ORIGINAL PAPER

M. Valárik · A. M. Linkiewicz · J. Dubcovsky

A microcolinearity study at the earliness *per se* gene *Eps-A^m1* region reveals an ancient duplication that preceded the wheat–rice divergence

Abstract Wheat flowering is controlled by numerous genes, which respond to environmental signals such as photoperiod and vernalization. Earliness per se (*Eps*) genes control flowering time independently of these environmental cues and are responsible for the fine tuning of flowering time. We recently mapped the *Eps-A^m1* gene on the end of *Triticum monococcum* chromosome arm 1A^mL. As a part of our efforts to clone *Eps-A^m1* we developed PCR markers flanking this gene within a 2.7 cM interval. We screened more than one thousand gametes with these markers and identified 27 lines with recombination between them. Recombinant lines were used to generate a high-density map and to investigate the microcolinearity between wheat and rice in this region. We mapped ten genes from a 149 kb region located at the distal part of rice chromosome 5 (*cdo393 – Ndk3*) on a 3.7 cM region on wheat chromosome one. This region is part of an ancient duplication between rice chromosomes 5 and 1. Genes present in both rice chromosomes were less similar to each other than to the closest wheat orthologues, suggesting that this duplication preceded the divergence between wheat and rice. This hypothesis was supported by the presence of 18 loci duplicated both in rice chromosomes 5 and 1 and in the colinear wheat chromosomes from homoeologous groups 1 and 3. Independent gene deletions in wheat and rice lineages explain the alternations of colinearity between rice chromosome 5 and wheat chromosomes 1 and 3. Colinearity between the end of rice chromosome 5 and wheat chromosome 1 was also interrupted by a small inversion, and several non-colinear genes. These results suggest that the distal region of the long arm of wheat chromosome 1 was involved in numerous changes that differentiated wheat and rice genomes. This comparative study provided sufficient markers to saturate the *Eps-A^m1* gene region and to precisely map this gene within a 0.9 cM interval flanked by the *VatpC* and *Smp* loci.

Keywords Flowering time · Earliness *per se* · Wheat · Rice · Duplication · Microcolinearity

M. Valárik · A. M. Linkiewicz · J. Dubcovsky (✉)
Department of Plant Sciences, University of California,
One Shields Avenue, Davis, CA 95616, USA
E-mail: jdubcovsky@ucdavis.edu
Tel.: +(530)-752-5159
Fax: +(530)-752-4361

Present address: A. M. Linkiewicz
Department of Biotechnology and Cytogenetics,
Plant Breeding and Acclimatization Institute Radzików,
05-870 Blonie, Poland

Introduction

Bread wheat *Triticum aestivum* L., is an allohexaploid species ($2n = 6x = 42$) originated from the hybridization between diploid wheat *Aegilops tauschii* Cosson ($2n = 2x = 14$, genome DD) and tetraploid *Triticum turgidum* L. ($2n = 4x = 28$, genomes AABB). The A genome of *T. turgidum* originated from diploid *T. urartu* ($2n = 2x = 14$, genomes AA), a species closely related to cultivated diploid *T. monococcum* L. ($2n = 2x = 14$, genomes A^mA^m; Dvorak et al. 1988, 1993). The B genome originated from a species related to *A. speltoides* ($2n = 2x = 14$, genomes SS; Dvorak and Zhang 1990; Huang et al. 2002).

Polyploid wheats are planted in a wide range of environments around the world, and this adaptability has been favored by their plasticity in the regulation of flowering time. Optimum yields in different environments can only be obtained when the plant flowers in a narrow window of time, which optimizes the available natural resources during growth and grain filling. If a

cultivar flowers too late, the grain might be filling under very hot conditions affecting quality. Late flowering cultivars might also require additional water, which may increase production costs in irrigated environments or may not be available in rain-fed environments. If a cultivar flowers too early, particularly in winter or spring cultivars planted in the fall (as in many Mediterranean regions), it may suffer late frosts, which can damage the sensitive reproductive meristem resulting in sterility and reduced yields.

A complex genetic network integrates the response of wheat to a diverse set of environmental signals and regulates the transition between the vegetative and reproductive meristem. Two important pathways within this network include the vernalization (*Vrn*) and photoperiod (*Ppd*) genes. The *Vrn* genes determine the requirement of a long period of cold temperatures to induce flowering and divide wheat into winter and spring classes. The *Ppd* genes determine the requirement of long days for flowering and divide wheat into photoperiod sensitive and insensitive cultivars. Allelic variation at the *Vrn* and *Ppd* genes can be responsible for large differences in flowering time if the plants are not exposed to the correct inductive conditions. A third group of genes, designated "earliness per se" (*Eps* hereafter) affects flowering time in a more subtle way. Allelic variation at the *Eps* genes generally results in differences of a few days in flowering time and their effect is not determined by vernalization or photoperiod (Snape et al. 2001).

The *Eps* genes are important for the fine tuning of flowering time and are under the continuous selection pressure of wheat breeders. Experiments with aneuploid and substitution lines of *Chinese Spring* grown under different vernalization and photoperiod combinations confirmed the presence of genes affecting flowering time in almost every chromosome of common wheat. Some of these chromosomes, such as homologous group 1, include more than one gene affecting heading time (Law et al. 1998). *Eps* genes also were identified on chromosomes from homoeologous groups 2 (Scarth and Law 1984; Sourdille et al. 2000; Worland and Law 1986), and 3 (Miura and Worland 1994). Miura and Worland (1994) showed the presence of a gene that increased the sensitivity to vernalization on 3B and one for promoting insensitivity to photoperiod on 3D. In addition, they found an *Eps* gene on the short arm of chromosome 3AS, which accelerated heading independently of vernalization and photoperiod (Shah et al. 1999). Two genes promoting flowering were detected on chromosome 4A and 4D (Hoogendoorn 1985) and one on chromosome arm 5AL close to the centromere. Additional *Eps* genes were detected on the distal regions of chromosome arms 5AL and 5DL (Kato et al. 1998; Sarma et al. 2000; Sourdille et al. 2000). Genes with a strong effect in delaying flowering have been reported for homoeologous group 6, but their effects on flowering time were affected by vernalization and photoperiod (Islam-Faridi et al. 1996). Finally, a major QTL for *Eps*

was reported on chromosome arm 7BS and a minor one on chromosomal arm 7DS (Sourdille et al. 2000).

Recently, we mapped an *Eps* gene in the distal region of chromosome 1A^{m}L in cultivated diploid wheat, *T. monococcum* L., which was designated *Eps-A^{m}1* (Bullrich et al. 2002). Even though allelic variation at this locus resulted in differences in flowering time of only a few days under natural conditions, those differences extended to several weeks when the plants where fully vernalized and then grown in a controlled environment under long day photoperiod and 16°C (Appendino and Slafer 2003). These phenotypic differences were large enough to map the *Eps-A^{m}1* gene as a single Mendelian locus 0.7 cM distal to *wg241*, the most distal marker of the 1A^{m}L RFLP map of *T. monococcum* at that time (Bullrich et al. 2002; Dubcovsky et al. 1996). This preliminary map was the starting point of our efforts to identify the *Eps-A^{m}1* gene using a positional cloning approach.

Positional cloning in the large genomes of wheat is now a feasible task (Feuillet et al. 2003; Huang et al. 2003; Yahiaoui et al. 2004; Yan et al. 2003, 2004; Turner et al. 2005). Large collections of mapped ESTs (Qi et al. 2004) and several bacterial artificial chromosome (BAC) libraries (Cenci et al. 2003; Lijavetzky et al. 1999; Liu et al. 2000; Ma et al. 2000; Moullet et al. 1999; Akhunov et al. 2005) are now available for wheat. In addition, a genome wide analysis of the mapped ESTs has shown that there are large blocks of conserved colinearity between the wheat and rice (*Oryza sativa* L.) genomes (Gale and Devos 1998; Sorrells et al. 2003). Therefore, the sequence of the rice genome can be used as a parallel road map to develop markers in targeted regions of the wheat genome. This strategy has been used to generate saturated high-density maps of several wheat genes (Distelfeld et al. 2004; Haen et al. 2004; Liu and Anderson 2003; Spielmeyer and Richards 2004; Yan et al. 2003).

In order to successfully use the rice information to saturate a target region in wheat it is necessary to validate the colinearity between the wheat and rice genes at a sub-centiMorgan level. In this study, we report the construction of a high-density genetic map for the wheat *Eps-A^{m}1* region using markers derived from predicted genes of the colinear rice region. Exceptions to microcolinearity between the wheat *Eps-A^{m}1* region and its corresponding region in the rice genome are discussed.

