

DNA MARKERS AND GENE EXPRESSION POLYMORPHISMS ASSOCIATED
WITH GROWTH HABIT QUANTITATIVE TRAIT LOCI IN *LEYMUS* WILDRYES

by

Parminder Kaur

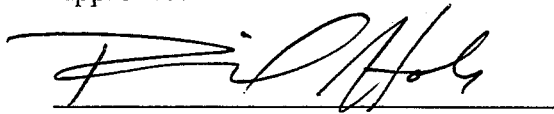
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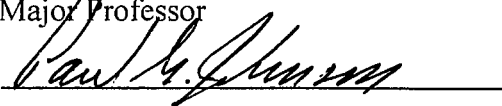
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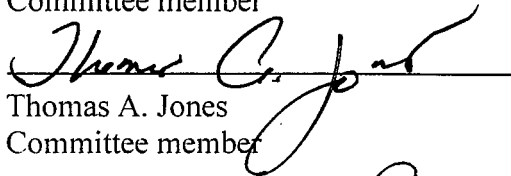
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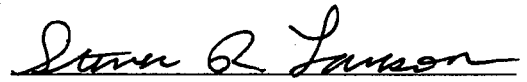
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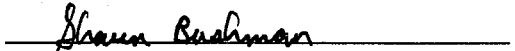
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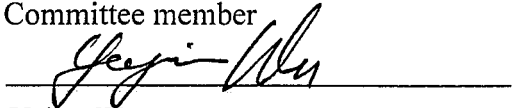
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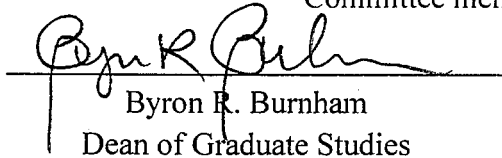
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ABSTRACT

DNA Markers and Gene Expression Polymorphisms Associated with Growth Habit

QTLs in *Leymus* Wildryes

by

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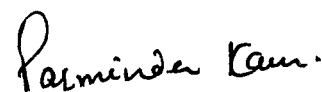
The genus *Leymus* includes about 30 long-lived perennial Triticeae grasses distributed throughout cold and/or semiarid regions of Americas, Europe, and Asia. *Leymus cinereus* and *L. triticoides* display divergent growth habit adaptations to different microenvironments in western North America. *L. cinereus*, a tall caespitose bunch grass, is commonly found near mountain and foothill waterways. *L. triticoides*, a relatively short rhizomatous grass, is restricted along stream banks or saline wetlands. Subterranean tiller and rhizome buds appear similar but display different responses to gravity and other cues governing branch angle and overall growth habit. In previous studies, two full-sib mapping families (TTC1 and TTC2) derived from *L. triticoides* x *L. cinereus* hybrids displayed growth habit QTLs on homoeologous regions of LG3a and LG3b in both allotetraploid families, suggesting that linkage group 3 controls much of the difference in rhizome spreading between two species. These TTC1 and TTC2 families segregated for

different regions of the putative growth habit QTL. Advanced backcross families were derived from TTC1 and TTC2 donor parents based on marker genotypes. Informative DNA markers were detected using 17 AFLP primer combinations in advanced backcross populations. Circumference QTL was localized to a 30 cM region on group LG3a of advanced backcross populations. The genetic map order of 23 LG3a and 17 LG3b *Leymus* EST or STS markers was strictly collinear with homologous sequences on rice chromosome 1. Mapping of three EST-SSR sequences on both LG3a and LG3b confirmed their homoeology. Overall gene expression patterns of negatively orthogeotropic (NOGT) and diageotropic (DGT) subterranean meristems were remarkably similar, confirming that *Leymus* orthogeotropic and diageotropic subterranean meristems develop from truly homologous meristems. One marker obtained from differentially expressing genes was genetically mapped on *Leymus* LG3. Although NOGT and DGT meristems eventually show substantial differentiation, these results identified a relatively small number of gene expression polymorphisms associated with early developmental differences involving the tropic differences between NOGT and DGT meristems.

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Parminder Kaur

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CHAPTER 1

INTRODUCTION

Perennial range grasses provide an important source of forage for livestock and wildlife throughout the world. Perennial grasses are also cultivated for soil stabilization, weed management, and turf. These grasses show divergent adaptations in terms of growth habit, salinity tolerance, phenology, seed dormancy, and many other traits. Growth habit, the genetic tendency of a plant to grow to a certain shape, height and spread, is a highly variable and economically important trait contributing to resiliency, competitiveness, grazing tolerance, and other functionally important attributes of perennial grasses. In general, grasses with caespitose and rhizomatous growth habits are adapted to xeric and mesic environments, respectively (Sims et al. 1978). Caespitose grasses form a compact tussock of upright aerial tiller stem branches (negative orthogeotropism), whereas sod-forming grasses typically spread via prostrate stolons or underground rhizome stem branches (diageotropism). The rhizomatous growth habit is also related to competitive ability and invasiveness of perennial grasses (Weaver 1963). Rhizomes provide protection from herbivory and trampling, storage tissues for vegetative propagation, and dispersal, but these rhizomes can also be a very problematic in perennial grasses where they contribute to weediness, e.g. quack grass (*Elymus repens*) (Holm et al. 1977).

The genus *Leymus*, perennial related to wheat (*Triticum*), barley (*Hordeum*), rye (*Secale*), and other annual and perennial Triticeae grasses, includes about 30 long-lived grass species with remarkable variation in growth habit, stature, and adaptation to harsh, cold, dry, and saline environments of Europe, Asia, and the Americas. *Leymus cinereus* (basin wildrye) and several other large-statured *Leymus* species, including *L. angustus*

(altai wildrye) and *L. racemosus* (mammoth wildrye), have high biomass accumulation potential across a wide range of high-elevation or high-latitude growing environments of western North America (Jefferson et al. 2002; Jensen et al. 2002; Lauriault et al. 2005), suitable for stockpiling fall and winter forage or bioenergy feedstocks.

Leymus has been used in wide-hybridization wheat breeding (Asay and Jensen 1996). *Triticum* and *Leymus* hybrids and wheat introgression lines containing *Leymus* chromosomes have been developed (Tsitsin, 1965; Mujeeb-Kazi et al. 1983; Plourde et al. 1992; Qi et al. 1997; Ananthawat – Jónsson et al. 1999; Ellneskog-Staam and Marker 2001), to transfer alien genes for salinity tolerance and viral and fungal disease resistance to wheat. More than half of the *Leymus* species are allotetraploid ($2n=4x=28$), but octoploid ($2n=8x=56$) and dodecaploid ($2n=12x=84$) variants may also arise due to interspecific hybridization (Ananthawat-Jónsson and Bödvarsdóttir 2001) or autoduplication within species.

