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ORIGINAL PAPER

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Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (*tin*) with rice chromosome 5S

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Abstract The capacity to tiller is a key factor that determines plant architecture. Using molecular markers, a single major gene reducing tiller number, formally named the tiller inhibition gene (*tin*), was mapped to the short arm of chromosome 1A in wheat. We identified a tightly linked microsatellite marker (*Xgwm136*) that may be useful in future marker-assisted selection. The *tin* gene was mapped to the distal deletion bin of chromosome 1AS (FLM value 0.86) and wheat ESTs which were previously mapped to the same deletion bin were used to identify 18 closely related sequences in the syntenic region of rice chromosome 5. For a subset of wheat ESTs that detected flanking markers for *tin*, we identified closely related sequences within the most distal 300 kb of rice chromosome 5S. The synteny between the distal chromosome ends of wheat 1AS and rice 5S appeared to be disrupted at the *hairy glume* locus and seed storage protein loci. We compared map position of *tin* with other reduced tillering mutants characterised in other cereals to identify possible orthologous genes.

Introduction

Tiller development is a key factor in determining plant architecture. Tillers are formed by the outgrowth of axillary buds that are located within the leaf axils on the basal internodes. They develop independently of the main culm and when fertile contribute significantly to grain yield. Several mutations have been reported in cereals that show an altered pattern of axillary bud development. In maize, the *teosinte branched1* (*tb1*) mutant causes a complete loss of apical dominance, allowing the unrestrained outgrowth of axillary buds (Doebley et al. 1997). In barley, the recessive *uniculm2* mutation on chromosome 6H initiates axillary buds normally but they fail to grow out and produce tillers (Babb and Muehlbauer 2003). In contrast, uniculm rice containing a mutation on chromosome 6 (*moc1*) lacked axillary buds altogether (Li et al. 2003). Additional recessive tillering mutants with reduced culm number (*rcn1*– *rcn5*) have been reported in rice, and three of these mutations, *rcn1*, *rcn2* and *rcn5*, have been mapped to rice chromosomes 6, 4 and 6, respectively (Gramene, http://www.gramene.org/index.html), *rcn1* and *rcn5* being independent of *moc1*.

Considerable genetic variation for the capacity to tiller exists within the wheat gene pool and low-tillering genotypes have been identified (Atsmon and Jacobs 1977). When grown under normal field conditions some of these low-tillering lines were uniculm and showed an enlarged spike and leaf morphology. Richards (1988), when studying the inheritance of one of these uniculm lines (line '492'), concluded that tiller number in line 492 was largely determined by a single recessive gene located on chromosome 1AS. The absence of tiller development in uniculm lines carrying the tiller inhibition gene (*tin*) was due to an altered pattern of axillary bud formation and outgrowth (W. Spielmeyer, unpublished). When near-isogenic lines were compared, low-tillering lines with the *tin* gene occasionally developed tillers from the first and second leaf axils whereas axillary buds failed to initiate and were absent in later leaf axils. Low-tillering lines in populations which segregated for the *tin* gene produced a greater harvest index, fewer sterile tillers and a larger grain size, demonstrating the agronomic potential of the *tin* gene in wheat (Richards 1988; Duggan et al. 2002).

As a first step towards isolating a candidate gene for *tin*, the gene was mapped in relation to molecular markers. Tightly linked flanking markers were identified that will assist in future development of a high resolution map. In addition, we investigated the relationship of the wheat region containing *tin* with the corresponding region in rice

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by comparing closely related EST sequences mapped in both species. Wheat ESTs that had been previously mapped to deletion bins provided a useful framework to identify closely related rice sequences and to establish the most likely syntenous region in rice for the wheat *tin* region.

Materials and methods

Plant material

The uniculm wheat line '492' and the oligoculm wheat line '380' are full sibs and originated from a cross between the cultivar 'Alpha' and a North African land race (Atsmon et al. 1986). A near-isogenic line referred to as 'Banks + *tin*' was produced by backcrossing (BC_4) the *tin* from a progenitor line of 492 into the Australian cultivar 'Banks' (Richards 1988). An additional backcross was made between Banks+ *tin* and Banks to produce 113 BC_5F_2 lines which were scored for tiller number and *hairy glume* (*Hg*). Pubescence of glumes was scored as presence/absence after the spike had fully emerged from the boot. *Hg* has previously been reported to be linked to *tin* on chromosome 1AS (Richards 1988). BC_5F_2 plants were grown in growth cabinets with a 14-h day length, 18/15°C day/night temperature treatment and with 300–350 μmol m^{-2} s^{-1} of photosynthetically active radiation. The tiller number was scored after leaf 7 was fully emerged and again during early booting. All F_2 lines were progeny tested at the F_3 generation (at least 16 individual plants) and some selected lines were scored again in the F_4 generation.

