Funct Integr Genomics (2008) 8:33–42
DOI 10.1007/s10142-007-0057-4

ORIGINAL PAPER

# Genomic targeting and mapping of tiller inhibition gene (*tin3*) of wheat using ESTs and synteny with rice

**Vasu Kuraparthy · Shilpa Sood · Bikram S. Gill**

Received: 25 May 2007 / Revised: 18 July 2007 / Accepted: 3 August 2007 / Published online: 22 September 2007


**Abstract** Changes in plant architecture have been central to the domestication of wild species. Tillering or the degree of branching determines shoot architecture and is a key component of grain yield and/or biomass. Previously, a *tiller inhibition* mutant with monoculm phenotype was isolated and the mutant gene (*tin3*) was mapped in the distal region of chromosome arm $3A^{m}L$ of *Triticum monococcum*. As a first step towards isolating a candidate gene for *tin3*, the gene was mapped in relation to physically mapped expressed sequence tags (ESTs) and sequence tag site (STS) markers developed based on synteny with rice. In addition, we investigated the relationship of the wheat region containing *tin3* with the corresponding region in rice by comparative genomic analysis. 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 *tin3* region. The *tin3* gene was mapped to a 324-kb region spanned by two overlapping bacterial artificial chromosomes (BACs) of rice chromosome arm 1L. Wheat–rice synteny was exceptionally high at the *tin3* region despite being located in the high-recombination, gene-rich region of wheat. Identification of tightly linked flanking EST and STS markers to the *tin3* gene and its localization to highly syntenic rice BACs will assist in the future development of a high-resolution map and map-based cloning of the *tin3* gene.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10142-007-0057-4) contains supplementary material, which is available to authorized users.

V. Kuraparthy · S. Sood · B. S. Gill (✉)
Wheat Genetic and Genomic Resources Center,
Department of Plant Pathology, Kansas State University,
Manhattan, KS 66506-5502, USA
e-mail: bsgill@ksu.edu

**Keywords** Comparative genomics · Genome mapping · Wheat · Tillering · Synteny · *Triticum monococcum*

## Introduction

While the angiosperm (flowering plant) lineage is thought to be about 200 million years old, the *Poaceae* family, which includes a very diverse set of cereal species, diverged from a common ancestor only about 50–70 million years ago (Kellogg 2001). This diversity is manifested in huge differences in nuclear DNA content, which varies from 430 Mb in rice to 5,700 Mb in diploid wheat *Triticum monococcum* (Arumuganathan and Earle 1991). Despite these large differences in genome size and chromosome number, extensive conservation of gene content and order, termed synteny or colinearity, was observed in the first comparative restriction fragment length polymorphism (RFLP) maps constructed for wheat and rice (Ahn et al. 1993; Van Deynze et al. 1995a, b). The genomes of distantly related cereals, such as oat, rice, and maize, can be divided into linkage blocks that have homology to corresponding segments of the wheat genome (Moore et al. 1995). The degree of genomic similarity observed at the macrolevel among grass genomes coupled with the assumption that the essential components of growth and development are conserved among plants led to the notion that comparative mapping experiments could serve as an efficient tool for transferring information and resources from well-studied genomes, such as that of rice, to related plants. Further, comparative low-resolution genetic mapping of shattering quantitative trait loci (QTL) in the orthologous genomic regions in sorghum, maize, and rice led to the notion that domestication of diverse cereals may have involved mutations in genes for same traits (Paterson

et al. 1995). This suggested that many structural and functional parallels appear to have persisted since the divergence of cereals from a common ancestor and synteny could allow the cross-referencing among plant genomes. The best examples of the use of macrolevel conservation of synteny in plants were the cloning of the *Rht* genes responsible for the green revolution in wheat (Peng et al. 1998) and the cloning of genes for the stay-green phenotype in rice based on the cotyledon color phenotype originally described by Mendel (Armstead et al. 2007).

Because genomics and gene discovery in hexaploid wheat is confounded by a genome size of approximately 17,300 Mb (Bennett and Leitch 1995) and an abundance (80%) of repetitive sequences (Wicker et al. 2001; SanMiguel et al. 2002; Li et al. 2004), the use of a small genome as a reference is a natural choice for positional cloning of agriculturally important genes using comparative genomic approaches (Yan et al. 2003). Currently, there are more than 550,000 wheat expressed sequence tag (EST) sequences, with 122,282 unique sequences (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=wheat) deposited in public databases, providing an excellent resource for mapping genes. A set of wheat deletion lines has been used to locate 7,873 unique ESTs into chromosome bins (Qi et al. 2004; http://wheat.pw.usda.gov/NSF/progress_mapping.html). Although the remaining EST sequences have not been physically or genetically mapped, existing synteny between rice and wheat, as well as other cereals, can be exploited to tentatively position ESTs in silico based on orthology with sequences in the rice genome, and wheat–rice comparative maps have been constructed for all the wheat chromosomes (Sorrells et al. 2003; La Rota and Sorrells 2004). Conserved synteny between wheat and rice has been used for gene mapping and gene discovery in wheat. The positional cloning of the wheat vernalization gene *VRN1* (Yan et al. 2003) is a good example of using information from the colinear regions in rice and sorghum to facilitate the cloning of a wheat gene. Most importantly, synteny between wheat and rice has been successful in some cases in finding new markers (Liu and Anderson 2003) and for fine mapping (Distelfeld et al. 2004) of the targeted gene or region in wheat.

To characterize the underlying genetic variation for tillering in wheat, we recently identified a tiller inhibition gene (*tin3*) in diploid wheat that was compromised in tillering ability (Kuraparthy et al. 2007). The *tin3* mutant produces a single monoculm phenotype compared to the wild-type *T. monococcum* with more than 30 tillers. Genetic and physical mapping suggested that the *tin3* gene was located in a high-recombination and gene-rich region of chromosome arm 3AL of wheat, suggesting it is feasible to clone the *tin3* gene using map-based cloning (Kuraparthy et al. 2007). In the present study, we report the genomic targeting and mapping of *tin3* gene using wheat ESTs and synteny with rice. Our goal is to achieve the map-based cloning of the *tin3* gene. The objectives of this study were to fine map the *tin3* gene, examine microcolinearity in the *tin3* region between wheat and rice, and explore the possible candidate genes for *tin3*.

