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# Genes controlling plant growth habit in *Leymus* (Triticeae): maize *barren stalk1* (*ba1*), rice *lax* panicle, and wheat *tiller inhibition* (*tin3*) genes as possible candidates

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**Abstract** *Leymus cinereus* and *L. triticoides* are large caespitose and rhizomatous perennial grasses, respectively. Previous studies detected quantitative trait loci (QTL) controlling rhizome spreading near the *viviparous1* (*vp1*) gene markers on linkage groups LG3a and LG3b in two families, TTC1 and TTC2, derived from *Leymus triticoides* × *Leymus cinereus* hybrids. The wheat *tiller inhibition* gene (*tin3*) is located on *Triticum monococcum* chromosome 3 A<sup>m</sup>L near *vp1*. Triticeae group 3 is reportedly collinear with rice chromosome 1, which also contains the maize *barren stalk1* and rice *lax* branching orthogene near *vp1*. However, previous studies lacked cross-species markers for comparative mapping and showed possible rearrangements of *Leymus* group 3 in wheat-*Leymus racemosus* chromosome addition lines. Here, we developed expressed sequence tag (EST) markers

from *Leymus* tiller and rhizomes and mapped sequences aligned to rice chromosome 1. Thirty-eight of 44 informative markers detected loci on *Leymus* LG3a and LG3b that were collinear with homoeologous sequences on rice chromosome 1 and syntenous in homoeologous group 3 wheat-*Leymus* and wheat-*Thinopyrum* addition lines. A SCARECROW-like GRAS-family transcription factor candidate gene was identified in the *Leymus* EST library, which aligns to the *Leymus* chromosome group 3 growth habit QTL and a 324-kb rice chromosome 1 region thought to contain the wheat *tin3* gene.

**Keywords** Addition lines · Growth habit · Comparative genomics · Tillers · Rhizomes · Perennial Triticeae

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## Introduction

Growth habit is a fundamentally important and adaptive trait in perennial grasses. Caespitose grasses form a compact tussock of tiller stem branches, whereas sod-forming grasses typically spread via prostrate stolon or subterranean rhizome stem branches. This growth habit variation evidently reflects different evolutionarily adaptations resulting from competition for moisture, nutrients, space, light, and, perhaps, other resources. Aggressive rhizomes are a problematic trait in opportunistic perennial grass weeds such as quackgrass (*Elymus repens*). In many cases, growth habit variation within and among perennial grass species also relates to ease of establishment, durability, yield, persistence, turf quality, soil protection, and other functional attributes of cultivated forage, turf, and biomass

crops. North American basin wild rye (*Leymus cinereus*) and creeping wild rye (*Leymus triticoides*) display divergent caespitose and rhizomatous growth habits, respectively. The circumference of tiller spreading of basin wild rye rarely exceeds 80 cm, whereas the circumference of some creeping wild rye plants can exceed 1,300 cm after only 3 years of growth (Larson et al. 2006). Creeping wild rye shows potential as a saline biomass crop (Suyama et al. 2007a, b). Basin wild rye is an unusually tall grass, exceeding 2 m, and is considered one of the largest native grasses in western North America. Interspecific hybrids of basin wild rye and creeping wild rye show a combination of traits, including rhizomatous tillers, which will be useful in the breeding of forage and biomass feedstocks.

*Leymus* is a polyploid Triticeae genus that includes about 30 long-lived, morphologically diverse perennial grass species, distributed throughout the temperate regions of Asia, Europe, and America (Dewey 1984). More than half of all *Leymus* species are allotetraploids ( $2n=4x=28$ ). *Psathyrostachys* and *Thinopyrum* were identified as possible diploid ancestors of *Leymus*, based on chromosome pairing (Dewey 1970; Dewey 1984; Löve 1984). The presence of *Psathyrostachys* (Ns) DNA in *Leymus* has been verified (Zhang and Dvorak 1991; Wang and Jensen 1994; Anamthawat-Jónsson 2005). However, extensive testing has failed to detect *Thinopyrum*-specific DNA in *Leymus* (Zhang and Dvorak 1991). The DNA content of the basic allotetraploid ( $2n=4x=28$ ) forms of *Leymus* is about 21.8 pg per cell or  $10.5 \times 10^9$  bp per haploid genome (Vogel et al. 1999). This is approximately midway between diploid barley (*Hordeum vulgare*) and hexaploid bread wheat (*Triticum aestivum*) Triticeae cereals (Vogel et al. 1999), and approximately 24 times greater than the 430-Mb rice genome (Yu et al. 2002; Goff et al. 2002).

Molecular genetic linkage maps containing 1,583 amplified fragment length polymorphism (AFLP) markers and 67 heterologous anchor markers have been constructed for two full-sib mapping families, TTC1 and TTC2, derived from two  $F_1$  *L. triticoides*  $\times$  *L. cinereus* hybrids crossed to one *L. triticoides* tester genotype (Wu et al. 2003). Colocalization of growth habit quantitative trait loci (QTL; LOD 3.3 to 5.4) near the *viviparous 1* (*Vp1*) gene markers on linkage groups (LG) LG3a and LG3b, in both TTC1 and TTC2 families, suggests that these growth habit QTLs may be located in homoeologous regions of the allotetraploid *Leymus* subgenomes (Larson et al. 2006). The *Vp1* orthogene is located on long arm of rice chromosome 1 and wheat chromosome 3 (Bailey et al. 1999). Chromosome group 3 is highly conserved among Triticeae grasses (Devos and Gale 2000) and is collinear with rice chromosome 1 (La Rota and Sorrells 2004). Markers flanking the wheat *tiller inhibition* gene (*tin3*), on the distal long arm of chromosome 3A<sup>m</sup>, are located within a 324-kb contig of

two bacterial artificial chromosomes (BACs) on the long arm of rice chromosome 1 (Kuraparthy et al. 2008). Likewise, the maize *barren stalk 1* (*Ba1*) and rice *lax* panicle orthogene is also located on the long arm of rice chromosome 1 and is also involved in the initiation and expression of axillary branches in both species (Komatsu et al. 2003; Gallavotti et al. 2004).