Materials and methods

Materials

The mapping populations used in this study are all derived from the cross between cultivated *T. monococcum* ssp. *monococcum* accession DV92 (spring) and wild *T. monococcum* ssp. *aegilopoides* accession G3116 (winter). DV92 and G3116 carry the *Eps-A^{m}1* alleles for late (*Eps-A^{m}1l* hereafter) and early (*Eps-A^{m}1e*) flowering, respectively (Bulrich et al. 2002).

The first mapping population included the 74 F_2 derived F_3 ($F_{2:3}$) lines used to construct a complete *T. monococcum* RFLP map (Dubcovsky et al. 1996), the second population consisted of 96 F_5 single-seed descent (SSD) plants, whereas the third population included 343 additional $F_{2:3}$ families, all from the same cross. The first population of 74 $F_{2:3}$ families and a set of single seed descent lines derived from them were previously characterized for differences in earliness per se. The effect of *Eps-A^m1* on flowering time was evaluated in two experiments including fully vernalized plants grown under long day conditions (Bullrich et al. 2002). A subset of 34 SSD lines was further evaluated in controlled chamber experiments at two different temperatures, and additional progeny tests were performed for critical recombinant lines (Bullrich et al. 2002). These detailed phenotypic data were used to map the *Eps-A^m1* gene relative to the markers developed in this study. The new recombinant lines obtained here from the second and third mapping populations will be useful to further delimit the position of the *Eps-A^m1* gene in future studies.

Methods

Genomic DNA extraction and Southern blot hybridization procedures were described before (Dubcovsky et al. 1994; Dvorak et al. 1988). We used the most distal RFLP markers in the wheat chromosome 1A^mL arm (Dubcovsky et al. 1996) to anchor the first colinear markers in the rice genome. Then we selected additional rice genes in this region from the rice genomic sequence, and used them to screen the wheat expressed sequenced tags (EST) database. Map locations of wheat ESTs and EST contigs within the physical bin map were obtained from http://www.wheat.pw.usda.gov/cgi-bin/westsql/contig.cgi. All sequence comparisons were made using the BLASTN, BLASTP or BLASTX algorithms (Altschul et al. 1997), and only alignments with *E* values lower than e^{-10} and aligned sequences longer than 100-bp were considered.

Since there is usually good structural conservation between orthologous genes in wheat and rice (Dubcovsky et al. 2001), we used the rice gene structure to predict the putative exon structure of the wheat ESTs. PCR primers were designed for each exon and PCR products including exons and introns were screened for length polymorphisms on 3.5% polyacrylamide vertical gels. If no size polymorphisms were detected, the PCR amplification products from both parental lines were cloned and sequenced. Single nucleotide polymorphisms (SNPs) between the parental lines were used to develop cleavage amplification polymorphic sequences (CAPS) or degenerate CAPS (dCAPS, Michaels and Amasino 1998) markers.

To investigate the possible presence of wheat chromosome segments in this region that were the result

from translocations from rice chromosome other than 5, we analyzed wheat ESTs mapped to the most distal bin of homoeologous group 1 (Peng et al. 2004). If a group of linked wheat ESTs were the result of a translocation, those markers would be likely linked in the rice genome. Therefore, we selected groups of wheat ESTs that were mapped to the same rice chromosome and investigated their linkage in rice.

To facilitate the description of the wheat and rice comparisons, the wheat chromosome numbers will be preceded by a 'W' and the rice ones by an 'R' throughout the text. In addition, we eliminated the "*X*" used in front of the anonymous DNA wheat loci (McIntosh et al. 2003) but that is absent in the corresponding rice names, to simplify the comparisons.

Results

Development of PCR markers flanking the *Eps-A^m1* gene

The *Eps-A^m1* gene was found to be linked to the RFLP locus *wg241* on W1L. Only the $F_{2:3}$ family #3 showed recombination between *Eps-A^m1* and *wg241* among the 74 $F_{2:3}$ families tested. Replicated progeny tests of family #3 under controlled environmental conditions confirmed that *Eps-A^m1* was 0.7 cM distal to *wg241* (Bullrich et al. 2002).

Since *wg241* was the most distal marker in the *T. monococcum* DV92 × G3116 mapping population and also in most wheat and barley (*Hordeum vulgare* L) RFLP maps (Langridge et al. 1995; Van Deynze et al. 1995) our initial priority was to develop a marker distal to *Eps-A^m1*. We first mapped the RFLP locus *gbxG259*, which was reported to be distal to *wg241* in the cross W-7984 with Opata85 in hexaploid wheat (Mingeot and Jacquemin 1999). However, in *T. monococcum gbxG259* was linked to *ksuE11*, approximately 10 cM proximal to *wg241*.

Since this marker was not distal to *Eps-A^m1*, we screened the recently developed BARC microsatellite markers for polymorphisms between our parental lines (Song et al. 2005). Two microsatellite markers, *barc287* and *barc17* (GenBank BV211560 and BV211528), were completely linked to each other at 2.7 cM distal to *wg241* in the first $F_{2:3}$ mapping population. The marker for the *barc287* locus was easier to score than *barc17* and was selected as a distal marker for the *Eps-A^m1* region. A PCR marker was also developed for the proximal *wg241* RFLP marker. WG241 primers (Table 1) amplified a 211 bp fragment, which included a polymorphic *Rsa*I restriction site.

Identification of critical recombinant chromosomes

The PCR markers for *wg241* and *barc287* were used to screen the different mapping populations for

Table 1 PCR markers developed for the wheat Eps-A^m1 high-density map

Marker type	Locus	Wheat EST	Primers	Restriction enzyme	Accession
CAP	wg241	BU100147	CTGCATGACTGTCAACTACGC CTCACCATATGCTGCTGACC	RsaI	DQ198537
CAP	VatpC	CB307863	AGGTTAGAGTTGCTGAATACGG CAAACGTATCAAGTGACTCGTAGC	BsmBI	DQ196178
LP[a]	Pp2c	CD875452	GCTTACGAGCATGT CTTCCCCTCCAGTT	–	–
CAP	Adk1	CK209708	GAAGTTGAGGCCCTGGATG CTGCAGCAGGACAAAGCTC	BstNI	DQ196179
LP	Pc13	BF473990	TGGAGTTGTCTCAAGTCTACGG CGGCATTGCAGATGTTTG	–	–
CAP	Cpe	CD490415	CCTCAATTGTGCTAACGACAT AAGGCAAGATAACGGTGGTG	BstNI	DQ196184
LP	CA600589	CA600589	TCCCTACTACCCTGGAGCTG ATGGCTGTCAGCGGCTTC	–	–
LP	Erd	CK161257	CAGACCCCGATGAATCAGTT AAGCCAATTCCGCATTTTC	–	–
CAP	Arp	CK210128	ATGTCTCTTGAGGGGACACG TTTCCTGTTATCATGCCTGAAC	BsmAI	DQ196182
LP	Smp	CA712687	GCAAGGGAAGAGAAAAGCAG TTTCTCAATCTCATGTTATCCTTCA	–	DQ196187
LP	Uge	BU099275	CAAGTATGGGGTGGAGGAGA AAGTTATTCCATGACCGACAAGA	–	–
LP	Pnp	CN012465	CTCCGGGTGCCTCAACTC TGATCTTTCTGCCAAAGTTCCT	–	–
LP	Ndk3	BQ806883	CTTGATGGCGATGAAGGTG ATGCTCCAGGATCAGGAGGT	–	–
MS[b]	barc17	–	TCGGCAGCAGCTAATGATA TTTGCCGGGGAATAAAGAAT	–	BV211528
MS	barc287	–	CGGATGGGTTACTTACTTAGGATG CGCAACTCCATTTCAGAATCATT	–	BV211560
CAP	CA608558	CA608558	GGATGTTCCCCTTCCTCCT TTCTCCTCCAGCATCCACTT	TseI	DQ196488
CAP	Cbp	CV761087	GGTCGCTCAGCTTCAAGAAC ATGCTGCAGTGCCAGTTTC	EcoRV	DQ308533
CAP	Mcr	BJ272732	CTGGGCTGTCATGCTTACAA CCCAACCTTGATCGGTATTC	EcoRV	DQ308534
CAP	Rl34	CV766923	TTACCATGAAATTTTGTGCCTTC CCCCTTGAGTTACTTCCCAAA	MspI	DQ308536
dCAP/CAP	Hip1	CK209851	GCCGATGTACCGACCAAAG CGCGGCCACGATGAT	MspI	DQ308539

[a] *LP* length polymorphism
[b] *MS* microsatellite marker (Song et al. 2005)
The last column includes GenBank accessions from sequences produced in this study (except for BV211528 and BV211560). Many of the length polymorphic markers were not sequenced.

recombination events within the Eps-A^m1 region. Three recombination events were found among the 74 $F_{2:3}$ plants used for the initial mapping, including F_2 plants #3, #46, and #69 (Table 2). The recombination event found in F_2 plant #3 was expected, because Bullrich et al. (2002) identified F_2 #3 as the only plant in this population with a crossover between Eps-A^m1 and $wg241$. These results indicated that Eps-A^m1 was approximately 0.7 cM distal to $wg241$ and 1.4 cM proximal to $barc287$.

