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ORIGINAL PAPER

Analysis of the barley chromosome 2 region containing the six-rowed spike gene *vrs1* reveals a breakdown of rice–barley micro collinearity by a transposition

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Abstract In cultivated barley (*Hordeum vulgare* ssp. *vulgare*), six-rowed spikes produce three times as many seeds per spike as do two-rowed spikes. The determinant of this trait is the Mendelian gene *vrs1*, located on chromosome 2H, which is syntenous with rice (*Oryza sativa*) chromosomes 4 and 7. We exploited barley–rice micro-synteny to increase marker density in the *vrs1* region as a prelude to its map-based cloning. The rice genomic sequence, covering a 980 kb contig, identified barley ESTs linked to *vrs1*. A high level of conservation of gene sequence was obtained between barley chromosome 2H and rice chromosome 4. A total of 22 EST-based STS markers were placed within the target region, and the linear order of these markers in barley and rice was identical. The genetic window containing *vrs1* was narrowed from 0.5 to 0.06 cM, which facilitated covering the *vrs1* region by a 518 kb barley BAC contig. An analysis of the contig sequence revealed that a rice *Vrs1* orthologue is present on chromosome 7, suggesting a transposition of the chromosomal segment containing *Vrs1* within barley chromosome 2H. The breakdown of micro-collinearity illustrates the limitations of synteny cloning, and stresses the importance of implementing genomic studies directly in the target species.

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Introduction

Modern day cereal species have evolved from a common ancestor over a 60 million year period (Devos 2005), with the result that a level of collinearity obtains between the model cereal rice and other major cereals studied to date (Gale and Devos 1998). Although chromosome number has not been conserved, the chromosomes of barley and wheat can nevertheless be reconstructed from rice chromosomes segments (Moore 1995; Devos 2005). Substantial sequence collinearity level exists in the coding regions of rice and both barley and wheat (Ramakrishna et al. 2002; Yan et al. 2003; Chantret et al. 2004). The complete rice genomic sequence (IRGSP 2005) and an extensive collection of barley ESTs (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) can be used together to accelerate the synteny-based positional cloning of barley genes (Perovic et al. 2004; Gottwald et al. 2004).

Uniquely for species in the Triticeae tribe, each rachis node in the *Hordeum* species carries three,

rather than one spikelet (Bothmer and Jacobsen 1985; Bothmer et al. 1995). The central spikelet is fertile and goes on to develop into a grain. In two-rowed barleys, the two lateral spikelets are reduced in size and sterile, but in six-rowed types, all three spikelets are fertile. Wild barley (*Hordeum vulgare* ssp. *spontaneum*) is two-rowed (*Vrs1Vrs1*). In wild barley, the three spikelets and their slender awns form a light and arrowhead-like dispersal unit that both facilitates seed dispersal by animals and aids seed burial (Bothmer et al. 1995; Zohary 1963). The mutation to the recessive six-rowed type (*vrs1vrs1*) is thought to have been selected after the domestication of cultivated barley because the six-rowed spike is a key trait for achieving a quantum increase in grain yield (Bothmer and Jacobsen 1985; Harlan 1995).

In this paper, we have explored the micro-collinearity in the rice and barley genomes in the region of *vrs1*. The *vrs1* locus is located on chromosome 2H (Griffee 1925; Robertson et al. 1965) in a 0.5 cM region defined by the marker loci cMWG699 and MWG865 (Komatsuda and Tanno 2004; He et al. 2004). This barley chromosome is a composite of rice chromosomes 4 and 7 (Moore 1995; Laurie 1997; Rostoks et al. 2005). Both *Ppd-H1*, a major determinant of the photoperiodic response (Dunford et al. 2002), and *sdw3*, a dwarfing gene (Gottwald et al. 2004) are located on the rice chromosome 7 portion of chromosome 2H, and a synteny-based approach proved useful in both cases as a means of increasing marker density around the target genes. Our objectives were to enrich the marker content around *vrs1* as a prelude to its map-based cloning, and to study the evolution of *Vrs1* by a comparison of its orthologues in barley and rice.