All five Triticeae group 3 anchor markers mapped in *Leymus*, including *Vp1*, were syntenous on LG3a and/or LG3b (Wu et al. 2003). However, DNA from wheat-*Leymus racemosus* monosomic addition line 'n' and disomic addition lines 'H' and 'J' cross hybridized with restriction fragment length polymorphism (RFLP) probes from wheat groups 1, 3, and 7 (Kishii et al. 2004). Thus, comparative mapping of the *Leymus* LG3 growth habit QTL was based on a limited number of cross-species anchor markers (Larson et al. 2006), and RFLP analysis of the wheat-*Leymus* chromosome addition lines suggests possible chromosome rearrangements in *Leymus* (Kishii et al. 2004).

Expressed sequence tag (EST) libraries provide an important resource for the development of highly polymorphic microsatellite markers with conserved polymerase chain reaction (PCR) primer annealing sites (Varshney et al. 2005) and functional association studies (Andersen and Lübberstedt 2003). Moreover, the availability of the entire rice genome DNA sequence (Dickson and Cyranoski 2001; Goff et al. 2002; Yu et al. 2002) has enabled researchers to align heterologous EST markers to the rice genome by in silico matching of conserved gene sequences (Sorrells et al. 2003; La Rota and Sorrells 2004; Perovic et al. 2004). Initial comparative mapping using heterologous RFLP probes in cereals suggests that large chromosome regions are conserved in the genomes of diverse *Poaceae* species (Ahn and Tanksley 1993; Hulbert et al. 1990; Van Deynze et al. 1995). Improvements in DNA sequencing technologies and bioinformatics have the potential to make the development of EST markers and applications of comparative mapping feasible for a greater diversity of organisms.

Identification of genes controlling functionally important traits in *Leymus* wild ryes and other perennial grasses will require utilization of genome sequence information in rice and other model grasses. The main objectives of this project were to develop polymorphic *Leymus* EST microsatellite markers from *L. triticoides*  $\times$  *L. cinereus* hybrids, align these markers to the rice genome, and use markers aligned to rice chromosome 1 to test the hypothesis that *Leymus* LG3a and LG3b growth habit QTLs are syntenous with the maize *Ba1*/rice *lax* panicle orthogene and wheat *tin3* gene (Kuraparthy et al. 2008). Another important objective of this study was to test amplification efficiency and synteny of *Leymus* EST microsatellite markers in the wheat-*L. racemosus* and wheat-*Thinopyrum* chromosome addition

lines (Qi et al. 1997; Kishii et al. 2004; Dvorak and Knott 1974; Zhang et al. 2002).

## Materials and methods

### *Leymus* mapping populations

The *Leymus* TTC1 and TTC2 mapping populations and parents, as described by Wu et al. (2003) and Larson et al. (2006), were used for the development, testing, and mapping of new *Leymus* EST markers. Briefly, TTC1 and TTC2 families were derived from one *L. triticoides* Acc641 plant (T-tester) pollinated by two different *L. triticoides* Acc:641 × *L. cinereus* Acc:636 hybrid plants (TC1 and TC2). The TTC1 and TTC2 families include 164 and 170 full-sib individuals, respectively.

### Development of *Leymus* EST library and rice genome alignment

The *Leymus* EST library was constructed, normalized, sequenced, filtered, and assembled using the same methods described by Anderson et al. (2007) with the minor modifications described below. Tissues from aerial tiller regrowth (less than 10 cm above ground) harvested under freezing cold temperatures about 2 h after sunrise and subterranean rhizomes were collected from the same *L. triticoides* × *L. cinereus* hybrids used to construct the TTC1 and TTC2 mapping families (Wu et al. 2003) and immediately flash-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  and ground to a fine powder in liquid nitrogen. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) reagent following the manufacturers' protocol. Total RNA was purified using Qiagen Midi columns (Qiagen, Valencia, CA, USA). The poly (A)<sup>+</sup> mRNA was converted to double-stranded cDNA using different *NotI*/oligo(dT)-tagged primers for two independent RNA samples as follows: (1) [5'-AACTGGAAGAATTCGCGGCCGCTCCGA(T)<sub>18</sub>V-3'] for aerial tiller regrowth and (2) [5'-AACTGGAAGAATTCGCGGCCGCTCGCA(T)<sub>18</sub>V-3'] for subterranean rhizomes. Double-stranded cDNA samples  $\geq 600$  bp were selected by agarose gel electrophoresis and an equal mass of subterranean branch meristem cDNA and aerial spring regrowth cDNA was used for cloning. The total number of white colony forming units before amplification was  $3 \times 10^6$ , whereas the total number of clones with insert was  $2 \times 10^6$  following normalization. A total of 15,000 clones were sequenced from the 5' end using the T7 primer and from the 3' end using the T3 or M13R primer.

All T3 and M13R primer reads were assembled into unigene contigs and aligned to the 12 chromosomes of rice

(*Oryza sativa*) using basic local alignment and search tool (BLAST)n (Altschul et al. 1997) with an *e* value threshold of 0.0001. Sequences of the 12 rice chromosomes were obtained from National Center for Biotechnology Information (NCBI) at [ftp://ftp.ncbi.nih.gov/genomes/Oryza\\_sativa/](ftp://ftp.ncbi.nih.gov/genomes/Oryza_sativa/) on December 7 of 2006.

### Development and genotyping of *Leymus* EST microsatellites

The SSRFINDER Perl scripts (Steven Schroeder, University of Missouri-Columbia), available at <http://www.maizemap.org/bioinformatics>, were used to scan all *Leymus* EST unigene sequences for microsatellite repeats and identify flanking primer annealing sites suitable for PCR amplification. The SSRFINDER scripts connect several development steps including the BLAST package, from NCBI, to search for microsatellite repeats and Primer3, from the Whitehead Institute (Cambridge, MA, USA), for primer design as described by Sharopova et al. (2002). All dinucleotide motifs with six repeats or greater and 3- to 6-nucleotide motifs with four repeat or greater were marked. Where possible, PCR primers flanking microsatellite repeats were designed based on the following criteria: (1) primer length ranging from 18 to 22 bp with 20 bp as the optimum; (2) product size ranging from 80 to 250 bp; (3) melting temperature between  $57^{\circ}\text{C}$  and  $63^{\circ}\text{C}$ ; (4) and GC% content between 20% and 80%.