A doubled haploid (DH) population of 110 lines from a cross between the Japanese winter wheat 'Fukuho-komugi' and line 380 was kindly provided by Dr. K. Suenaga. This population was scored for tiller number in the glasshouse during the summer season under natural day length and 20/15°C day/night temperature treatment. At least four DH plants/line were assessed for tiller number after leaf 7 was fully emerged.

Mapping of the tiller inhibition gene

Microsatellite and RFLP markers from chromosome 1AS were screened on parental lines and polymorphic markers were mapped in the above families. Primer sequences and amplification conditions for microsatellite markers *Xgwm136* and *Xwmc24* are published in Röder et al. (1998) and Graingenes (http://wheat.pw.usda.gov/index.shtml). RFLP probes WHS179 was provided by Drs. V. Mohler (Freising, Germany), MWG2245 by A. Graner (IPK Gatersleben, Germany) and BCD1434 and BCD1072 by M. Sorrells (Cornell University, USA). Primer sequences for the *XPsp2999* (Glu-3A) marker were published in Devos et al. (1995). Microsatellite and RFLP markers were positioned into physical intervals on chromosome 1AS in 'Chinese Spring' delineated by deletion bins 1AS-3 (FLM value 0.86), 1AS-1 (FLM value 0.47) and 1AS-5 (FLM value 0.2) (http://www.ksu.edu/wgrc/Germplasm/Deletions/group1.html). Linkage analysis and interval mapping were performed using MapManager (Manly et al. 2001).

Cloning of wheat ESTs

Primers designed on the basis of published EST sequences were used to amplify Chinese Spring genomic sequences that were gel purified (Qiagen, Germany) and cloned into the pGemT Easy Vector system (Promega). Insert identity was confirmed by DNA sequencing and cloned fragments were amplified by PCR to produce probes for DNA gel-blot analysis.

Wheat and rice sequence comparisons

Wheat ESTs previously mapped to deletion bin 1AS-3 were downloaded from http://wheat.pw.usda.gov/NSF/progress_mapping.html. Blast searches (threshold limit of $E<10x-8$ was applied) were carried out at the nucleotide level to identify related rice sequences on BAC clones using the NCBI (http://www.ncbi.nlm.nih.gov) and Gramene (http://www.gramene.org/index.html) databases. A physical contig of BAC clones on rice chromosome 5S was assembled using the FPC physical map from Gramene and overlaps were confirmed using pair-wise Blast comparisons.

Results

Segregation and mapping of the tiller inhibition gene

F_3 families from an advanced backcross segregating family (BC_5 F_2) derived from a cross between a progenitor of the low-tillering line 492 and a free-tillering Australian cultivar Banks were scored for culm number. Under conditions of long days and low temperatures, the parental line 492 produced 1–2 culms whereas Banks produced between 5 and 8 culms. Segregation of the tiller phenotype amongst 113 F_2 lines showed a 1:3 ratio of low-tillering (1–2 culms) to free-tillering lines (3–8 culms) consistent with the expected segregation ratio of the single recessive gene that was previously described as *tin* (1–2 culms:3–8 culms = 33:80, X^2_1=1.06, P= 0.30, Fig. 1) . Individual F_3 lines were classified into homozygous low-tillering, segregating and homozygous free-tillering classes with observed ratios consistent with the 1:2:1 Mendelian segregation for a single recessive gene (low-tillering: segregating:free-tillering = 25:61:21, X^2_2= 2.40, P= 0.30). Within the low-tillering lines which were predicted to be homozygous for *tin*, some F_3 families produced lines with an extreme uniculm phenotype while other families had limited tillering consistently producing on average 2–3 culms/plant. The difference in tiller number amongst