Leymus cinereus is the largest (up to 2 m tall) native grass and most abundant *Leymus* species in the Great Basin, Rocky Mountain, and Intermountain regions of the western North America, where grazing livestock rely heavily on natural or low-maintenance forage production. These *Leymus* wildryes are capable of producing high biomass and valuable forage on many saline/alkaline sites where few other species are adapted. However, caespitose *L. cinereus* is susceptible to damage by intense grazing of early-season and fall regrowth. Once abundant on the floodplains of major rivers, alluvial gullies, and other watered areas with deep, well-drained soils in the Intermountain regions, *L. cinereus* has been eliminated from much of its former range due to over-

grazing, harvesting, and cultivation of field crops. Cultivars of *L. cinereus* are commonly used in rangeland seed mixtures, but have limited use in pastures or hay crops.

The second most common *Leymus* species in western North America is *L. triticoides* (creeping wildrye). *Leymus triticoides* is a shorter (0.3-0.7 m) but highly rhizomatous grass specifically adapted to poorly drained alkaline sites in the Intermountain region. *Leymus triticoides* is cultivated using vegetative propagules as a saline biomass crop in California, but poor seed production limits its widespread use.

Leymus cinereus and *L. triticoides* are self-sterile out-crossing species that hybridize in nature (Jensen et al. 1990), although some interspecific hybrids are unable to produce seeds. Artificial hybrids of *L. cinereus*, *L. triticoides*, and other North American *Leymus* species display regular meiosis and stainable pollen (Stebbins and Walters 1949; Dewey 1972; Hole et al. 1999). One major difference between these two species is their response towards gravitropism because, as *L. cinereus* is caespitose and *L. triticoides* is rhizomatous. Contrasting growth habit, plant height, seed dormancy, salt tolerance, and other functionally important traits make these two perennial grasses a particularly useful genetic system to evaluate the inheritance of these traits. Experimental breeding populations have been developed to construct high-density molecular genetic linkage maps for two full-sib families, TTC1 and TTC2, derived from two interspecific *L. triticoides* x *L. cinereus* F1 hybrids, TC1 and TC2, backcrossed to an *L. triticoides* T-tester. The F1 hybrids are robust plants showing a heterotic combination of tall plant height, large stems and leaves, prolific seed production, and good seed germination from *L. cinereus* with vigorous proliferation of tillers, rhizomes, relatively good establishment

(after seed germination), regrowth potential, and plant resiliency from *L. triticoides* (Fig. 1-1). Admixed breeding populations derived from interspecific hybrids of *L. cinereus* and *L. triticoides* have excellent potential for high biomass production, reduced susceptibility to grazing or harvest, and better regrowth potential.

One of the initial requirements of utilizing genomic approaches in plant improvements is the availability of DNA sequence information. *Leymus* ESTs were mapped on the previously developed linkage maps of TTC1 and TTC2 families (Wu et al. 2003). This mapping information was used to compare the collinearity and homology of *Leymus* EST sequences with related genomes such as wheat and rice. EST-SSRs have become an attractive choice to complement existing molecular markers such as SSRs because EST-SSRs provide an important source for the development of functional molecular markers that can be used in molecular mapping, or the comparative mapping studies (Anderson and Lübberstedt 2003; Perovic et al. 2004). A large amount of the genomes of several species consist of repetitive DNA that may be a major obstacle in sequencing these genomes. Due to the abundance of repetitive DNA, the key approach for systematic gene identification in various species is expressed sequence tag (EST) sequencing. The most commonly developed markers from an EST databases are simple sequence repeat (SSR) and sequence tagged site (STS) markers. The EST-derived SSRs are useful as molecular markers because they represent expressed or transcribed sequences, and thus can be used to assay functional diversity in natural populations and their putative function can be deduced from homology searches (Varshney et al. 2005). These markers have a higher transferability rate to related species than genomic SSRs

that can be used for comparative mapping and genomic investigations of natural populations. The ability to transfer mapped STS markers within the Poaceae family has been demonstrated both between cereal species (Erpelding et al. 1996) and among cereal and forage grass species (Taylor et al. 2001). *Leymus* EST library was used to compare the ESTs in *Leymus* with rice to compare the location of growth habit QTLs in *Leymus* with rice sequences related to growth habit, and to map these sequences as anchor markers (mapped in wheat, barley, rice or other well characterized species) on genetic linkage maps of TTC1 and TTC2 developed by Wu et al. (2003).

Linkage group 3 is highly conserved among Triticeae grasses (Devos and Gale, 2000). Collinear from end to end with rice chromosome 1, Triticeae group 3 is also the most conserved of all chromosome groups when compared with rice (La Rota and Sorrells 2004). Additionally rice chromosome 1 also controls the rhizome (Hu et al. 2003) and tiller (Li et al. 1999) angle QTLs. Recently a tillering inhibition gene (*tin3*) in wheat has been located on the long arm of chromosome 3A^m by Kuraparthi et al. (2007). A gene *LAX* is known to be involved in the formation of axillary meristems during the reproductive phase in rice (Komatsu et al. 2003) and has been mapped on rice chromosome 1 (Kinoshita and Takahashi 1991). Another homologous sequence of *LAX*, known as *barren stalk 1* (*bal*), is involved in the initiation of aerial lateral meristems during vegetative and reproductive development in maize, and the map positions of *bal* and *lax* are in syntenic regions of chromosomes 3L of maize and 1L of rice, respectively (Gallavotti et al. 2004). Thus, major genes controlling growth habit have been located on rice chromosome 1. The ESTs aligning to rice chromosome 1 were used as starting point

to map these *Leymus* ESTs on linkage group 3 of TTC1 and TTC2 families developed by Wu et al. (2003), and to discover a major growth habit QTL (Larson et al. 2006).

Molecular genetic linkage maps of *Leymus* include 1583 AFLP markers and 67 cross-species anchor markers (i.e., markers mapped in other grass species) on 14 linkage groups of allotetraploid *Leymus* ($2n=4x=28$) (Wu et al. 2003; Larson et al. 2006) based on synteny of corresponding markers in closely related wheat (*Triticum* spp.), barley (*Hordeum vulgare*), and cereal rye (*Secale cereale*) (Wu et al. 2003; Larson et al. 2006). Moreover, genome-specific markers have been used to distinguish several homoeologous linkage groups corresponding to the **Ns** and **Xm** genomes of *Leymus* (Wu et al. 2003). The **Ns** genome originates from *Psathyrostachys* (Dewey 1984; Zhang and Dvořák 1991; Wang and Jensen 1994; Wang et al. 1994; Ananthawat-Jónsson and Bödvarsdóttir, 2001), whereas the origin of **Xm** genome is considered to be unknown (Wang and Jensen 1994; Wang et al. 1994; Sun et al. 1995). Löve (1984) speculated that the other (**Xm**) genome originates from *Thinopyrum bessarabicum*, but chromosome pairing in *Thinopyrum* x *Leymus* hybrids has been ambiguous (Sun et al. 1995) or very low (Wang and Jensen 1994; Zhang and Dvořák 1991). However, hybridization of *Leymus* DNA with repeated nucleotide sequences from other Triticeae species suggests that *Leymus* is a segmental autopolyploid, **Ns1Ns1Ns2Ns2**, derived from two distinct *Psathyrostachys* species (Zhang and Dvořák 1991).