## Materials and methods

### Plant material

For mapping the physically mapped wheat ESTs and sequence tag site (STS) markers developed based on synteny with rice, an $F_2$ population of 88 plants reported in Kuraparthy et al. (2007) was used. For additional low-resolution molecular mapping, the *tin3* mutant was crossed as a female with *T. monococcum* subsp. *aegilopoides* (TA4342-95) and an $F_2$ population of 476 plants was used for segregation analysis. To study whether there are any local chromosomal rearrangements at the *tin3* locus, we tested the map order of the orthologous alleles of the linked markers of *tin3* in the diploid D-genome donor species *Aegilops tauschii*. An $F_2$ population of 118 plants derived from a cross between AL8/78 and TA1604 was used for molecular mapping.

### EST/STS mapping and wheat–rice synteny

Physically mapped wheat ESTs of the deletion bin 3AL5-0.78-1.00 of Chinese Spring (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) and markers developed based on synteny with rice were used for molecular mapping of *tin3*. Both the ESTs and STS markers were mapped as RFLPs for molecular mapping. DNA isolation, Southern blotting, and hybridization were done as reported in Kuraparthy et al. (2007). DNA of the parents of the diploid wheat mapping populations was digested with six restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I, and *Xba*I) for polymorphism study.

Comparative genomic analysis using physically and genetically mapped ESTs with the rice annotated bacterial artificial chromosome (BAC)/P1 artificial chromosome (PAC) sequence was done to establish the synteny and macrocolinearity in the genomic region encompassing the *tin3* gene of wheat. Full-length cDNA or tentative contig sequence of the mapped ESTs were extracted using The Institute for Genomic Research (TIGR) wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/). These sequences were then used to search the rice genome database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using BLASTn (Altschul et al. 1997) to identify the syntenic rice BAC/PACs. Sequences in the target region

of the rice genome were also used as queries in BLASTn (Altschul et al. 1997) searches of the wheat EST database (http://tigrblast.tigr.org/tgi/) to identify additional unmapped wheat ESTs that are potentially linked to *tin3* and to develop STS markers (Table 2) for further mapping and genomic targeting of *tin3*. Predicted rice sequences of the syntenic rice BACs were further subjected to tBLASTx searches against the wheat EST clusters using the Gramene (Ware et al. 2002) database (http://www.gramene.org/Multi/blastview) for confirming the true orthology. In the BLASTn searches, a significant match was declared when there was at least 65% nucleotide identity for at least half of the query sequence but not less than 150 bases, and with an *e* value of less than e-20. For tBLASTx searches, significance was declared when there was at least 40% amino acid identity over at least half of the TC or EST sequence, but no less than 200 amino acids, and an *e* value of less than e-11. Whenever there were several significant matches for a single predicted rice gene sequence, only the best match was reported.

### Development of STS markers for RFLP analysis

Primer design was done with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and amplicons of 160–400 bp were targeted. Polymerase chain reaction (PCR) amplifications were performed in 25-µl reactions with 2.5 µl of 10× magnesium-free PCR buffer, 1.5 µl of magnesium chloride (25 mM), 2.0 µl of deoxyribonucleotide triphosphates (dNTPs) (2.5 mM each dNTP), 1 µl each forward and reverse primer (10 pmol/µl), and 100 ng of DNA in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). Primer annealing temperatures varied from 50 to 60°C depending on the primers. All PCR products were resolved in 1% agarose gels with 1× Tris-borate EDTA buffer and visualized using ethidium bromide staining. After verifying the fragment sizes of the PCR products from EST–STS markers in agarose gels, fragments were eluted using a NucleoTrap® Gel Extract Kit (BD Biosciences Clontech, Palo Alto, CA, USA) as per the manufacturer's instructions. Purified PCR product was quantified using a NanoDrop ND-1000 UV-VIS spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) and ligated to $p^{Gem}$-T Easy Vector System I (Promega, Madison, WI, USA) according to the manufacturer's instructions. For all PCR products, excess salts were removed by drop dialysis using 0.025 µm dialysis membranes (Millipore, Billerica, MA, USA). The ligated mixture was transformed into competent cells of *Escherichia coli* strain DH10B (Invitrogen, Carlsbad, CA, USA) by electroporation using a Cell-Porator (Life Technologies, Invitrogen). The transformation product was then mixed with SOC medium and incubated in a shaker for 1 h at 37°C. Approximately 8–15 µl of this incubated mixture was inoculated on Luria–Bertani (LB) media containing carbenicillin and X-gal. Ten to 20 white colonies of each transformant were selected and grown in liquid LB containing 50 mg/ml carbenicillin for 8–12 h. Plasmid DNA was isolated from three well-grown cultures for each transformant using a Qiagen Plasmid Mini Kit (Qiagen, Santa Clarita, CA, USA) as per the manufacturer's instructions. Plasmid inserts were sequenced and positive clones were identified through sequence alignment using bl2seq of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi), and positive clones were PCR amplified using the standard M13 primer. The PCR product was purified using QIAquick PCR Purification kit (Qiagen) as per the manufacturer's instructions. About 50 ng of purified PCR product was used for genetic mapping. All markers that showed polymorphism between *tin3*/*Tm18* of *T. monococcum* and *T. aegilopoides* were mapped genetically in the $F_2$ mapping population. Southern hybridization and RFLP mapping was done as reported previously (Kuraparthy et al. 2007).

### Linkage analysis

The computer program Mapmaker (Lander et al. 1987), version 2.0 for Macintosh, was used to calculate linkage distances using the Kosambi mapping function (Kosambi 1944) with an LOD threshold of 3.00.

