitative trait (Fig. 1). The average number of detectable recombination events in the barley RIL populations (Fig. 1a–d) ranged from 6.7 for the P/T population to 16.3 for the W/A population. The A/S and the W/A populations are crosses involving similar parents (the WI2875-1 parent is designated as Sloop-sibling) but differ in both the direction of the cross and in the population type. The DH A/S population (Fig. 1e) showed, on average, fewer recombination events (mean of 11.2) than the W/A RIL population (mean of 16.3). The three wheat DH populations (Fig. 1f–h) showed an average of between 15.8 recombination events for the 2/J population, to 29.5 for the C/H population. The rice RIL population B/A (Fig. 1i) displayed an average of 18.0 recombination events.

### QTL identification

With the exception of the P/T barley population, QTL were identified in each of the populations examined (Table 2). Between two and five QTL were detected in each of the individual wheat DH populations. In the 2/J population, a QTL on the short arm of chromosome 2A (Qrec.2-Ja-2A) was the most significant QTL, explaining 10.6% of the

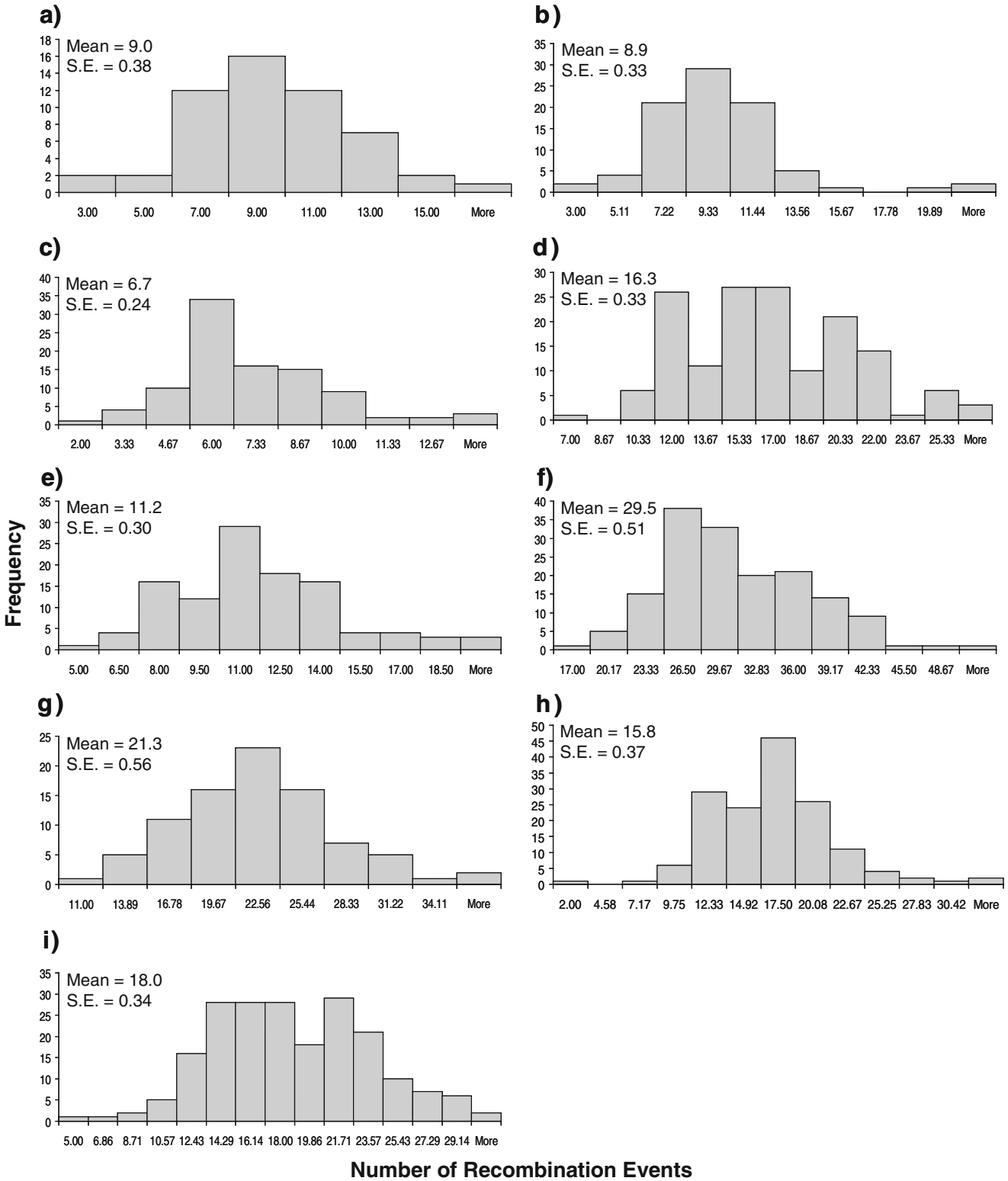


**Fig. 1** Histograms of recombination frequency in each of the mapping populations examined. **a** F/S, **b** F/C, **c** P/T, **d** W/A, **e** A/S, **f** C/H, **g** W/M, **h** 2/J, **i** B/A. Barley (**a**–e), wheat (**f**–**h**) and rice (**i**) populations are shown. The mean and standard error (S.E.) of each population are indicated