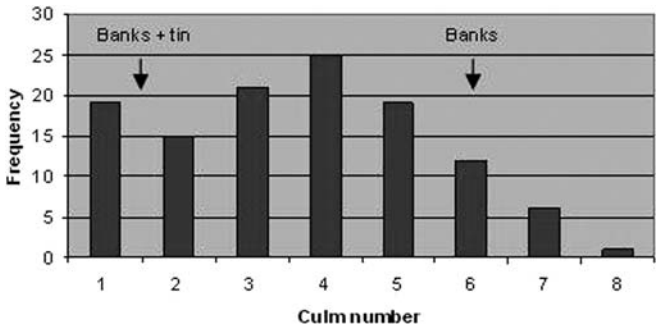

Fig. 1 Frequency distribution of culm number in an F_2 population generated from a cross between the Australian free-tillering cultivar Banks and Banks + *tin* developed by backcrossing the tiller inhibition gene from the low-tillering line 492 into Banks

homozygous low-tillering lines was confirmed by progeny testing in the F_4 generation. Similarly, differences in tiller number amongst homozygous F_3 families that were predicted not to carry *tin* were also heritable. Thus, it appears that after five backcrosses background genes modifying tiller number were still segregating amongst F_3 families.

The *tin* gene was mapped to the short arm of chromosome 1A flanked on the distal side by *Hg* and *XPsp2999*, a PCR-based marker for *Glu-A3* (3.8 cM) and on the proximal side by the RFLP marker *Xwhs179* (2.4 cM) (Fig. 2). This map position is in agreement with previous results that located *tin* to the distal end of chromosome 1AS within an approximate genetic distance of 10 cM from *Hg* (Richards 1988). In this study, we report the identification of a microsatellite marker (*Xgwm136*) that is tightly linked to the *tin* gene. In the F_2 segregating family *Xgwm136* cosegregated with *tin* indicating that the marker is located within 1.3 cM ($P<0.05$) of the gene (Hanson 1956). The *Xgwm136* marker detected multiple alleles; an approximately 300 bp fragment was present in Banks and a larger fragment of approximately 350 bp present in the near-isogenic line Banks+ *tin* that was cosegregating with the *tin* gene. Line 492, the source of *tin* in this segregating family, also contained the 350 bp fragment (Fig. 3).

To confirm linkage of the microsatellite marker *Xgwm136* with *tin* in a different genetic background we scored the culm number of DH lines that were derived from a cross between the oligoculm line 380 and the free-tillering cultivar Fukuho-komugi. Although the culm number between the parental lines was clearly different (line 380 produced 1–3 culms and Fukuho-komugi between 6 and 8 culms), individual DH lines were not separated into discrete classes (Fig. 4). The more free-tillering phenotype of parental line 380 and the segregation of other major genes for vernalization requirement, flowering time and plant height with pleiotropic effects on tiller number contributed to the difficulties of classifying DH lines into discrete groups.

The microsatellite marker *Xgwm136* amplified the same size fragment (350 bp) from line 380 that was linked to *tin* in line 492 (Fig. 3). Single marker regression analysis

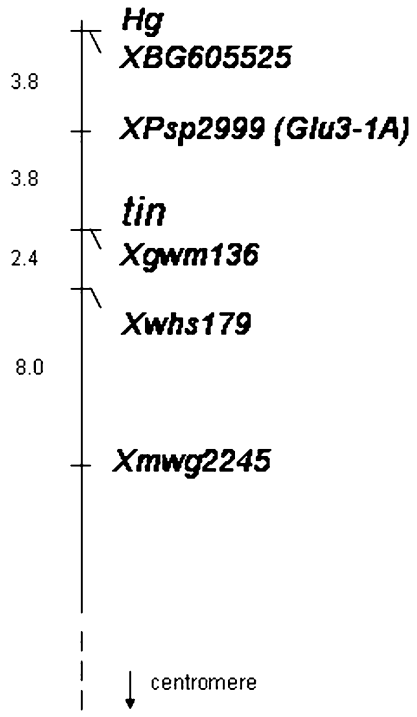

Fig. 2 Genetic linkage map of the distal end of the short arm of chromosome 1A of wheat carrying the *tin* gene. The map was generated using 113 F_2 lines derived from a cross between Banks and Banks+ *tin*. Numbers on the left indicate genetic distance in centiMorgan