## Results

### Candidate gene mapping

The *lateral suppressor* of tomato (Schumacher et al. 1999), *no apical meristem protein* of petunia (Souer et al. 1996), *monoculm1* of rice (Li et al. 2003), and *teosinte branched1* of maize (Doebley et al. 1997) are the cloned genes involved in lateral branching or tillering in plants. These candidate genes were analyzed for their association with the *tin3* gene or to its colinear rice chromosomal regions. BLASTn searching of these sequences against rice genomic sequence database showed that lateral suppressor (*Ls*) of tomato (GRAS family transcription factor protein) and no apical meristem (NAM) of petunia (a novel class of proteins with conserved N-terminal domain) showed significant homology to sequences on the rice chromosome arm 1L. Of the two other candidate gene sequences, *monoculm1* of rice, which is a GRAS family transcription factor, was located on chromosome arm 6L of rice, and the orthologue of *teosinte branched1* of maize was mapped on chromosome 3 of rice. Wheat homologues of *Ls* and *NAM* were used to develop STS markers for molecular mapping

of *tin3* (Table 2). Although *XSTS-WNAM5* produced multiple bands, the polymorphic fragment was not linked to *tin3* in the $F_2$ population at an LOD of 3.0. The STS marker (*XSTS-WLS6*) developed from lateral suppressor (*Ls*) of tomato mapped 37.1 cM proximal to *tin3* and cosegregated with a previously reported RFLP marker *Xbcd372* on chromosome arm $3A^mL$ (Fig. 1).

## EST and wheat–rice synteny-based mapping

Out of the 25 ESTs of deletion bin 3AL5-0.78-1.00, 22 (88%) were polymorphic between the diploid parents, *tin3* and *T. aegilopoides*. From these, only 11 polymorphic EST markers were included in the map because few ESTs produced multiple bands where the polymorphic fragments mapped elsewhere in the genome and the remaining ESTs, although produced single/double bands, the polymorphic fragments of these ESTs were not mapped on chromosome arm $3A^mL$. Combined with the previously mapped markers of Kuraparthy (2007) and Kuraparthy et al. (2007), these markers gave a genetic map length of 75.3 cM (Fig. 1). Comparative genomic analysis of the genetically mapped markers with the rice genome sequence showed that eight had significant homology to the colinear rice genome

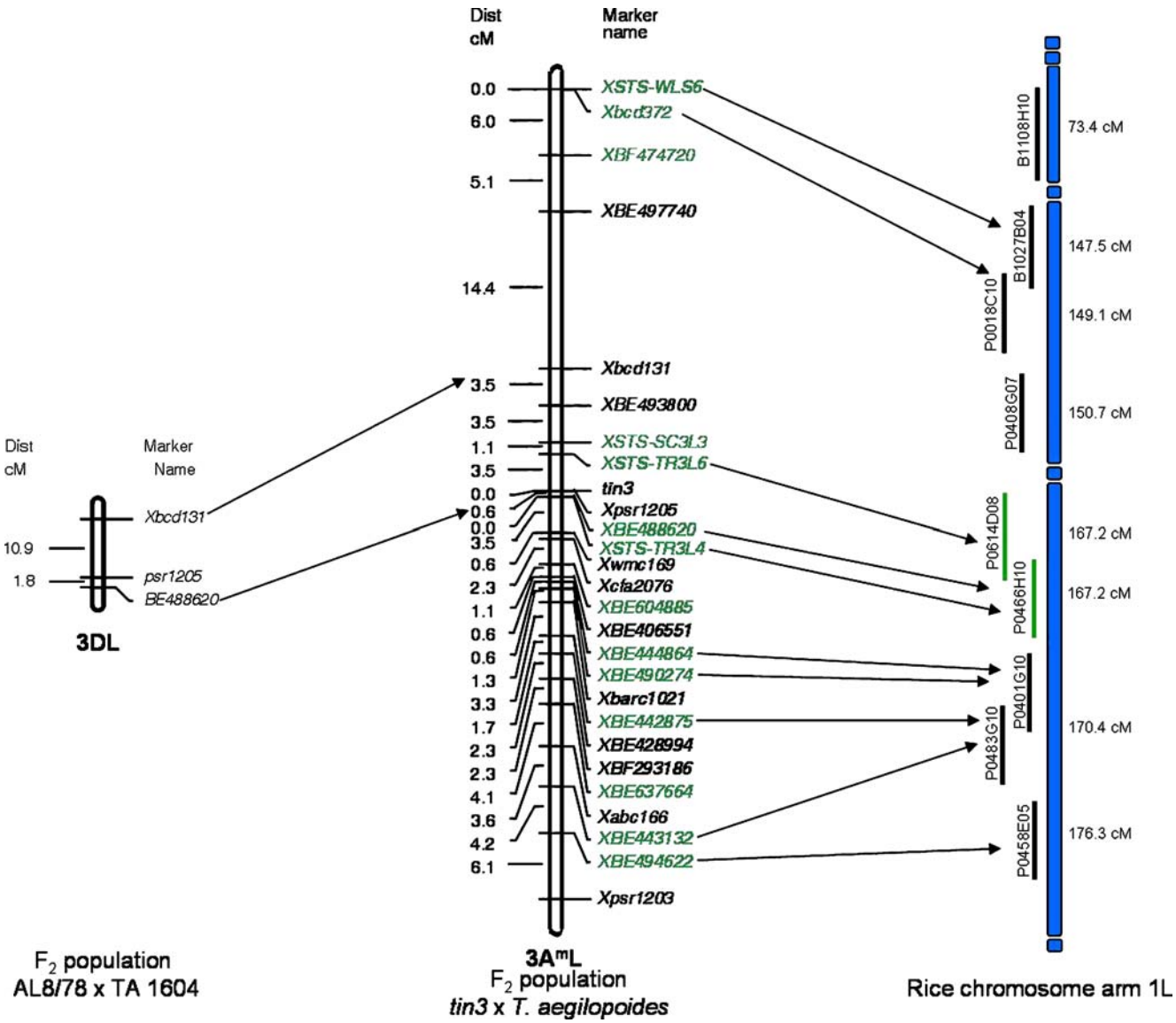


**Fig. 1** Genetic mapping of *tin3* gene using wheat ESTs and STS markers and its alignment with the BAC/PAC-based physical map of colinear rice chromosome 1L and the diploid D genome map. The top of each map is towards the centromere and the bottom is towards the telomere. In the *T. monococcum* map, all the markers that were mapped in the present study are in *bold*, and the markers that are highly syntenic to the colinear rice genomic sequence are indicated in *green*. Orthologous genes among the species are connected by *arrows*. Syntenic rice BAC/PACs spanning the *tin3* orthologous region are indicated in *green filling*. All STS markers were developed based on syntenic rice genomic sequences