**Table 2** Overview of QTL for recombination frequency detected in the populations examined

| Population | Type | Ind. | Markers | Chr. | Q | LRS | % PE | Flanking markers | Effect |
|---|---|---|---|---|---|---|---|---|---|
| Wheat | | | | | | | | | |
| 2/J | DH | 153 | 151 | 2AS | Qrec.2-Ja-2A | 18.6 | 10.6 | gwm425-gwm558 | 1.51 |
| | | | | 2DS | Qrec.2-Ja-2D | 12.0 | 10.2 | wmc112-gwm107 | 1.96 |
| W/M | DH | 95 | 347 | 2DS | Qrec.W2Me-2D.1 | 7.1 | 5.4 | wmc112-cfd56 | 1.22 |
| | | | | 2DC | Qrec.W2Me-2D.2 | 8.9 | 6.8 | p8-185W-cfd11a | −1.39 |
| | | | | 6DL | Qrec.W2Me-6D | 10.4 | 8.8 | p31-120-gdm098 | 1.56 |
| | | | | 7BL | Qrec.W2Me-7B.1 | 6.3 | 5.3 | p39-139-p1-13 | −1.96 |
| | | | | 7BL | Qrec.W2Me-7B.2 | 14.6 | 11.5 | p1-13-P53-109 | 3.10 |
| C/H | DH | 159 | 710 | 3DS | Qrec.CrHa-3D | 6.3 | 3.4 | gwm183-P35/M50-3 | 1.15 |
| | | | | 5BC | Qrec.CrHa-5D | 21.9 | 11.6 | gwm67-psr911 | 4.58 |
| | | | | 6AL | Qrec.CrHa-6A.1 | 8.7 | 5.9 | wmc163 - cdo1091a | −1.59 |
| | | | | 6AL | Qrec.CrHa-6A.2 | 16.8 | 8.2 | cdo1091a-P38/M51-4 | 1.91 |
| Barley | | | | | | | | | |
| W/A | RIL | 153 | 258 | 1HL | Qrec.WIAl-1H | 11.6 | 7.0 | Hor1-bcd22 | −1.02 |
| | | | | 3HL | Qrec.WIAl-3H | 13.3 | 6.8 | bcd131b-P11/M62-183 | −1.09 |
| | | | | 5HL | Qrec.WIAl-5H | 5.7 | 3.2 | P12/M54-psr637 | −0.76 |
| | | | | 6HL | Qrec.WIAl-6H | 7.7 | 4.1 | bcd269-P14/M51-166 | 0.85 |
| | | | | 7HS | Qrec.WIAl-7H.1 | 14.1 | 7.2 | abg704-P14/M47-315 | −1.38 |
| | | | | 7HS | Qrec.WIAl-7H.2 | 17.3 | 9.4 | bcd129-wg789 | 1.80 |
| F/C | RIL | 86 | 212 | 2HS | Qrec.FoCI-2H.1 | 18.5 | 15.1 | bPb-5489-abc311 | 1.33 |
| | | | | 2HS | Qrec.FoCI-2H.2 | 23.2 | 19.5 | MWG889a-ABG461C | −1.49 |
| | | | | 6HL | Qrec.FoCI-6H | 14.5 | 11.8 | bPb-1466-bPb5778 | 1.22 |
| F/S | RIL | 52 | 213 | 2HL | Qrec.FrSt-2H | 7.0 | 7.8 | bPb-8255-bPb-6048 | 0.83 |
| | | | | 3HC | Qrec.FrSt-3H | 6.5 | 7.2 | bPb-9746-bPb-5012 | −0.78 |
| | | | | 5HL | Qrec.FrSt-5H | 7.9 | 9.9 | bPb-1661-bPb-5845 | 0.90 |
| | | | | 6HL | Qrec.FrSt-6H | 12.8 | 14.0 | bPb-1466-bPb-0606 | −1.11 |
| P/T | RIL | 96 | 135 | – | – | – | – | – | – |
| A/S | DH | 110 | 224 | 6HL | Qrec.AlSl-6H | 6.9 | 5.5 | Ebmac874-P13/M51-205 | 0.75 |
| Rice | | | | | | | | | |
| B/A | RIL | 205 | 166 | 1AS | Qrec.BaAz-1A | 6.3 | 4.0 | RG532-RG400 | 0.97 |
| | | | | 3AS | Qrec.BaAz-3A | 11.6 | 5.3 | RG745-a12455 | 1.14 |
| | | | | 7AL | Qrec.BaAz-7A | 11.0 | 5.0 | C451-RG650 | 1.09 |
| | | | | 11AC | Qrec.BaAz-11A.1 | 7.0 | 4.2 | RZ141-G44 | −1.16 |
| | | | | 11AC | Qrec.BaAz-11A.2 | 9.1 | 5.4 | a12375-c189 | 1.14 |

The QTL are sorted according to species and individual populations. The population type (RIL or DH), number of individuals in each population (*Ind.*), number of non-redundant markers used in the QTL analysis (*Markers*), chromosomal location (*Chr.*), QTL designation (*Q*), likelihood ratio statistic (*LRS*), the percent phenotypic variance explained by the QTL (*% PE*), flanking markers, and the additive effect (positive or negative values indicate different parental contributions) are shown. QTL were identified in each population examined with the exception of the P/T population

phenotypic variance. In the W/M populations, the most significant QTL was located on the long arm of chromosome 7B (Qrec.W2Me-7B.2), which explained 11.5% of the phenotypic variance. This QTL was closely linked in repulsion with another QTL in the same region (Qrec. W2Me-7B.1). In the C/H population, the most significant QTL was located in the centromeric region of chromosome 5B (Qrec.CrHa-5B) and explained 11.6% of the phenotypic variance. Similarly with the W/M QTL that were linked in repulsion, we also detected QTL linked in repulsion on the long arm of chromosome 6A in the C/H population (Qrec. CrHa-6A.1 and -6A.2).