Materials and methods

Plant materials

The two-rowed barley cvs. ‘Kanto Nakate Gold’ (KNG, JP 15436) and ‘Golden Promise’ (GP, JP 15923) and the six-rowed cv. ‘Azumamugi’ (AZ, JP 17209) are maintained in the Gene Bank, NIAS, Tsukuba, Japan. A two-rowed deficiens type ‘Debre Zeit 29’ (DZ, OU E525) was obtained from the Research Institute for Bioresources, Okayama University, Kurashiki, Okayama, Japan, and a six-rowed mutant ‘New Golden M13’ (NGM13), derived from two-rowed cv. ‘New Golden’ (NG, JP 15718) (Makino et al. 1995) was obtained from Dr. T. Makino, National Institute of Crop Science, Tsukuba, Japan. A set of 99 F_{12} recombinant inbred lines, generated from the cross AZ × KNG, was employed for primary mapping, and high-resolution mapping was carried out using a selection of 79 individuals defined by particular recombination events from three segregating populations one of 6,269 gametes (Komatsuda and Tanno 2004), one of 3,562 gametes from the cross AZ × GP, and one of 3,262 gametes from AZ × KNG (See Supplemental Table S1). The set of wheat (cv. Chinese Spring)—barley (cv. Betzes) chromosome addition lines (CALs), were kindly provided by Dr. A. K. M. R. Islam, Department of Plant Science, Waite Institute, University of Adelaide, Australia. Each of the six CALs contains a single pair of barley chromosome in wheat (chromosome 1H is not represented—Shepherd and Islam 1981).

Identification of a barley–rice syntenous region

Barley markers linked to *vrs1* (Komatsuda and Tanno 2004) were located on the rice genome by BLASTN searches (http://tigrblast.tigr.org/euk-blast/index.cgi?project = osa1). Barley ESTs giving the best match to rice orthologues were selected using the Gramene database (http://www.gramene.org/Oryza_sativa/index.html). High copy number ESTs were excluded using TIGR Plant Repeat Database (http://tigrblast.tigr.org/euk-blast/index.cgi?project = plant.repeats). ESTs from wheat and other cereal species were exploited where no matching barley ESTs were available. In the final step the rice genomic DNA lying between the annotated genes was analysed by gene prediction database located at http://www.gramene.org/Oryza_sativa/index.html and further gene prediction was done at http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi, based on barley model and these were included as a source of potential sequence for marker development.

Conversion of ESTs to PCR markers

Plant DNA was extracted as described by Komatsuda et al. (1998). PCR primers of length 21 nt were designed with Oligo5 software (W. Rychlick, National Bioscience, Plymouth, MN, USA) and synthesized commercially (Bex, Tokyo, Japan). PCR amplifications were carried out in 10 µl reactions, each containing 0.25 U Ex*Taq* polymerase (Takara, Tokyo, Japan), 0.3 µM of each primer, 200 µM dNTP, 1.0–4.0 mM $MgCl_2$, 1 × PCR buffer 25 mM TAPS pH 9.3 (at 25°C), 50 mM KCl, 1 mM 2-mercaptoethanol and 20 ng genomic DNA. The PCR programme consisted of a denaturation step of 94°C/5 min, followed by 30 cycles of 94°C/30 s, 50–68.5°C/30 s and 72°C/0.5–5 min, and a final incubation step of 72°C/7 min. Reaction products

were electrophoresed on either agarose (Agarose ME, Iwai Kagaku, Tokyo, Japan) or a MetaPhor agarose (Cambrex Bio Science Rockland Inc., Rockland, MA, USA) gels, depending on amplicon size, and were visualized by ethidium bromide staining.

Development of CAPS (cleaved amplified polymorphic sequence) markers

PCR products were purified using the QIAquick PCR purification kit (Qiagen, Germantown, MD, USA) and subjected to cycle sequencing using a Big Dye kit (Applied Biosystem, Foster, CA, USA). Sequencing reactions were purified by Sephadex G-50 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and analysed with an ABI prism 3100 genetic analyzer (Applied Biosystem). Sequence data were aligned by ClustalW software (http://www.ebi.ac.uk/clustalw/). Polymorphic restriction endonuclease sites were identified by applying the Restriction Maps option at http://arbl.cvmbs.colostate.edu/molkit/mapper/.