sequence (Table 1, Fig. 1). Three EST markers (*XBE428994*, *XBE406551*, and *XBF293186*) showed high homology with noncolinear rice sequences (Table 1). Although the other two EST markers (*XBE604885* and *XBE637664*) had less homology, they showed hits with the genomic sequences of the rice chromosome arm 1L (Table 1). The order of the genetically resolved colinear ESTs is consistent with the physical order of the orthologous rice sequences on chromosome arm 1L (Fig. 1). Furthermore, the orientation of the telomeric end of the wheat chromosome 3L corresponded well with the telomeric end of chromosome arm 1L of rice where the distal region of 3L correspond to the distal region of chromosome arm 1L of rice (Fig. 1). This indicated that macrocolinearity at the orthologous region proximal to the *tin3* gene is well conserved between wheat and rice.

The EST marker (*XBE488620*) tightly linked to *tin3* in the genetic map showed high sequence homology to the BAC P0466H10, which was mapped distally at 167.2 cM on the long arm of rice chromosome 1 (Fig. 1, Table 1). Because *XBE488620* mapped distal to the *tin3*, and the centromere to telomeric end orientation of the syntenic wheat–rice orthologous regions corresponded well (Fig. 1), we selected the rice gene sequences within BAC P0466H10 and its proximally mapped overlapping BAC P0614D08 for further comparative genomic analysis and genomic targeting of *tin3*. Annotated gene sequences from rice BACs were used as queries in the BLASTn searches of the wheat EST database (http://tigrblast.tigr.org/tgi/). Of the 13 gene sequences of BAC P0466H10, 7 (53.8%) showed significant homology to wheat ESTs, whereas 47.36% (9) of the total 19 gene sequences of the BAC P0614D08 were significantly homologous to wheat ESTs (Table 3). These results tentatively suggested that microcolinearity is also well conserved between wheat and rice in the genomic region spanning *tin3* gene of wheat. Only one wheat EST homologous to the rice gene armadillo repeat-containing protein (TC240391) of BAC P0466H10 was physically mapped on the homoeologous group-5 chromosomes by the wheat NSF-EST mapping project (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). For further genomic targeting of the *tin3* gene, STS markers were developed from the wheat ESTs that were showing high homology with rice gene sequences from the above two BACs. Of the three STS markers developed, two were from P0466H10 and one was based on sequences from P0614D08 (Table 3). All three STS markers showed polymorphism with one or more restriction enzymes used. Genetic mapping in the $F_2$ population showed that the STS marker *XSTS-TR3L4* developed from Rho-GTPase-activating protein cosegregated

**Table 1** Syntenic relationships of the wheat ESTs and RFLP markers genetically mapped in the region encompassing *tin3* gene with rice genome sequence

| EST/TC/ RFLP marker | Marker | Syntenic relationship with rice | | | | | |
|---|---|---|---|---|---|---|---|
| | | *e* value | | BLASTn | | | |
| | | tBLASTx | BLASTn | Rice BAC(s) | Rice chromosome | Genetic position on chromosome 1 (cM) | Physical position on chromosome1 (bp) |
| TC237943 | *XSTS-WLS6* | 1.4e-238 | 6.6e-287 | B1027B04 B1065E10 | 1 | 147.5 | 38389995–38756345 |
| TC268056 | *Xbcd372* | 3.1e-135 | 2.7e-122 | B1027B04 P0018C10 | 1 | 149.1 | 38389995–38756345 |
| TC253444 | *XSTS-TR3L6* | 9.3e-113 | 3.6e-63 | P0614D08 | 1 | 167.2 | 41807392–41925745 |
| PSR1205 | *XPSR1205* | 7.6e-05 | 0.0016 | OSJNBb0069N01 | 4 | na | na |
| TC254523 | *XBE488620* | 6.2e-88 | 3.7e-135 | P0466H10 | 1 | 167.2 | 41925746–42095418 |
| TC269390 | *XSTS-TR3L4* | 1.7e-104 | 4.2e-118 | P0466H10 | 1 | 167.2 | 41925746–42095418 |
| TC251929 | *XBE604885* | 3.4e-65 | 2.3e-30 | B1108H10 | 1 | 73.4 | 18609821–18709760 |
| TC271773 | *XBE406551* | 5.8e-21 | 1.5e-30 | OSJNBa0095N06 | 3 | na | na |
| TC26924 | *XBE444864* | 6.6e-78 | 4.4e-64 | P0401G10 | 1 | 170.4 | 42582381–42723237 |
| TC238164 | *XBE490274* | 5.4e-43 | 1.6e-35 | P0401G10 | 1 | 170.4 | 42582381–42723237 |
| TC236864 | *XBE442875* | 1.1e-39 | 3.8e-59 | P0401G10 P0483G10 | 1 | 170.4 | 42582381–42723237 |
| TC262720 | *XBE428994* | 5.3e-77 | 3.9e-92 | OSJNBb0081K01 | 3 | na | na |
| BF293186 | *XBF293186* | 1.4e-67 | 2.3e-75 | OSJNBa0025B05 | 7 | na | na |
| TC257361 | *XBE637664* | 1.2e-90 | 3.0e-2 | P0408G07 OSJNBa0048I01 | 1 | 150.7 | 38756346–38874806 |
| TC272982 | *XBE443132* | 1.5 | 0.00079 | P0483G10 | 1 | 170.4 | 42723238–42865873 |
| TC253918 | *XBE494622* | 2.5e-154 | 3.0e-178 | P0458E05 | 1 | 176.3 | 42865874–43007184 |