QTL were detected in four of the five barley populations that were examined. Six QTL were detected in the W/A RIL population. The most significant QTL was located on the short arm of chromosome 7H (Qrec.WIAl-7H.2) and explained 9.4% of the phenotypic variance. Another QTL linked in repulsion to the 7H QTL in the W/A population was observed (Qrec.WIAl-7H.1). Three QTL were detected in the F/C RIL population. The most significant barley QTL was located on the short arm of chromosome 2H (Qrec. FoCI-2H.2) and explained 19.5% of the phenotypic variance. Again, another QTL was linked in repulsion to the 2H QTL in the F/C population (Qrec.FoCI-2H.1). The most significant QTL of the four identified in the F/S RIL population on the long arm of chromosome 6H (Qrec.FrSt-6H) explained 14.0% of the phenotypic variance. However, no QTL were detected in the P/T RIL population, and only

one on the long arm of chromosome 6H (Qrec.AlSl-6H) was detected in the A/S DH population (explaining 5.5% of the phenotypic variance).

In the rice B/A RIL population, four QTL were detected. The most significant of these was located on rice chromosome 3 (Qrec.BaAz-3A) and explained 5.3% of the phenotypic variance. As observed for some of the wheat and barley populations, two QTL linked in repulsion (on chromosome 11; Qrec.BaAz-11A1 and -11A.2) were detected in the rice population. Thus overall, we observed QTL that were linked in repulsion in five of the nine populations examined.

## Comparative mapping

We were most interested in those QTL that appeared to reside in common regions within or between each of the species examined. Two such regions were identified from the QTL analysis. The first region was located on chromosome 2D in wheat and was identified in the 2/J and W/M populations. Alignment of markers flanking the QTL in each of the populations with the consensus map of Appels (2003) revealed that the QTL were located in the same position on chromosome 2D (Fig. 2). Sorrells et al. (2003) have reported that wheat group 2 chromosomes share synteny with rice chromosomes 4 and 7. Supporting this, we also identified a QTL on chromosome 7 of the B/A rice population. To assess if this rice QTL shared synteny with the wheat 2D QTL, we identified EST markers that had been bin-mapped to the short arm of 2D of wheat from GrainGenes (http://wheat.pw.usda.gov) and performed a BLASTN analysis using The Institute for Genomic Research's rice database against all bacterial artificial chromosome (BAC) and phage artificial chromosome (PAC) sequences in Genbank (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1). The chromosome location of the BAC/PAC with the