BAC screening

Pooled DNA was extracted from the cv. Morex BAC library (Yu et al. 2000) using a standard method (Sambrook and Russell 2001), and this was used as a template for PCR screening as described elsewhere (Stein et al. 2005). Chromosome walking was achieved with markers based on BAC sequences, obtained by shotgun sequencing of the contig surrounding *vrs1* (Komatsuda et al. 2007).

Sequence analysis

The EMBOSS package (http://emboss.sourceforge.net/) was used for sequence analysis. Transposable elements were characterized by BLASTN and BLASTX search against the TREP database (http://wheat.pw. usda.gov/ITMI/Repeats). The presence of putative genes in the remaining sequences was identified by a BLASTX search against all rice and Arabidopsis proteins, and by a BLASTN search against all Triticeae ESTs. Regions in which neither genes nor repetitive sequences could be identified were submitted to RiceGAAS, the rice genome automated annotation system (http://ricegaas.dna.affrc.go.jp). Rice genomic sequences were obtained from the public rice genome sequence (Version 3 at http://www.tigr.org). For the efficient processing of large datasets, de novo Perl programs were written. Annotated sequences were presented visually using the Perl Tk module.

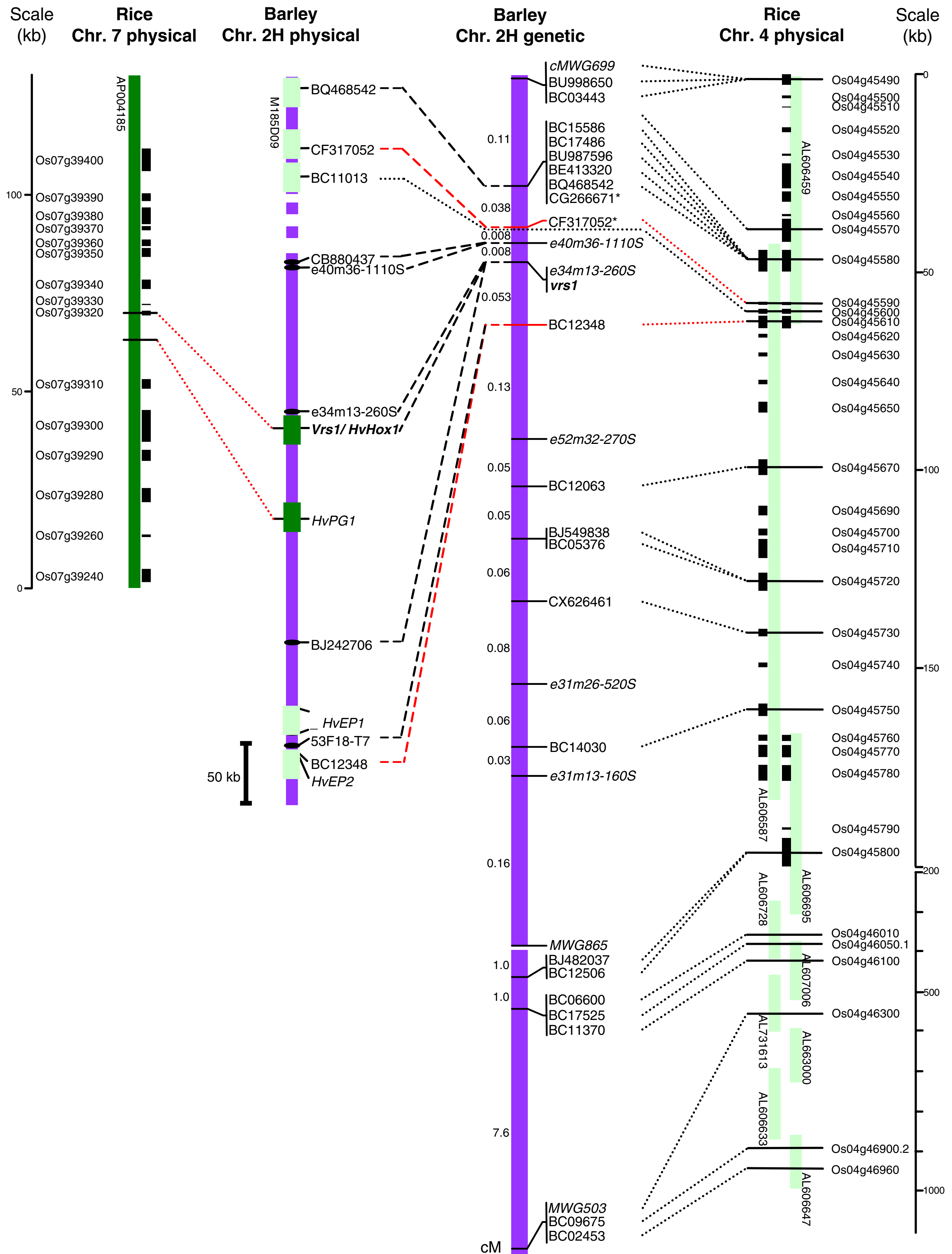
Results

Saturation of the *vrs1* region using barley EST markers

The two flanking markers cMWG699 and MWG503 share homology with rice genes Os04g45490 and Os04g46300 (respective e-values 7.3e-39 and 8.8e-44), which lie in a 980 kb contig of nine rice BAC clones on rice chromosome 4 (Fig. 1). Initially, 20 barley ESTs with homology to sequence within this contig were selected to assemble an outline comparative map. These were then supplemented with 20 barley ESTs, chosen on the basis of sequence homology to sequences within BAC clones OSJNBa0091D06 (accession AL606459) and OSJNBa0011L07 (accession AL606587), and nine barley ESTs mapping to the region defined by the overlap of these two BACs. The identity of all 49 ESTs and the experimental details relating to their conversion to a CAPS format are provided in Supplementary Table S2.

