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Assaf Distelfeld · Cristobal Uauy · Sofia Olmos · Ana R. Schlatter · Jorge Dubcovsky · Tzion Fahima

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

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Abstract The conservation of the linear order (colinearity) of genetic markers along large chromosome segments in wheat and rice is well established, but less is known about the microcolinearity between both genomes at subcentimorgan distances. In this study we focused on the microcolinearity between a 2.6-cM interval flanked by markers *Xcdo365* and *Xucw65* on wheat chromosome 6B and rice chromosome 2. A previous study has shown that this wheat segment includes the *Gpc-6B1* locus, which is responsible for large differences in grain protein content (GPC) and is the target of a positional cloning effort in our laboratories. Twenty-one recombination events between *Xcdo365* and *Xucw65* were found in a large segregating population (935 gametes) and used to map 17 genes selected from rice chromosome 2 in the wheat genetic map. We found a high level of colinearity between a 2.1-cM region flanked by loci *Xucw75* and *Xucw67* on wheat chromosome 6B and a 350-kb uninterrupted sequenced region in rice chromosome arm 2S. Colinearity between these two genomes was extended to the region proximal to *Xucw67* (eight colinear RFLP markers), but was interrupted distal to *Xucw75* (six noncolinear RFLP markers). Analysis of different comparative studies between rice and wheat suggests that microcolinearity is more frequently disrupted in the distal region of the wheat chromosomes. Fortunately, the region encompassing the *Gpc-6B1* locus showed an excellent conservation between the two genomes, facilitating the saturation of the target region of the wheat genetic map with molecular markers. These markers were used to map the *Gpc-6B1* locus into a 0.3-cM interval flanked by PCR markers *Xucw79* and *Xucw71*, and to identify five candidate genes within the colinear 64-kb region in rice.

Keywords *Triticum turgidum* var. *dicoccoides* · Grain protein content · Colinearity · Genetic map · Rice

A. Distelfeld · T. Fahima
Institute of Evolution,
University of Haifa,
Mount Carmel, 31905 Haifa, Israel

C. Uauy · S. Olmos · J. Dubcovsky (✉)
Department of Agronomy and Range Science,
University of California,
One Shields Avenue, Davis, CA 95616-8515, USA
e-mail: jdubcovsky@ucdavis.edu
Tel.: +1-530-7525159
Fax: +1-530-7524361

A. R. Schlatter
INTA EEA Pergamino,
Buenos Aires, Argentina

Introduction

Comparative mapping in plants has provided evidence for conservation of markers and gene order (colinearity) between related genomes. The first study concerning comparative mapping in grasses was reported between the three homoeologous genomes of hexaploid wheat (Chao et al. 1989). Since then, the use of restriction fragment length polymorphism (RFLP) markers has shown considerable colinearity of marker order between the grass species in spite of a 40-fold variation in genome size and over 50 million years of evolutionary divergence time (Devos and Gale 2000; Feuillet and Keller 2002; Paterson et al. 2000). Based on these studies, the genomes of wheat (*Triticum*) and barley (*Hordeum*) were dissected in a limited number of large colinear chromosome segments from rice (*Oryza sativa*; Kurata et al. 1994; Moore et al. 1995; Van Deynze et al. 1995a). Rice is a particularly valuable reference because its small diploid genome has been almost completely sequenced (Goff et al. 2002; Yu et al. 2002).

Small rearrangements of gene content, order and orientation have been found in some of the few available studies of microcolinearity between wheat and rice (Bennetzen and Ramakrishna 2002; Feuillet and Keller 2002; SanMiguel et al. 2002). Therefore, it is important to validate the microcolinearity between a specific target region in the wheat genome and the corresponding region in rice before attempting to use the later genome as a

stepping-stone for the positional cloning of a wheat gene. In this study we explore the microcolinearity between the *Gpc-6B1* locus in wheat (Joppa et al. 1997; Olmos et al. 2003) and rice. This locus is known to affect grain protein content (GPC) in wheat, an important economic trait that determines the nutritional quality and the baking properties of this crop.

An allele for high GPC was identified in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*, referred hereafter as DIC) accession FA15-3 from Israel (Avivi 1978). Substitution lines of the chromosomes of this DIC accession in the cultivar Langdon (*Triticum turgidum* var. *durum*, referred hereafter as LDN) showed that a locus for high GPC was present on chromosome 6B (Joppa and Cantrell 1990). Joppa et al. (1997) developed a population of recombinant substitution lines (RSLs) from a cross between the substitution line LDN(DIC 6B) × LDN, and used these lines to map a QTL for GPC on the proximal region of the short arm of chromosome 6B. The segment from DIC containing that QTL was shown to be also present in hexaploid wheat varieties derived from crosses with DIC accession FA15-3, and selected for high GPC (Khan et al. 2000; Mesfin et al. 1999).