*na* not applicable, *TC* tentative consensus

with the *XBE488620* and mapped 0.6 cM distal to *tin3* (Fig. 1). The gene sequence of Rho-GTPase-activating protein (TC269390) in rice was located 2.77 kb proximal to the homologous sequence (pyrroline-5-carboxylate reductase, TC254523) of wheat EST marker *XBE488620*; their cosegregation in the genetic map suggests the conserved microcolinearity. The other STS marker *XSTS-TR3L17* produced multiple bands, and the polymorphic fragments were not linked to *tin3* and mapped elsewhere in the genome. However, the STS marker *XSTS-TR3L6* developed from wheat ESTs that were homologous to gene sequence of BAC P0614D08 (Table 2) mapped 3.5 cM proximal to the *tin3* gene in the genetic map (Fig. 1). Genetic map order of these wheat STS markers was consistent with the physical order of the orthologous rice sequences on chromosome arm 1L, further confirming the conserved microcolinearity at the *tin3* region. Because *XSTS-TR3L6* mapped proximally and *XBE488620* mapped distally, *tin3* gene was localized in the rice genomic region spanning two overlapping BACs, P0614D08 and P0466H10 of chromosome 1L.

Because macro- and microcolinearity at the genomic region of *tin3* gene spanned by two overlapping BACs P0614D08 and P0466H10 of chromosome 1L were well conserved, this genomic region in rice was analyzed for the presence of possible candidate genes involved in tillering or lateral branching in plants. Three candidate genes involved in lateral branching or tillering in plants were identified, NAM-like protein, Rho-GTPase-activating protein-like, and a GRAS family transcription factor containing protein (Table 3). Because the wheat STS marker (*XSTS-TR3L4*) homologous to Rho-GTPase-activating protein-like of P0466H10 was mapped 0.6 cM distal to *tin3*, this gene cannot be a candidate for *tin3*. BLASTn searching of the remaining two candidate sequences as queries in the wheat EST database (http://tigrblast.tigr.org/tgi/) showed that the level of homology of the ESTs detected by the rice GRAS family transcription factor (CA721360, *e* value 1.3e-27) and NAM-like protein (CV771545, *e* value 3.5e-0.06) was not significant. However, we were unsuccessful in developing STS markers from these ESTs for candidate gene mapping of *tin3*.

## Mapping in the D-genome

The additional low-resolution $F_2$ mapping population of 476 plants segregated 369 wild-type and 107 mutant phenotypes, which was a good fit for the monogenic segregation ratio of 3:1. Only the mutant $F_2$ plants (107 plants) were used for mapping using previously reported linked marker *Xpsr1205*. The *tin3*-specific allele of the marker *Xpsr1205* cosegregated with the mutant phenotype in all the mutant $F_2$ plants (Supplementary Fig. 1), suggesting that *Xpsr1205* is a very tightly linked marker to *tin3*.

Because marker *Xpsr1205* cosegregated with mutant phenotype in the high-resolution mapping population of 468 $F_2$ plants, the genomic region of *tin3* was studied for possible chromosomal rearrangements and suppressed recombination. An $F_2$ population of the diploid D genome derived from *A. tauschii* accessions AL8/78 × TA1604 was used to map the markers *Xbcd131*, *Xpsr1205*, and *XBE488620*, which were mapped in the genomic region of *tin3* gene. Marker order and relative marker distances in the diploid D genome map were the same as in the *tin3* $F_2$ mapping population, suggesting that there were no DNA rearrangements in the genomic region of *tin3*. However, the genetic distance between markers *Xpsr1205* and *XBE488620* was much longer in the D genome than in the A genome (Fig. 1). Because the marker alleles of *Xpsr1205* and *XBE488620* showed clear monogenic codominant segregation in the A genome, relatively less genetic distance between markers *Xpsr1205* and *XBE488620* in the A genome compared to the D genome indicated that the recombination could be mildly suppressed in the *tin3* region in the A-genome mapping population. However, it is also possible that the markers could be physically farther apart in *A. tauschii* than in *T. monococcum* because intergenic sequences are highly variable among genomes due to retroelements and other genomic processes.

**Table 2** Wheat–rice synteny based STS markers used for targeting the *tin3* gene

| Marker | Source | Forward primer (5′→3′) | Reverse primer (5′→3′) | $T_A$ (°C) | Fragment size (bp) |
|---|---|---|---|---|---|
| *XSTS-TR3L4* | TC269390 | CCACACTAGGCAGGCTCTTC | CAGCAAGATGCAGAGGATCA | 60 | 195 |
| *XSTS-TR3L6* | TC261350 | ATGGCTTCTACGCATGGAGT | TGTTGATATGGCGAGCTGAG | 60 | 220 |
| *XSTS-TR3L17* | TC244162 | TGATGAACATGACAGCAGCA | TTCTTTATGGCGAGCAATCC | 60 | 199 |
| *XSTS-WNAM5* | TC218530 | TACGGCGAGAAGGAGTGGTA | ACTCGTGCATGATCCAGTTG | 60 | 214 |
| *XSTS-WMEPC* | TC256332 | TGTTAAGAGGGATGGCCTTG | GAAAGGCAATGGAATGTCGT | 50 | 230 |
| *XSTS-WLS6* | TC237943 | CAGCTGTCAGCAATGCAAAT | TCGTGATCATCCACACCAGT | 60 | 224 |

*TC* tentative consensus

**Table 3** Annotated rice sequences in the syntenic rice BACs P0466H10 and P0614D08 which spans the *tin3* gene of wheat and their wheat orthologues based on the best BLASTn and tBLASTx hits in the wheat gene indices.