The EST microsatellite primers were tested for PCR amplification in 10- $\mu\text{l}$  reactions prepared using 1× PCR buffer, 0.2 mM dNTPs, 0.5  $\mu\text{M}$  of each primer, 1.25 units jump start Taq DNA polymerase, 0.1  $\mu\text{mol}$  [R110] dCTP, and 50–100 ng of DNA. PCR touch-down protocol was used with the following profile: (1) initial denaturation at  $95^{\circ}\text{C}$  for 90 s, (2) five cycles of  $95^{\circ}\text{C}$  for 1 min,  $65^{\circ}\text{C}$  for 1 min, decreasing annealing temperature  $1^{\circ}\text{C}/\text{cycle}$ ,  $72^{\circ}\text{C}$  for 1 min, (3) 30 cycles of  $95^{\circ}\text{C}$  for 1 min,  $60^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 1 min, (4) and a final extension of  $72^{\circ}\text{C}$  for 6 min. The relative mobility of PCR amplicons was analyzed by capillary electrophoresis using GS500 LIZ internal size standard and ABI 3730 genetic analyzer (PE Applied Biosystems Inc., Foster City, CA, USA) and Genescan software (PE Applied Biosystems). The relative mobility of PCR amplicons was compared and classified, by genotype, using Genographer version 1.6.0 (Benham et al. 1999).

### Linkage and QTL analyses

New EST microsatellite markers were initially assigned to one of 14 possible linkage groups (map nodes) in the TTC1 and/or TTC2 families (Wu et al. 2003; Larson et al. 2006)



using the ‘Create Groups Using a Map Node’ and ‘Assign Ungrouped Loci to Strongest Cross Link (SCL) Groups’ functions of JoinMap 4.0 (Van Ooijen 2006). A SCL threshold of 20 LOD was used to assign new (ungrouped) EST microsatellite markers to previous linkage groups. The TTC1 and TTC2 linkage groups were joined using the ‘Combine Groups for Map Integration’ function, of JoinMap 4.0, to create integrated map nodes for the LG3a and LG3b linkage groups. Consensus maps for LG3a and LG3b linkage groups were calculated by of ‘Regression Mapping’ using only linkages with a recombination frequency smaller than 0.4, linkage LOD greater than 1.0, goodness-of-fit jump threshold of 5.0, ripples after each added locus, and Haldane’s mapping function with a third round to force any remaining markers on the map. However, only those AFLP markers that were previously mapped in both populations, fitted in second-round regression mapping, and required to fill gaps between new EST and other previously mapped anchor were used for further analyses and presentation in this report. The TTC1 and TTC2 growth habit QTL, previously detected on LG3a and LG3b (Larson et al. 2006), were realigned to the simplified LG3a and LG3b consensus maps using MapQTL 5.0 (Van Ooijen 2004) and the same phenotypic data described by Larson et al. (2006). Map diagrams and QTL graphs were developed using MapChart 2.2 (Voorrips 2002).

#### Identification and alignment of rice chromosome 1 BAC clones containing *Leymus* EST microsatellite markers and other reference sequences

Rice BAC clones containing those *Leymus* EST sequences aligned to rice chromosome 1 and mapped in the *Leymus* TTC1 and TTC2 families were identified using a BLAST search against the rice genome database (<http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1>). The resulting rice BAC clones containing mapped *Leymus* EST ortholoci were aligned to other rice BAC clones and contigs containing the wheat *tin3* gene (Kuruparthi et al. 2008) and rice *lax* panicle (Komatsu et al. 2003)/maize *barren stalk 1* (Gallavotti et al. 2004) orthogenes using physical map data from the TIGR rice genome database ([http://www.tigr.org/tigr-scripts/osa1\\_web/gbrowse/rice/](http://www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice/)), which is slightly different from the NCBI database ([ftp://ftp.ncbi.nih.gov/genomes/Oryza\\_sativa/](ftp://ftp.ncbi.nih.gov/genomes/Oryza_sativa/)) used for other *Leymus* ESTs alignments described above. Rice BAC clones containing the CentO (RCS2) centromere-specific retrotransposons sequences from GenBank accession AF058902 (Cheng et al. 2002; Dong et al. 1998) were identified using a BLAST search against the rice genome database (<http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1>). The resulting rice BAC clones were aligned to the rice

chromosome 1 physical map and the *Leymus* LG3a and LG3b genetic maps using physical map data from the TIGR rice genome database ([http://www.tigr.org/tigr-scripts/osa1\\_web/gbrowse/rice/](http://www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice/)).

#### Testing *Leymus* EST markers on other Triticeae species and chromosome addition lines

One or more accessions were used to represent five other Triticeae grasses including *L. racemosus* (D-2949, DJ-4116, PI 565037, PI 108491, PI 313463, PI 531811, and PI531812), *Psathyrostachys juncea* (cv. Bozoisky, cv. Cabree, cv. Mankota, cv. Swift, Syn-A, and cv. Vinal), *Thinopyrum bessarabicum* (AJC, D3584, PI 532711, PI53712, and PI531710) and *Thinopyrum elongatum* (D3610, PI 531719, KJ243, and PI 547326), and *T. aestivum* (Chinese Spring). The PI lines were obtained from the United States Department of Agriculture National Plant Germplasm System and other accessions are available at Forage and Range Research Laboratory.

The wheat-*L. racemosus* chromosome addition lines described by Qi et al. (1997) were provided by the Wheat Genetic and Genomic Resource Center (Kansas State University, KS, USA). The wheat-*L. racemosus* chromosome addition lines described by Kishii et al. (2004) were provided by Professor Hisashi Tsujimoto (Tottori University, Japan). Wheat-*Thinopyrum* addition lines (Dvorak and Knott 1974; Zhang et al. 2002) were provided by A. Mujeeb-Kazi (CIMMYT, Mexico). The reported number of chromosomes for each chromosome addition line (Qi et al. 1997; Kishii et al. 2004) was verified by our own cytological analysis of plants used in this study.

## Results and discussion

### Development of *Leymus* EST microsatellite markers and consensus maps

A total of 28,786 successful *Leymus* EST reads were obtained from 15,000 clones and are available in the NCBI GenBank Nucleotide EST database as accession numbers EG37452 to EG403327. The 28,786 *Leymus* ESTs were assembled or partitioned into 6,217 contigs and 5,064 singletons with a total of 11,281 unigenes. A total of 9,389 (87.6%) of the 11,281 *Leymus* EST unigenes showed at least one significant match to the overall rice genome, but only 1,370 (14.6%) of these unigene alignments were located on rice chromosome 1. A subset of 1,798 (15.9%) of the 11,281 *Leymus* EST unigenes met criteria for microsatellite PCR primer design (Table S1). A total 1,575 (83.2%) of the 1,798 *Leymus* EST microsatellite markers were aligned to

the rice genome (Table S1), but only 227 (14.4%) of these 1,575 *Leymus* EST microsatellite markers aligned to rice chromosome 1.