In a previous study, we mapped the *Gpc-6B1* locus as a single Mendelian factor within a 2.6-cM region between loci *Xcdo365* and *Xucw67* by using recombinant substitution lines and a large number of replications in three different field studies (Olmos et al. 2003). The specific objective of the present study was to investigate the microcolinearity of the 2.6-cM region on wheat chromosome arm 6BS and the corresponding region on rice chromosome 2, and to generate new markers and candidate genes within the target region.

Materials and methods

Plant material

The mapping population included 85 homozygous RSLs developed by L.R. Joppa from the cross LDN(DIC-6B) × LDN, 134 F_2 plants

Table 1 Triticeae ESTs and primers used to generate probes for the RFLP analysis

Locus name	TriticeaeEST	Primer sequences	Wheat PCR product (bp)	Rice PCR product (bp)	Rice physical location	TBLASTN E value
Xucw65	BF473522	AGGGGAATCGTTCCTTTCTG GGAGCATGTCAAACACACGA	340	315	AP005288	e^{-144}
Xucw66	BG606570	CCATGATGCATCTCATACCG GGCTGTTCTGAAGGAGGTCA	1,000	808	AP004087	e^{-111}
Xucw67	BE515435	GCCCTATCTCTTGCTGCACT CCGGATATAATCAGCCTCCA	310	309	AP004112	0.0
Xucw68	BE426401	TGGTGGACCTCTTTTGAAGG CAGCAGAATGTGGAGCAAGA	700	794	AP004184	e^{-61}
Xucw69	BG604419	GTGAGGCTTTGGCGTACAAG GCCACCATTTATTGGACGAT	320	303	AP005721	$2e^{-28}$
Xucw70	BG418640	CGACTCGGCCTACTCGTG AATGAACGCACTGACAGCAG	210	192	AP005647	e^{-79}
Xucw71	BU995216 BQ753500	CATTTGGCAATGCAACTGAG TCAACCCTTTTAAGCAATTTGAA	1,450	1,499	AP004061	e^{-118}
Xucw73	BF484238	GGTGCCTGAAGAGACAAAGG CCATTGCACACGTCAAAATC	300	266	AP004061	e^{-121}
Xucw74	BG907620	TTCTCGCAGTTGGCTCCTAT ATGTCTCTCCCAGACGTTCG	570	503	AP004061	0.0
Xucw75	BE231038	CAACTTCAAGCGTGGTCTGA CCCCCTTGATCATCACAACT	200	196	AP004061	e^{-102}
Xucw76	BG908065	GAGGATGGCACAGTTCGATT CCTTCACAGCCAACGGTAAT	1,500	1,656	AP003974	$3e^{-61}$
Xucw77	BE443025	ACGTTACTTGCCCGATCTGT GCTCTCTTCGCAGCATCTTT	370	328	AP004088	e^{-118}
Xucw78	BF484919	ACTGCTGGGTCACAAGCATA TTGCAAAGACAACAGGAGCTT	370	425	AP004113	e^{-100}
Xucw79	CA643341	GTGGGGTACGTGGGGAAG GTTCCATGGTTTCAAGCTCA	4,610	1,705	AP004061	e^{-24}
Xucw80	BF619095	ACAACCTGGAAACAGCTTCG CATCCTTTTGCATCCGGTAG	270	300	AP005294	e^{-159}
Xucw81	BG416625	GATCGTGATGGGGAAGAAGA GCTTGAGGATGCGGTACAC	910	577	AP005294	$6e^{-44}$
Xucw82	BF267402	CAGGGTCCTGCTCGACTG CTGAAGTTGGTGACGCACAC	500	492	AP005721	$6e^{-64}$

from the cross RSL65 × LDN used in the mapping of the GPC gene within the *Xcdo365* and *Xucw67* interval (Olmos et al. 2003), and 291 new F_2 plants from the same cross (a total of 935 gametes). The recombinant plants from the two first populations were previously evaluated for GPC in three field experiments (Olmos et al. 2003), but the new recombinant lines have not been characterized for GPC yet. All 935 gametes were used in the present wheat-rice colinearity study.