A total of 8 recombination events between $wg241$ and $barc287$ were detected among the 96 SSD lines, and 16 more were found among the 343 F_2 lines from the third mapping population. Using a weighted average from the three mapping populations, the genetic distance between flanking markers $wg241$ and $barc287$ was estimated more precisely as 2.7 cM (Fig. 1c).

Table 2 Graphical genotypes of recombinant chromosomes with available Eps-A^m1 phenotypic information (Bulrich et al. 2002)

DNA	3	46	54	58	67	69
cdo393	H	B	H	B	H	H
If2	H	B	B	B	A	H
wg241	H	B	B	H	A	H
CA6085558	H	B	B	H	A	H
VatpC	H	B	B	H	A	H
EpsAm1[a]	A	B	B	H	A	H
Adk1	A	B	B	H	A	H
Smp	A	B	B	H	A	B
Uge	A	B	B	H	A	B
Pnp	A	H	B	H	A	B
Ndk3	A	H	B	H	A	B
barc287	A	H	B	H	A	B

[a] From Bulrich et al. (2002)
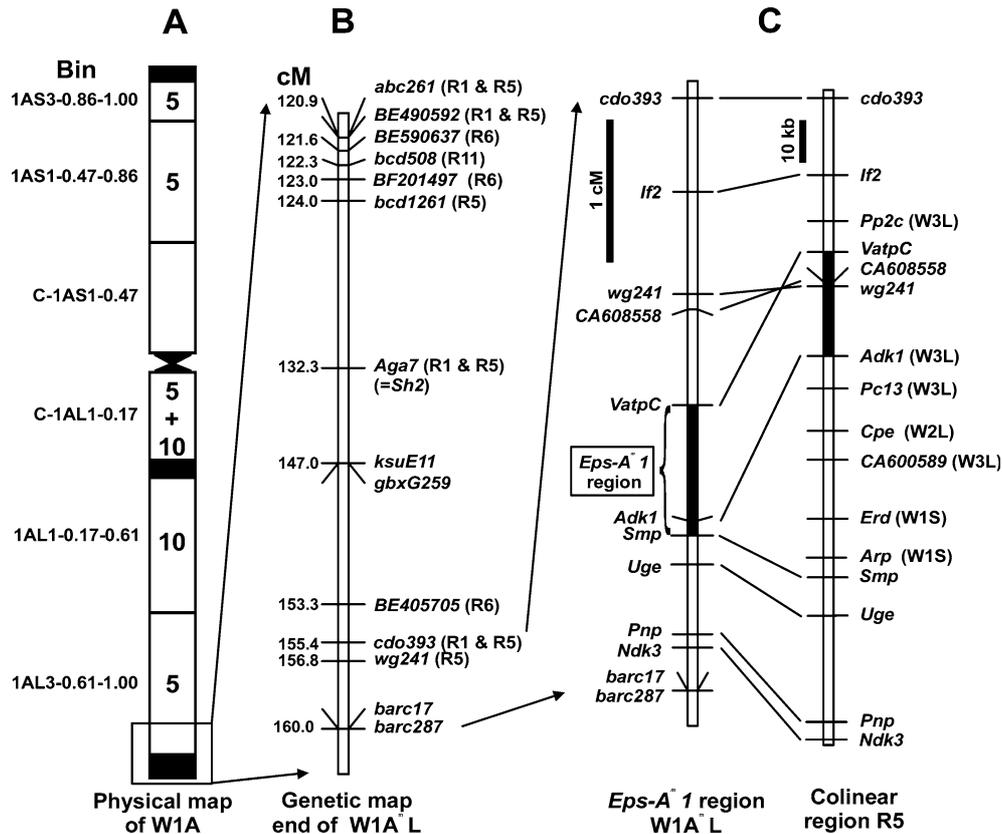
Fig. 1 Comparison between the long arms of rice chromosome 5 (R5L) and wheat chromosome 1A (W1L) in the *Eps-A^m^1* gene region. **a** Physical bin map of W1A. (http://wheat.pw.usda.gov/wEST/binmaps/). *Numbers* inside the wheat chromosome indicate the rice colinear chromosomes. **b** Linkage map of the distal region of *T. monococcum* 1A^m^L map. *Numbers within brackets* indicate the rice chromosome including the genes with highest similarity. *Numbers on the left-hand side* indicate the position of the markers in centi Morgans starting from the most distal marker in the short

arm (Dubcovsky et al. 1996). **c** Microcolinearity between the telomeric region of *T. monococcum* 1A^m^L linkage map and the distal regions of rice chromosome 5. Microsatellite markers *barc287* and *barc17* have no rice homologues. Note the inversion in the *VatpC – wg241* region. The *black rectangles* indicate the current *Eps-A^m^1* candidate gene region based on phenotypic data from Table 2. Note also the interspersed presence of R5 genes with high similarity to W1L and W3L genes

Development of wheat markers using the rice genome as template

R5 region colinear with W1AL: Previous studies have shown that W1 and R5 chromosomes are colinear, except for the centromeric regions of W1, which is mainly colinear with R10 (Gale and Devos 1998; Peng et al. 2004; Sorrells et al. 2003; Van Deynze et al. 1995). Therefore, we first selected rice genes from the distal region of the long arm of R5 as a source of new markers (Fig. 1a).

The overall colinearity between R5 and W1 was confirmed by mapping six genes from the distal region of R5L arm on the distal 40 cM of W1L arm (*abc261*, *BE490592*, *bcd1261*, *Aga7*, *cdo393*, and *wg241*). Other markers were mapped on R6 (*BE590637*, *BF201497*, and *BE405705*) and R11 (*bcd508*). These results indicate that colinearity between R5 and W1 is interrupted by several non-colinear markers. As expected wheat genomic DNA clones (*ksuE11* and *gbxG259*) and microsatellite markers (*barc17* and *barc287*) showed no significant similarity to sequences in the rice genome (Fig. 1b).

We selected the two most distal RFLP markers from W1L, *cdo393* and *wg241* (Dubcovsky et al. 1996), as the starting points to anchor the colinear region in R5. The sequences of the wheat probes used to map these two loci were significantly similar to two genes located within the same rice BAC AC130728 (Table 3), mapped 0.4 Mb proximal to R5L telomere (Fig. 2). These two genes were separated by 40 kb in rice and were 1.4 cM apart in the genetic map of *T. monococcum* (Dubcovsky et al. 1996). The colinearity between rice BAC AC130728 and the distal region of W1L was confirmed by mapping three additional rice genes from the same rice BAC (*If2*, *VatpC*, and *CA608558*, Table 3) on the distal region of 1A^m^L (Fig. 1c). An additional gene from BAC AC130728, *Pp2C*, was 85% identical (at the protein level) to wheat EST BE445496 mapped on the centromeric bin of homoeologous W3L and on the distal region of the W3A^m^L linkage map (Table 3).