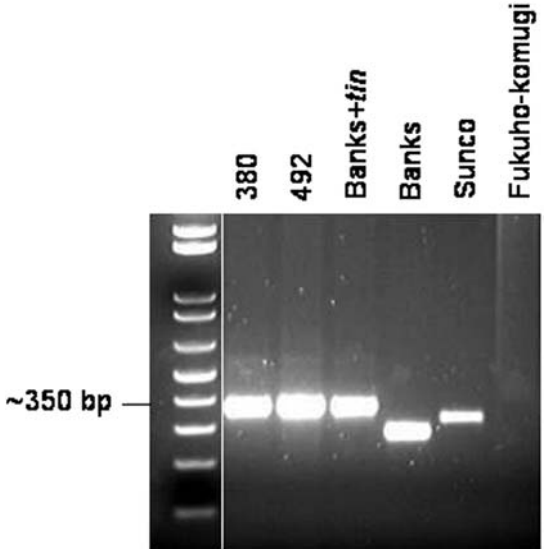

Fig. 3 PCR products generated with microsatellite marker *Xgwm136* from chromosome 1AS of wheat. Low-tillering wheat lines *380*, *492* and *Banks* + *tin* contain diagnostic 350 bp fragment. Free-tillering wheat cultivars *Banks*, *Sunco* and *Fukuho-komugi* contain smaller sized bands or a null allele

between the *Xgwm136* marker and culm number resulted in a highly significant association ($P<0.00001$) indicating that this marker is also useful in the 380 genetic background to predict the presence of *tin* and a low-tillering phenotype. To confirm marker/trait association

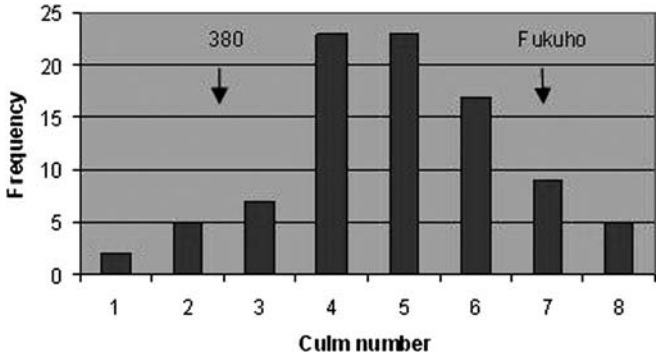

Fig. 4 Frequency distribution of culm number in DH population generated from a cross between the Japanese free-tillering cultivar *Fukuho-komugi* and oligoculm line *380*

within the distal region of chromosome 1AS, we constructed a linkage map by scoring the DH family for *Hg* and additional microsatellite and RFLP markers (Fig. 5). The resulting map was used for interval mapping to confirm that *Xgwm136* was located within the highly significant confidence interval (LOD 4.8) that was predicted to contain the *tin* gene.

Molecular markers linked to *tin* were positioned within a physical interval of chromosome 1AS by assaying deletion lines developed in Chinese Spring (Endo and Gill 1996). These lines contain deletions of different fraction length measurements (FLM) from the telomeric end of chromosome 1AS (1AS-3, 1AS-1 and 1AS-5). Using PCR and RFLP analysis we located molecular markers linked to *tin*, including the microsatellite marker *Xgwm136*, to the most distal deletion bin 1AS-3 (0.86−1.00) (Fig. 2). By combining the genetic and physical mapping information we estimate that *tin* is located distal with respect to the deletion breakpoint 1AS-3 (FLM 0.86) and within a physical interval of less than 14% of the chromosome arm.