Growth habit/circumference quantitative trait loci (CIRC QTLs) have been detected on TTC1 and/or TTC2 families on LG3a, LG3b, LG5Xm and LG6a, with *L. cinereus* alleles showing its negative effect (Larson et al. 2006). The most consistent and

significant CIRC QTLs were detected on linkage group LG3a and LG3b of TTC1 and TTC2 families (Fig. 1-2) (Larson et al. 2006). This CIRC QTL covers approximately 70 cM region of the long arm of linkage group 3 of *Leymus*. An anchor locus VP1 (A transcription factor viviparous-1 encoded by VP1 gene) induces and maintains seed dormancy (McCarty et al. 1991). VP1 has been mapped to orthologous loci in wheat, maize, and rice (Bailey et al. 1999) in both TTC1 and TTC2 families near QTL regions of LG3a and LG3b, suggesting that QTLs are homoeologous. In addition to the homologous and possibly homoeologous QTLs detected on LG3a and LG3b, in both TTC1 and TTC2 families, a TTC1-specific CIRC QTL was detected on LG6a and a TTC2-specific CIRC QTL was detected on LG5Xm (Larson et al. 2006). Thus, two CIRC QTLs were common to both TTC1 and TTC2 families and two were unique to only one family. But some other methods needed to be developed to validate and precisely locate the gene(s) or CIRC QTL(s) related to growth habit in these grasses e.g. using advanced backcross populations.

Four different advanced backcross (BC2) populations were developed to validate and refine the location of CIRC QTLs on linkage group 3 in perennial *Leymus* wildryes. Advanced backcross-QTL analysis is a useful method for QTL detection and inheritance because this may fix the epistatic interactions between QTLs and other donor genes, leading to silencing of the measured QTL effects (Pillen et al. 2003). The AFLP technique (Vos et al. 1995) is a robust and efficient method of constructing high-density molecular genetic linkage maps in higher plant species with complex genomes (Vuylsteke et al. 1999).

Gene expression is the fundamental mechanism that controls developmental processes in a plant (Ji et al. 2004). Recent developments in techniques for large-scale analysis of gene expression, such as cDNA and oligonucleotide microarrays or gene chips, facilitate the search for genes associated with developmental variation (Aharoni et al. 2000, Girke et al. 2000, Zhu et al. 2001). Ji et al. (2004) hypothesized that the sequence conservation between closely related species can be used for cross-species hybridizations, without constructing or designing the gene-chips specific to each species of interest. Based on the unique feature of the Affymetrix GeneChip®, where multiple probes represent each gene, it was hypothesized that sequence conservation within genera or families may be high enough to generate sufficient signals from some of the probes for expression analysis (Ji et al. 2004). Recently, a number of studies of this kind have been reported in plants (Potokina et al. 2004; Lall et al. 2004; Kirst et al. 2004; Hazen et al. 2005).

As tillers and rhizomes arise from the same initial lateral axillary bud, but display different growth habit in response to gravity, we hypothesized that the negatively orthogeotropic (NOGT) and diageotropic (DGT) subterranean meristems arise due to differential gene expression towards gravity in the axillary buds and apical meristems in the shoots. We anticipate that gene expression studies in the *Leymus* segregating populations will be useful to identify the differentially expressing genes controlling growth habit and architecture and to investigate divergent adaptations of *Leymus* and other perennial Triticeae.

The aims of my research were (i) to evaluate different chromosome regions of the

70 cM *Leymus* LG3a growth habit QTL in four advanced backcross populations derived from one TTC1 and three TTC2 recombinant lines, (ii) to use these linkage maps to compare the location of *Leymus* rhizome QTLs with gene sequences and other DNA markers mapped to homoeologous chromosomes in wheat, barley, rice, and other Poaceae species, (iii) to identify ESTs spanning or flanking *Leymus* LG3a rhizome QTLs from perennial *Leymus* wildryes for future gene discovery purposes, compare these sequences with the rice genome, and map these ESTs in *Leymus* wildryes to compare the collinearity of the markers on *Leymus* group 3 with rice chromosome 1 and wheat group 3, and (iv) to compare gene expression profiles between negatively orthogeotropic and diageotropic branch meristems using TTC1 and TTC2 families.

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Fig. 1-1. *L. cinereus*, *L. triticoides*, and F1 hybrid in the field.