The eight recombination events found between *wg241* and *VatpC* placed the *VatpC* locus 0.8 cM distal to *wg241* and *CA608558* between the previous two loci (Fig. 1c). This order is different from the order of the

Table 3 Comparison between wheat expressed sequence tags (ESTs) and rice proteins (BLASTX)

Wheat chromosome	Mapped or the best matches of wheat ESTs	Rice chromosome	Rice gene	Percent identity[a]	E value[a]
W1L	BU100147 = $wg241$	R5L	AAT44306 = Unknown protein	51	$1e^{-47}$
W1L	BE404147 = $Ndk3$	R5L	AAV59386 = $Ndk3$, Nucleoside Diphosphate Kinase 3	92	$2e^{-21}$
W1L	BE606965 = Pnp	R5L	AAV59385.1 = Pnp, Putative nuclear protein [b]	85	$8e^{-21}$
W1L	BG274687 = Uge	R5L	AAV59383 = Uge, UDP-glucose 4-epimerase	89	$2e^{-59}$
W1L	CA712687 = Smp	R5L	AAV59381 = Smp, putative small nuclear ribonucleoprotein	87	$2e^{-41}$
W1L	CK210736 = $VatpC$	R5L	AAT44305 = $VatpC$, Vacuolar ATP synt. subunit C	88	e^{-146}
W1L	AA231911 = $cdo393$	R5L + R1L	AAT44300 (BAD53405) UV-damaged DNA binding protein	88	$4e^{-33}$
W1L	BE499389-BE424894 = $If2$	R5L + R1L	AAT44302 (BAD53222) = $If2$, Translation initiation factor 2	89	$1e^{-40}$
W1L	CA608558 = $CA608558$	R5L + R1L	BAD45309 Unknown gene [c]	86	$4e^{-22}$
W1L	X14350 = $Aga7$ = $Sh2$	R5L + R1L	AAF21886 (BAD68891) = $Sh2$, ADP-glucose pyrophosphorylase	75	0.0
W1L	BE500439	R5L + R1L	AAU44105 (BAD61286) = Putative CTP synthase	84	$9e^{-85}$
W1L	BE426661	R5L + R1L	AAU44105 (BAD68695) = Putative CTP synthase	90	e^{-111}
W1L	BE518048	R5L + R1L	AAS16898 (BAD61401) = Mitogen-activated protein kinase 7-like	82	$9e^{-44}$
W1L + W3L[d]	CV066916 = $abc261$ (BE422866)	R5L + R1L	AAT77404 (BAB90499) = Ribosomal protein L18a-like	94	$2e^{-90}$
W1L + W3L	CK209708 (BE500510) = $Adk1$	R5L + R1L	AAT44307 (BAD45137) = $Adk1$, Putative protein kinase	85	$6e^{-93}$
W1L + W3L	BE500394	R5L + R1L	AAS16887 (BAB21097) = Putative LEA protein	73	$1e^{-58}$
W1L + W3L	BM140321 (BG313297)	R5L + R1L	AAT39260 (BAC10696) = Putative threonine synthase	96	$3e^{-94}$
W1L + W3L	BE490592 (BE423472)	R5L + R1L	AAT69584 (BAC00625) = Putative malate dehydrogenase	94	$4e^{-86}$
W1L + W3L	BE499131 (EST contig 17177)	R5L + R1L	AAT07616 (BAB63635.1) = Putative actin 1	96	$1e^{-51}$
W1L + W3L	BE442818 (EST contig 2251)	R5L + R1L	AAV31342 (BAD88145) = ATP-NAD kinase-like	58	$6e^{-51}$
W1L + W3L	BF291485	R5L + R1L	AAS90659 (P0471B04) = Putative galactosyltransferase	96	e^{-100}
W1L + W3L	BE445579	R5L + R1L	AAU90110 = Putative LRR-containing F-box protein	86	$4e^{-70}$
W1L + W3L	BE495786	R5L + R1L	BAB86453 = Putative AAA-metalloprotease FtsH	53	$8e^{-45}$
W1L + W3L	BE495028	R5L + R1L	AAT69643 (BAB89640) = Putative calmodulin	99	$1e^{-70}$
W1L + W3L	BE499664 (BF473231)	R5L + R1L	AAT94032 (BAB92274) = Beta-tubulin	97	$2e^{-93}$
W1L + W3L	BE488529 (BF201516)	R5L + R1L	AAT44276 (BAD82068) = Putative transcription initiation factor	79	$3e^{-77}$
W1L + W3L	BF146193 (BE636979)	R5L + R1L	AAU44062 (BAB89453) = Putative 24 kDa seed maturation protein	74	$2e^{-50}$
W1L + W3L	BF291740 (BF145392)	R5L + R1L	AAT39244 = Putative phosphoethanolamine N-methyltransferase	90	$3e^{-92}$
W1L + W3L	BG313297	R5L + R1L	BAC10696 = Threonine synthase-like	97	$6e^{-85}$
W1L + W3L	BE490596	R5L + R1L	AAT85199 (BAD82521) = Putative ATP synthase beta chain	98	$2e^{-93}$
W1L + W3L	BE499209	R5L + R1L	AAT39152 (BAB92793) = Putative ATP synthase beta subunit	87	$4e^{-55}$
W1L + W3L	BE500792	R1L	BAD81673 = Glycogenin-like protein	86	$3e^{-54}$
W1L	BE405705	R6L	BAD54128 = Putative CPRD2	61	$2e^{-65}$
W1L	BF201497	R6L	BAD36006 = Unknown protein	64	e^{-36}
W1L	BE590637	R6L	BAD37496 = Putative cytochrome P450	72	$5e^{-46}$
W3L	BE445496 = $Pp2c$	R5L + R1L	AAT44303 (B1097D05.38) = $Pp2c$, Protein phosphatase 2C	85	$4e^{-87}$
W3L	BJ256544 = $Pc13$	R5L	AAT44308 = $Pc13$, Peptidase C13 family	70	$3e^{-67}$
W3L	CA600589	R5L	AAV59378 = Unknown gene	70	$1e^{-76}$
W3L	BM138163	R1L	BAD45217 = Root hair defective 3 GTP-binding protein	81	$1e^{-416}$
W3L	BE404709	R1L	BAD53164 = RNA-binding protein-like	85	$2e^{-56}$
W3L	CV761087 = Cbp	R1L	BAD45140 = Cbp, Putative calmodulin-binding protein	62	e^{-55}

Table 3 (Contd.)

Wheat chromosome	Mapped or the best matches of wheat ESTs	Rice chromosome	Rice gene	Percent identity[a]	E value[a]
W3L	BJ272732 = *Mcr*	R1L	BAB90009 = *Mcr*, Putative mitochondrial carrier	90	e^{-104}
W3L	CV766923 = *Ri34*	R1L	BAB91779 = *Rl34*, LIN1 protein-like	78	e^{-55}
W3L	CK209851 = *Hip1*	R1L	BAB91906 = *Hip1*, Hypothetical protein	78	e^{-119}
W1S	CK210128 = *Arp*	R5L	AAV59380 = *Arp*, Adhesion regulating protein	84	e^{-117}
W1S	CK161257 = *Erd*	R5L	AAV59379 = *Erd*, Dehydration related protein	73	e^{-132}
W2	BE445284 = *Cpe*	R5L	AAV59377 = *Cpe*, Putative sodium/calcium exchanger	74	$4e^{-24}$

[a]For loci duplicated in R5L-R1L or in W1L – W3L the accession number indicated in parentheses represent the R1L or W3L locus. In these cases, percent identity and E values correspond to comparisons including the W1L and/or the R5L copy

[b]AAV59385.1 annotation is missing last two exons (confirmed by rice EST CF309058) that are the ones similar to wheat EST BE606965

[c]The duplicated gene in R5L is present but not annotated (AC130728: 111177 to 112168 bp) therefore, the accession of the corresponding R1L gene is provided

[d]Only the ESTs from the shorter bin W1BL3-0.85-1.00 were analyzed except for those duplicated in W1L and W3L, which include distal bins W1AL3-0.61-1.00 and W1DL2-.041-1.00

same genes in rice (*VatpC* – *CA608558* – *wg241*) suggesting the presence of a small paracentric inversion in this region (Fig. 1c).

Flowering data for the critical $F_{2:3}$ families (Table 2) indicated that *Eps-A^m1* was located between *VatpC* and *barc287*; therefore, additional markers were developed for this region. The first selected gene was the Nucleoside diphosphate kinase 3 (*Ndk3*) that was located 94 kb distal to *wg241* on R5 and was 92% identical (at the protein level) to wheat EST BE404147, which was mapped on the distal bin of W1L. A PCR marker for wheat *Ndk3* (Table 1) was mapped 0.3 cM proximal to *barc287* and distal to *Eps-A^m1*. Based on this data the candidate region for *Eps-A^m1* was reduced to a 1.7 cM region flanked by *VatpC* and *Ndk3*.