RFLP procedures

Plant nuclear DNA isolations, Southern blots, and hybridization procedures have been described before (Dubcovsky et al. 1994; Dvorak et al. 1988). Polymorphism between parental genotypes LDN(DIC-6B) and LDN was detected using DNAs digested with 24 different restriction enzymes (*Asc*I, *Apa*I, *Ava*II, *Bam*HI, *Bfa*I, *Bgl*I, *Bst*EII, *Bst*NI, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hha*I, *Hin*dIII, *Msp*I, *Nco*I, *Nde*I, *Sac*I, *Sau*3AI, *Ssp*I, *Stu*I, *Sty*I and *Xba*I). New RFLP loci mapped with polymorphic EST clones were assigned UCW numbers.

RFLP markers

Sequences of rice BACs were obtained from the International Rice Genome Project (http://rgp.dna.affrc.go.jp). The rice BAC sequences were used to screen the Triticeae EST database at http://www.ncbi.nlm.nih.gov/ using the BLASTN program. Specific pairs of primers were designed for each EST based on the gene structure predicted by the alignment of the Triticeae ESTs with the rice genomic sequence. The specific primers were used to amplify PCR products from genomic DNA of tetraploid wheat, which were then purified and used as probes for hybridization (Table 1). Sources of the probes for the *XNor*, *Xucw64*, *Xcdo365*, *XgbxR004*, and *Xpsr113* loci have been previously described (Olmos et al. 2003).

PCR-based markers

PCR markers were developed for two wheat ESTs that showed no polymorphism with any of the 24 restriction enzymes listed above. First, conserved primers were designed from the available wheat ESTs to amplify gene regions including introns. The PCR products were cloned, and eight different colonies were screened with restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Mse*I to identify clones corresponding to the two different genomes. One clone per genome was sequenced and inter-genome polymorphisms were used to design genome-specific primers. These genome-specific primers (or the polymorphic restriction sites) were tested in nulli-tetrasomic lines N6AT6B and N6BT6A (Sears 1966) to assign the two types of clones to the A and B genomes. Specific primers for the B genome were used to amplify the LDN and LDN(DIC-6B) alleles from genomic DNA. The amplified products were sequenced and the observed polymorphisms were used to develop PCR markers.

Gene annotation

Predicted open reading frames (ORFs) and potential exon/intron boundaries for the rice region were obtained from the TIGR automatic annotation (http://www.tigr.org) and Gramene (http://www.gramene.org). The potential identities of predicted coding regions were tested by searches against the non-redundant protein and DNA databases, and against the EST database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) using the BLASTN, BLASTX, and BLASTP programs. Predicted ORFs were used to search by TBLASTX the cereal repeats database at TIGR (http://www.tigr.org/tdb/e2k1/osa1/blastsearch.shtml) and the TREP database at Grain Genes (http://wheat.pw.usda.gov/ggpages/Repeats/index.shtml) to eliminate predicted proteins with similarities to retro elements.

Results

RFLP markers

RFLP markers *Xpsr113* and *Xpsr8* were previously shown to detect loci on the short arm of wheat chromosome 6 (Jia et al. 1996) and on rice chromosome 2 (Harushima et al. 1998). These two markers define a 28-cM segment in wheat 6BS that includes the QTL for GPC (Joppa et al. 1997; Khan et al. 2000), and a 29-cM region in rice 2S that served as the start point for the present wheat-rice comparative study (Fig. 1).

A clear contraction of the genetic distances was observed in the wheat genetic map relative to the rice genetic map in the proximal region of the chromosomes (Fig. 1, 2). RFLP marker *Xpsr113* was mapped 14 cM distal to the centromere in rice (Harushima et al. 1998) and 7 cM distal to the centromere in wheat (Marino et al. 1996). If the comparison is extended to the centromere-*Xucw67* interval, the genetic distance in wheat (11 cM) is one-third of the genetic distance in rice (35 cM, Fig. 1).