In all, 40 of the 49 ESTs were successfully PCR amplified as a single product, and an analysis of the wheat–barley CALs allowed 19 of these to be assigned to chromosome 2H. Three EST amplicons had a chromosomal location other than 2H, and these were excluded from further investigation (See Supplemental Table S2). With respect to the remaining 18 ESTs, CAL analysis was not able to deliver a chromosomal location because either the amplicons from cv. Betzes and cv. Chinese Spring co-electrophoresed, or no product was produced from cv. Betzes. Resequencing of the amplicons produced from templates of the mapping parents AZ and KNG showed that 23 of the sequences possessed at least one single nucleotide polymorphism, and all but one of these were convertible to CAPS markers (See Supplemental Table S2).

Twenty of the informative barley ESTs (e-value < e-10) were successfully mapped to an interval between cMWG699 and MWG503 including *vrs1* (Fig. 1). The linear order of these markers in barley and rice was identical. When high-resolution mapping was used BC12348 (an EST contig consisting of AJ468022 and CB881790) placed 0.053 cM distal to *vrs1*. The mapping narrowed the location of *vrs1* to a 0.06 cM interval between e40m36-1110S and BC12348 (Fig. 1). In addition, one barley EST was located on chromosome 6H probably due to the large e-value (0.19), and another barley EST was not mapped due to the instability of PCR amplification (See Supplemental Table S2).



Scale (kb)
Rice Chr. 7 physical
Barley Chr. 2H physical
Barley Chr. 2H genetic
Rice Chr. 4 physical
Scale (kb)
AP004185
Os07g39400
Os07g39390
Os07g39380
Os07g39370
Os07g39360
Os07g39350
Os07g39340
Os07g39330
Os07g39320
Os07g39310
Os07g39300
Os07g39290
Os07g39280
Os07g39260
Os07g39240
M185D09
BQ468542
CF317052
BC11013
CB880437
e40m36-1110S
e34m13-260S
Vrs1/ HvHox1
HvPG1
BJ242706
HvEP1
53F18-T7
BC12348
HvEP2
50 kb
cMWG699
BU998650
BC03443
BC15586
BC17486
BU987596
BE413320
BQ468542
CG266671*
CF317052*
e40m36-1110S
e34m13-260S
vrs1
BC12348
e52m32-270S
BC12063
BJ549838
BC05376
CX626461
e31m26-520S
BC14030
e31m13-160S
MWG865
BJ482037
BC12506
BC06600
BC17525
BC11370
MWG503
BC09675
BC02453
cM
0.11
0.038
0.008
0.008
0.053
0.13
0.05
0.05
0.06
0.08
0.06
0.03
0.16
1.0
1.0
7.6
Os04g45490
Os04g45500
Os04g45510
Os04g45520
Os04g45530
Os04g45540
Os04g45550
Os04g45560
Os04g45570
Os04g45580
Os04g45590
Os04g45600
Os04g45610
Os04g45620
Os04g45630
Os04g45640
Os04g45650
Os04g45670
Os04g45690
Os04g45700
Os04g45710
Os04g45720
Os04g45730
Os04g45740
Os04g45750
Os04g45760
Os04g45770
Os04g45780
Os04g45790
Os04g45800
Os04g46010
Os04g46050.1
Os04g46100
Os04g46300
Os04g46900.2
Os04g46960
AL606459
AL606587
AL606728
AL606695
AL607006
AL731613
AL663000
AL606633
AL606647
0
50
100
150
200
500
1000