| Locus identifier | Putative function | Wheat EST/TC[a] | *e* value | |
|---|---|---|---|---|
| | | | Wheat BLASTn | Wheat tBLASTx |
| BAC: P0466H10 | | | | |
| LOC_Os01g71830 | Glucan endo-1, 3-beta-glucosidase GV, putative, expressed | TC253444[b] | 7.8e-124 | 3.5e-119 |
| LOC_Os01g71850 | Putative far-red impaired response protein | TC272356 | 2.0e-35 | 9.0e-55 |
| LOC_Os01g71860 | Glycosyl hydrolases family 17 protein, expressed | TC235738 | 6.4e-130 | 1.9e-111 |
| LOC_Os01g71870 | LigA, putative | N/A | NS | NS |
| LOC_Os01g71930 | Glycosyl hydrolases family 17 protein, expressed | TC238272 | 2.2e-61 | 6.8e-80 |
| LOC_Os01g71950 | Expressed protein | NS | NS | NS |
| LOC_Os01g71960 | ERCC4 domain containing protein, expressed | TC244162[b] | 1.5e-101 | 1.9e-58 |
| LOC_Os01g71970 | GRAS family transcription factor containing protein, expressed | N/A | NS | NS |
| LOC_Os01g71980 | Rho-GTPase-activating protein-like, expressed | TC269390[b] | 4.2e-118 | 3.8e-109 |
| LOC_Os01g71990 | Pyrroline-5-carboxylate reductase, putative, expressed | TC254523[b] | 3.7e-135 | 4.5e-102 |
| LOC_Os01g72000 | Armadillo repeat-containing protein, putative, expressed | TC240391[c] | 3.9e-125 | NS |
| LOC_Os01g72009 | Expressed protein | N/A | NS | NS |
| LOC_Os01g72020 | BOP2, putative, expressed | N/A | NS | NS |
| BAC: P0614D08 | | | | |
| LOC_Os01g71620 | Expressed protein | N/A | NS | NS |
| LOC_Os01g71624 | Expressed protein | N/A | NS | NS |
| LOC_Os01g71630 | Expressed protein | N/A | NS | NS |
| LOC_Os01g71650 | Glucan endo-1,3-beta-glucosidase, acidic isoform precursor, putative | TC251667 | 5.7e-65 | 1.5e-70 |
| LOC_Os01g71670 | Glucan endo-1,3-beta-glucosidase GII precursor, putative, expressed | TC235738 | 3.3e-149 | 2.3e-128 |
| LOC_Os01g71680 | Glucan endo-1,3-beta-glucosidase GII precursor, putative | TC235738 | 9.3e-113 | 8.9e-105 |
| LOC_Os01g71690 | RecA protein, expressed | TC261350[b] | 2.7e-108 | 9.6e-76 |
| LOC_Os01g71700 | Amino-acid permease C1039.01, putative, expressed | N/A | NS | NS |
| LOC_Os01g71710 | Amino-acid permease C74.04, putative, expressed | N/A | NS | NS |
| LOC_Os01g71720 | GABA-specific permease, putative, expressed | TC240455 | 7.6e-89 | 4.9e-78 |
| LOC_Os01g71740 | Amino-acid permease C584.13, putative, expressed | TC270002 | 1.5e-81 | NS |
| LOC_Os01g71760 | Amino-acid permease C584.13, putative, expressed | N/A | NS | NS |
| LOC_Os01g71770 | Heterogeneous nuclear ribonucleoprotein 27C, putative, expressed | TC236826 | 1.3e-114 | 4.9e-103 |
| LOC_Os01g71780 | Nucleotide binding protein, putative, expressed | CK210942 | 1.2e-32 | 4.5e-79 |
| LOC_Os01g71790 | NAM-like protein, putative, expressed | N/A | NS | NS |
| LOC_Os01g71800 | 1-*O*-acylceramide synthase precursor, putative | N/A | NS | NS |
| LOC_Os01g71810 | Glucan endo-1,3-beta-glucosidase GV, putative, expressed | TC253444[b] | 1.1e-122 | 1.9e-113 |
| LOC_Os01g71820 | Glucan endo-1,3-beta-glucosidase GV, putative, expressed | TC253444[b] | 5.1e-124 | 4.3e-119 |
| LOC_Os01g71599 | Hypothetical protein | N/A | NS | NS |

*TC* tentative consensus, *NS* no significant similarity. *N/A* TC sequence not available
[a] Designations of ESTs (Genbank) and TCs (TIGR) as of December 2006
[b] Closest EST-based STS marker placed in the linkage maps
[c] Mapped to 5BL, 5DL and 4AL by wheat NSF-EST mapping project

## Discussion

As a first step towards isolating a candidate gene, the *tin3* gene was mapped in relation to physically mapped ESTs and STS markers developed based on synteny with rice. 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 *tin3* with the corresponding region in rice by comparative genomic analysis. 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 *tin3* region.

Of all the homoeologous groups in wheat compared to rice chromosomes, the homoeologous group-3 chromosomes have been shown to be the best conserved (Sorrells et al. 2003; La Rota and Sorrells 2004). Our results agree with this notion, and the data in this study confirm the synteny reported for 3AL of wheat and 1L of rice (La Rota and Sorrells 2004; Sorrells et al. 2003). Of the total ESTs and STS markers mapped in the genetic map (Fig. 1), 68%