A total of 44 (19.8%) of the 227 *Leymus* EST microsatellites aligned to rice chromosome 1 showed segregating polymorphisms in the TTC1 and/or TTC2 families. All 44 of these informative *Leymus* EST microsatellites were successfully assigned to one of 14 possible linkage groups in the *Leymus* and were then aligned to corresponding rice chromosome 1 BACs (Table 1). Nine markers detected more than one polymorphic amplicon, including five markers that did not map to the same linkage group (Table 1). A total of 38 (86%) of the 44 new *Leymus* EST microsatellite markers, aligned to rice chromosome 1, detected 23 loci on LG3a and 17 loci on LG3b (Fig. 1). Only three (about 7.9%) of the 38 *Leymus* EST microsatellite markers (Ltc0323, Ltc0195, and Ltc0276) mapped to both LG3a and LG3b. Although six of the 44 mapped *Leymus* EST microsatellites aligned to rice chromosome 1 did not map to LG3a or LG3b, there was no other discernable pattern of synteny or rearrangement of these six markers, on other linkage groups of *Leymus*. These results confirm our hypothesis that most of the *Leymus* EST markers aligned to rice chromosome 1 are syntenous on *Leymus* LG3a and LG3b.

All 44 *Leymus* EST microsatellite markers mapped in the TTC1 and/or TTC2 families were also tested for PCR amplification on five other Triticeae species (Table 1). The numbers of *Leymus* EST microsatellite markers that showed successful amplification in the five other Triticeae species varied as follows: 35 (80%) in allotetraploid *L. racemosus*, 29 (66%) in diploid *P. juncea*, 20 (45%) in diploid *Th. bessarabicum*, 20 (45%) in diploid *Th. elongatum*, and 25 (57%) in hexaploid bread wheat (*T. aestivum*). These data indicate relatively good transferability of the EST markers from North American *Leymus* species to Eurasian giant wild rye (*L. racemosus*) and Russian wild rye (*P. juncea*). These data also indicate that congeneric North American and Eurasian *Leymus* species show relatively high genetic similarity and are consistent with the interpretation (Zhang and Dvorak 1991; Wang and Jensen 1994; Anamthawat-Jónsson 2005) that the allotetraploid *Leymus* contains at least one *Psathyrostachys* genome. The numbers of *Leymus* LG3a and LG3b EST markers showing successful amplification in the other Triticeae genera and species were partitioned as follows: 14 LG3a and ten LG3b in diploid *P. juncea*, eight LG3a and ten LG3b in diploid *Th. bessarabicum*, eight LG3a and eight LG3b in diploid *Th. elongatum*, and 11 LG3a and ten LG3b in hexaploid bread wheat (*T. aestivum*; Table 1). Thus, EST markers mapped to *Leymus* homoeologous group LG3a tended to show some preferential amplification in diploid *P. juncea*. However, EST markers mapped to *Leymus* homoeologous group

LG3b do not show preferential amplification in any one of the other Triticeae genera tested.

Testing *Leymus* homoeologous group 3 ESTs on the wheat-*Leymus* and wheat-*Thinopyrum* chromosome addition lines

Twenty-six of the 38 *Leymus* LG3a and LG3b EST microsatellite markers were informative in at least one of the 16 wheat-*Leymus* chromosome addition lines (Table 2). Twenty-three (88%) of these 26 EST-based amplicons were observed in either the H or NAU524 wheat-*L. racemosus* chromosome addition lines. These results indicate that most of the *Leymus* EST markers aligned to rice chromosome 1 are syntenous in the H or NAU524 wheat-*L. racemosus* chromosome addition lines.

Most of the 26 EST-based amplicons observed in the wheat-*L. racemosus* chromosome addition lines were specific to H or NAU524 (Table 2), which suggests that these lines may contain different *Leymus* group 3 homologs. In particular, 15 (58%) and 12 (46%) of these EST-based amplicons were observed in the H and NAU524 wheat-*L. racemosus* chromosome addition lines, respectively. Only four (11.5%) EST-based amplicons (Ltc290, Ltc305, Ltc195, and Ltc268) were detected in both of H and NAU524 wheat-*L. racemosus* chromosome addition lines. The frequency of markers detected on both H and NAU524 lines (15.4%) is similar to the frequency of markers detected on both LG3a and LG3b (7.9%, Table 1) and one of the three markers detected in both H and NAU524 (Ltc195) was also detected on both LG3a and LG3b. These data suggest that wheat-*L. racemosus* chromosome addition lines H and NAU524 represent different subgenomes of *Leymus*. However, the actual ratios of LG3a:LG3b EST markers were 6:7 and 6:4 in the wheat-*Leymus* chromosome addition lines H and NAU524, respectively, excluding EST markers that were mapped to both LG3a and LG3b (Table 2). Thus, EST markers that were genome specific in the TTC1 and TTC2 families, were not unique to either of the H or NAU524 wheat-*L. racemosus* chromosome addition lines.

Only six of the 38 *Leymus* LG3a and LG3b EST microsatellite markers were informative in at least one of the 14 wheat-*Th. bessarabicum* and wheat-*Th. elongatum* chromosome addition lines. Nevertheless, five (83%) of these six EST-based markers were observed in the homoeologous group 3 addition lines including Ltc195, Ltc247, and Ltc257 in wheat-*Th. bessarabicum* (Zhang et al. 2002) and Ltc247 and Ltc323 in wheat-*Th. elongatum* (Dvorak and Knott 1974). The Ltc322 marker amplified only in wheat-*Th. bessarabicum* group 1. Although transfer efficiency of the *Leymus* EST markers to *Thinopyrum* was not as good as it was to *L. racemosus* or *P. juncea*, these

**Table 1** Summary of *Leymus* EST microsatellite marker mapping, cross-species amplification, and BLASTn hits to rice (*Oryza sativa*) chromosome 1 bacterial artificial chromosome (BAC) clones