BLAST searches using the rice BAC sequences covering the region between *Xpsr8* and *Xpsr113* detected significant similarity ($E < e^{-20}$) with 64 wheat and barley ESTs. We successfully amplified 30 genes from tetraploid wheat genomic DNA and used them as RFLP probes. The size of the successfully amplified PCR product from

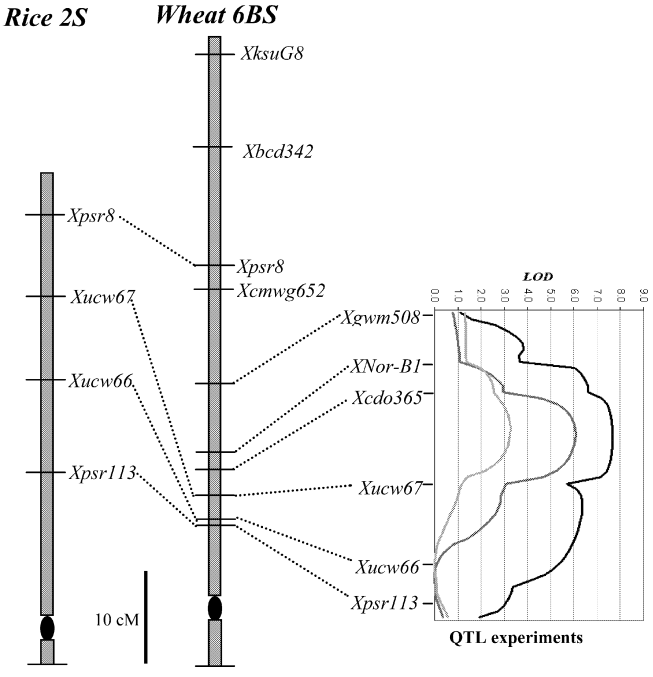

Fig. 1 *Left* Colinearity between rice chromosome arm 2S and wheat genetic maps of chromosome arm 6BS. *Right* QTL analyses for grain protein content (Olmos et al. 2003). Centromeres are indicated with a *black oval*

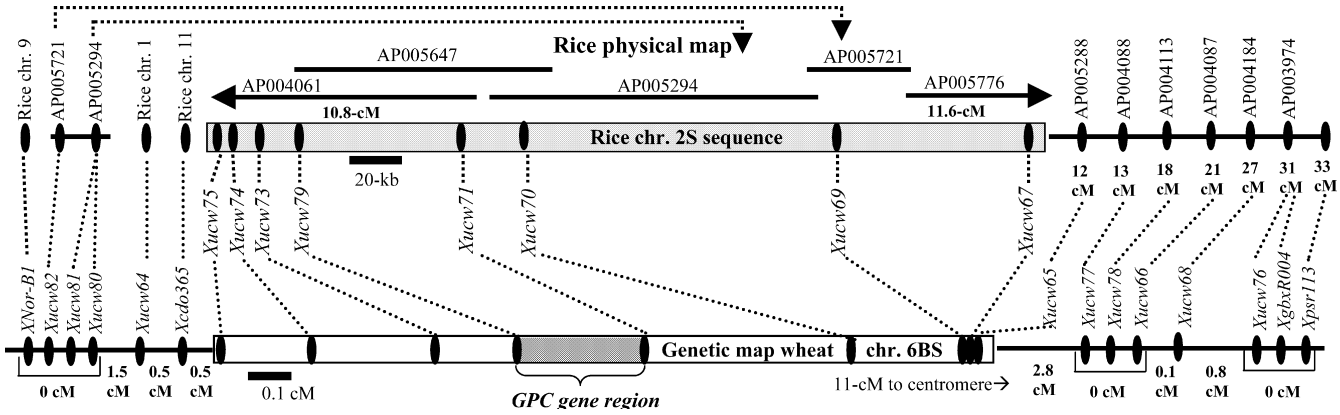

Fig. 2 Microcolinearity between the *Gpc-B1* region in wheat chromosome 6B and rice chromosome 2. Lines representing BAC clones *above* the rice chromosome are proportional to the length and position of the clones in the rice physical map. The positions of the genes in the rice genomic sequence (*gray rectangle*) were compared with the genetic distances in the colinear region in wheat (*white rectangle*). *Lines outside the rectangles* indicate the comparison of the genetic maps of wheat and rice adjacent to the *Gpc-B1* region. Determination of GPC content in the critical recombinant lines was used to map the *Gpc-B1* locus between *Xucw79* and *Xucw71* (*dark gray region*)

Table 2 PCR-based markers for genes *Xucw71* and *Xucw79*

Locus name and marker type	Primer name	Primer sequences	Restriction enzyme
Xucw71 CAPS	UCW71-BF	TGGACTTTCTATTTCTCCGTACC	*Bsm*I
	UCW71-R	TCAACCCTTTTAAGCAATTTGAA	
Xucw79 Allele specific	UCW79-LDNF	CCGATGCGATCTTAGCACAT[a]	–
	UCW79-LDNR	GCTTTGTGTTTTCCTGGCTA	
	UCW79-DICF	GACCGATGCGATCTTAGCATAC[a]	
	UCW79-DICR	CCCTGTTCCGGTTTTCCTAT	
Xucw79 dCAPS	UCW79-dCAPsF	AGATAACGACCGATGCGATCTTAGTA[b]	*Acc*I
	UCW79-dCAPSR	TCCTTTTTCCGATTTTCTTTGTGT	