Fourteen genes were predicted within the 94 kb region between *wg241* and *Ndk3* in R5, ten of which showed no homology to repetitive elements and had significant similarity to wheat ESTs. Among these ten genes, four (*Pnp*, *Uge*, *Smp*, and *Adk1*, Table 3) were mapped in the targeted region of W1L, confirming the colinearity between W1 and R5 (Fig. 1c). *Adk1* was also 74% identical (E = $4e^{-78}$) to wheat EST BE500510 mapped on the centromeric bin of W3L. The graphical genotypes of critical $F_{2:3}$ families with available *Eps-A^m1* phenotypic data (Table 2) showed that *Eps-A^m1* was located within a 0.9 cM region flanked by loci *VatpC* and *Smp* and completely linked to the *Adk1* locus (Table 2, Fig. 1c). Unfortunately, none of the other rice genes present in the *wg241* – *Ndk3* interval in R5 were useful to develop additional markers for the *Eps-A^m1* candidate region. This result suggested the possibility that this W1L region was colinear to a segment from a different rice chromosome.

R5 genes not colinear with W1AL: Two genes from the *wg241*- *Smp* region in R5 were mapped on W3A^mL. These genes include a putative vacuolar processing enzyme belonging to the Peptidase C13 family (*Pc13*), and an unknown gene designated *CA600589* (Table 3). The *CA600589* locus was mapped on the centromeric bins of W3L whereas the *Pc13* locus was mapped on the distal

region of W3L by both deletion and linkage maps. In summary, wheat probes similar to rice genes from the *EpsA^m1* region were mapped on W3L in the distal region (*Pc13*), the proximal region (*Adk1* and *CA600589*), or both (*Pp2C*). A possible explanation for these results is the presence of a duplication involving these two regions, but further research is necessary.

Two other genes, *Arp* and *Erd*, which cover a 12 kb region on R5 close to the *Smp* gene were mapped on the short arm of W1 (Table 3, Fig. 1c). However, these two genes were mapped 54 cM apart in wheat suggesting that they were not likely part of a single segmental translocation. Finally, the Cation/proton exchanger 1b (*Cpe*, GenBank AB112770.1) located between *Pc13* and *CA600589* in R5 was mapped on *T. monococcum* chromosome 2A^m between markers *ksuE3* and *Chs1.2*.

Development of wheat markers from the wheat physical map

Since the colinearity between wheat and rice was interrupted in the critical region between *VatpC* and *Smp*, we decided to use wheat ESTs mapped on the most distal physical bins of the long arm of homoeologous group W1 (Peng et al. 2004) to search for additional markers for this region. We hypothesized that if the wheat segment between *VatpC* and *Smp* was orthologous to a rice chromosome segment located in a different chromosome, we should find several wheat ESTs with significant similarity to a rice chromosome region different from the colinear one on R5.

We first selected wheat ESTs mapped on the W1BL3-0.85-1.00 bin, which has the most distal break point interval (bin) among the distal bins on the long arm of homoeologous group one chromosomes. Among the 63 low copy-number ESTs mapped on bin W1BL3-0.85-1.00, 34 showed significant similarity to genes located on ten different rice chromosomes. As expected, most of these ESTs (16) were similar to genes on R5. The other rice chromosomes, including more than one gene with

Fig. 2 Comparison of duplicated chromosome segments in R5 (10.8 Mb) and R1 (22.8 Mb) including the region colinear to the wheat *Eps-A^m1* gene. These segments are in opposite orientation relative to the centromeres. Note that the segment between *Pp2C* and *Adk1* is 21 fold longer in R1 than in R5 (*black rectangles*). Loci *wg241*, *VatpC*, *Pc13*, and *CA600589* from R5 were not found in the colinear region on R1. Numbers at the sides of the chromosomes indicate the position of the genes on the respective rice chromosomes in Mb. Rice loci are designated with the corresponding wheat EST names (Table 3) to facilitate comparisons with Fig. 1
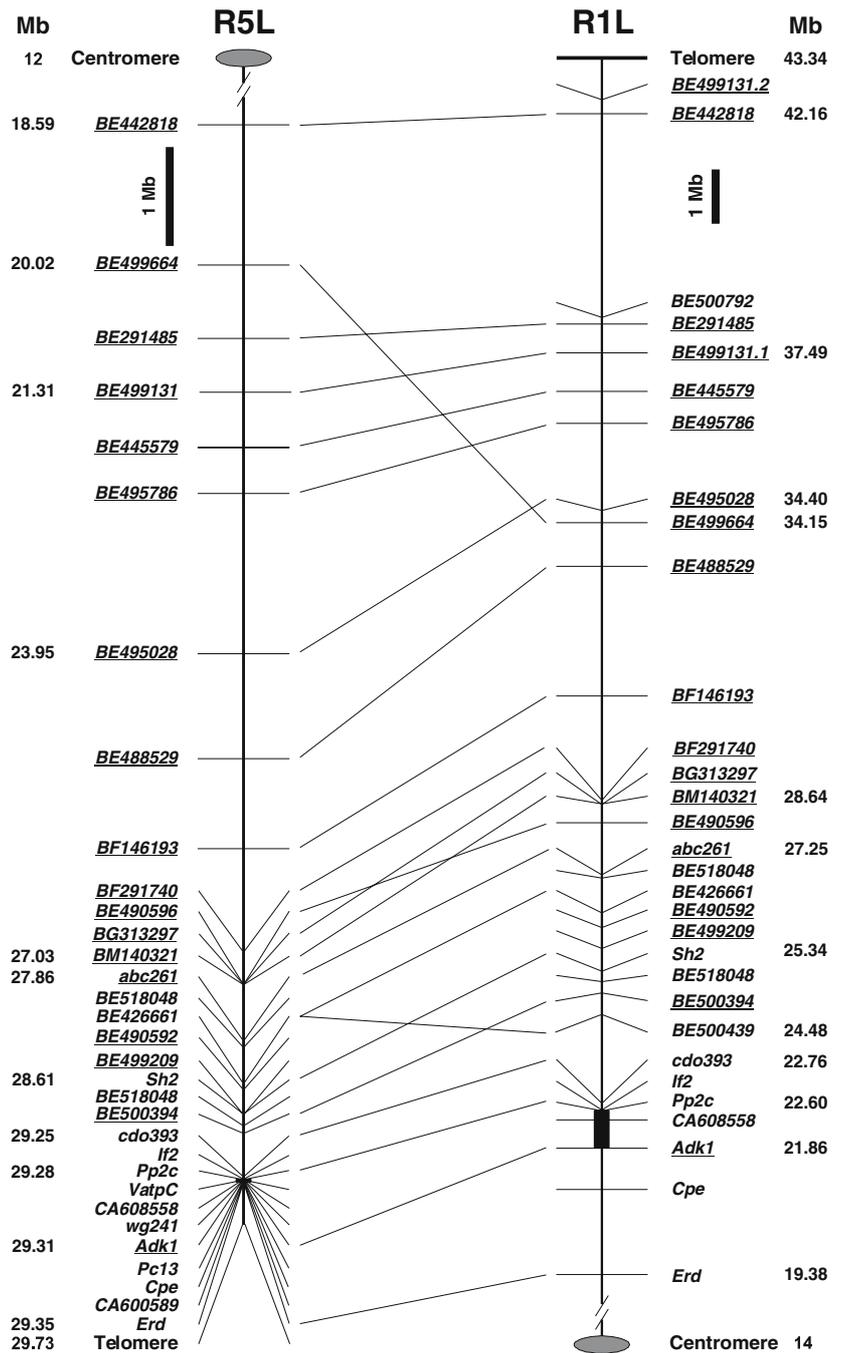
significant similarity to wheat ESTs from the distal W1L bins included R4 (3 ESTs), R6 (5 ESTs), and R10 (5 ESTs). ESTs included in R4 and R10 were scattered along the rice chromosomes and therefore, were likely not useful to identify segments of the rice genome corresponding to *VatpC–Smp* region on W1AL.

The five R6 genes with significant similarity to wheat ESTs from the distal W1L bins were within a 16 Mb region in the rice genome. Their order in R6 was BF201497 (14.4 Mb), BE405705 and BF474998 (20.7 Mb), BE590637 (26.0 Mb), and BF200636 (29.8 Mb). We mapped BE405705, BF201497, and

BE590637 on the distal region of W1AL in *T. monococcum*, but they were all located proximal to *cdo393* (Fig. 1b) and therefore, were not useful to identify additional markers within the *Eps-A^m1* region.

Evidence for a duplication involving wheat chromosomes 1 and 3

Six wheat ESTs mapped on the 1BL3-0.85-1.00 bin (BE490592, BE500439, BE500394, BE426661, BM140321, and BE518048) showed significant similarity

with rice genes located in both R5 and R1 (Table 3). This was not an unexpected result since a duplication involving segments from R1 and R5 chromosomes was reported before (Guyot and Keller 2004). Since R1 and R5 are colinear with W3 and W1, respectively (Sorrells et al. 2003), it is possible that the same rice duplication is present in W3 and W1.