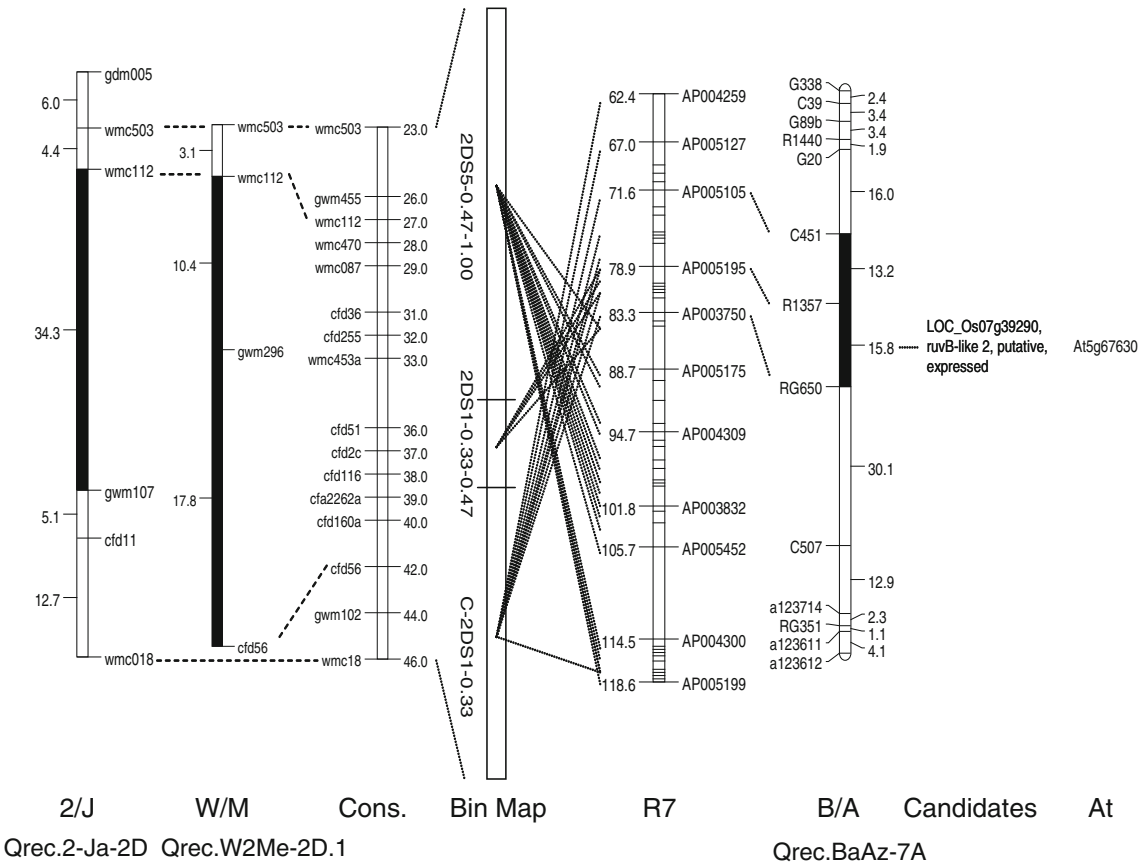


**Fig. 2** Comparative mapping of a chromosome 2D QTL identified in two wheat populations, with chromosome 7 of rice. QTL were identified in the 2/J and W/M populations. The consensus map (*Cons.*) of Appels (2003) was used to align the wheat maps. Bin-mapped wheat EST markers (*Bin Map*) were used as queries to BLASTN against rice PACs and BACs to obtain physical locations on rice chromosome 7 (*R7*). The alignment of the wheat ESTs with the rice BACs/PACs revealed an inversion of this region in wheat. Sequence data for markers flanking the rice B/A population chromosome 7 QTL were used to identify the physical location of this QTL. For clarity, not all BACs or PACs are shown on the physical map. The location of the QTL in the mapping populations are indicated by the *filled bars*. The most probable candidate gene (see text) identified underneath this QTL, and an Arabidopsis (At) orthologue is shown. Cumulative or interval distances between markers are in centimorgans (cM)

highest *E* value was used to align the wheat EST sequences with rice chromosomes. Of the 256 EST sequences, 114 (45%) displayed significant alignments to rice chromosome 7, with the majority aligning to the long arm. By using sequence data for markers that flank the rice QTL, we found that the rice chromosome 7 QTL does share syntenous regions with the two wheat populations (Fig. 2).

To identify candidate genes for this conserved region, we obtained brief information about the gene models from TIGR using markers that flanked the rice QTL and searched for genes that might be predicted to have a role in affecting genome-wide recombination events. Numerous candidate genes were identified, including many DNA binding proteins, transcription factors and proteins of unknown function (data not shown). Between the flanking markers of the rice QTL, we identified a *RuvB-like 2* gene, which is worthy of further investigation as this gene might be responsible for influencing genome-wide recombination events.

The second region of interest, located on chromosome 6H, was detected in three of the five barley populations (Fig. 3). We used the consensus map of Wenzl et al. (2006) to assist in aligning markers in the F/S, F/C and A/S populations. The QTL that were identified in the three populations were also in the same genomic location, as evidenced by flanking markers in common between the populations. In order to identify candidate genes for this region, we performed a BLASTN analysis using barley markers for which sequence data was available against rice BACs and PACs as described above. Again, numerous