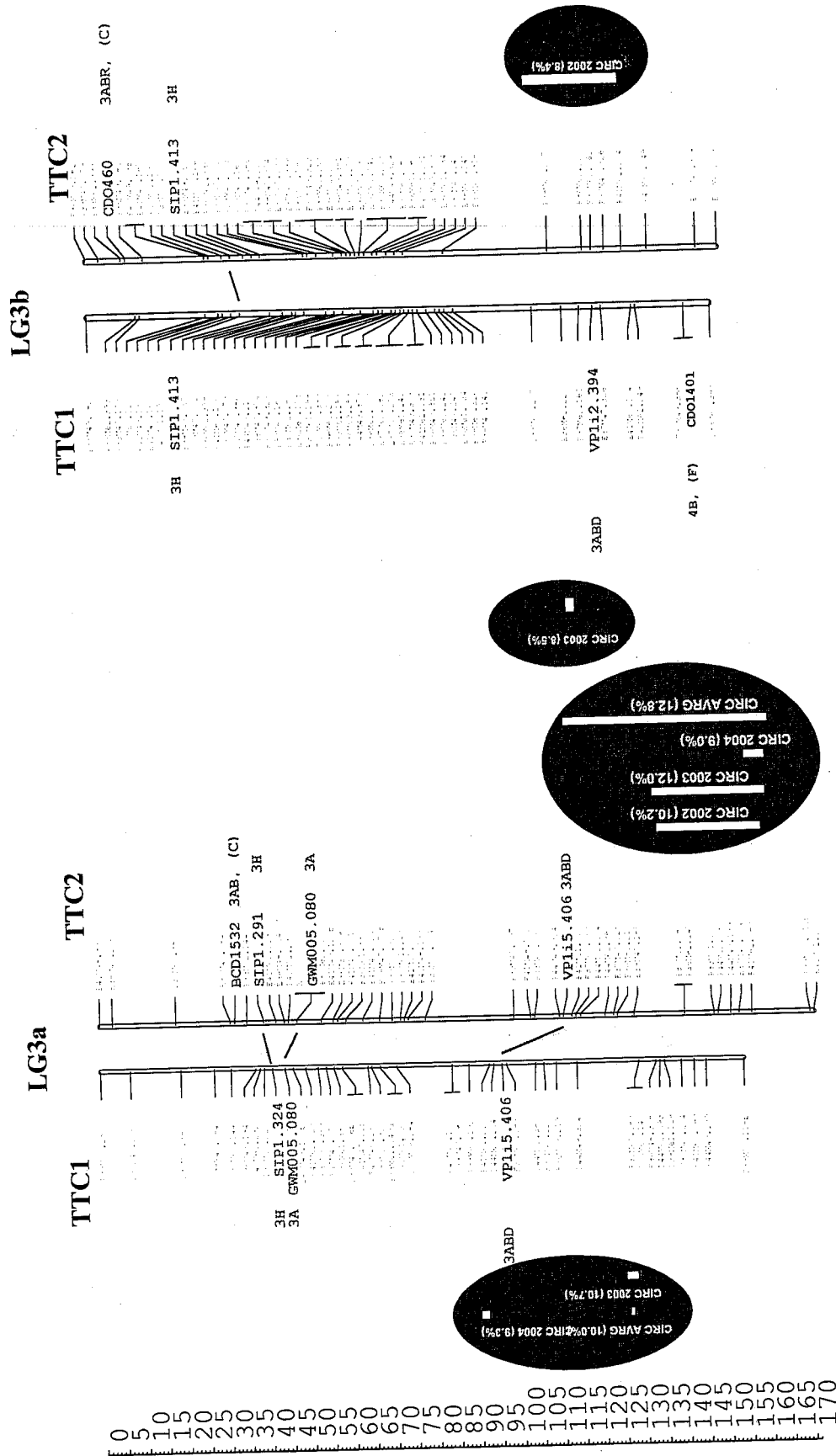


Fig 1-2. LG3a and LG3b growth habit QTL regions of two full-sib mapping families, TTC1 and TTC2 of allotetraploid *Leymus* (Larson et al. 2006).

CHAPTER 2
IDENTIFICATION OF *LEYMUS* HOMOEODOUS LINKAGE
GROUP 3 AND CHROMOSOME ADDITION LINES TO WHEAT BASED ON
IN SILICO ALIGNMENT OF *LEYMUS* EST MARKERS AND
RICE CHROMOSOME 1

Abstract

Studies have shown the synteny of the Triticeae linkage group 3 and rice chromosome 1. The objectives of the project were a.) the development and mapping of ESTs in perennial *Leymus* wildryes and b.) comparative analysis of the collinearity of the expressed sequence tag microsatellite markers (EST-SSRs) on *Leymus* linkage group 3 with rice chromosome 1 and wheat linkage group 3. A total of 228 of 1,798 *Leymus* EST-SSRs were aligned to rice chromosome 1. From 67 polymorphic EST-SSRs in *Leymus*, the genetic map order of 23 LG3a and 17 LG3b *Leymus* EST or STS markers was strictly collinear with homologous sequences on rice chromosome 1. Mapping of three EST-SSR sequences on both LG3a and LG3b also confirmed the homoeology of two linkage groups. A total of 44% of the ESTs present on rice chromosome 1 showed significant sequence homology with wheat EST sequences. About 32% of all *Leymus* ESTs homologous to rice chromosome 1 were also homologous to wheat group 3 ESTs at a threshold value of E^{-12} . Identification of *Leymus* linkage group 3 chromosomes in wheat-*Leymus* addition lines using *Leymus* linkage group 3 ESTs refuted the assertion of partitioning chromosome 3 into two parts.

Introduction

Conservation of gene content and order (collinearity) in related genomes has been studied in several model species such as rice and *Arabidopsis* in the past few years. The information obtained from these model plants is successfully being used in the related plant species (Devos and Gale 1997, 2000). This allows us to design effective strategies for map-based gene cloning from plant species with large and complex genomes. Several exceptions of collinearity among grass genomes exist (Guyot et al. 2004; Li and Gill 2002; Ramakrishna et al. 2002), which may result from changes in chromosome number and genome size or sequence changes such as insertions, deletions, duplications, and translocations, making the use of these model species for cross-species comparisons with other grasses a complicated process (Sorrells et al. 2003).

Leymus wildryes are medium-to large-statured perennial Triticeae grasses, members of Poaceae, and comprising important staple crops such as rice (*Oryza* spp.), maize (*Zea mays*), barley (*Hordeum vulgare*) and wheat (*Triticum* spp.). Cool-season *Leymus* grasses are remarkably well adapted to harsh conditions such as polar, saline, desert, and erosion-prone environments in North America. The genus *Leymus* comprises about 30 long-lived perennial grass species, distributed throughout the temperate regions of Asia, Europe, and America (Dewey 1984). *Leymus cinereus* and *L. triticoides* are allotetraploid ($2n=4x=28$), and display disomic inheritance (Wu et al. 2003). *Leymus triticoides* is characterized by the presence of aggressive subterranean rhizomes and are well adapted to poorly drained, alkaline sites of the western United States. Conversely, *L. cinereus* is a tall conspicuous bunchgrass growing strictly upright with high biomass

potential adapted to well-drained soils of western North America (Wu et al. 2003; Larson et al. 2006). Hybrids of *L. cinereus* and *L. triticoides* display regular meiosis and stainable pollen (Dewey 1972; Hole et al. 1999).

High-density molecular genetic linkage maps have been constructed for two full-sib mapping families, TTC1 and TTC2 (Wu et al. 2003). The 164-sib TTC1 and 170-sib TTC2 were derived from two different *L. triticoides* x *L. cinereus* F1 hybrids, TC1 and TC2, crossed to the same clones of *L. triticoides* T-tester genotype. The initial parents were derived from two outcrossing and inherently heterogeneous accessions; Acc:636 and Acc:641 (*L. cinereus* and *L. triticoides*, respectively). Thus TC1 and TC2 hybrids are genetically different (Wu et al. 2003). The TTC1 and TTC2 families segregate for growth habit and other characteristics of the *L. cinereus* and *L. triticoides* source populations and thus can be used for gene discovery research and development of molecular markers in perennial grasses. Over 1,583 AFLP markers and 67 anchor markers were previously mapped to the *Leymus* TTC1 and/or TTC2 molecular genetic linkage maps (Wu et al. 2003; Larson et al. 2006). Recently, Larson et al. (2006) exploited the two families to discover quantitative trait loci (QTLs) controlling growth habit, plant height, and flowering traits. Growth habit QTLs near the VP1 loci on linkage group LG3a and LG3b, in both TTC1 and TTC2 families, suggest that the growth habit QTL is controlled by homoeologous copies of the same gene on Triticeae homoeologous group 3 (Larson et al. 2006). However, only two anchor markers (SIP1 and VP1) were available to align LG3a and LG3b to each other and other Poaceae species (Wu et al. 2003).