To test this hypothesis we screened 335 ESTs from the three distal bins of homoeologous group 1 (W1AL3-0.61-1.00, W1BL3-0.85-1.00, and W1DL2-0.41-1.00) and from markers mapped in the distal region of the *T. monococcum* map against wheat ESTs mapped on W3L (http://www.wheat.pw.usda.gov/wEST/blast). The finding of 18 loci duplicated in both R1L – R5L and W1L – W3L (Table 3, Fig. 2 underlined loci) confirmed that the same duplication was present in both species. We found seven additional loci (BE403428, BE606632, BF474841, BF291831, BG262238, BF475035, and BM135222) duplicated in W1L – W3L that had no significant similarity to known rice genes under the threshold used in this study; and one W1L – W3L EST (BE500792) which was found only on R1L (Table 3).

The addition of the rice genes corresponding to the duplicated ESTs from the larger distal bins W1AL3 and W1DL2 extended the duplicated rice region to 22.8 Mb on R1L (19.4 – 42.5 Mb) and 10.8 Mb on R5L (18.6 – 29.4 Mb, Fig. 2). These regions included 19 loci in the same order in both chromosomes, but in inverted orientation relative to the centromeres (Fig. 2). The exceptions to the R1L – R5L colinearity included two rice genes corresponding to wheat ESTs BE499664 and BE490596 and a duplication involving rice genes corresponding to wheat ESTs BE500439 and BE426661 (79% identical to each other at the protein level; Fig. 2).

Additional support for the idea that the R1 – R5 duplication preceded the wheat rice divergence was obtained by comparing the protein sequence coded by five genes located in both R1 and R5 (*cdo393, If2, Pp2C, CA608558,* and *Adk1*) with the closest wheat homologue. The identity between the corresponding R1 and R5 proteins (average $60.4 \pm 3.7\%$) was significantly smaller ($P = 0.04$) than the identity of the rice proteins to the best matching wheat protein (average $75.4 \pm 5.0\%$).

The chromosome region involved in the R1 – R5 duplication included the *Pp2c – Adk1* region colinear to the wheat *Eps-A^{m}1* region. This region is 21 fold longer in R1L (744 kb) than in R5L (35 kb) suggesting the possibility of a large deletion in R5 (Fig. 2, black rectangles). We selected four genes from this region on R1L and mapped them on the 74 $F_{2:3}$ *T. monococcum* population to test the possibility of generating additional markers for the *Eps-A^{m}1* region. Loci *Cbp, Mcr,* and *Rl34* (Table 3) were mapped completely linked to each other on the centromeric region of W3A^{m}L, and 1 cM proximal to *CA600589*. Two separate *Hip1* loci were mapped on W3A^{m}L (Table 1): the first one in the centromeric region and the second one distal to *cdo127*.

Discussion

Colinearity between W1L and R5L

The origin of the grass family was dated approximately 55 – 70 million years ago and its monophyletic origin is supported by morphological and molecular similarities (Kellogg 2001) and by the presence of large blocks of colinear markers among the different grass subfamilies (Ahn et al. 1993; Devos 2005; Gale and Devos 1998; Keller and Feuillet 2000; Moore et al. 1995; Van Deynze et al. 1995). The conservation of gene order within the grass family provides a unique opportunity to transfer information from the completely sequenced rice genome to other grass species.

Here we took advantage of the good colinearity reported before between W1 and R5 chromosomes (Peng et al. 2004; Sorrells et al. 2003; Van Deynze et al. 1995) to develop markers for the W1L *Eps-A^{m}1* region using genes from R5. Although most of the genes we selected from R5L were mapped on W1L, numerous exceptions to the R5–W1 colinearity were encountered. A similar result was reported by (Peng et al. 2004) who found several wheat ESTs from the most distal bin on W1L with high similarity to rice genes located on five different rice chromosomes in addition to R5.

Detailed studies at the sequence level have shown numerous exceptions to the rice–Triticeae colinearity. Some of these changes are caused by structural rearrangements, such as insertions, deletions, duplications, and inversions. In other cases single non-colinear markers are flanked by perfectly colinear markers suggesting the existence of mechanisms that do not involve structural rearrangements (Dubcovsky and Dvorak 1995).

Frequent exceptions to colinearity in telomeric regions

The ends of the chromosomes seem to be particularly rich in colinearity exceptions. This increase in the number of exceptions seems to be associated with the higher gene density and higher rates of recombination observed in the telomeric regions of the large genomes of the Triticeae species (Akhunov et al. 2003b). High recombination rates were also associated with a higher frequency of colinearity interruptions among wheat homoeologous chromosomes in the distal regions relative to the centromeric regions (Akhunov et al. 2003a). Detailed sequence analyses of wheat and barley BAC clones have confirmed the presence of frequent breakages in colinearity with rice in the distal regions of the chromosomes (Brunner et al. 2003; Feuillet and Keller 1999; Kilian et al. 1997; Li and Gill 2002; Scherrer et al. 2005).

A similar observation was made here for the distal ends of R5L and W1L (Fig. 1b). A small inversion altered the orientation of the *VatpC, CA608558,* and

wg241 loci in W1L, but not in R5 suggesting the presence of a small paracentric inversion (Fig. 1c). An additional interruption to the W1L – R5L colinearity was the presence of three wheat ESTs, which showed high similarity to genes located on R6 (Fig. 1B), and two wheat ESTs with significant similarity to W1S. The latter does not seem to be the result of a segmental duplication because the two W1S ESTs were mapped more than 50 cM apart on W1S.

An additional interruption of colinearity in the W1L–R5L distal region which might have a different origin, included the presence of rice genes with significant similarity to ESTs mapped on W3L (Table 3). Rice genes colinear to W1L and W3L were interspersed in a way that was difficult to explain by structural rearrangements (Fig. 1c). An alternative explanation for this interspersed pattern could be an ancient duplication followed by differential deletions of genes in the wheat and rice lineages (Paterson et al. 2004).

The ancestral R1–R5 duplication is also present in W3–W1

Analyses of duplications in the rice genome have revealed the presence of a massive duplication event that involved at least half of the rice genes (Guyot and Keller 2004; Paterson et al. 2004; Vandepoele et al. 2003; Wang et al. 2005). Sizes of the duplicated rice blocks vary from 0.1 to 17 Mb with more than half of them longer than 1 Mb (Guyot and Keller 2004). One of these duplications involves the R1 and R5 chromosomes in the region colinear to the wheat $EpsA^m1$ region and; therefore, is particularly relevant for our study. The R1–R5 duplication was first discovered in comparative RFLP maps (Kishimoto et al. 1994) and was later confirmed in genome wide comparisons (Guyot and Keller 2004; Paterson et al. 2004; Wang et al. 2005). The R1 – R5 duplication includes a 22.8 Mb region in R1 and a 10.8 Mb region in R5 (Fig. 2) making it one of the longest duplicated regions in rice.

According to Paterson et al. (2004), a large duplication event including the R1–R5 duplication occurred approximately 70 millions years ago, before the divergence of the grass lineages. This duplication was followed by large genome rearrangements (Wolfe 2001) and "diploidization" (loss) of many duplicated genes during evolution (Paterson et al. 2004; Wang et al. 2005). Since the large grass subfamilies evolved as separate lineages for approximately two-thirds of the time (50 million years) from the large duplication, most of the gene deletions are unique for the different lineages (Paterson et al. 2004), obscuring the relationships between the duplicated segments in different species.

According to the previous evolutionary scenario, which places the ancestral duplication before the radiation of the grasses, the same duplication observed in rice chromosomes R5 and R1 should be present in wheat chromosomes W1 and W3, which are colinear with the previous rice chromosomes (Sorrells et al. 2003). This hypothesis was confirmed here by the discovery of 18 wheat ESTs from W1L duplicated in W3L and in rice R1L – R5L chromosomes (Fig. 2, underlined loci). In addition, seven ESTs mapped only on W1L were significantly similar to genes in both R1L and R5L (Table 3).

The identity between the proteins coded by the genes that are still present in both the R1 and R5 duplicated segments was significantly lower (60%) than the identity between the proteins coded by the same rice genes and their best matches in the wheat EST collection (75%). This result indicates that this duplication occurred before the divergence of the wheat and rice lineages, confirming the data from Paterson et al. (2004).