Leymus EST marker ID	Expected amplicon size (EST)	Amplicons observed in <i>Leymus</i> TTC1 and/or TTC2 families (linkage map group)	Observed amplicons ( <i>Leymus racemosus</i> )	Observed amplicons ( <i>Psathyrostachys juncea</i> )	Observed amplicons ( <i>Thinopyrum bessarabicum</i> )	Observed amplicon ( <i>Thinopyrum elongatum</i> )	Observed amplicons ( <i>Triticum aestivum</i> )	Rice BAC	Physical location of rice BAC clones on rice chromosome 1 (bp)	BLASTn e value
Ltc0290	150	146, 148 (3a)	152, 155	151	–	151	147	P0436E04	173730–300776	4.6e-51
Ltc0292	155	129, 148 <sup>a</sup> (3b), 153 <sup>b</sup> (3b), 158	137	146	–	–	118	P0482C06	436799–540380	2.6e-85
Ltc0295	147	148, 150 <sup>a</sup> (3a)	137	–	–	–	–	OSJNB0032H19	659189–740492	1.5e-25
Ltc0299	137	244, 256 <sup>b</sup> (3a), 261 <sup>a</sup> (3a)	238	–	413	–	199	P0698A04	1105711–1169567	1.9e-35
Ltc0305	109	100, 109 (3a)	109	109	108, 110	111, 113	105, 106	P0409B08	1437631–1566827	2.5e-23
Ltc0306	160	146 (3a), 152, 160	–	146, 149	145	145, 148	–	P0409B08	1437631–1566827	1.9e-38
Ltc0308	127	127, 147 <sup>b</sup> (3b), 153, 160, 166	160	–	–	–	–	P0480E02	2081895–2218552	1.5e-43
Ltc0309	143	140 (3a), 144	144	–	–	–	–	P0480E02	2081895–2218552	1.1e-30
Ltc0315	128	121 (3b), 125, 127, 130	128	125, 127	133, 135	133, 135	133, 135	B1189A09	3200350–3347934	1.0e-94
Ltc0322	110	99, 107, 123 (3a)	99, 109	99	100	124	106	OSJNBa0089K24	3724618–3878397	2.2e-41
Ltc0323	149	256 (3b), 260, 262, 266 <sup>b</sup> (3a)	260	284	258, 263	258, 261, 263	258, 263	OSJNBa0089K24	3724618–3878397	1.6e-66
Ltc0329	102	84 (3b), 90, 107	94	87, 93	96	96	89, 96	OSJNBa0026L17	4878766–5041775	4.2e-79
Ltc0334	160	231, 239 (3b)	157, 162, 231	231	322	–	236	P0665D10	6401809–6510374	6.0e-34
Ltc0336	87	80, 86 (7a)	82	80	–	82, 85	79	P0483F08	6579030–6666616	3.2e-10
Ltc0345	111	111 (3b)	–	–	–	–	–	OSJNBa0086P08	7617987–7732538	2.3e-34
Ltc0354	113	107 <sup>b</sup> (4Xm), 109, 111, 185 <sup>a</sup> (4Xm)	–	–	–	–	–	B1153F04	12113700–2190836	1.4e-79
Ltc0376	117	114, 138 <sup>a</sup> (2a)	–	–	–	150	136	B1109A06 (CentO)	16937732–17086376	2.4e-105
Ltc0379	212	209, 211 <sup>b</sup> (3a), 213	–	209, 213	–	–	206	B1061G08	17086377–17174060	1.4e-101
Ltc0384	118	109, 113, 116 (3b)	112, 115	113	104, 112	104, 112	104, 112	OSJNBa0029L04 (CentO)	17231137–17348328	5.0e-35
Ltc0389	152	147 <sup>b</sup> (6b), 149, 157 <sup>a</sup> (2b)	146, 149	–	–	–	–	P0520B06	18259563–18353569	5.4e-21
Ltc0399	156	149 (3b), 153	148, 153, 154	148, 153	147, 148, 152, 154	152	148, 152, 154	B1144D11	19135755–19266682	3.2e-33
Ltc0401	89	84 <sup>b</sup> (3a), 87	82, 88, 91	–	83, 88	84, 88	86, 90	B1097D05	21426366–21521135	4.8e-21
Ltc0413	106	80, 89, 106 (3b)	85, 87, 89, 90	–	82, 88	88	81, 84, 88	P0046B10	22852255–22959879	1.4e-40
Ltc0195	145	136, 150 (3a), 140 (3b)	137, 149	136	143, 146	143, 146	142	P0046B10	24519755–24648769	3.6e-33
Ltc0207	142	137 (3a), 139	252	–	134	134	134	P0686E09	24751587–24906778	3.1e-45
								OSJNB0063G05	27560927–27735754	1.6e-81
								P0034C09	2881559–28977377	2.8e-63
								P0481E12	31584509–31740870	1.8e-28