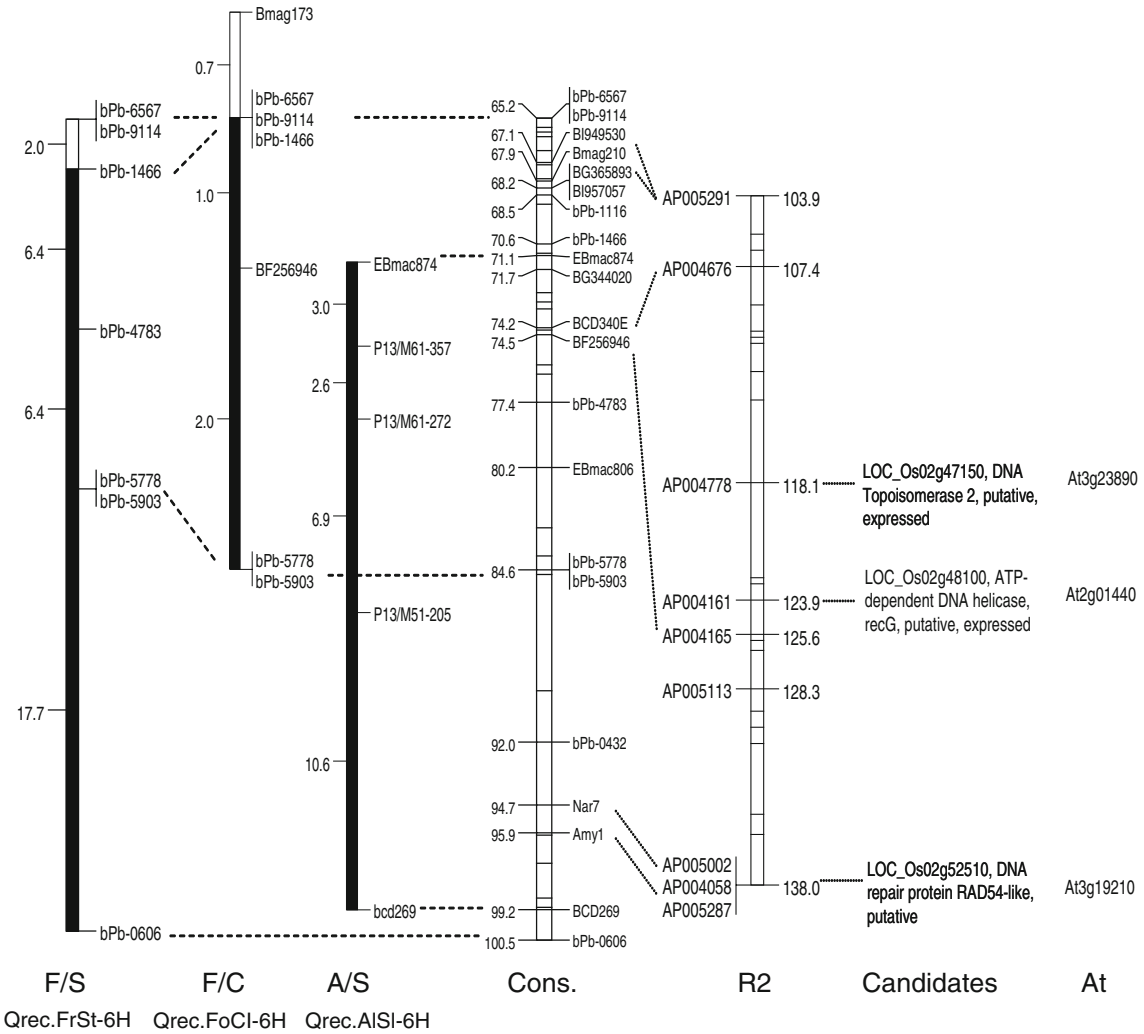


**Fig. 3** Comparative mapping of a chromosome 6H QTL identified in three barley populations, with chromosome 2 of rice. QTL were identified in the F/S, F/C and A/S populations. The consensus map (*Cons.*) of Wenzl et al. (2006) was used to align the barley maps. Barley markers for which sequence data was available were used as queries to BLASTN against rice PACs and BACs to obtain physical locations on rice chromosome 2 (*R2*). For clarity, not all BACs or PACs are shown on the physical map. The location of the QTL in the mapping populations are indicated by the *filled bars*. Three candidate genes were identified (see text), and Arabidopsis (*At*) orthologues for each are shown. Cumulative or interval distances between markers are in centimorgans (cM)

genes were identified, with a DNA topoisomerase 2, a DNA helicase (*RecG*) and *RADIATION SENSITIVE 54* (*RAD54*) being the most interesting (Fig. 3). Under the same criteria used in our comparative genetics analysis, putative orthologues for each of these genes were identified in wheat and barley with significant TBLASTX values (data not shown).

## Transcriptomics analysis of genes that underlie the identified QTL

To identify further genes that underlie these QTL and that may have been overlooked in our candidate gene analysis (either because they were non-annotated or because they did not seem likely candidates for contributing to genome-wide recombination), we performed a BLASTN analysis of the 350 genes that were up-regulated during meiosis identified by Crismani et al. (2006). Nine of these Affymetrix probes hit to the QTL with a BLASTN significance threshold $>E^{-20}$: six on the rice chromosome 2 region and three on the rice chromosome 7 region. The rice chromosome 2 genes included a non-annotated expressed protein (LOC_Os02g44230); a histone H4 variant (LOC_Os02g45940); an aspartyl tRNA synthetase (LOC_Os02g46130); the same DNA topoisomerase 2 that we identified in our candidate gene search (LOC_Os02g47150); an actin-binding protein (LOC_Os02g50570); and a phosphate-induced (phi-like) protein (LOC_Os02g52040). The rice chromosome 7 genes included a histone H2A.2 variant (LOC_Os07g36130); an isopentenyl-diphosphate delta-isomerase II (LOC_Os07g36190); and a non-annotated expressed protein (LOC_Os07g37850). Putative orthologues for each of these genes were also identified in barley. We removed the aspartyl tRNA synthetase, the phi-like protein and the isopentyl-diphosphate delta-isomerase II from further analysis as these metabolism genes are unlikely to contribute to genome-wide recombination. The other genes we identified in our candidate gene analysis (*RecG* and *RAD54* on rice chromosome 2; and *RuvB* on rice chromosome 7) were not represented on the Affymetrix GeneChip® used by Crismani et al. (2006). This is due to the wheat Affymetrix GeneChip® only being representative of the wheat database sequence entries at the time of production (2004).

We examined the expression profiles for the remaining candidates using the staged meiotic data of Crismani et al. (2006; Fig. 4). All of these candidates were categorised as being meiotically regulated by Crismani et al. (2006). With the exception of the Chr_7 Expressed Protein candidate, no candidates showed any sharp decreased or increased expression over the first four meiotic stages (pre-meiosis to telophase II). While the Chr_7 Expressed Protein candidate did show an eightfold increase over these first four stages, it is unlikely to have a role in genome-wide recombination control given that its expression peaks during immature pollen. In general, all of the candidates were moderately to highly expressed across the seven stages, with reduced expression being triggered at the cessation of meiosis (tetrads to mature anthers) for all candidates located on chromosome 2.

As the other three candidates (*RecG* and *RAD54* on rice chromosome 2 and *RuvB* on rice chromosome 7) were not represented on the wheat Affymetrix GeneChip®, we examined the expression of putative Arabidopsis ortho-