The duplicated wheat markers in the *T. monococcum* map cover a region of 35 cM (*abc261 – wg241*, Fig. 1b). However, the duplicated region in rice extends far beyond *abc261* (Fig. 2), suggesting that the colinear region in the wheat map might be also much larger. The higher level of differentiation between the duplicated segments in wheat compared to the well conserved duplication in rice (Fig. 2) may reflect more dynamic chromosomal rearrangements in wheat relative to rice, as suggested by other authors (Li and Gill 2002; Scherrer et al. 2005).

The presence of an ancient W1–W3 duplication, followed by differential deletions of genes in the wheat and rice lineages, provides a simple explanation for the observed pattern of R1 or R5 genes with significant similarity to wheat genes located in both W1L and W3L (Table 3, Fig. 1). This duplication also provides an alternative explanation for the differences observed between wheat and rice in the *Sh2 – A1* region (Li and Gill 2002). The *Sh2 – X1 – X2 – A1* region is conserved in R1L, sorghum, and maize (Bennetzen and Ramakrishna 2002), but in wheat the *X2* and *A1* genes were mapped on W3L (the expected colinear region) whereas the *Sh2* and *X1* genes were mapped in W1L. These results were originally explained by a putative translocation of the *Sh2 – X1* region to W1L (Li and Gill 2002). However, the discovery that the *Sh2 – A1* region is included within the W1L – W3L duplication suggests an alternative explanation. Under this alternative hypothesis, the four genes were originally present in both W1L and W3L, and the *Sh2* and *X1* genes were then deleted in W3L whereas the *X2* and *A1* genes were deleted in W1L. These results provide a good example of the importance of the knowledge of these ancient duplications for a correct interpretation of gene evolution in cereals.

Implications of the colinearity study on the positional cloning of $EpsA^m1$

The good overall colinearity between R5 and W1 helped us to make rapid progress in saturating the $EpsA^m1$ region with markers (Fig. 1c). However, after finishing the mapping of all the available markers in the colinear R5 regions there was still a gap of 0.8 cM in wheat

between markers *Adk1* and *VatpC*, suggesting the possibility of additional genes in this interval that were not present in R5.

The use of ESTs previously mapped in the distal region of wheat homoeologous group one provided an independent source of markers for the targeted region. The selection of multiple ESTs with similarity to closely located genes in rice chromosomes different from R5 proved to be an efficient strategy to identify a segment of W1L colinear with R6, which was outside of our target region.

The discovery of the R1 – R5 ancestral duplication provided a potential source of additional markers for the *Eps-A^m1* region. The critical region flanked by loci *Pp2c* and *Adk1* in R1 is 21 fold longer (744 kb) than the colinear region in R5 (35 kb) (Fig. 2, black rectangles). These differences in size are also reflected in the number of genes present in both regions. While there were no additional genes between the *Adk1 – wg241* region in R5, the corresponding region on R1 included more than 100 putative genes. If a deletion occurred in R5 after the wheat–rice divergence, some of the genes in the corresponding region in R1 might have homologues in both W1L and W3L. However, if the deletion event occurred before the rice–wheat divergence, it will not be possible to generate markers from the R1 region for W1L, because the deletion would be present in both species. Unfortunately, the four markers developed so far from rice genes located in the *Adk1–wg241* R1 region have been all mapped on W3, suggesting that the second hypothesis is more likely. We are currently testing additional genes from the *Adk1–wg241* R1 region, but if they all map on W3L we will abandon this region as a potential source of markers for W1L.

The markers identified so far, were sufficient to delimit a 0.9 cM candidate region for the *Eps-A^m1* gene. This map position was established using only the flowering data from our previous study (Table 2, Bullrich et al. 2002). Progeny tests for the critical nine recombinant lines within the *VAtpC–Smp* interval identified here will help us to define more precisely the position of *Eps-A^m1*, with a precision of approximately 0.1 cM.

In summary, this microcolinearity study confirmed the power of the rice genomic sequence to predict gene order of markers in wheat, but also highlighted the numerous exceptions that interrupt this microcolinearity. The presence of ancient duplications shared by the different grass genomes, differential deletions and insertions in the duplicated segments, and structural rearrangements further complicate the use of the rice genome as a road map for positional cloning in the large genomes of the Triticeae.

The existence of the R1–R5 and the W1–W3 duplications suggests the possibility of a duplication of the *Eps-A^m1* gene itself, in one or both species. Until now, we have not identified any obvious candidate for *Eps-A^m1* in the two colinear regions in rice. However, even if interesting candidate genes were identified within the rice colinear regions, a final sequencing of the wheat physical maps would be necessary to rule out the possibility of alternative candidate genes absent in the rice genome. There are now good examples of positional cloning projects in wheat and barley, where the target gene was absent from the colinear region in rice (Brueggeman et al. 2002; Yan et al. 2004).

References

Ahn S, Anderson JA, Sorrells ME, Tanksley SD, (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483-490

Akhunov ED, Akhunova AR, Linkiewicz AM, Dubcovsky J, Hummel D, Lazo G, Chao SM, Anderson OD, David J, Qi LL, Echalier B, Gill BS, Miftahudin, Gustafson JP, La Rota M, Sorrells ME, Zhang DS, Nguyen HT, Kalavacharla V, Hossain K, Kianian SF, Peng JH, Lapitan NLV, Wennerlind EJ, Nduati V, Anderson JA, Sidhu D, Gill KS, McGuire PE, Qualset CO, Dvorak J, (2003a) Synteny perturbations between wheat homoeologous chromosomes caused by locus duplications and deletions correlate with recombination rates along chromosome arms. Proc Natl Acad Sci USA 100:10836–10841

Akhunov ED, Goodyear AW, Geng S, Qi LL, Echalier B, Gill BS, Miftahudin, Gustafson JP, Lazo G, Chao SM, Anderson OD, Linkiewicz AM, Dubcovsky J, La Rota M, Sorrells ME, Zhang DS, Nguyen HT, Kalavacharla V, Hossain K, Kianian SF, Peng JH, Lapitan NLV, Gonzalez-Hernandeiz JL, Anderson JA, Choi DW, Close TJ, Dilbirligi M, Gill KS, Walker-Simmons MK, Steber C, McGuire PE, Qualset CO, Dvorak J, (2003b) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res13:753–763

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ, (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Appendino ML, Slafer GA, (2003) Earliness *per se* and its dependence upon temperature in diploid wheat lines differing in the major gene *Eps-A^m1* alleles. J Agr Sci 141:149–154

Bennetzen JL, Ramakrishna W, (2002) Numerous small rearrangements of gene content, order and orientation differentiate grass genomes. Plant Mol Biol 48:821–827

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A, (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Nat Acad Sci USA 99:9328–9333

Brunner S, Keller B, Feuillet C, (2003) A large rearrangement involving genes and low-copy DNA interrupts the microcolinearity between rice and barley at the *Rph7* locus. Genetics 164:673–683

Bullrich L, Appendino ML, Tranquilli G, Lewis S, Dubcovsky J, (2002) Mapping of a thermo-sensitive earliness per se gene on *Triticum monococcum* chromosome 1A^m. Theor Appl Genet 105:585–593

Cenci A, Chantret N, Xy K, Gu Y, Anderson OD, Fahima T, Distelfeld A, Dubcovsky J, (2003) Construction and characterization of a half million clones Bacterial Artificial Chromosome (BAC) library of durum wheat. Theor Appl Genet 107:931–939

Devos KM, (2005) Updating the 'Crop circle'. Curr Opin Plant Biol 8:155–162

Distelfeld A, Uauy C, Olmos S, Schlatter AR, Dubcovsky J, Fahima T, (2004) Microcolinearity between a 2-cM region encompassing the grain protein content locus *Gpc-6B1* on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct Integr Genomics 4: 59–66

Dubcovsky J, Dvorak J, (1995) Genome identification of the *Triticum crassum* complex (Poaceae) with the restriction patterns of repeated nucleotide sequences. Am J Bot 82:131–140

Dubcovsky J, Galvez AF, Dvorak J, (1994) Comparison of the genetic organization of the early salt stress response gene system in salt-tolerant *Lophopyrum elongatum* and salt-sensitive wheat. Theor Appl Genet 87:957–964

Dubcovsky J, Luo MC, Zhong GY, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J, (1996) Genetic map of diploid wheat, *Triticum monococcum* L, and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Dubcovsky J, Ramakrishna W, SanMiguel P, Busso C, Yan L, Shiloff B, Bennetzen J, (2001) Comparative sequence analysis of colinear barley and rice BACs. Plant Physiol 125:1342–1353