Ltc0209	114	100, 107, 114 (3b)	117	100, 104, 107, 119	111, 115	115	111, 115	P0481E12	31584509–31740870	3.0e-33
Ltc0210	152	116 <sup>a</sup> (3a), 157	134	–	–	–	–	P0435B05	31902303–31971564	3.2e-65
Ltc0211	141	137 (3b)	139	–	–	–	–	OJ1414_E05	32067039–32160774	1.3e-36
Ltc0214	158	157 <sup>b</sup> (3a), 175, 185	174	185	–	–	–	OSJNB0053G03	32613923–32703206	7.4e-80
Ltc0222	133	88, 122 (3a), 129	122	130, 154	–	–	–	P0699H05	33998805–34125370	5.3e-61
Ltc0240	110	103 (3a), 105, 107	–	100	–	–	–	P0446G04 (LAX)	35863726–35946918	8.4e-50
Ltc0247	150	130, 138, 149 (3b)	150	130, 147	122	120, 122	122	P0414E03	36824637–36959813	2.4e-16
Ltc0248	158	158 (3a), 167, 174, 183	158, 186, 173	155, 156, 169, 177	186	186	160	P0491F11	38154182–38264508	1.6e-102
Ltc0253	115	100, 111, 114 <sup>b</sup> (3a), 121, 129	147	100, 104, 157	–	–	110	B1027B04	38517882–38683218	1.6e-38
Vp1-i2	401	394 <sup>a</sup> (3b)	–	–	–	–	–	P0035F12	39460876–39611269	8.1e-11
Vp1-i5	694	HhaI 406 (3a)	–	–	–	–	–	P0470A12	40026335–40167787	1.4e-149
Ltc0256	143	128 <sup>a</sup> + 129 <sup>b</sup> (1a), 138, 139	–	130, 137	–	–	–	P0470A12	40026335–40167787	1.4e-149
Ltc0257	160	160 (3b), 164, 167, 172, 188	160	164, 167, 172, 175, 179, 188	169	–	–	P0470A12	40026335–40167787	5.3e-45
Ltc0261	155	148 (3a), 156	–	–	–	–	–	OSJNBa0034K07	40257629–40411155	1.3e-33
Ltc0265	145	137, 145, 150 (3a)	145	137, 145, 150	–	–	152	P0026C12	40455601–40595978	3.9e-66
Ltc0268	99	89 <sup>a</sup> (3b), 95, 101 <sup>b</sup> (3b), 107	95, 97, 101	95, 101, 107	89	89	97	P0482D04	40854476–40999058	6.5e-60
Ltc0270	154	160, 162, 166 <sup>b</sup> (3a)	147, 151, 161	149, 153, 155, 159, 162, 165	–	–	–	P0506E04	41199726–41262318	2.4e-36
Ltc0276	208	199 <sup>b</sup> (3b), 206 <sup>a</sup> (3a)	–	–	–	–	–	P0506E04	41199726–41262318	2.3e-40
Ltc0277	217	200 <sup>b</sup> (2b), 205, 209, 212	210	212	–	–	–	P0614D08	41807392–41925745	1.4e-119
Ltc0279	151	147, 153, (3a)	154	146	–	–	–	(XSTS-TR3L6)	–	–
Ltc0281	158	99, 152, 156, 160 (3a), 167	168	156, 164, 167	158	158	158	P0466H10	41925746–42095418	1.2e-30
								(XBE48620)	–	–
								B1147A04	42095419–42241167	5.1e-60
								B1147A04	42095419–42241167	1.9e-107
								P0401G10	42582381–42723237	3.2e-25
								P0483G10	42723238–42865873	6.7e-71

Rice BAC clones containing the rice *CentO* centromere-specific retrotransposon sequences (AY058902), the wheat *tin3* gene markers (XSTS-TR3L6 and XBE48620), the maize *barren stalk 1* (Ba1)/rice *lax* panicle orthogenes are also included for reference.

<sup>a</sup> Amplicon mapped only in TTC1 family

<sup>b</sup> Amplicon mapped only in TTC2 family

data demonstrate that the *Thinopyrum* amplicons are probably orthologous to the *Leymus* EST sequences from which they were derived. Thus, these *Leymus* EST markers may have important applications in other Triticeae species.

#### Alignment of *Leymus* LG3a and LG3b growth habit QTLs to rice chromosome 1

The new LG3a consensus map, which combines data from the TTC1 and TTC2 families, includes a total of 34 AFLP markers and four anchor markers (Vp1-i5, BARC71.095, SIP1.324, and GWM005.080) previously mapped by Wu et al. (2003) in addition to 23 new EST microsatellite markers (Fig. 1). Although Ltc0210 and Ltc0379 were linked and assigned to LG3a (Table 1), these two markers did not integrate with other LG3a markers. The new LG3b consensus map, combining data from the TTC1 and TTC2 families, includes a total of 31 AFLP markers and two anchor markers (Vp1-i2 and SIP1.413) previously mapped by Wu et al. (2003) in addition to 17 new EST microsatellite markers. Thus, 38 different *Leymus* EST microsatellite markers, aligned to rice chromosome 1, detected 37 independent loci on LG3a or LG3b, excluding Ltc0210 and Ltc0379. The relative orders of the new LG3a and LG3b EST markers were very similar in both TTC1 and TTC2 families (Results not shown), as were the relative orders of other markers previously mapped in both TTC1 and TTC2 families (Wu et al. 2003). Moreover, the relative genetic map order of new *Leymus* EST microsatellite markers on the LG3a and LG3b consensus maps is very similar to the relative order of homoeologous BAC clone sequences of rice (Fig. 1). These data provide additional support for the hypotheses that Triticeae group 3 is highly conserved (Devos and Gale 2000) and collinear with rice chromosome 1 (La Rota and Sorrells 2004).

Relatively small recombination distances between Ltc322 and Ltc401 markers on *Leymus* LG3a and between Ltc345 and Ltc384 markers on *Leymus* LG3b correspond to relatively large physical distances surrounding the centromere-specific CentO retrotransposon sequences on rice chromosome 1 (Fig. 1). Moreover, Wu et al. (2003) mapped a dense cluster of AFLP markers surrounding the LG3b E37M60.339 marker, located between Ltc345 and Ltc384, most of which have been omitted from this simplified map (Fig. 1). Previous studies in wheat and barley have demonstrated that recombination rates (cM/Mb) are suppressed near the centromere and tend to dilate exponentially with increasing distance from the centromere (Werner et al. 1992; Lukaszewski and Curtis 1993; Delaney et al. 1995; Künzel et al. 2000; Akhunov et al. 2003). We speculate that the dense cluster of AFLP markers surrounding the *Leymus* LG3b E37M60.339 marker (Wu et al. 2003), aligned to the rice chromosome 1 centromere (Fig. 1), can be attributed to