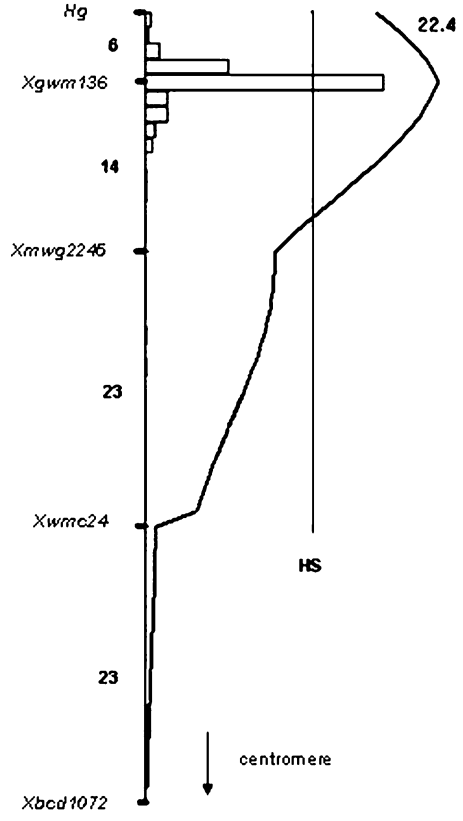

Fig. 5 Interval mapping of culm number on the short arm of chromosome 1A of wheat. The linkage map was developed from 110 DH lines derived from a cross between the free-tillering cultivar Fukuho-komugi and the oligoculm line 380. A significant QTL was detected for culm number, with the peak of the confidence interval (LOD=4.8, LRS=22.4) coinciding with position of microsatellite marker *Xgwm136*. The peak of the confidence interval generated after bootstrap resampling and shown as a histogram also coincided with the map location of *Xgwm136*. A permutation test was conducted (2,000 permutations) to establish the highly significant threshold for the QTL shown as line HS. Numbers on the left indicate the genetic distance between markers in centiMorgan

Relationship of wheat chromosome 1AS with rice chromosome 5S

The placement of *tin* into a defined physical interval of chromosome 1AS provides the framework for utilising the wheat EST mapping information generated by a US National Science Foundation-funded project. This project has so far mapped over 7,000 wheat unigenes into 101 deletion bins defined by different size deletions on all chromosome arms of wheat (Endo and Gill 1996; http://wheat.pw.usda.gov/NSF/progress_mapping.html). Using the publicly available data set, we identified 114 wheat ESTs that were mapped within the target region of 1AS-3 on chromosome 1AS. Closely related rice sequences were identified for 65 out of 114 (57%) wheat ESTs by BlastN searches of the rice database hosted by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) or the Gramene database (http://www.gramene.org/index.html). Significant matches were found on all rice chromosomes, except for chromosome 9, but the largest number of matches per chromosome occurred on rice chromosome 5 (25/65, 38%) (Table 1). This result is consistent with previous findings that reported a high level of synteny between chromosome 5 of rice and chromosome group 1 of wheat (Moore et al. 1995; Sorrells et al. 2003). Most of the 25 closely related rice sequences are located within PAC/BAC clones that are contained within ordered contigs on chromosome 5 of rice (R5) (http://www.gramene.org/index.html). Fourteen of these 25 rice sequences were located within PAC/BAC clones that were present within approximately 1.5 Mb at the end of the short arm of R5. The remainder of sequence matches were detected within clones present between approximately 7.9 and 30.7 Mb on R5 (Table 1).

In summary, we started with 114 wheat ESTs, which were located within the distal deletion bin 1AS-3 of wheat and found a closely related rice sequence in the database for approximately half of these ESTs (65/114). Approximately a third of all rice sequences (25) were located within the expected syntenic region on rice chromosome 5 and a subset of 14 sequences identified a rice contig at the

Table 1 BlastN search results of wheat ESTs in deletion bin 1AS-3 (0.86–1.00) with rice sequences on chromosome 5. The first 16 wheat ESTs identified closely related rice genes within a contiguous sequence of approximately 1.5 Mb at the end of the short arm of rice chromosome 5