◀ **Fig. 1** Genetic and physical maps of *vrs1* on barley chromosome 2HL, compared with the part of rice chromosomes 4 and 7. A portion of rice chromosome 4 syntenic with the barley *vrs1* region is represented by nine rice BAC clones (*right*). A rice BAC from chromosome 7, including Os07g39320 (*Oshox14*), the rice orthologue of *Vrs1*, is depicted on the *left*. The scale of the maps has been reduced in the lower parts. In the barley genetic map, markers in *italic* are STSs derived from RFLPs and AFLPs, the *other* markers are EST-derived. Markers with *asterisks* were derived from rice and maize ESTs. Rice open reading frames are shown in *black boxes* next to the BACs. *Broken lines* connect barley physical and genetic maps, *dotted lines* connect barley markers with their rice orthologues. *Red lines* define the region where the insertion/deletion of *Vrs1*/*Oshox14* occurred

Marker development using orthologous ESTs

Two further rice genes (Os04g45580, Os04g45590) were targeted for conversion to barley markers. For Os04g45590, rice EST CF317052 was converted to an STS marker, which mapped 0.016 cM proximal to *vrs1* (Fig. 1). Two maize ESTs (CG266671 and BH406686) share homology with the sequence of Os04g45580. CAL analysis showed that copies of the former are present on chromosomes 2H and 6H, and CG266671 was mapped within the cluster containing BQ468542 on chromosome 2H (Fig. 1, See Supplemental Table S3). BH406686 was assigned by CAL analysis to chromosomes 2H, 3H and 6H (See Supplemental Table S3).

Marker development using rice genomic sequence

The 17 kb of genomic sequence separating Os04g45580 and Os04g45610 was targeted for the generation of further barley markers (Fig. 1). GENEMARK.HMM predicted two distinct putative open reading frames (ORFs) GM1, GM2. The former was located by CAL analysis to four separate barley chromosomes, including chromosome 2H (See Supplemental Table S4). When the chromosome 2H GM1 sequence was cloned, six distinct repetitive sequences were revealed, indicating the unsuitability of this sequence for mapping. GM2 was assigned to both chromosomes 2H and 4H; and the former copy included a ferrochelatase sequence, which had been previously mapped to chromosome 5HL (Genbank accession AF020791). In the Gramene database we found a small (about 170 bp) predicted gene GRME00000251530 (TIGR GeneModel), but this sequence was not amplifiable from barley. Thus, no non-genic rice sequence was convertible to a barley marker, a result agreeing with Dubcovsky et al. (2001) proposal that sequence conservation between rice and barley is restricted mainly to coding regions.

Characterization of the 518 kb contig containing *vrs1*

A chromosome walk defined the location of *vrs1* within a contig of six BAC clones (Komatsuda et al. 2007). The contig spanned eight recombination events between CB880437 and BC12348 (Fig. 1). The topmost 137 kb segment of the contig (between CB880437 and e34m13-260S) had one recombination event in the high resolution mapping population, corresponding to a genetic to physical distance ratio of 0.058 cM/Mb. The lowermost 95 kb segment (between BJ242706 and BC12348) had seven recombinations corresponding to a 0.56 cM/Mb ratio, whereas, the central 220 kb segment between e34m13-260S and BJ242706, and harbouring *vrs1*, did not show any recombination.