[a] The underlined base pairs are SNPs between LDN and DIC
[b] The underlined T is a degenerate base pair (original sequence is C) that generates a unique *Acc*I restriction site in DIC or a unique *Rsa*I site in LDN

wheat gDNA was generally similar to that predicted based on rice genomic sequences with the exception of UCW79, which had an expected size of 1,705 bp based on the rice sequence but amplified a 4.5-kb segment in wheat due to the presence of a larger first intron (Table 1).

Seventeen RFLP probes and PCR markers showed polymorphism between the parental lines and were mapped in wheat using the recombinant lines (Table 1, Fig. 2). In addition to the new markers, Fig. 2 also includes loci *XNor-B1*, *Xucw64*, *Xcdo365*, *XgbxR004*, and *Xpsr113* selected from a previous mapping study (Olmos et al. 2003). In the current study, we mapped eight new markers (six RFLP and two PCR markers) within the 2.6-cM interval delimited by loci *Xcdo365* and *Xucw65* previously shown to include the *Gpc-B1* locus (Fig. 2).

PCR markers

To develop a PCR marker for locus *Xucw71* we amplified a 1,450-bp fragment from LDN using the primers indicated in Table 1. The amplified product was cloned and two groups were identified, sequenced, and assigned to A and B genomes using nulli-tetrasomic lines. Sequence differences between the A and B genome were used to design B-genome-specific primer UCW71-BF (Table 2). This primer was used in combination with UCW71-R (Table 2, same as conserved *Xucw71* reverse primer indicated in Table 1) to amplify and sequence a 1-kb segment from the B genome of DIC and to compare it with the previous sequence of LDN. Comparison of the two B-genome sequences revealed the presence of one single nucleotide polymorphism (SNP) that was used to develop a cleavage amplified polymorphic sequence (CAPS) marker. Digestion of the amplified product with restriction enzyme *Bsm*I produced fragments of 385 bp in LDN and 429 bp in DIC that were used to map *Xucw71* in the critical recombinant plants (Fig. 3A).

To develop a PCR marker for locus *Xucw79* we amplified and cloned a 4.5-kb fragment from LDN and DIC using the primers indicated in Table 1. PCR fragments from different transformed colonies were tested for polymorphism with a panel of restriction enzymes. We identified two types of clones from each parental line using a polymorphism detected with restriction enzyme *Mse*I. The same polymorphism was tested in the nulli-

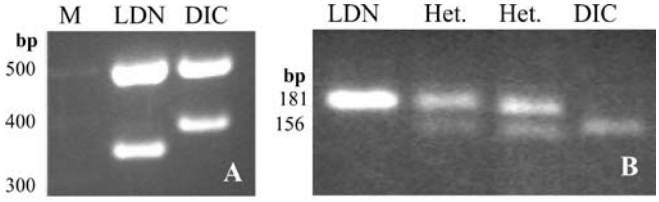

Fig. 3 **A** Cleavage amplified polymorphic sequence (CAPS) marker for locus *Xucw71*. PCR products were digested with restriction enzyme *Bsm*I. **B** Degenerate CAPS marker for locus *Xucw79*. PCR products were digested with restriction enzyme *Acc*I (LDN, Langdon; DIC, *Triticum turgidum* var. *dicoccoides*; Het., heterozygous plants)

tetrasomic lines for homoeologous group 6, and the two types of sequences were assigned to the A and B genome. We sequenced the B-genome clones from DIC and LDN and found two close SNPs between LDN (CAT) and DIC (TAC) that were used to develop allele-specific primers UCW79-LDNF and UCW79-DICF (Table 2). Two different reverse primers (UCW79-LDNR and UCW79-DICR) were used to generate products of different size (204 and 293 bp, respectively) that could be analyzed simultaneously in the same gel.