Locus	EST	BlastN	Rice chromosome	BAC clones	Accession	Distance (Mb)	FPC contig	BlastX description
WHE2239_A11_B21ZS	BG605525	E−41	5	P0036D10	AC073405	0.1	102	Unknown protein (*Oryza sativa*)
WHE0563_B07_C13ZE	BE492937	E−48	5	P0036D10	AC073405	0.1	102	Putative protein kinase [*Oryza sativa* (japonica cultivar-group)]
WHE1258_C09_F18ZS	BE494877	E−14	5	P0036D10	AC073405	0.1	102	Hypothetical protein
WHE1214_H05_O10ZS	BE405749	E−29	5	P0668H12	AC084818	0.2	102	Tubulin folding cofactor E (*Arabidopsis thaliana*)
WHE2205_A11_A21ZS	BF291707	E−9	5	OSJNBb0035 J08	AC104285	0.4	102	Unknown protein
WHE1123_B11_D21ZS	BE443931	E−12	5	OSJNBa0068 N01	AC129716	0.5	103	Hydroxymethylglutaryl-COA synthase
WHE2473_E02_J03ZS	BG607867	E−25	5	P0016H04	AC079356	0.8	103	Receptor-like kinase Xa21-binding protein 3 (*Oryza sativa*)
WHE0437_B05_C09ZS	BE405834	E−31	5	OSJNBb0041A22	AC093921	1	103	Inorganic pyrophosphatase-like protein
WHE1117_B07_C13ZS	BE444266	E−49	5	OSJNBb0041A22	AC093921	1	103	Hypothetical protein
WHE0925_B07_C13ZS	BF473056	E−69	5	OSJNBb0041A22	AC093921	1	103	Actin-depolymerizing factor 1 (ADF 1)
WHE2161_G03_N05ZS	BF294059	E−50	5	OSJNBb0041A22	AC093921	1	103	Putative Cys2/His2 zinc-finger protein [*Oryza sativa* (jap.)]
WHE1113_F04_K07ZS	BE443103	E−30	5	OSJNBb0041A22	AC093921	1	103	DNA-binding protein HEXBP (hexamer-binding protein)
WHE2204_A04_B08ZS	BF291787	E−14	5	OJ1654_B10	AC108504	1.1	103	EGF-like domain and laminin EGF-like (*Caenorhabditis elegans*)
WHE1212_C05_F10ZS	BE405518	E−13	5	P0699E04	AP001111	1.4	103	Unknown protein [*Oryza sativa* (japonica cultivar-group)]
WHE1788_F07_K14ZS	BF484129	E−57	5	P0699E04	AP001111	1.4	103	Unknown protein [*Oryza sativa* (japonica cultivar-group)]
WHE0364_B07_C14ZS	BE490041	E−15	5	OJ1001_G01	AC093088	1.5	103	APETALA2 protein homolog HAP2 (*Hyacinthus orientalis*)
WHE2154_E04_J08ZS	BF293744	E−9	5	OSJNBa0074P11	AC135914	8.4		*Zea mays* c2 gene for chalcone synthase
WHE2154_E04_J08ZS	BF293744	E−11	5	OSJNBb0067H15	AC136226	8.6		*Zea mays* c2 gene for chalcone synthase
WHE1258_C09_F18ZS	BE494877	E−16	5	P0680F01	AC137624	10.4		Unknown protein
WHE2156_a01_a02zS	BF293621	E−35	5	P0411C02	AC135922	15.6		Putative protein
WHE1114_G01_M02ZS	BE443002	E−32	5	P0589A05	AC135926	15.8		Putative signal peptidase subunit [*Oryza sativa* (japonica cultivar-group)]
WHE0861_H04_P07ZS	BE591042	E−62	5	OSJNBa0088 M05	AC136222	22.9		Plasma membrane associated protein (*Hordeum vulgare*)
WHE1458_C01_F02ZS	BE446624	E−9	5	OJ1281H05	AC117265	28.3		C2H2 zinc finger protein (*Oryza sativa*)
WHE0088_B08_C16ZS	BE424946	E−61	5	OJ1118C04	AC108523	30.7		Latex-abundant protein-like protein [*Oryza sativa* (japonica cultivar-group)]
WHE1783_F01_K01ZS	BF202657	E−26	5	OJ1575E09	AC104714			Unknown protein (*Oryza sativa*)