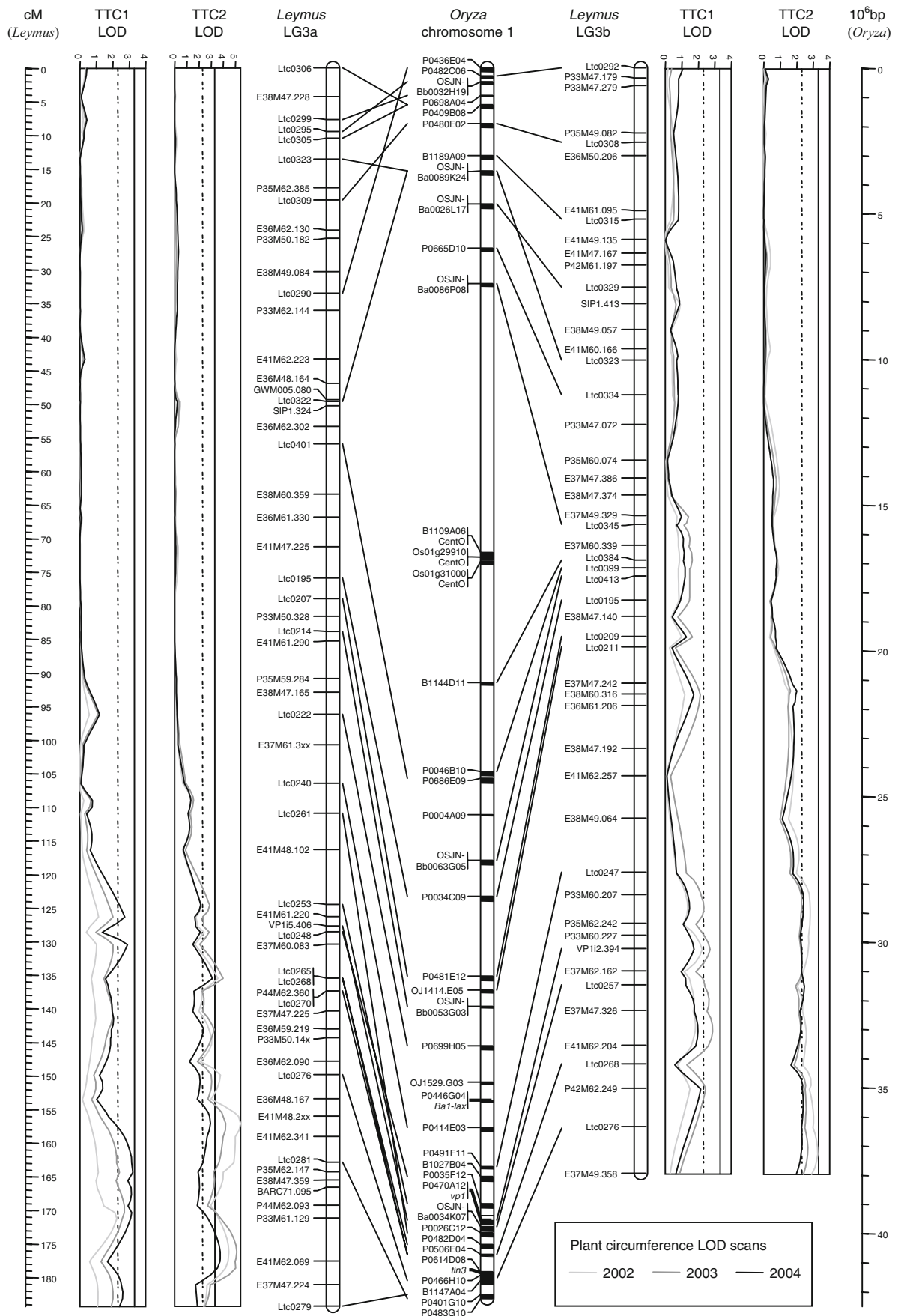
**Fig. 1** Comparisons of new *Leymus* LG3a and LG3b genetic (recombination) linkage consensus maps; TTC1 and TTC2 plant circumference (growth habit) QTL scans; and physical map of rice (*Oryza*) chromosome 1 based on the best BLASTn alignments between *Leymus* EST microsatellite markers (Ltc) and corresponding rice BAC clones. The approximate 5% chromosome-wide and genome-wide LOD thresholds are shown as *dashed lines* (LOD=2.3) and *solid lines* (LOD=3.3). The recombination distances for the new *Leymus* LG3a and LG3b consensus maps is shown (in centiMorgan units) on the *left side* of figure, whereas the physical location and coverage of *Oryza* chromosome 1 BAC clones is shown (in base pair units) on the *right side* of figure

reduced recombination in a physically large centromere region of *Leymus* LG3b. Presumably, the *Leymus* LG3a centromere also aligns with the rice chromosome 1 centromere between Ltc322 and Ltc401 (Fig. 1).

Relatively large recombination distances in the LG3a and LG3b growth habit QTL regions correspond to relatively small physical region in rice (Fig. 1). In particular, the TTC1 and TTC2 LG3a growth habit (plant spreading circumference) QTLs show relatively high LOD values associated with the *Leymus* Ltc248, Ltc253, *vp1*, Ltc265, Ltc268, Ltc270, Ltc276, Ltc279, and Ltc281 markers located between 120 and 185 cM (Fig. 1). These nine *Leymus* LG3a markers were aligned to a 4.3 Mb region located between rice BAC clones P1027B04 and P0483G10 (Table 1). Similarly, the TTC1 and TTC2 LG3b growth habit QTL showed relatively high LOD values associated with the *Leymus* Ltc247, *vp1*, Ltc257, Ltc268, and Ltc276 markers located between 120 and 165 cM (Fig. 1). These five *Leymus* LG3b markers were aligned to a 4.1 Mb region located between rice BAC clones P0491F11 and B1147A04 (Table 1). Thus, the *Leymus* LG3a and LG3b growth habit QTLs, of both TTC1 and TTC2 families, correspond approximately to the same relatively small 4.7 Mb region located between rice BAC clones P0491F11 and P0483G10. Although growth habit QTLs occupy 35% and 37% of LG3a and LG3b recombination distances in *Leymus*, respectively, these regions correspond to only 11% of the DNA comprising rice chromosome 1. There is some evidence that gene densities and recombination rates may be unusually high on the distal long arm of Triticeae group 3 (Künzel et al. 2000; Erayman et al. 2004; Varshney et al. 2006). However, the limited amount of Triticeae genome sequence available does not allow reliable statements on the overall gene density and recombination rates (Stein 2007).

#### Identification of growth habit candidate genes

The 4.7 Mb region located between rice BAC clones P0491F11 and P0483G10, which aligned to the *Leymus* LG3a and LG3b growth habit QTLs, includes sequences homoeologous to the *XSTS-TR3L6* and *XBE48620* markers flanking the wheat *tin3* gene (Kuraparthy et al. 2008) but



**Table 2** Description of PCR amplicons (estimated number of base pairs) from wheat-*Leymus racemosus* addition lines obtained using primers designed from 26 EST/microsatellite markers, isolated from *L. triticoides* x *L. cinereus* hybrids, that were mapped to the homoeologous LG3a and LG3b linkage groups using the TTC1 and TTC2 backcross families derived from *L. triticoides* x *L. cinereus* hybrids