Linkage group 3 is highly conserved among Triticeae grasses (Devos and Gale

2000). Collinear from end to end with rice chromosome 1, Triticeae linkage group 3 is also the most conserved of all chromosome groups when compared with rice (La Rota and Sorrells 2004). Rice chromosome 1 also controls the rhizome (Hu et al. 2003) and tiller (Li et al. 1999) angle QTLs and the putative transmembrane auxin efflux carrier and DnaJ-like genes (Sasaki et al. 2002) originally identified in *Arabidopsis pin*-forming (Gälweiler et al. 1998) and *arg1* (altered response to gravity) (Sedbrook et al. 1999) mutants, respectively. Recently, the tillering inhibition gene (*tin3*) in wheat was located on long arm of chromosome 3A^m by Kuraparthi et al. (2007a), and the STS marker (*XSTS-WLS6*) developed from lateral suppressor (*Ls*) of tomato mapped 37.1 cM proximal to *tin3* (Kuraparthi et al. 2007b). A gene, *LAX*, is known to be involved in the formation of axillary meristems during the reproductive phase in rice (Komatsu et al. 2003) and has also been mapped on rice chromosome 1 (Kinoshita and Takahashi 1991). Another homologous sequence of *LAX* known as *barren stalk 1* (*bal*) is involved in initiation of aerial lateral meristems during vegetative and reproductive development in maize, and the map positions of *bal* and *lax* are in syntenic regions of chromosomes 3L of maize and 1L of rice, respectively (Gallavotti et al. 2004).

A large amount of the genomes of grass species consist of repetitive DNA, a major obstacle in genome sequencing. Thus, a key approach for systematic gene identification in various species is the use of EST sequencing. For example, the rice (*Oryza sativa*) EST database has 1,211,418 ESTs, the wheat (*Triticum aestivum*) EST database has 1,050,932 ESTs, and the barley (*Hordeum vulgare* ssp. *vulgare*) EST database has 437,713 ESTs that have been deposited in dbEST

(http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html, dbEST release 092107).

The EST databases are expected to cover a significant portion of the transcriptome of a given species. Also, ESTs provide an important source for the development of functional molecular markers that can be used in molecular mapping or comparative mapping studies (Andersen and Lübberstedt 2003; Perovic et al. 2004). The most commonly developed markers from EST database are simple sequence repeats (SSRs) and other sequence tagged sites (STSs) markers. The EST-derived SSRs are useful as molecular markers because they represent expressed or transcribed sequences, and can be used to assay functional diversity in natural populations and to deduce putative function from a homology search (Varshney et al. 2005). These markers have a higher transferability rate to related species than genomic SSRs. The ability to transfer mapped STS markers within the Poaceae family has been demonstrated both between cereal species (Erpelding et al. 1996) and among cereal and forage grass species (Taylor et al. 2001).

Wheat-*Leymus* addition lines have been developed previously by Qi et al. (1997) and Kishii et al. (2004). Using the PCR-based molecular markers, specific alien chromosomes or chromosomal fragments can be detected. As each addition line of wheat has one particular chromosome of *Leymus*, these addition lines can be used to confirm the presence of *Leymus* ESTs or specific gene sequences on specific chromosomes. For instance, to test the *Leymus* ESTs mapped on linkage group 3 of *Leymus* on these addition lines or to locate genes for a specific function on a particular chromosome of *Leymus* added to wheat. These wheat-*Leymus* addition lines can also be used for wheat improvement such as transferring the disease resistance and other desirable genes from

Leymus to wheat. *Leymus* is evolutionarily distant to wheat, and has many characteristics that do not appear in wheat. For example, besides its salt and drought tolerance (McGuire and Dvorak 1981), *L. racemosus* is highly resistant to wheat scab (also called *Fusarium* head blight), which is one of the serious wheat diseases (Ban 2002). Thus, these desirable characteristics can be transferred from *Leymus* to wheat by producing wheat-*Leymus* addition lines, and the trait of interest is located to a particular chromosome.

Psathyrostachys and *Thinopyrum* were initially identified as donors of N and J genomes to *Leymus* (Dewey 1970, 1984, Löve 1984), but cytogenetic and molecular evidences disparaged the role of *Thinopyrum* in the origin of *Leymus* (Zhang and Dvorak 1991, Sun et al. 1995). However, *Psathyrostachys* is still considered to be the donor of Ns genome to *Leymus* (Zhang and Dvorak 1991, Wang and Jensen 1994). *Thinopyrum bessarabicum* contains the E^b or J genome, whereas, *Th. elongatum* possesses the E^c or E genome (Wang et al. 1995). Several wheat-*Th. bessarabicum*, and wheat-*Th. elongatum* addition lines developed in the past may provide important sources to test the alignment of linkage group 3 *Leymus* ESTs on the *Thinopyrum* genome and quantify the relationships, or lack thereof, between *Thinopyrum* and *Leymus*.

We hypothesized that the *Leymus* linkage group 3 is homologous to rice group 1. The main objective of this project was to develop ESTs from perennial *Leymus* wildryes for future gene discovery purposes and to compare these sequences with the rice genome. Second objective was to map these ESTs in *Leymus* wildryes, and to compare the collinearity of these markers on *Leymus* linkage group 3 with rice chromosome 1 and wheat linkage group 3.

Materials and Methods

Leymus EST library construction

Leymus EST libraries were constructed, normalized, sequenced, filtered, and assembled at W.M. Keck Center for Comparative and Functional Genomics, University of Illinois, Urbana, IL, using the same methods described by Anderson et al. (2007) with the following modifications.