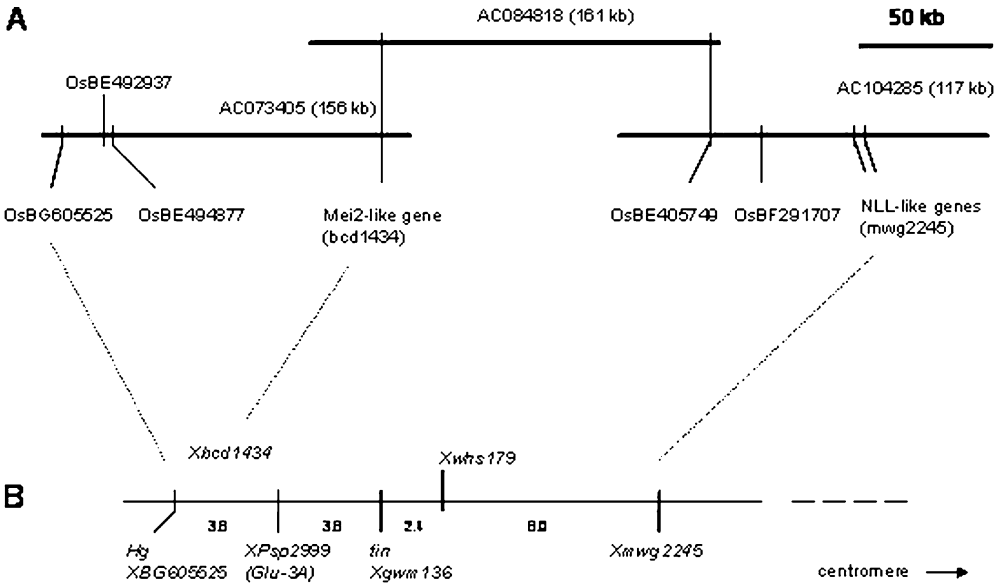

Fig. 6a, b Comparative mapping of rice chromosome 5 with the distal end of the short arm of chromosome 1A of wheat containing the *tin* gene. **a** BAC contig (AC073405–AC104285) at the distal end of the short arm of chromosome 5 of rice containing 7 rice sequences closely related to wheat or barley ESTs BG605525, BE492937, bcd1434, BE405749, BF291707 and mwg2245 that were positioned within the same terminal deletion delineated by 1AS-3 on wheat chromosome 1AS. **b** Three out of seven wheat ESTs (BG605525, bcd1434 and mwg2245) with homologs present in the rice contig [as shown in (**a**), OsBG605525, Meiosis2-like gene, NLL gene] were genetically mapped in the same order to the distal region of chromosome 1AS flanking the tiller inhibition gene *tin*. The map position of *Xbcd1434* was inferred on the basis of published maps on Graingenes (http://wheat.pw.usda.gov/index.shtml)

end of the short arm of R5. This rice sequence constitutes approximately 1.5 Mb and is a putative orthologous region for at least part of the wheat chromosome 1AS between deletion breakpoint 0.86 and the telomere.

Relationship of the wheat region which contains the tiller inhibition gene to the corresponding region in rice

Having identified a putative orthologous region in rice for at least part of the sequence within wheat bin 1AS-3, we defined the rice sequence that corresponded to the region flanking the *tin* gene in more detail. Two RFLP markers, *Xbcd1434* and *Xmwg2245,* have been previously mapped to deletion bin 1AS-3 (Gill et al. 1996; Guyot et al. 2004) and shown to flank *tin* (Fig. 6). These markers are detected by gene sequences that are predicted to encode proteins with significant homology to a MEI2-like protein (bcd1434) and a nodulin-like-like protein (NLL) (located within AF326781, 198,531–198,911 bp) . Stein et al. (2000) described the construction of a BAC contig spanning the linked leaf rust resistance gene (*Lr10*) locus in *Triticum monococcum* and reported that duplicated loci of *Xmwg2245* were present within a 170 kb of *T. monococcum* sequence . Rice genes predicted to encode MEI2-like and NLL proteins were located within adjoining PAC clones AC084818 and AC104285 contained within the most distal contig of 1.5 Mb on chromosome 5S (Fig. 6). Duplicated NLL genes were also present within 7 kb of the rice sequence (AC104285). It is therefore possible that the distal end of R5 and the region in wheat that contains *tin* are orthologous. To further investigate this possibility, we located rice sequences corresponding to the wheat ESTs onto the three distal rice clones of chromosome 5S (AC073405, AC084818, AC104285) (Fig. 6). The most distal rice sequence, closely related to the wheat EST BG605525, was positioned at approximately 10 kb from the end of AC073405. We located four additional rice sequences that were closely related to wheat ESTs BE492937, BE494877, BE405749 and BF291707 between the rice ortholog of BG605525 and the location of the duplicated NLL genes on AC104285 (Fig. 6). Using published sequence information, we cloned two wheat ESTs (BG605525 and BE405749) from this region and used them as probes for DNA hybridisation. BE405749 failed to detect any polymorphism between the parental lines, Banks and Banks + *tin*, but BG605525 could be mapped as an RFLP marker in wheat and showed tight linkage to the *Hg* locus. The marker order in wheat, *XBG605525*, *Xbcd1434*, *Xmwg2245*, is thus conserved compared with the corresponding sequences in the rice contig AC073405–AC104285. It is therefore likely that this region on 1AS of wheat shares a common ancestral origin with the most distal end of the short arm of rice chromosome 5.