Dvorak J, McGuire PE, Cassidy B, (1988) Apparent sources of the A genomes of wheats inferred from the polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30:680–689

Dvorak J, Di Terlizzi P, Zhang H-B, Resta P, (1993) The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21–31

Dvorak J, Zhang HB, (1990) Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci USA 87: 9640–9644

Feuillet C, Keller B, (1999) High gene density is conserved at syntenic loci of small and large grass genomes. Proc Natl Acad Sci USA 96:8265–8270

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B, (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100:15253–15258

Gale MD, Devos KM, (1998) Comparative genetics in the grasses. Proc Natl Acad Sci USA 95:1971–1974

Guyot R, Keller B, (2004) Ancestral genome duplication in rice. Genome 47:610–614

Haen KM, Lu HJ, Friesen TL, Faris JD, (2004) Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. Crop Sci 44:951–962

Hoogendoorn C, (1985) A reciprocal F1 analysis of the genetic control of the time of ear emergence, number of leaves and number of spikelets in wheat (*Triticum aestivum* L.). Euphytica 34:545–558

Huang L, Brooks SA, Li WL, Fellers JP, Trick HN, Gill BS, (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Huang S, Sirikhachornkit A, Su X, Faris JD, Gill BS, Haselkorn R, Gornicki P, (2002) Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. Proc Natl Acad Sci USA 99: 8133–8138

Islam-Faridi MN, Worland AJ, Law CN, (1996) Inhibition of ear-emergence time and sensitivity to day-length determined by the group 6 chromosomes of wheat. Heredity 77:572–580

Kato K, Miura H, Akiyama M, Kuroshima M, Sawada S, (1998) RFLP mapping of the three major genes, *Vrn1*, *Q* and *B1*, on the long arm of chromosome 5A of wheat. Euphytica 101:91–95

Keller B, Feuillet C, (2000) Colinearity and gene density in grass genomes. Trends Plant Sci 5:246–251

Kellogg EA, (2001) Evolutionary history of the grasses. Plant Physiol 125:1198–1205

Kilian A, Chen J, Han F, Steffenson B, Kleinhofs A, (1997) Towards map-based cloning of the barley stem rust resistance gene *Rpg1* and *Rpg4* using rice as an intergenomic cloning vehicle. Plant Mol Biol 35:187–195

Kishimoto N, Higo H, Abe K, Arai S, Saito A, Higo K, (1994) Identification of the duplicated segments in rice chromosomes 1

and 5 by linkage analysis of cDNA markers of known function. Theor Appl Genet 88:722–726

Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S, (1995) A consensus linkage map of barley. Mol Breeding 1:389–395

Law CN, Suarez E, Miller TE, Worland AJ, (1998) The influence of the group 1 chromosomes of wheat on ear-emergence times and their involvement with vernalization and day length. Heredity 80:83–91

Li WL, Gill BS, (2002) The colinearity of the *Sh2/A1* orthologous region in rice, sorghum and maize is interrupted and accompanied by genome expansion in the Triticeae. Genetics 160:1153–1162

Lijavetzky D, Muzzi G, Wicker T, Keller B, Wing R, Dubcovsky J, (1999) Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. Genome 42:1176–1182

Liu S, Anderson JA, (2003) Targeted molecular mapping of a major wheat QTL for *Fusarium* head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Liu YG, Nagaki K, Fujita M, Kawaura K, Uozumi M, Ogihara Y, (2000) Development of an efficient maintenance and screening system for large-insert genomic DNA libraries of hexaploid wheat in a transformation-competent artificial chromosome (TAC) vector. Plant J 23:687–695

Ma Z, Weining S, Sharp PJ, Liu C, (2000) Non-gridded library: a new approach for BAC (bacterial artificial chromosome) exploitation in hexaploid wheat (*Triticum aestivum*). Nucleic Acids Res 28:e106

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R, (2003) Catalogue of Gene Symbols for Wheat. In: Pogna NE, Romano M, Pogna E, Galterio G (eds) Proceedings of 10th international wheat genetic symposium. Instituto Sperimentale per la Cerealicoltura, Rome, Paestum, Italy, http://www.wheat.pw.usda.gov/ggpages/wgc/2000upd.html.

Michaels SD, Amasino RM, (1998) A robust method for detecting single-nucleotide changes as polymorphic markers by PCR. Plant J 14:381–385

Mingeot D, Jacquemin JM, (1999) Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98:1132–1137

Miura H, Worland AJ, (1994) Genetic control of vernalization, day-length response, and earliness, by homoeologous group-3 chromosomes in wheat. Plant Breed 113:160–169

Moore G, Devos KM, Wang Z, Gale MD, (1995) Grasses, line up and form a circle. Curr Biol 5:737–739

Moullet O, Zhang H-B, Lagudah ES, (1999) Construction and characterization of a large DNA insert library from the D genome of wheat. Theor Appl Genet 99:305–313

Paterson AH, Bowers JE, Chapman BA, (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci USA 101:9903–9908

Peng JH, Zadeh H, Lazo G, Gustafson JP, Chao S, Anderson OD, Qi LL, Echalier B, Gill BS, Dilbirligi M, Sandhu D, Gill KS, Greene RA, Sorrells ME, Akhunov ED, Dvorak J, Linkiewicz AM, Dubcovsky J, Hossain K, Kianian SF, Mohmand A, Miftahudin, Wennerlind E, Anderson JA, Pathan MS, Nguyen HT, McGuire PE, Qualset CO, Lapitan NLV, (2004) Chromosome bin map of expressed sequence tags in homoeologous group 1 of hexaploid wheat and homoeology with rice and Arabidopsis. Genetics 168:609–623

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin, Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NLV, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO,

Gill BS, (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Sarma RN, Fish L, Gill BS, Snape JW, (2000) Physical characterization of the homoeologous Group 5 chromosomes of wheat in terms of rice linkage blocks, and physical mapping of some important genes. Genome 43:191–198

Scarth R, Law CN, (1984) The control of the day-length response in wheat by the Group-2 chromosomes. Plant Breed 92:140–150

Shah MM, Gill KS, Baenziger PS, Yen Y, Kaeppler SM, Ariyarathne HM, (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:1728–1732

Scherrer B, Isidore E, Klein P, Kim JS, Bellec A, Chalhoub B, Keller B, Feuillet C, (2005) Large intraspecific haplotype variability at the Rph7 locus results from rapid and recent divergence in the barley genome. Plant Cell 17:361–374

Snape JW, Butterworth K, Whitechurch E, Worland AJ, (2001) Waiting for fine times: genetics of flowering time in wheat. Euphytica 119:185–190

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB, (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sorrells ME, La Rota CM, Bermudez-Kandianis CE, Greene RA, Kantety R, Munkvold JD, Miftahudin, Mahmoud A, Gustafson PP, Qi LL, Echalier BE, Gill BS, Matthews 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 KG, Kalavacharla V, Kianian SF, Choi DW, 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

Sourdille P, Snape JW, Cadalen T, Charmet G, Nakata N, Bernard S, Bernard M, (2000) Detection of QTLs for heading time and photoperiod response in wheat using a doubled-haploid population. Genome 43:487–494

Spielmeyer W, Richards RA, (2004) Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (tin) with rice chromosome 5S. Theor Appl Genet 109:1303–1310

Turner A, Beales J, Faure S, Dunford RP, Laurie DA, (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310:1031–1034

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R, (1995) Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38:45–59

Vandepoele K, Simillion C, Van de Peer Y, (2003) Evidence that rice and other cereals are ancient aneuploids. Plant Cell 15:2192–2202

Wang XD, Shi X, Hao BL, Ge S, Luo J, (2005) Duplication and DNA segmental loss in the rice genome: implications for diploidization. New Phytol 165:937–946

Wolfe KH, (2001) Yesterday's polyploids and the mystery of diploidization. Nat Rev Genet 2:333–341

Worland AJ, Law CN, (1986) Genetic analysis of chromosome 2D of wheat. I. The location of genes affecting height, day-length insensitivity, hybrid dwarfism and yellow-rust resistance. Plant Breed 96:331–345

Akhunov ED, Akhunova AR, Dvořák J, (2005) BAC libraries of Triticum urartu, Aegilops speltoides and Ae. tauschii, the diploid ancestors of polyploid wheat. Theor Appl Genet 111:1617–1622

Yahiaoui N, Srichumpa P, Dudler R, Keller B, (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene Pm3b from hexaploid wheat. Plant J 37:528–538

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J, (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J, (2003) Positional cloning of wheat vernalization gene VRN1. Proc Natl Acad Sci USA 100:6263–6268