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) reagent following the manufacturers' protocol. Samples were purified using the RNA cleanup procedure with Qiagen Midi columns (Qiagen, Valencia, CA). The poly (A)⁺mRNA was converted to double-stranded cDNA using different NotI/oligo(dT)-tagged primers for two independent RNA samples. Tag [5'-

AACTGGAAGAATTCGCGGCCGCTCCGA(T)₁₈V-3'] was used for purple and green aerial regrowth (less than 10 cm above the ground) from field evaluations of the *Leymus* TTC1 and TTC2 rhizomes (Larson et al. 2006), harvested under freezing cold temperatures about 2 hours after sunrise, and tag [5'-

AACTGGAAGAATTCGCGGCCGCTCGCA(T)₁₈V-3'] for NOGT, DGT, and plagiotropic subterranean branch meristems of TTC1 and TTC2 plants sampled during March of 2005. Tissues were immediately flash-frozen in liquid nitrogen and stored at -80°C and ground to a fine powder in liquid nitrogen. Double-stranded cDNA samples ≥ 600 bp were selected by agarose gel electrophoresis. 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 (cfu) before amplification was 3x10⁶,

whereas the total number of clones with insert was 2×10^6 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.

EST sequencing and analysis

The primary library was sequenced from the 3' end, whereas normalized libraries were screened from both 5' and 3' ends. We obtained a total of 28,786 successful sequence reads from 15,000 clones, with 6,217 contigs and 5,064 singletons. Thus the total number of unique sequences was 11,281. These unique sequences were compared with the genomes of other Triticeae species.

Alignment of *Leymus* EST-SSRs on the rice and wheat genomes

All the *Leymus* ESTs developed were aligned with the rice genome. Only the EST-derived SSRs aligned to rice chromosome 1 were used for the mapping of these EST-SSRs in *Leymus* for this project.

Physical size of rice chromosome 1 (~42.3 Mb) was aligned to genetic linkage map of *Leymus* LG3 (~192 cM). The physical locations of *Leymus* EST markers aligned to two ends of rice chromosome 1 (Ltc 290 at 183,358, and Ltc 281 at 42,487,921) were used to calculate the total length of the rice physical map (i.e. ~42.3 Mb). Then the conversion factor of 1 cM/220 kb was used to set the physical length of rice chromosome 1 equal to the length of the genetic linkage map of *Leymus* LG3. Thus the sub-start sequence regions of the 228 EST-SSR loci aligning to rice chromosome 1 were divided

by 220,000 (220 kb/cM) to locate the position of these loci on rice chromosome 1 in cM, and were compared with the mapped positions of these loci on *Leymus* LG3 mapped EST-SSRs to study the synteny of *Leymus* group 3 sequences with rice chromosome 1.

Leymus EST sequences were used to search the rice genome database (<http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1>) using BLASTn to identify syntenic rice BAC/PACs. Physical location for each BAC was identified using rice genome browser in TIGR rice genome database (http://www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice/). The SSRs containing ESTs aligned to rice chromosome 1 during the present study were also used for a BLASTN homology search with the wheat ESTs available at GrainGenes (<http://wheat.pw.usda.gov/GG2/blast.shtml>). The significant matches were determined using a cut-off E-value of 10^{-12} .

Primer design and SSR amplification

Each of the 11,281 unigenes was analyzed for microsatellite repeats. A microsatellite was defined as a sequence containing a minimum of three repeats of a motif comprising two to six nucleotides, with a total length of at least 12 nucleotides. Primers flanking microsatellite repeats were designed using SSRFINDER software (<http://www.maizemap.org/bioinformatics>) 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 (T_m) between 57°C and 63°C; (4) and GC% content between 20% and 80%.

These EST-derived SSR loci were tested on the two interspecific hybrids (TC1

and TC2) of *L. cinereus* and *L. triticoides*, one *L. triticoides* t-tester, and *Psathyrostachys juncea*. The following protocol was used for PCR amplification. Reaction volume of 10 μ l was prepared using 1X PCR buffer, 0.2 mM dNTPs, 0.5 μ M of each primer, 1.25 units jump start Taq DNA polymerase, 0.1 μ mol [R110] dCTP, and 50-100 ng of DNA. PCR touch-down protocol was used with the following profile: 1) initial denaturation at 95°C for 90 sec, 2) 5 cycles of 95°C for 1 min, 65°C for 1 min, decreasing annealing temperature 1°C/cycle, 72°C for 1 min, 3) 30 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, 4) and a final extension of 72°C for 6 min. Analysis of SSR variation was done using capillary electrophoresis with GS500 LIZ internal size standard and ABI 3730 genetic analyzer according to manufacturer's instructions (PE Applied Biosystems Inc., Foster City, CA). The segregation of these markers was analyzed using a combination of Genescan (PE Applied Biosystems) and Genographer version 1.6.0 (Benham et al. 1999) software.

Genetic analysis and map construction

The LG3a and LG3b genetic linkage maps of full-sib mapping populations TTC1 and TTC2 were constructed based on the segregation of DNA markers among gametes from the TC1 and TC2 hybrids using the backcross (BC1) population model of JoinMap 4.0 (van Ooijen and Voorrips 2006). In practice, this analysis was based on DNA fragments present in the TC1 and/or TC2 hybrids, absent in the T-tester, and segregating in the TTC1 and/or TTC2 progenies. These markers have a 1:1 expected segregation ratio (assuming disomic inheritance of monoallelic fragments) corresponding to gamete

genotypes. All the AFLP markers that mapped on linkage group 3 (Wu et al. 2003) and *Leymus* EST-SSR markers were integrated to construct molecular genetic linkage maps. Linkage groups were evaluated by increments of 2 LOD values from 4 to 40. Linkage analyses were performed using (i) unknown linkage phase and (ii) a mixture of inferred and unknown linkage-phase designations. The relative order, goodness of fit, and distances between all markers (AFLP and EST-SSRs) were determined by three cycles of map construction. The marker maps were rippled with the sequential addition of each marker. Map distances among markers were determined using Haldane's mapping function. The first two cycles placed well-fitting markers with χ^2 values less than 5.00. A third cycle forced remaining markers onto the map. Maps were drawn using MapChart 2.1 (Voorrips 2002).

Leymus ESTs on wheat-*L. racemosus*,
wheat-*Th. bessarabicum*, and
wheat-*Th. elongatum* addition lines

Leymus ESTs that mapped to chromosome 3 in *Leymus* were also tested on wheat-*L. racemosus*, wheat-*Th. bessarabicum*, and wheat-*Th. elongatum* addition lines. Seeds of wheat-*L. racemosus* addition lines were generously provided by Dr. BS Gill of KSU, Manhattan, KS, USA (Qi et al. 1997), and Prof. H Tsujimoto of Tottori University, Tottori, Japan (Kishii et al. 2004). Seven different lines of *Triticum aestivum* and two of *Th. bessarabicum* (Savul. and Rayss) Á. Löve were provided by Dr. A. Mujeeb-Kazi (CIMMYT, Mexico), and a complete set of *T. aestivum* with a pair of *Th. elongatum* chromosomes were provided by the Wheat Genetics Resource Center (KSU, Manhattan.