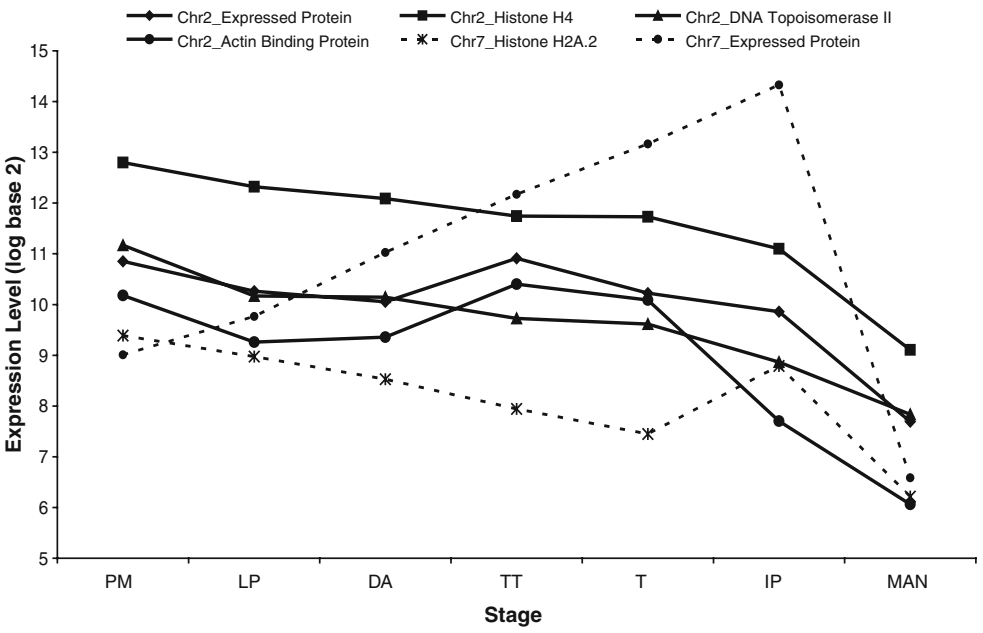


**Fig. 4** Meiotic expression profiles for six candidate genes which underlie the identified QTL associated with genome-wide recombination. *Solid lines* represent those candidates identified on chromosome 2 (rice), while *dashed lines* represent chromosome 7 (rice) candidates. *PM* pre-meiosis, *LP* leptotene to pachytene, *DA* diplotene to anaphase I, *TT* telophase I to telophase II, *T* tetrads, *IP* immature pollen, *MAN* mature anthers

logues for these candidates using the NCBI Expression Profile Viewer. This enabled us to obtain evidence as to whether these Arabidopsis genes were expressed in meiotic tissues. *RecG* expression was found to be up-regulated in flower and root tissue, while *RuvB* was found to be up-regulated in flower tissue only (data not shown). Data for *RAD54* was not available from the NCBI Expression Profile Viewer. However, Osakabe et al. (2006) have previously provided evidence that, while *AtRAD54* transcripts were found in all tissues examined, the highest levels were found in flower buds.

## Discussion

Much of our current knowledge of meiosis comes from research on model organisms such as yeast. Orthologues of key recombination genes first identified in yeast have since been identified and characterised in the model plant, Arabidopsis. By using DNA sequence data for many characterised yeast and Arabidopsis meiotic genes, we found that most of the yeast genes and all that have published Arabidopsis homologues (Electronic Supplementary Material S1) appear to have orthologous counterparts in wheat, rice and barley. Those that did not included key genes involved in DSB formation as well as genes such as *RADIATION SENSITIVE 52*, which plays a key role in homologous recombination in yeast (Paques and Haber 1999). Interestingly, we only found evidence for the presence of two of the three subunits of REPLICATION PROTEIN A (*REPLICATION FACTORS A1* and *A2)*. Although the wheat and barley genomes have not been completely sequenced, both rice and Arabidopsis have been, and therefore, it is unlikely that wheat and barley will have any of the yeast genes we could not identify in either the sequenced rice or Arabidopsis genomes. Investigating some of the more primitive land plants, and/or simpler eukaryotes, might enable a more accurate point of divergence of meiotic processes to be calculated. We acknowledge that more stringent, domain-based searching may have revealed further conservation and note that DNA sequence divergence for some meiotic genes (for example ZYP1; Higgins et al. 2005; and ASY1; Boden et al. 2007) is high across species. Genes with a high level of sequence divergence may have been missed due to the stringency we imposed to determine putative candidates from the BLAST searches. Even so, our data clearly shows that a large percentage of known meiotic genes are conserved at the DNA sequence level across diverse organisms.

Based on our comparative genetics results, it appeared reasonable to assume that a QTL mapping approach (similar to Esch et al. 2007) would reveal conserved genomic regions that influence the number of genome-wide recombination events in wheat, rice and barley genomes. We addressed this by scoring the number of detectable recombination events in mapping populations of each of the three cereal species. In all populations, a continuous distribution was evident, which is characteristic of quantitative traits. Within each population, there was a broad range in the number of recombination events. Comparing the A/S DH population with the closely related W/A RIL population, we found that the DH population on average showed fewer recombination events (mean of 11.2) than the RIL population (mean of 16.3). This result was expected due to the smaller number of meioses the DH population underwent compared to the RIL population.

In the majority of organisms, the average number of recombination events has been reported to range from one to three per chromosome (Sanchez-Moran et al. 2007), of which at least one (the so-called obligatory crossover; Mezard et al. 2007) is required to ensure correct segregation of homologues. The average number of recombination events reported in this study fits within this range, although we acknowledge that the average number of recombination events does vary between species and even within chromosomes (Mezard 2006). Some lines appeared to have considerably fewer or a considerably greater number of recombination events than the population average. Such lines might be useful for breeding programmes wishing to select individuals from crosses that contain a higher or lower amount of a particular parental genotype in their programmes.

QTL for recombination frequency were detected in all but one of the nine populations examined (the P/T barley population). Although it is possible that the P/T population did not segregate for recombination frequency QTL, this appears unlikely as all other populations examined did segregate. QTL linked in repulsion were detected in five of the nine populations examined and in each of the crop species. Reports of QTL for the same trait linked in repulsion appear to be rare in ‘classical’ QTL analyses but are common for a number of the recombination QTL we detected. While the significance of this observation is unknown, further studies might provide an answer to the biological basis of this finding.