were colinear to rice chromosome 1, where these markers also showed very high level of homology to their orthologous rice sequences. The conservation of the order of the genetically resolved wheat EST and STS markers relative to their orthologous sequences in the rice genome suggest that the macrocolinearity in the *tin3* region is well conserved. Comparative genomic analysis of the annotated rice sequences from rice BACs P0466H10 and P0614D08 as queries in BLASTn searches of the wheat EST database TIGR wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/) showed that 73–75% of rice sequences had corresponding homologues in wheat, tentatively suggesting the conserved microcolinearity at the *tin3* genomic region (Table 3). It is interesting to note that the level of homology at the *tin3* genomic region is less than what was observed at the *R-A1* gene region. This observation is in agreement with previous reports suggesting that wheat genomic regions with high recombination rates show perturbations in synteny with rice (Akhunov et al. 2003) because the *tin3* gene was mapped distal to the *R-A1* gene or to its linked distal markers on the distal region of the chromosome arm 3AL (Kuraparthy 2007; Kuraparthy et al. 2007). Nevertheless, the level of homology within the *tin3* gene region was still much higher than what was expected based on a general observation that colinearity among the wheat genomes is better in the proximal regions of the chromosomes than in the distal regions (Akhunov et al. 2003). The ends of the chromosomes were found to be particularly rich in colinearity exceptions because these regions were 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. 2003; SanMiguel et al. 2002; Yan et al. 2003; Distelfeld et al. 2004). Breaks in wheat/rice microcolinearity were frequently observed in studies involving the distal regions of the wheat genome, such as the *Lrk/Tak* region (Feuillet and Keller 1999), the *Sh2/X1/X2/A1* region (Li and Gill 2002), or the *Rpg1* region (Kilian et al. 1997). Comparative genomics analysis at the whole genome level between wheat and rice also indicated an increase in the divergence of gene sequences physically located at or near the telomeric ends of wheat chromosomes (See et al. 2006). Although *tin3* is located at the distal high-recombination gene-rich region of wheat, high level of conservation of wheat–rice synteny at this region suggest that the present results are an exception to the above observations. Exceptions to the idea that distal regions are less conserved than proximal regions were also reported by Chantret et al. (2004). These exceptional observations are of considerable interest in understanding the biological and evolutionary processes in the cereal genome evolution. The partial genetic map of ESTs and STS markers and anchoring this map to rice sequence is the first step to characterize this important region in cereals.

Since the initial comparative mapping experiments with rice, wheat, and maize (Ahn et al. 1993) and the recently constructed genome-wide comparative mapping between wheat and rice (Sorrells et al. 2003; La Rota and Sorrells 2004), conserved wheat–rice synteny was used in gene mapping and gene discovery. The rice genome sequence was a potentially valuable tool for map-based cloning of the vernalization gene VRN1 (Yan et al. 2003) and a grain protein content gene (Distelfeld et al. 2004; Uauy et al. 2006). However, more extensive use of wheat–rice synteny has been the use of colinear regions of rice as a useful source of markers for saturation and high-resolution mapping of target genes in wheat (Distelfeld et al. 2004; Mateos-Hernandez et al. 2005; Valárik et al. 2006). Identification of flanking markers to *tin3*, mapping of *tin3* to specific genomic location in wheat chromosome arm 3AL, and the genomic targeting of *tin3* to two overlapping BACs P0466H10 and P0614D08 suggested that physically mapped EST sequences of wheat and wheat–rice synteny was very helpful in molecular mapping and targeting of the *tin3* gene. Liu and Anderson (2003) were also able to leverage the synteny of chromosome 3BS of wheat and 1S of rice to enrich the markers near the QTL for resistance to FHB. Because the markers flanking *tin3* were from highly homologous sequences of these two BACs with the size of 176.53 kb (P0466H10) and 147.5 kb (P0614D08), the physical size of the *tin3* BAC region in rice could be less than 324 kb. Because the microcolinearity is also conserved within the BAC region orthologous to *tin3* gene, the present results could pave the way for further high-resolution mapping, candidate gene analysis, and molecular cloning of *tin3* in wheat.

The degree of genomic similarity observed at the macrolevel among grass genomes coupled with the assumption that the essential components of growth and development are conserved among plants led to the notion that comparative mapping experiments could serve as an efficient tool for transferring information and resources from well-studied genomes, such as that of rice, to related plants. Further comparative low-resolution genetic mapping of shattering QTL in the orthologous genomic regions in sorghum, maize, and rice led to the notion of convergent domestication of cereal crops by independent mutations at orthologous loci (Paterson et al. 1995). However, the recent mapping data (Li and Gill 2006) and our studies reported here including comparative mapping of candidate genes for tillering loci do not support this hypothesis. The candidate homolog of a major gene, lateral suppressor (GRAS family transcription factor) of tomato mapped 37.1 cM proximal to the *tin3* gene (Fig. 1). In rice, a major gene controlling

tillering was found to be a GRAS family transcription factor (Li et al. 2003). Its noncolinear map location on chromosome 6 of rice with respect to *tin3* of wheat suggests that different genes or genetic systems are involved in the tillering of cereal crops. The *Ls* and *Moc1* genes encode putative transcriptional regulators of the plant-specific GRAS family (Bolle 2004). Recent evidence also suggests that members of the GRAS gene family encode transcriptional regulators that have diverse functions in plant growth and development, such as gibberellin signal transduction, root radial patterning, axillary meristem formation, phytochrome A signal transduction, and gametogenesis (Bolle 2004). Consistent with these observations, bioinformatic analysis identified 57 GRAS genes in rice (Tian et al. 2004). This partly indicates that the paralogous sequences of GRAS genes could have different functions in different plants, thereby challenging the convergent domestication of crop plants with respect to tillering or lateral branching. Although convergent domestication has not been unequivocally demonstrated for major domestication traits in cereals, functional gene orthologs have been reported for some plant traits. For example, the green revolution genes *Rht-B1/Rht-D1* and maize *dwarf-8* are orthologues of Arabidopsis *Gibberellin insensitive* gene (GAI) (Peng et al. 1998) and genes involved in the vernalization in wheat and barley (Yan et al. 2006; Yan et al. 2004; Fu et al. 2005). Cloning and characterization of *tin3* in wheat could not only shed more light on the genetics and domestication of tillering in wheat and cereals but could also lead to better understanding of the grass evolution.

Most of the cloned genes involved in the lateral branching or tillering are found to be transcription factors (Schumacher et al. 1999; Li et al. 2003; Lynn et al. 1999; Otsuga et al. 2001; Doebley et al. 1997). In the genomic region of *tin3* there are two putative transcription factors, the GRAS family transcription factor-like protein in BAC P0466H10 and the NAM-like protein genes in P0614D08 (Table 3). However, absence of a high level of sequence similarity expected for these two developmental genes makes the candidate gene approach difficult for cloning the *tin3* in wheat. Nevertheless, genomic targeted mapping of *tin3* in the specific syntenic rice BACs delimited by closely linked markers of *tin3* is a first step in molecular cloning of *tin3* in wheat.