Of the 518,343 bp combined sequence (accession number EF067844), over 82% consists of nested transposable elements (Komatsuda et al. 2007), leaving only seven ‘islands’ of a few kb in size which can be classified as non-repetitive sequence (Fig. 2). Sequences showing homology to known genes were identified in islands 3, 6 and 7, whereas, the other islands contain no such sequences. *Vrs1*/*HvHox1*, in island 3, belongs to a homeobox gene family. The *Vrs1* gene encodes a 222 amino acid polypeptide, including a homeodomain of three helices and a leucine zipper motif in its centre (Fig. 3). Orthologue of *Vrs1* was *Oshox14* (OS07g39320), which was located on rice chromosome 7 (Fig. 1). The *Oshox14* sequence includes three exons and two introns, with an exon–intron structure identical to that of *Vrs1* (Fig. 3). The barley and rice amino acid sequences are largely identical, containing a small number of conserved amino acid changes. *HvEP2* (island 7) is an expressed gene with unknown function. Ψ*HvEP1* (island 6) probably arose as a result of the duplication of *HvEP2* (or vice versa), with the sequence having been degenerated and interrupted by several insertion events involving transposable elements (Fig. 2). No genic sequence is present either in the 305 kb interval between *vrs1* and *HvEP2*, or in the 164 kb region proximal to *vrs1*. Island 5 contains a series of sequence motifs (referred to as *HvPG1*), present also in rice chromosome 7, which are reminiscent of exon/intron junctions (See Supplemental Fig. S1). However, the *HvPG1* sequence has no similarity with any known genes or ESTs. Interestingly, the rice orthologue of *HvPG1* is located only 5.7 kb from *Oshox14* on rice chromosome 7 (Fig. 1).

Genome rearrangements flanking the *vrs1* region

Screening of cv. Morex BAC library using barley markers BQ468542, CF317052 and BC11013 identified

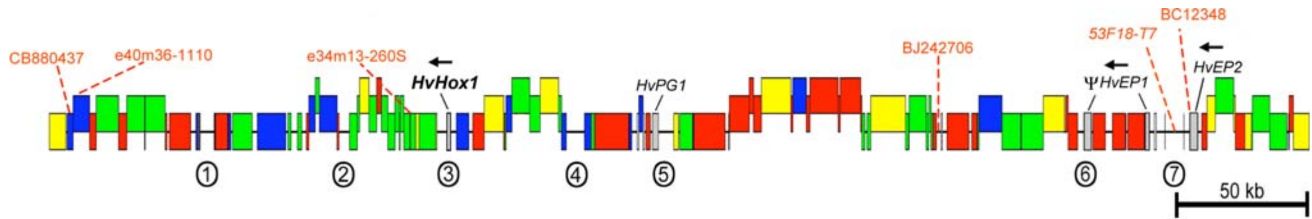


Fig. 2 Graphical annotation of a 518 kb BAC contig covering the *vrs1* region of six-rowed barley cv. Morex. Genes are depicted as *grey* and transposable elements as *coloured* boxes (red: *copia*; yellow: *gypsy*; green: *athila*; blue: CACTA). Repetitive elements that are inserted into others are raised to illustrate the nesting level. Seven segments lacking transposable elements, and thus candidate gene islands are *numbered*. Transcriptional orientation of genes is indicated by *arrows*, the pseudogene *HvEP1* is indicated by Ψ, and the positions and names of markers are displayed in *red*

7, 10 and 11 BAC clones, respectively, but none of them overlapped with the *vrs1* contig (Fig. 1, See Supplemental Table S5). The physical order of these three markers was determined based on the screening result (See Supplemental Table S5). Although a lack of polymorphism prevented genetic mapping of BC11013 (corresponding to Os04g45600), the marker was placed on barley BAC M185D09, which lies physically proximal to *vrs1* (Fig. 1). This analysis suggested the insertion/deletion of *Vrs1*/*Oshox14* occurred between Os04g45600 and Os04g45610 (Fig. 1).

Discussion

An orthologue of *Vrs1* is present on rice chromosome 7

All rice genes that could be converted to barley markers in the *Vrs1* region defined a collinear region on rice chromosome 4 (Fig. 1) but a rice orthologue of *Vrs1* was missing in this collinear rice interval. The best homologue, *Oshox14*, was located on rice chromosome 7. Since barley chromosome 2H is known to resemble as shuffling of rice chromosomes 4 and 7 (Moore 1995; Laurie 1997; Rostoks et al. 2005) this gene is likely representing the putative orthologue of *Vrs1*.