The allele-specific primers required a careful optimization since occasional faint amplifications were observed in the negative alleles. Therefore, to confirm the mapping results we developed a degenerate CAPS marker (Michaels and Amasino 1999). Primer UCW79-dCAPSF (Table 2) includes a degenerate T in the second base from the 3′ end (original sequence is C), creating a unique *Acc*I restriction site in the amplified product from DIC and a unique *Rsa*I restriction site in the amplified product from LDN. We used this primer in combination with the B-genome-specific primer UCW79-dCAPSR (Table 2) to amplify a 181-bp fragment that after digestion with *Acc*I produced a polymorphism between DIC (156 bp + 25 bp) and LDN (181 bp; Fig. 3B). This polymorphism was used to map *Xucw79* in the critical recombinant plants (Fig. 2).

Microcolinearity between wheat chromosome 6 and rice chromosome 2

Twenty-one recombination events were identified between *Xcdo365* and *Xucw67* among the 935 gametes included in this study (0.107 cM per recombination event; Fig. 2). These recombination events were used to develop a high-density map of the markers identified in this region and to establish the sub-cM colinearity with rice. The eight wheat RFLP loci mapped in the 2.1-cM interval between *Xucw75* and *Xucw67* were colinear with a 350-kb region in rice encompassing five overlapping BACs (Fig. 2). These five rice BACs were previously assigned by anchor RFLP markers to a region between 10.8 and 11.6 cM in the genetic map of rice chromosome arm 2S (http://www.tigr.org/tigr-scripts/IRGSP/assignMap.pl?chr=2&site= All&markerSource=All). Eight additional RFLP markers mapped proximal to *Xucw67* in the wheat genetic map were also colinear with the corresponding genes in rice, as inferred from the genetic location assigned to the rice BACs including these genes (Fig. 2).

Table 3 Genotypes of the subset of recombinant substitution lines (*RSLs*) previously characterized for grain protein content (GPC; Olmos et al. 2003). Eight additional markers were mapped between previous flanking markers *Xcdo365* and *Xucw65* (D, DIC alleles; L, LDN alleles). A change between gray and white cells indicates a recombination event

RSL	Xcdo 365	Xucw 75	Xucw 74	Xucw 73	Xucw 79	Gpc-6B1*	Xucw 71	Xucw 70	Xucw 69	Xucw 67	Xucw 65
113	L	D	D	D	D	D	D	D	–	D	D
116	L	D	D	D	D	D	D	D	D	D	D
8	L	L	L	D	D	D	D	D	D	D	D
121	L	L	L	L	L	D	D	D	D	D	D
28	D	D	D	D	D	D	L	L	L	L	L
110	D	D	D	D	D	D	D	L	L	L	L
117	D	D	D	D	D	D	D	L	L	L	L
119	D	D	D	D	D	D		D	L	L	L

[a] From Olmos et al. 2003

Contrasting with the almost perfect colinearity observed in the proximal region, the six markers mapped distal to *Xucw75* in wheat were not colinear with rice. The translated protein for *Xcdo365* (AA231882, 0.5 cM distal to *Xucw75*) was significantly similar (87% similar $E = 9e^{-97}$) to the rice protein Glu-tRNA (Gln) amidotransferase subunit B (5030.t00010), located in rice chromosome 11. The translated protein for *Xucw64* (AA231882, 1 cM distal to *Xucw75*) was significantly similar (98% similar $E = e^{-52}$) to rice protein protoheme IX farnesyltransferase (4984.t00024) located in rice chromosome 1. The location of the *Xucw64* locus on rice chromosome 1 was confirmed by hybridization of the UCW64 probe with Nipponbare BAC filters. The BAC end sequences of the ten positive clones showed significant similarity with sequences from rice chromosome 1. Finally, the NOR locus that was mapped 2.5 cM distal to *Xucw75* on wheat chromosome arm 6BS was previously mapped on rice chromosome 9 (Kurata et al. 1994). Three additional wheat ESTs linked to the *XNor-B1* locus were colinear with rice BACs AP005294 and AP005721, and therefore out of colinearity with the other markers of rice chromosome 2 identified in the region. The presence of a large inversion in one of the genomes was ruled out because other markers located within this region were in the same order in wheat and rice (e.g. *Xucw75*, *Xucw74*, *Xucw73*, *Xucw71*, *Xucw70*, and *Xucw69*).

Delimitation of the GPC gene region

Table 3 shows the graphical genotypes (Young and Tanksley 1989) of the eight homozygous RSLs with available information for the *Gpc-6B1* allele present on those lines (Olmos et al. 2003). In this table, white cells with an “L” indicate Langdon alleles, whereas gray cells with a “D” indicate DIC alleles. The *Gpc-6B1* locus was

mapped within a 0.3-cM interval defined by flanking loci *Xucw71* and *Xucw79*.