Discussion

A gene of major effect on tiller number (*tin*) in wheat was mapped to the distal region on the short arm of chromo-

some 1A. A tightly linked microsatellite marker (*Xgwm136*) was identified that may be useful in selecting for the gene in future marker-assisted breeding. From mapping tiller number in two separate segregating families we conclude that sib lines 492 and 380 carry the same *tin* gene. Line 380 probably contained additional modifier genes that produced more tillers than line 492.

The cloning and mapping of wheat unigenes to individual deletion bins provides a useful resource to generate new markers for linkage maps. It also enables comparative studies between the wheat and rice genomes. We used the map positions and sequence relatedness of 18 ESTs to infer that the distal end of wheat chromosome 1AS carrying the *tin* gene was orthologous to the terminal end of rice chromosome 5S. These results were consistent with previous reports of synteny between chromosome group 1 of wheat and chromosome 5 of rice (Moore et al. 1995; Sorrells et al. 2003). Although these regions may have a common ancestory, the majority of wheat ESTs in deletion bin 1AS-3 detected closely related sequences that were located on non-syntenic rice chromosomes. ESTs are often part of gene families or contain conserved motifs that encode proteins with similar functional domains. Depending on the evolutionary history, a large number of sequence matches between species may not be orthologous but instead reflect duplication/translocation events followed by sequence divergence, thus explaining at least in part the frequency of apparent 'non-syntenic' matches. To use comparisons at the protein level may overcome some of these limitations, however focusing on the greatest homology and most frequent matches within a given deletion bin to one particular chromosomal region in rice is probably a reliable indicator of synteny. Using this approach we established a syntenic relationship between wheat 1AS and rice 5S, though the majority of wheat ESTs in the deletion bin 1AS-3 showed greatest similarity to rice ESTs that were located elsewhere in the genome.

Rice also contains a single, major gene conferring pubescent glumes (*Hg*). *Hg* was previously positioned on rice chromosome 3 (R3), tightly linked to RFLP marker *Xcdo20* and positioned approximately 1.7 Mb from the end of the short arm of R3 (Gramene). If *Hg* genes in rice and wheat share a common ancestor, we would predict that both genes may be located within syntenic regions. Five wheat ESTs from deletion bin 1AS-3 detected closely related rice sequences on chromosome 3 (data not shown). However, none of the rice sequences mapped near the predicted location of *Hg* on the short arm of chromosome rice 3. As no other common markers were identified between wheat chromosome 1AS and rice chromosome 3, it is possible that the rice gene has relocated to a non-syntenic region in a small-scale translocation event. Our limited knowledge of micro-synteny that exists between small physical intervals may have prevented us from identifying orthologous genes on chromosome 1AS of wheat and chromosome 3 of rice. Synteny may also be disrupted at the *Glu-3A* locus in wheat which comprises a large gene family encoding low molecular weight glutenins, one of the most abundant seed storage proteins. A microsatellite marker assaying one of the glutenin genes was flanked by markers *XBG605525* and *Xmwg2245*. We therefore expected that the corresponding region in rice also contains related seed storage protein genes. However, no seed storage protein genes were present within the distal contig on rice chromosome 5S suggesting that these regions in wheat and rice have diverged. The genetic distance of approximately 18 cM between markers in wheat was much greater than between markers on the corresponding rice map (~3 cM). It is likely that the physical distance in wheat is also substantially greater than in rice as was observed in several recent studies (Yan et al. 2003; Feuillet et al. 2003; Brunner et al. 2003). We conclude that although a high level of synteny was found between wheat and rice, including at least 18 closely related gene sequences, the most distal region on rice chromosome 5S and wheat 1AS also show significant divergence, as seen for genes encoding pubescent glumes or seed storage proteins.

Defining the map position of the *tin* gene on chromosome 1A relative to molecular markers allowed us to investigate *tin* in relation to tillering mutants that were identified and mapped in other cereals. Based on these results, we conclude that *tin* and reduced tillering mutations on rice chromosome 4 and 6 (*rcn1*, *rcn2*, *rcn5* and *moc1*) and the barley *uniculm2* mutant on chromosome 6H are located on non-syntenic chromosomes. A wheat sequence that was closely related to the gene that encodes the *tb1* mutation in maize hybridised to a single RFLP band on chromosome group 4 of wheat (W. Spielmeyer, unpublished). It appears that no ortholog within a known syntenic region has so far been described in other cereals for the *tin* in wheat.

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