The most significant finding of our QTL analysis was the discovery of two conserved regions that contribute to genome-wide recombination. The first conserved region was identified in two wheat populations (the 2/J and W/M populations) on chromosome 2D and a syntenic region on rice chromosome 7 (in the B/A population). The second was found in three barley populations (the F/C, F/J and S/A populations). In addition to the synteny observed for the wheat 2D and rice chromosome 7 QTL (Fig. 2), we also identified QTL on the short arm of chromosome 2A in the 2/J wheat population and on the short arm of chromosome

2H in the F/C barley population (see Table 2). Similarly, QTL were also identified in the C/H wheat population on the long arm of chromosome 6A and in the W/M population on the long arm of chromosome 6D. Given the synteny between wheat and barley (Linde-Laursen et al. 1997) and the sequenced rice genome (Goff et al. 2002), it is possible that the same gene(s) underlie these QTL as well. However, due to a lack of markers in common between the maps, we were unable to definitively align these QTL.

Esch et al. (2007) used RIL populations for their work on mapping QTL for recombination frequency in maize, wheat, Arabidopsis and mouse. We also used RIL population data but did not confine our study to RIL populations alone, choosing to also include $F_1$-derived DH populations to identify recombination frequency QTL. Esch et al. (2007) point out several limitations to using RIL populations, some of which include QTL segregating away from linked markers as a result of individuals undergoing several rounds of meiosis; that the number of recombination events is the sum of male and female meioses over several generations; and that the final phenotype may not always accurately reflect the recombination history of the line. Although DH populations will have less recombination events compared to RILs, using DH populations enables problems associated with QTL segregating away from linked markers during the several generations of selfing that RILs undergo to be overcome.

Of the candidates identified under our QTL and that were represented on the wheat Affymetrix GeneChip®, the non-annotated expressed protein and the DNA topoisomerase II are the most interesting with respect to putative roles in increasing genome-wide recombination events. *SPO11* (a DNA topoisomerase II) is an enzyme that produces DNA double strand breaks at the onset of meiosis in organisms including yeast and Arabidopsis (Keeney et al. 1997; Hartung and Puchta 2000). However, the DNA topoisomerase II enzyme that we identified in the present study is not a homologue of *SPO11*, and further work is therefore required to ascertain the meiotic role that this candidate has in wheat, rice and barley. Similarly and based on the preliminary results we have presented for the non-annotated expressed protein, further research will enhance our understanding of this candidate also.

While *RAD54*, *RuvB* and *RecG* were not represented on the wheat Affymetrix GeneChip®, each of these are also strong candidates that might influence genome-wide recombination. Arabidopsis *RAD54* has been shown to display increased expression in flower buds and interacts with Arabidopsis *RAD51* in a yeast-two-hybrid assay, which implies a meiotic role (Osakabe et al. 2006). Intriguingly, Shaked et al. (2005) have shown that expressing yeast *RAD54* in Arabidopsis results in a 27-fold enhancement in gene-targeting, thus making *RAD54* a strong candidate for increasing genome-wide recombination events in the current study. Using expression profiles for *RuvB* and *RecG* from the NCBI EST Expression Profile Viewer, we found that these genes were expressed in Arabidopsis floral tissues. *RuvB* is a member of the RuvABC complex which promotes branch migration and resolution of Holliday junctions in *Escherichia coli* (reviewed in West 1997). *RecG* is a junction-specific DNA helicase that promotes branch migration and is required for normal levels of recombination and DNA repair in the same organism (Whitby and Lloyd 1995). Meddows et al. (2004) have provided evidence that double-strand break repair in *E. coli* can proceed via two alternative pathways—one which depends upon the RuvABC Holliday junction resolvase and another that relies upon the RecG protein. Bacterial genes such as these have not been studied in plants and therefore offer further novel targets that may be investigated.

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## References

Appels R (2003) A consensus molecular genetic map for wheat—a cooperative international effort. In Proceedings of the 10th International Wheat Genetics Symposium(Ed, Progna, N. E.) Paestum, Italy, pp 211–214

Barr AR, Jefferies SP, Broughton S, Chalmers KJ, Kretschmer JM, Boyd WJR, Collins HM, Roumeliotis S, Logue SJ, Coventry SJ, Moody DB, Read BJ, Poulsen D, Lance RCM, Platz GJ, Park RF, Panozzo JF, Karakousis A, Lim P, Verbyla AP, Eckermann PJ (2003) Mapping and QTL analysis of the barley population Alexis x Sloop. Aust J Agr Res 54:1117–1123

Boden SA, Shadiac N, Tucker EJ, Langridge P, Able JA (2007) Expression and functional analysis of Ta*ASY1* during meiosis of bread wheat (*Triticum aestivum*). BMC Mol. Biol. 8:65

Bovill WD, Ma W, Ritter K, Collard BCY, Davis M, Wildermuth GB, Sutherland MW (2006) Identification of novel QTL for resistance to crown rot in the doubled haploid wheat population 'W21MMT70' x 'Mendos'. Plant Breed 125:538–543

Brevis JC, Chicaiza O, Khan IA, Jackson L, Morris CF, Dubcovsky J (2008) Agronomic and quality evaluation of common wheat near-isogenic lines carrying the leaf rust resistance gene *Lr47*. Crop Sci 48:1441–1451