Gene cloning experiments relying on cereal–rice micro-collinearity has generated three different scenarios. The rice orthologues of wheat *Vrn1* (Yan et al. 2003), and barley *ror2* (Collins et al. 2003), *Rym4/5* (Stein et al. 2005) and *Ppd-H1* (Turner et al. 2005) are all present in the collinear region of the rice genome. However, the orthologues of maize *Adh1* (Tarchini et al. 2000) and Triticeae *Sh2* (Li and Gill 2002) lie in a non-homeologous rice chromosome. Finally, both barley *Rpg1* (Brueggeman et al. 2002) and wheat *Ph1* (Griffiths et al. 2006) lack any rice orthologue. The rice orthologue of *Vrs1* was found in a syntenous chromosome (rice 7), but it is out of the collinear region (Fig. 1).

Collinearity can be disrupted by small inversions, tandem duplications, single or multiple gene indels and transpositions (Devos 2005). It remains to be determined whether the transposition of *Vrs1* took place in the barley or the rice genome. *Vrs1* and *HvPG1* (and their rice orthologues) are closely linked to one another, so were probably transposed together. After the separation of barley and rice, one of the following two hypotheses is plausible: (1) the chromosomal

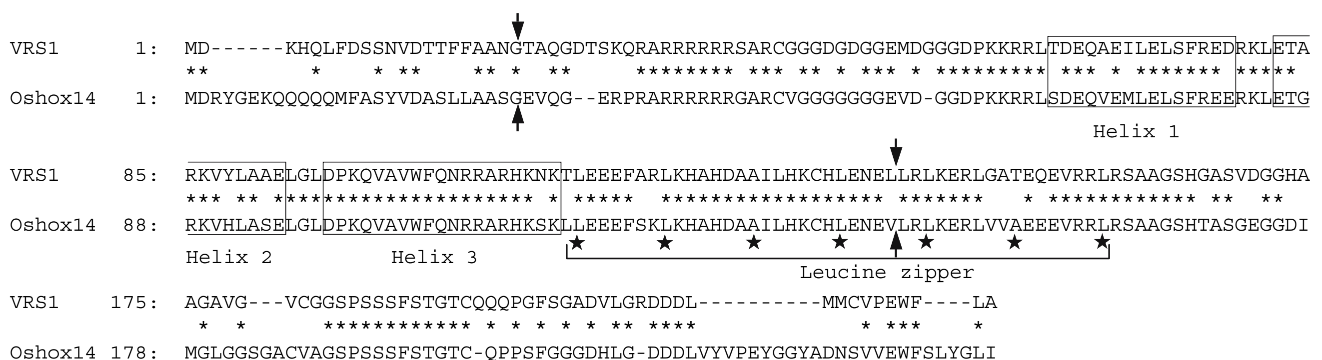


Fig. 3 Comparison of the deduced amino acid sequences of VRS1 and Oshox14. The three homeodomain helical sequences are *boxed*, and a leucine zipper region is *underlined*. Residues in common between the two sequences are indicated by *asterisks*. *Hyphens* indicate a gap introduced to facilitate alignment. *Arrows* indicate the positions of introns

segment containing *Vrs1* was transposed within chromosome 2H and (2) the chromosomal segment containing *Oshox14* was transposed from chromosomes 4 to 7. We favour the former hypotheses because overall, the rice genome has retained its integrity more strongly than has the barley one (Bennetzen and Ma 2003). *Vrs1* might have evolved a function (the suppression of lateral spikelet development) that does not exist in other cereals. We found that barley ortholog of Os07g39280, which is co-located with *Oshox14* in the rice BAC AP004185 (Fig. 1), was mapped around the centromere of barley chromosome 2H (Rostoks et al. 2005). Location of this barley orthologue (scsnp06322) may indicate the original location of *Vrs1* if the rearrangement happened in barley (hypothesis 1). Since deletions for *Vrs1* are viable (Komatsuda et al. 2007), it is plausible that *Vrs1* evolved from a 'copy' of an indispensable 'master' gene, which is still present in its ancestral location on chromosome 2H. The elucidation of the chromosomal locations of *Vrs1* orthologues in related genera of Triticeae is required to provide an evidential basis to distinguish between these two alternative hypotheses.