Chromosomes	44	44	43	44	44	44	44	44	44	43	44	44	44	44	42	46	
Putative group	2	5	4	4+5	1+3	5	3+7	6	2	3+5+7	5	5	6	2	2	3+7	
EST marker	<i>Leymus</i> group	A <sup>a</sup>	C <sup>a</sup>	E <sup>a</sup>	F <sup>a</sup>	H <sup>a</sup>	I <sup>a</sup>	J <sup>a</sup>	k <sup>a</sup>	l <sup>a</sup>	n <sup>a</sup>	NAU-502 <sup>b</sup>	NAU-504 <sup>b</sup>	NAU-512 <sup>b</sup>	NAU-516 <sup>b</sup>	NAU-551 <sup>b</sup>	NAU-524 <sup>b</sup>
Ltc290	3a	–	–	–	–	152	–	–	–	–	–	155	155	–	–	–	155
Ltc292	3a, 3b	–	–	–	–	137	–	–	–	–	–	–	–	–	–	–	–
Ltc295	3a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	137
Ltc299	3a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	238
Ltc305	3a	–	–	–	–	109	–	–	–	–	–	–	–	–	–	–	109
Ltc308	3b	–	–	–	–	160	–	–	–	–	–	–	–	–	–	–	–
Ltc315	3b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	128
Ltc322	3a	–	–	–	–	99	–	–	–	–	–	–	–	–	–	–	–
Ltc323	3a, 3b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	260
Ltc329	3b	–	–	–	–	94	–	–	–	–	–	–	–	–	–	–	–
Ltc334	3b	–	–	–	–	231	–	–	–	–	–	–	–	–	–	–	–
Ltc384	3b	–	–	–	–	115	–	–	–	–	–	–	–	–	–	–	–
Ltc401	3a	–	–	–	–	82	–	–	–	–	–	–	–	–	–	–	–
Ltc413	3b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	57
Ltc195	3a, 3b	–	–	–	–	137	–	–	–	–	–	–	–	–	–	–	149
Ltc207	3a	–	–	–	–	252	–	–	–	–	–	–	–	–	–	–	–
Ltc209	3b	–	–	–	–	117	–	–	–	–	–	–	–	–	–	–	–
Ltc210	3a	–	–	–	–	134	–	–	–	–	–	–	–	–	–	–	–
Ltc211	3b	–	–	–	–	139	–	–	–	–	–	–	–	–	–	–	–
Ltc214	3a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	174
Ltc247	3b	–	–	–	–	–	–	150	–	–	150	–	150	–	–	–	150
Ltc248	3a	158	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ltc257	3b	–	–	–	–	–	–	–	–	160	–	–	–	–	–	–	–
Ltc268	3b	–	–	95	–	101	–	–	–	–	–	–	–	–	–	–	95
Ltc279	3a	–	154	–	–	–	–	–	–	–	–	154	–	–	–	–	–
Ltc281	3a	–	–	–	–	–	–	161	–	–	–	–	–	–	–	–	168

Markers are ordered based on alignment to rice chromosome 1 (see Table 1).

<sup>a</sup>Wheat-*L. racemosus* chromosome addition lines described by Kishii et al. (2004).

<sup>b</sup>Wheat-*L. racemosus* chromosome addition lines described by Qi et al. (1997).

does not include the maize *barren stalk 1* (*Ba1*) and rice *lax* panicle orthogene (Fig. 1 and Table 1). Although the maize *barren stalk 1* (*Ba1*) and rice *lax* panicle orthogene may be an important factor in the formation of axillary branches in *Leymus*, results of this research suggest that it is not a primary factor controlling differences in the circumference of rhizome spreading between basin wildrye (*L. cinereus*) and creeping wildrye (*L. triticoides*). Conversely, the wheat *tin3 XSTS-TR3L6* and *XBE48620* marker sequences aligns quite precisely to growth habit QTL peaks located on *Leymus* LG3a and LG3b in both TTC1 and TTC2 families (Fig. 1).

Although the wheat *tin3* gene has not been positively identified, it is likely to be involved in the formation of subterranean branches in other Poaceae species and we speculate that it may be a fundamentally important factor controlling growth habit variation in perennial *Leymus* wildryes based on alignments to the LG3 growth habit

QTLs. Kuraparthi et al. (2008) used targeted genomic mapping to refine the location of the wheat *tin3* gene to a 324-bp rice BAC contig (Table 1), which contains 32 annotated genes including two candidate genes similar to NAM-like (LOC\_Os01g71790) and SCARECROW-like GRAS-family (LOC\_Os01g71970) transcription factor proteins involved in lateral branching in other plants. However, Kuraparthi et al. (2008) did not find any wheat ESTs that showed significant similarity to either of these rice NAM-like or GRAS-family candidate genes and were unable to amplify an orthologous sequence from wheat. We did not find any *Leymus* EST sequences showing significant similarity to the rice NAM-like protein (LOC\_Os01g71790) candidate gene. However, BLASTn searches of the rice SCARECROW-like GRAS-family candidate gene (LOC\_Os01g71970) against the *Leymus* EST library identified one 1,500-bp contig, BG01\_2.3579.C1.Contig4507 of two overlapping GenBank sequences, EG386855 and EG387126 read from a single

clone), with 88% similarity. Conversely, BLASTn searches between *Leymus* BG01\_2.3579.C1.Contig4507 and the entire GenBank nucleotide collection showed significant matches ( $e < 0.0001$ ) to a maize SCARECROW-like sequence (AY367051) and a number of rice sequences, with greatest similarity to the same rice SCARECROW-like GRAS-family transcription factor locus (LOC\_Os01g71970) identified as a wheat *tin3* candidate gene (Kuraparthi et al. 2008). No other plant DNA sequences in the NCBI GenBank nucleotide collection showed significant similarity ( $e = 3.3e-233$ ) to this *Leymus* EST. Our original rice genome alignments (Table S1) also matched the *Leymus* BG01\_2.3579.C1.Contig4507 sequence to the same SCARECROW-like GRAS-family transcription factor locus (LOC\_Os01g71970) identified as a wheat *tin3* candidate gene (Kuraparthi et al. 2008). Thus, we believe that *Leymus* BG01\_2.3579.C1.Contig4507 is orthologous to the rice SCARECROW-like GRAS-family transcription factor locus, LOC\_Os01g71970. However, we have been unable to map this gene in *Leymus*. The alignment of this SCARECROW-like GRAS-family transcription factor to the wheat *tin3* gene (Kuraparthi et al. 2008) and the *Leymus* LG3 growth habit QTLs (Fig. 1), and the presence of this otherwise uncommon EST in *Leymus* rhizome and tiller meristems, pinpoints this locus as an intriguing and plausible candidate gene.

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