KS). Seeds of these addition lines were grown in a greenhouse, and DNA was extracted from seedlings using the Qiagen DNeasy® plant mini kit (Qiagen Inc., Valencia, CA). The same PCR protocol and reaction conditions were used for PCR amplification as described above for EST amplification in *Leymus*. Eight different *L. racemosus* accessions (D2949, DJ38801, DJ4116, PI108491, PI313463, PI531811, PI531812, and Volga) were bulked for this analysis. DNA of a wheat cultivar 'Chinese Spring', bulked from three Chinese Spring lines (CS1 self, CS2 self, and CS7), was also included for comparison. For the *Thinopyrum* testing, DNA of *Th. bessarabicum* and *Th. elongatum* were also included as controls.

Results

Alignment of *Leymus* ESTs with grass genomes

Table 2-1 shows the number of query hits of *Leymus* ESTs with barley, wheat, rice, maize, and other grass genomes. From the 9,671 significant hits, 9,389 unambiguously aligned to the rice genome. From these 9,389 hits, 1,370 were aligned to rice chromosome 1, and 228 EST-SSRs out of 1,370 sequences aligned to rice chromosome 1 at a cut-off E-value of E^{-05} .

Mapping of EST-SSRs in *Leymus*

A total of 1,798 primer pairs were designed and 228 aligned to rice chromosome 1. These 228 EST-derived SSRs were tested on the TC1 and TC2 hybrids and *L.*

*triticoide*s t-tester to select the polymorphic markers. A total of 67 EST-SSR markers were found to be polymorphic in the parental testing (Table 2-2). These polymorphic markers were genotyped in the TTC1 and TTC2 progenies. A total of 40 EST-based SSR markers detected 44 loci, 19 and 15 markers were mapped on linkage groups LG3a and LG3b, respectively, of the TTC1 family, and 22 and 18 markers were mapped on LG3a and LG3b, respectively of the TTC2 family (Tables 2-2, 2-3). Four, six, and three markers were specific only to TTC1 LG3a, TTC2 LG3a, and TTC2 LG3b, respectively (Table 2-3). A total of 16 markers were mapped on both TTC1 LG3a and TTC2 LG3a, and 15 were mapped on both TTC1 LG3b and TTC2 LG3b. Thus, a total of 44 marker loci were mapped on TTC1, TTC2 LG3a, and LG3b (Fig. 2-1). Markers Ltc270, Ltc272, and Ltc379 (Table 2-2) were linked to group 3 of *Leymus*, but their mapping order could not be resolved and hence have not been shown in map.

Most of the markers had amplified products in the expected size range, but small differences were observed in some cases between expected and observed size of amplification products based on mobility relative to size standard. Three SSR-containing sequences (Ltc299, Ltc323, and Ltc334) mapped on LG3 of *Leymus* were over 20 base pairs more than expected sizes due to probable introns within amplicons (Table 2-3). Markers Ltc195, Ltc276, and Ltc323 were mapped on both LG3a and LG3b groups of *Leymus* (Fig. 2-1). Fifteen *Leymus* EST-SSRs were mapped on linkage groups other than linkage group 3 of *Leymus* (Table 2-2). The remaining 12 *Leymus* EST-SSRs were unable to map to any of the *Leymus* linkage groups.

Alignment of *Leymus* ESTs with rice genome

Leymus linkage group 3 ESTs, when aligned to rice showed significant sequence collinearity with rice chromosome 1 (Fig. 2-2). Gene orders between rice chromosome 1 and *Leymus* linkage group 3 were highly conserved, with rearrangements being limited largely to small transpositions, small putative inversions, or mapping inconsistencies that may arise from occasional genotyping errors or possibly be attributed to the mapping errors. A total of 101 ESTs (44%) present on rice chromosome 1 showed significant sequence homology with wheat genome sequences. About 32% of all 228 *Leymus* ESTs homologous to rice chromosome 1 were also homologous to wheat group 3 ESTs at a threshold E-value of E^{-12} . Out of 40 *Leymus* ESTs that were mapped on *Leymus* linkage group 3, 14 (35%) were found to be homologous to wheat linkage group 3 at a threshold E-value of E^{-12} (Table 2-3).

Amplification of *Leymus* ESTs from wheat-*L. racemosus* addition lines

Table 2-4 lists all wheat-*L. racemosus* addition lines and the EST-SSRs that were polymorphic on these addition lines. From 41 marker loci mapped to linkage group 3 of *Leymus*, a total of 37 EST-SSRs were tested on wheat-*L. racemosus* addition lines. Four *L. racemosus* loci were different from those in TC1 and TC2 hybrids. For example, Ltc 207 showed polymorphism at 252 bp in *L. racemosus* and wheat-*L. racemosus* addition lines, but showed different polymorphic loci at 137 bp in TC1 and TC2 hybrids. Similar was the case with Ltc209, Ltc290, and Ltc329. A total of 11 EST-SSRs produced two

bands showing different patterns of polymorphism, and 37 showed one or more bands that showed identical patterns of polymorphism on the lines tested. Thus, a total of 48 marker loci were observed on wheat-*Leymus* addition lines. From these 48 markers, 38 were present in *L. racemosus*, 13 in 96-1027 (a double disomic addition line), 14 in monosomic wheat-*Leymus* addition line 20"+1"H, 13 in disomic wheat-*Leymus* addition line 21"+1"H. Only 2 ESTs were present in 21"+1"J, where H and J belong to chromosome 3L of *L. racemosus* according to Kishii et al. (2004). Only one EST was detected in 21"+1"n, and 1 in 21"+1"A, where 'n' and 'A' allegedly denote chromosome 3S, and chromosome 3L, respectively, of *L. racemosus* (Table 2-4) (Kishii et al. 2004). A double disomic addition line 96-1027 reportedly contains *L. racemosus* chromosomes 3 and 7 (Qi et al. 1997).

Amplification of *Leymus* ESTs from wheat-*Thinopyrum* addition lines

From the *Leymus* EST-SSR markers mapped on linkage group 3 of *Leymus*, seven EST-SSRs produced 12 loci in either *Th. bessarabicum*, *Th. elongatum*, or both (Table 2-5). Only six *Leymus* ESTs mapped on linkage group 3 in *Leymus* aligned to linkage group 3 in wheat-*Th. bessarabicum* (E^b genome) addition lines, and two linkage group 3 *Leymus* ESTs aligned to linkage group 3 in wheat-*Th. elongatum* (E^c genome) addition lines. One *Leymus* EST, Ltc 247, was mapped on group 1, 3, and 5 of *Th. elongatum* using wheat-*Th. elongatum* addition lines. Two ESTs mapped on linkage group 1 and linkage group 2, each using wheat-*Th. bessarabicum* addition lines (Table 2-5).