The wheat region between *Xucw71* and *Xucw79* corresponds to a 64-kb region in rice located within Nipponbare BAC AP004061 (Fig. 2). Preliminary automatic annotation of this BAC resulted in 24 putative genes (TIGR, assembly OJ1407_E09). Wheat marker *Xucw79* corresponds to rice locus 2474.t0010 (heterotrimeric G-protein gamma subunit 2) and *Xucw71* to rice locus 2474.t0022 (Ca^{2+}/H^{+} antiporter-related protein). Eleven putative genes were predicted in the rice region between 2474.t0010 and 2474.t0022 that is colinear to the wheat chromosome segment including the *Gpc-6B1* gene. Four of these predicted proteins have significant similarities to known transposable elements in the Triticeae Repetitive Element Database or in TIGR Cereal Repeats databases (loci 2474.t0015:$7e^{-13}$, 2474.t0016:$2e^{-41}$, 2474.t0018:$2e^{-54}$, and 2474.t0019:$1e^{-9}$), and two were short hypothetical proteins with no significant similarity to any known protein (loci 2474.t0020 and 2474.t0021). Therefore, in addition to flanking genes 2474.t0010 and 2474.t0022, five genes were identified in the rice region colinear to the *Gpc-6B1* candidate region in wheat including loci 2474.t0011 (putative porphobilinogen synthase), 2474.t0012 (leucine-rich repeat/extensin 1), 2474.t0013 (similar to expressed protein NP_565196 from *Arabidopsis*), 2474.t0014 (similar to RNA recognition motif-containing protein NP_200621.1 from *Arabidopsis*), and 2474.t0017 (putative auxin-independent growth promoter).

Discussion

Wheat-rice microcolinearity

Initial comparisons of rice RFLP maps with wheat and barley maps showed the presence of extensive blocks of colinear markers (Gale and Devos 1998; Maroof et al. 1996; Van Deynze et al. 1995a). An almost complete colinearity was reported between rice chromosome 2 and wheat chromosome 6 (Gale and Devos 1998; Sorrells et al. 2003). A recent study including 400 wheat ESTs assigned to 22 deletion bins on wheat homoeologous group 6, showed that in 20 out of the 22 bins the number of matches with orthologous genes on rice chromosome 2 was higher than the combined matches with the other 11 rice chromosomes (Sorrells et al. 2003). The exceptions were bins 6BS-5 (3 matches with rice 2 and 10 matches with other rice chromosomes) and 6DS-4 (1 match with rice chromosome 2 and 3 with other rice chromosomes). The numerous exceptions to the colinearity between wheat chromosome 6 and rice chromosome 2 within the 6BS-5 bin are particularly relevant for this study, because the *Gpc-6B1* gene was mapped within this bin (Olmos et al. 2003). An earlier study (Van Deynze et al. 1995b), reported that three RFLP markers (*Xcdo365*, *Xcdo270*, and *XNor-B1*) from wheat chromosome arm 6BS were not syntenic with rice chromosome 2. Results from our study suggest that the interruption in the colinearity between rice chromosome 2 and wheat chromosome 6 occurred somewhere within the 0.5-cM region that separates colinear marker *Xucw75* and non-colinear marker *Xcdo365*. This region of low wheat-rice colinearity is close to the nucleolar organizer locus (*XNor*), a region that shows a particular lack of colinearity, even within the Triticeae species (Dubcovsky and Dvorak 1995). Except for the interruption in colinearity at the distal end of the analyzed segment, the nine wheat genes included in the proximal 2.1-cM region between *Xucw75* and *Xucw67* showed good microcolinearity with a 350-kb region in rice chromosome 2. A similar example of microcolinearity between wheat and rice was found in the *Vrn1* region in chromosome 5AL (Yan et al. 2003). Thirteen genes were found in perfect colinear order with the exception of two tandem gene duplications in wheat and barley and one gene inversion in barley (Dubcovsky et al. 2001; Yan et al. 2003). The *Vrn1* and *Gpc-B1* loci are located at similar positions within the physical maps of their respective chromosomes. The 6BS-5 bin is proximal to the NOR between fraction lengths (FL) 0.5 and 0.7 (considering the satellite as part of the total length; Gill et al. 1993). The *Vrn1* region is located between the breakpoints in deletion lines 5AL-6 and 5AL-17 between FL 0.68 and 0.78 (Sutka et al. 1999). Excellent colinearity between wheat and rice was also described for the *Ph1* region, located close to the centromere of the long arm of wheat homoeologous group 5 (Roberts et al. 1999).