**Acknowledgement** We thank Drs. Allan Fritz, Justin Faris, and Scot Hulbert for reviewing this manuscript. We extend a special note of thanks to Duane Wilson and Jon Raupp for their excellent technical assistance. We also thank Dr. Wanlong Li for sharing the D-genome mapping population used in the present study. Research supported by USDA and Kansas Wheat Commission. This paper is contribution number 07-283-J from the Kansas Agricultural Experimentation Station, Kansas State University, Manhattan, KS.

## 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, Goodyear JA et al (2003) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res 13:753–763

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Armstead I, Donnison I et al (2007) Cross-species identification of Mendel's *I* locus. Science 315:73

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Report 9:208–218

Bennett MD, Leitch IJ (1995) Nuclear DNA amounts in angiosperms. Ann Bot 76:113–176

Bolle C (2004) The role of GRAS proteins in plant signal transduction and development. Planta 218:683–692

Chantret N, Cenci A, Sabot F, Anderson O, Dubcovsky J (2004) Sequencing of the *Triticum monococcum hardness* locus reveals good microcolinearity with rice. Mol Genet Genomics 271:377–386

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

Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386:485–488

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

Fu D, Szűcs P, Yan L, Helguera M, Skinner JS, Zitzewitz J, Hayes PM, Dubcovsky J (2005) Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol Gen Genomics 273:54–65

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

Kosambi D (1944) Estimation of map distances from recombination values. Ann Eugen 12:172–175

Kuraparthy V (2007) Genomic targeting and mapping of agronomically important genes in wheat. Ph.D. dissertation, Kansas State University

Kuraparthy V, Sood S, Chunneja P, Dhaliwal HS, Gill BS (2007) Identification and mapping of tiller inhibition gene (*tin3*) in wheat. Theor Appl Genet 114:285–294

Lander E, Green P, Barlow A, Daley P, Stein L et al (1987) MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

La Rota M, Sorrells ME (2004) Comparative DNA sequence analysis of mapped wheat ESTs reveals the complexity of genome relationships between rice and wheat. Funct Integr Genomics 4:34–36

Li W, 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

Li W, Gill BS (2006) Multiple genetic pathways for seed shattering in the grasses. Funct Integr Genomics 6:300–309

Li X, Qian Q, Fu Z et al (2003) Control of tillering in rice. Nature 422:618–621

Li W, Zhang P, Fellers JP, Friebe B, Gill BS (2004) Sequence composition, organization and evolution of the core Triticeae genome. Plant J 40:500–511

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

Lynn K, Fernandez A, Aida M, Sedbrook J, Tasaka M, Masson P, Barton MK (1999) The PINHEAD/ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. Development 126:469–481

Mateos-Hernandez M, Singh RP, Hulbert SH, Bowden RL, Huerta-Espino J, Gill BS, Brown-Guedira G (2005) Targeted mapping of ESTs linked to the adult plant resistance gene *Lr46* in wheat using synteny with rice. Funct Integr Genomics 6:122–131

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

Otsuga D, DeGuzman B, Prigge MJ, Drews GN, Clark SE (2001) REVOLUTA regulates meristem initiation at lateral positions. Plant J 25:223–236

Paterson AH, Lin Y et al (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718

Peng J, Richards D et al (1998) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261

Qi LL, Echalier B et al (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

SanMiguel P, Ramakrishna W, Bennetzen JL, Buss CS, Dubcovsky J (2002) Transposable elements, genes and recombination in a 215-kb contig from wheat chromosome $5A^m$. Funct Integr Genomics 2:70–80

Schumacher K, Schmitt T, Rossberg M, Schmitz G, Theres K (1999) The Lateral suppressor (Ls) gene of tomato encodes a new member of the VHIID protein family. Proc Natl Acad Sci U S A 96:290–295

See DR, Brooks S, Nelson JC, Guedira GB, Friebe B, Gill BS (2006) Gene evolution at the ends of wheat chromosomes. Proc Natl Acad Sci U S A 103:4162–4167

Sorrells ME, La Rota M et al (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Souer E, van Houwelingen A, Kloos D, Mol J, Koes R (1996) The no apical meristem gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordial boundaries. Cell 85:159–170

Stuurman J, Jaggi F, Kuhlemeier C (2002) Shoot meristem maintenance is controlled by a GRAS-gene mediated signal from differentiating cells. Genes Dev 16:2213–2218

Tian C, Wan P, Sun S, Li J, Chen M (2004) Genome-wide analysis of the GRAS gene family in rice and *Arabidopsis*. Plant Mol Biol 54:519–532

Uauy C, Distelfeld A, Fahima T, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1301

Valárik M, Linkiewicz AM, Dubcovsky J (2006) microcolinearity study at the earliness *per se* gene *Eps-$A^m$1* region reveals an ancient duplication that preceded the wheat–rice divergence. Theor Appl Genet 112:945–957

Van Deynze AE, Dubcovsky J et al (1995a) Molecular-genetics maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38:45–59

Van Deynze AE, Nelson JC, Yglesis ES, Harrington SE, Braga DP, McCouch SR, Sorrells ME (1995b) Comparative mapping in grasses. Wheat relationships. Mol Gen Genet 248:744–754

Ware DH, Jaiswal P et al (2002) Gramene, a tool for grass genomics. Plant Physiol 130:1606–1613

Wicker T, Stein N, Albar L, Feuillet C, Schlagenhauf E, Keller B (2001) Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L.) reveals multiple mechanisms of genome evolution. Plant J 26:307–316

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

Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J (2004) Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor Appl Genet 109:1677–1686

Yan L, Fu D, Li C et al (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc Natl Acad Sci U S A 103:19581–19586