Childs KL, Hamilton JP, Zhu W, Ly E, Cheung F, Wu H, Rabinowicz PD, Town CD, Buell CR, Chan AP (2007) The TIGR plant transcript assemblies database. Nucleic Acids Res 35:D846–D851

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. Science 282:699–705

Collard BCY, Grams RA, Bovill WD, Percy CD, Jolley R, Lehmensiek A, Wildermuth GB, Sutherland MW (2005) Development of molecular markers for crown rot resistance in wheat:

mapping of QTLs for seedling resistance in a 2-49 × Janz population. Plant Breed 124:1–6

Cox BS, Parry JM (1968) The isolation, genetics and survival characteristics of ultraviolet-light sensitive mutants in yeast. Mutat Res 6:37–55

Crismani W, Baumann U, Sutton T, Shirley N, Webster T, Spangenberg G, Langridge P, Able JA (2006) Microarray expression analysis of meiosis and microsporogenesis in hexaploid bread wheat. BMC Genomics 7:267

Esch E, Syzmaniak JM, Yates H, Pawlowski WP, Buckler ES (2007) Using crossover breakpoints in recombinant inbred lines to identify quantitative trait loci controlling the global recombination frequency. Genetics 177:1851–1858

Goff SA, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun W-l, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296:92–100

Hartung F, Puchta H (2000) Molecular characterisation of two paralogous SPO11 homologues in *Arabidopsis thaliana*. Nucleic Acids Res 28:1548–1554

Higgins J, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FCH (2005) The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. Gene Dev 19:2488–2500

Keeney S, Giroux CN, Kleckner N (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell 88:375–384

Krogh BO, Symington LS (2004) Recombination proteins in yeast. Annu Rev Genet 38:233–271

Lehmensiek A, Eckermann PJ, Verbyla AP, Appels R, Sutherland MW, Daggard G (2005) Curation of wheat maps to improve map accuracy and QTL detection. Aust J Agr Res 56:1347–1354

Linde-Laursen I, Heslop-Harrison JS, Shepherd KW, Taketa S (1997) The barley genome and its relationship with the wheat genomes. A survey with an internationally agreed recommendation for barley chromosome nomenclature. Hereditas 126:1–16

Meddows TR, Savory AP, Lloyd RG (2004) RecG helicase promotes DNA double-strand break repair. Mol Microbiol 52:119–132

Mezard C (2006) Meiotic recombination hotspots in plants. Biochem Soc T 34:531–534

Mezard C, Vignard J, Drouaud J, Mercier R (2007) The road to crossovers: plants have their say. Trends Genet 23(2):91–99

Osakabe K, Abe K, Yoshioka T, Osakabe Y, Todoriki S, Ichikawa H, Hohn B, Toki S (2006) Isolation and characterization of the *RAD54* gene from *Arabidopsis thaliana*. Plant J 48:827–842

Paques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol R 63:349–404

Price AH, Tomos AD (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.). II: mapping quantitative trait loci using molecular markers. Theor Appl Genet 95:143–152

Primig M, Williams RM, Winzler EA, Tevzadze GC, Conway AR, Hwang SY, Davis RW, Esposito RE (2002) The core meiotic transcriptome in budding yeasts. Nat Genet 26:415–423

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715

Roeder GS (1997) Meiotic chromosomes: it takes two to tango. Gene Dev 11:2600–2621

Sanchez-Moran E, Santos J-L, Jones GH, Franklin FCH (2007) ASY1 mediates AtDMC1-dependent interhomolog recombination during meiosis in *Arabidopsis*. Gene Dev 21:2220–2233

Sears ER (1976) Genetic control of chromosome pairing in wheat. Annu Rev Genet 10:31–51

Shaked H, Melamed-Bessudo C, Levy AA (2005) High-frequency gene targeting in *Arabidopsis* plants expressing the yeast *RAD54* gene. P Natl Acad Sci U S A 102:12265–12269

Sorrells ME, La Rota M, Bermudez-Kandianis CE, Greene RA, Kanteny R, Munkvold JD, Miftahudin, Mahmoud A, Ma X, Gustafson PJ, Qi LL, Echalier B, Gill BS, Mathews DE, Lazo GR, Chao S, Anderson OD, Edwards H, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dvorak J, Zhang D, Nguyen HT, Peng J, Lapitan NLV, Gonzalez-Hernandez JL, Anderson JA, Hossain K, Kalavacharla V, Kianian SF, Choi D-W, Close TJ, Dilbirligi M, Gill KS, Steber C, Walker-Simmons MK, McGuire PE, Qualset CO (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Van Os H, Stam P, Visser RGF, Van Eck HJ (2005) RECORD: a novel method for ordering loci on a genetic linkage map. Theor Appl Genet 112:30–40

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTL. Heredity 93:77–78

Wang S, Basten CJ, Zeng Z-B (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna J, Cakir M, Poulsen D, Wang J, Raman R, Smith K, Muehlbauer G, Chalmers K, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206

West SC (1997) Processing of recombination intermediates by the RuvABC proteins. Annu Rev Genet 31:213–244

Whitby MC, Lloyd RG (1995) Branch migration of three-strand recombination intermediates by RecG, a possible pathway for securing exchanges initiated by 3′-tailed duplex DNA. EMBO J 14:3302–3310

Zickler D, Kleckner N (1999) Meiotic chromosomes: integrating structure and function. Annu Rev Genet 33:603–754