More frequent interruptions in the wheat-rice microcolinearity were observed in studies of more distal regions of the wheat chromosomes. The *Lrk/Tak* locus located on the distal region of the short arms of homoeologous group 1 is a duplication from an ancestral locus on the short arm of chromosome 3 that is also present in the colinear regions in rice and maize (Feuillet and Keller 1999). A similar situation was found in the *Sh2* locus (same as *XAga7* in wheat) that was mapped in the distal region of the long arm of wheat homoeologous group 1. The *Sh2/X1/X2/A1* region is conserved in rice, sorghum and maize (Bennetzen and Ramakrishna 2002), but the *Sh2/X1* genes were translocated to a non-colinear region of the long arm of homoeologous group 1. The wheat *X2/A1* genes remained in the long arm of homoeologous group 3 in a location that is colinear with the *Sh2/X1/X2/A1* region in rice chromosome 1 (Li and Gill 2002). The *Lrk/Tak* duplication and the *Sh2/X1* translocation represent interruptions of the general colinearity previously described between wheat chromosome 1 and rice chromosomes 5 and 10 (Sorrells et al. 2003; Van Deynze et al. 1995a). The *Rpg1* region at the very end of the short arm of chromosome 7H in barley also showed several exceptions to the microcolinearity with rice. A 10- to15-kb region including three common probes moved to a non-colinear location after the rice-barley divergence (Kilian et al. 1997). In addition, the barley stem rust-resistance gene *Rpg1* was not present in the colinear region of rice (Brueggeman et al. 2002).

The better wheat-rice microcolinearity observed in the studies including proximal regions of the chromosomes compared with those including more distal regions is in agreement with general evolutionary trends observed along the large Triticeae chromosomes. These studies have shown that new loci originated by duplication and transposition, and fixed deletions are more frequent in high recombination regions at the distal ends of the wheat chromosomes (Akhunov et al. 2003a, 2003b). As a result of these trends, the distal regions of the wheat chromosome arms have been evolving faster than the proximal regions (Akhunov et al. 2003a). We hypothesize that this evolutionary trend is responsible for the higher frequency of observed exceptions to colinearity and microcolinearity between wheat and rice in the distal chromosome regions relative to the more proximal regions. A practical corollary of this trend is that the use of the rice genome sequence as a stepping-stone in positional cloning projects in wheat will be more reliable in the proximal regions than in the more variable distal regions of the wheat chromosomes.

Positional cloning of the *Gpc-6B1* gene

The long-term objective of our laboratories is to clone the high GPC gene to provide a better understanding of the physiological processes involved in the allelic differences in GPC in wheat. The microcolinearity established between the 2.1-cM region between *Xucw75* and *Xucw67* on wheat 6BS and the 350-kb region in rice chromosome 2 (Fig. 2) is a first step towards this goal. The five unmapped rice genes identified between *Xucw79* and *Xucw71* are excellent candidates to generate new molecular markers within the critical region. These five genes will require the development of PCR markers because we found no RFLP polymorphism between the parental lines. Development of PCR markers in this population is complicated by the polyploid nature of pasta wheat and by the low level of sequence polymorphism between DIC and LDN in this region (≈1 SNP/1 kb).

None of the five genes identified in the 64-kb rice region colinear to the *Gpc-6B1* candidate gene region in wheat is known to be involved in nitrogen metabolism or nitrogen transport. Therefore, it is difficult to identify a clear candidate gene at this stage. In addition, we cannot rule out the presence of additional genes in the wheat sequence colinear with rice. Examples of non-colinear wheat segments inserted in otherwise colinear regions have been discussed above (Feuillet and Keller 1999; Kilian et al. 1997; Li and Gill 2002). Sequencing of the complete candidate region in wheat will be necessary to test this hypothesis. This task will be facilitated by the recent construction of a BAC library for a tetraploid wheat recombinant line carrying the DIC allele for high GPC (Cenci et al. 2003).

In summary, the combination of the rice genome sequence and the extensive Triticeae EST collections available today were powerful tools to accelerate our positional cloning efforts in wheat. Even if the *Gpc-B1* turns out to be a non-colinear wheat gene inserted in the region, the rice sequence would still have been an invaluable resource to saturate the region with molecular markers. A similar approach has been used successfully to saturate other genomic regions in barley and wheat (Kilian et al. 1997; Roberts et al. 1999; Yan et al. 2003).

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