Genes of common descent can acquire distinct functions or specificities in different species. A prime example of this relates to *tb1* function in maize and foxtail millet (Doust et al. 2004). The gene is a major factor in the control of tillering in the former species but not in the latter. *Vrs1* encodes a transcription factor that includes a homeodomain with a closely linked leucine zipper motif (HD-ZIP), and its expression suppresses the development of lateral spikelets (Komatsuda et al. 2007). The role of *Oshox14*, whose function remains to be determined, may be closer to that played by the ancestral gene, which is clearly not the suppression of lateral spikelets. It is of course possible that the transposition event itself created a novel function for *Vrs1*, but such speculation awaits the functional analysis of *Vrs1* orthologues in other cereal species, which will allow an understanding of when, how and in which genome the transposition happened.

The *vrs1* is associated with a cold-spot of recombination and a low-gene density

Plant genomes all exhibit recombination 'hot-spots' and 'cold-spots', where recombination rates per kb are much higher or lower than the genome average (Schnable et al. 1998; Lichten and Goldman 1995). One of the most comprehensive barley genetic maps cover 1,245 cM (Kleinhofs et al. 1993) distributed over seven linkage groups. The barley haploid genome size is ~4,900 Mb (Arumuganathan and Earle 1991), yielding an average of ~4 Mb/cM. However, as in other species, recombination is unevenly distributed in barley (Künzel et al. 2000). Across the 1.08 Mb *Mla* BAC contig, it ranges from 0.2 to 5.7 cM/Mb (Wei et al. 1999), and across the 217 kb *Rph7* BAC contig from 0.4 to 13.3 cM/Mb (Brunner et al. 2003). Across the 650 kb *Rym4/5* contig, it ranges from 0.033 to 1.25 cM/Mb leaving more than 300 kb contig without recombination from >7,500 gamets (Stein et al. 2005). Künzel et al. (2000) categorized barley chromosomal subregions into those of suppressed recombination (<0.23 cM/Mb), high recombination (0.23–1.0 cM/Mb), and very high recombination (>1.0 cM/Mb, 'hot-spots'). According to the criteria, *vrs1* is located in a region of suppressed recombination. In this study the 463 kb harbouring *vrs1* has a map distance of 0.061 cM (0.13 cM/Mb), leaving the 220 kb interval including *vrs1* without any recombination events from >13,000 gametes.

Recombination in a given region can be suppressed by a high level of recombination in a neighbouring one through chiasma interference. The 95 kb region between BJ242706 and BC12348, distal to *vrs1*, showed a considerably higher rate of recombination than the adjacent region. This enhanced rate of recombination may be due to the presence of the two similar loci *ΨHvEP1* and *HvEP2*, located just 17 kb from one another (Fig. 2). Further research is needed to determine whether crossing-over events are either distributed evenly within the 95 kb segment, or are concentrated within a small region between these two loci (i.e. in a recombination hot-spot) in the seven recombinant lines detected.

Recombination is known to be concentrated within, or in the neighbourhood of genes (Schnable et al. 1998). Cytogenetic observations have shown that gene density is higher in distal regions of the chromosome arm (Gill et al. 1996; Künzel et al. 2000; Akhunov et al. 2003). The presence of 'gene islands' appears to be commonplace in grasses having a large genome (SanMiguel et al. 1996; Feuillet and Keller 1999; Tikhonov et al. 1999; Wicker et al. 2001). An average density of one gene every 20 kb was detected in a gene island of barley (Caldwell et al. 2004). However, not all genes are located within gene clusters. Thus, for example, only two genes (*Rym4/5* and *Hv-MLL*) co-locate to a 439 kb barley contig (Stein et al. 2005). We have shown here that *vrs1* is not located within a gene cluster; but rather is isolated from neighbouring genes. The location of *vrs1* in the gene poor region might be consistent with the hypothesis that *vrs1* was moved to this region through transposition in barley.

The synteny-based approach we have described here was successful in developing a high-density genetic map necessary for the map-based cloning of *Vrs1*. Even though the rice *Vrs1* orthologue is not in collinear position with that of barley, synteny-based marker-enrichment significantly assisted in the construction of a physical map for barley *vrs1*. This study illustrates the advantages of the use of rice as a tool for positional cloning in other grass species, and also stresses the importance of implementing genomic studies directly in the target species in the case of breakdown of collinearity.

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