Discussion

Because of the high level of sequence conservation within genes, EST-based genetic maps offer the opportunity of to compare conserved genome organization between related species like grasses and rice. Initial observations of genome collinearity among grass species detected via mapping RFLP probes in related species (Ahn and Tanksley 1993; Hulbert et al. 1990; Van Deynze et al. 1995) postulated that the genomes of *Poaceae* consist of conserved linkage blocks that evolved from a progenitor of the rice genome. Subsequent refinement of the study led to the conclusion that the gross chromosomal organization of the *Poaceae* genomes can be traced back over more than 70 million years of evolution and that any extant grass genome can be reconstituted from 30 linkage blocks of the rice genome (Devos and Gale 2000).

The present study was aimed at the mapping of *Leymus* EST-derived SSRs to *Leymus* linkage group 3 and its alignment and comparison with rice chromosome 1 to study the collinearity of the two groups. An EST library was constructed from developing tiller and rhizome tissues of the interspecific hybrid between *L. cinereus* (basin wildrye) and *L. triticoides* (beardless wildrye). One of the concerns in using cDNA sequences for microsatellite development is the unknown final size of the PCR product that could vary depending on the number and size of intron sequences (Scott 2001). This may be the reason that three EST-SSRs (Ltc 299, Ltc323, and Ltc334) did not produce amplified products in the expected size range of the 40 EST-SSR marker loci that were integrated along with the AFLP markers and other anchor markers (Wu et al. 2003) into the group 3 genetic linkage map of tetraploid *Leymus*. From a total of 67 *Leymus* ESTs aligning to

rice chromosome 1, 13 were mapped on linkage groups other than linkage group 3 of *Leymus* and 17 did not get mapped to any of the *Leymus* group, which can be attributed to their best alignment match on rice chromosomes other than rice chromosome 1.

Previously Larson et al. (2006) discussed the homoeology of LG3a and LG3b of *Leymus* based on the presence of the VP1 locus in the growth habit QTL regions of both these linkage groups. Mapping different alleles of three EST-SSRs, Ltc195, Ltc276, and Ltc323 on both LG3a and LG3b confirm the homoeology of LG3a and LG3b. Comparative mapping of linkage group 3 of *Leymus* EST-SSRs with group 1 of rice showed a consistent mapping order.

Homoeologous groups in *Leymus* have arbitrarily been assigned letters 'a' and 'b'. An attempt was made to analyze whether *P. juncea* donates either 'a' or 'b' group to *Leymus* (Table 2-3). However, the amplicons present in *P. juncea* were evenly distributed on LG3a and LG3b of *Leymus*, and no definitive conclusion was reached.

Most of the mapped ESTs on *Leymus* linkage group 3, and rice group 1 showed significant homology with wheat linkage group 3 when compared to wheat genome. Thus the study also confirms the previous data concerning relationships between the wheat and the rice genomes (Munkvold et al. 2004, Sorrells et al. 2003). The earlier studies have reported that chromosome 3 of Triticeae is collinear from end to end with rice chromosome 1. Furthermore, Triticeae linkage group 3 is the most conserved of all chromosome groups in comparison to rice (La Rota and Sorrells 2004). A few discrepancies of the *Leymus* linkage group 3 ESTs not present on wheat linkage group 3 can be attributed to incomplete genome sequencing mapping of wheat.

Rice BACs/PACs identified by Kuraparthi et al. (2007b) near the *tin3* gene were integrated into the rice chromosome 1, aligned to *Leymus* linkage group 3. Five rice BACs (B1108H10, B1027B04, P0408G07, P0614D08, P0466H10) identified on rice chromosome 1 and linked to *tin3* by Kuraparthi et al. 2007b were integrated into rice chromosome 1 used for alignment with *Leymus* group 3 (Fig. 2-2). Although the exact location of *tin3* is not known, it has been located on either P0614D08 or P0466H10 (Kuraparthi et al. 2007b). These BACs (P0614D08 or P0466H10) are located in the growth habit QTL region of TTC1 and TTC2 families (Larson et al. 2006) and thus *tin3* can be expected to be located in the growth habit QTL region of group 3 of *Leymus*.

The EST-SSR profiles of *L. racemosus* chromosomes in the addition lines were quite consistent with the previous studies (Qi et al. 1997; Kishii et al. 2004). It has been reported that chromosomes 'H' and 'J' of *L. racemosus* are homologous to linkage group s1, 3, and, 7, and chromosome 'n' is a mixture of linkage groups 3 and 7 of wheat. Chromosome 'A', originally reported to represent linkage group 2 of *Leymus* also showed the presence of one marker on 3L (Kishii et al. 2004). Line 96-1027, a double disomic addition line ($2n=46$), also carries chromosome 3 of *L. racemosus*. Failure to map all of the *Leymus* EST markers mapped in *L. cinereus* x *L. triticoides* hybrids to *L. racemosus* (Table 2-4) can be attributed to the species-specific differences among *L. cinereus*, *L. triticoides*, and *L. racemosus*. However, the addition line with chromosome 'J' ($21"+1"J$) of *L. racemosus* showed polymorphism only with 2 *Leymus* linkage group 3 EST-SSRs, throwing doubt on the conservation of this chromosome. Kishii et al. (2004) described the 'H' and 'J' chromosomes to be less conserved and complex, as both 'H'

and 'J' exhibited polymorphisms matching the markers of linkage group 1 (short arm), linkage group 3 (long arm), and linkage group 7 (short arm) of wheat. Chromosome 'J' also had the markers of linkage group 7 (long arm). However, i) the presence of 14 *Leymus* linkage group 3 EST-SSRs on addition lines having chromosome 'H' of *L. racemosus* and ii) the presence of only one polymorphic marker on addition line 21"+1"n, where 'n' reportedly belongs to short arm of group 3 may not support the lack of conservation in this chromosome and refutes the decision to split group 3 of *Leymus* (Kishii et al. 2004). Kishii et al. (2004) discussed the evolutionary separation of the long and short arms of *L. racemosus* linkage group 3 by translocation within the group or through the fusion of two arms originally belonging to different groups.

Some bands present in either *Th. bessarabicum* (Ltc323) or *Th. elongatum* (Ltc 195) were not present in any wheat-*Thinopyrum* addition line (Table 2-5). Amplification of these EST-SSRs in *Th. bessarabicum*, and *Th. bessarabicum*, but absence in any addition line can be attributed to the use of different accessions in the two studies. Presence of only 12 out of 48 *Leymus* linkage group 3 ESTs on Wheat-*Thinopyrum* addition lines showed only 25% genome conservation between *Leymus* and *Thinopyrum*.

Previously developed linkage maps of *Leymus* (Wu et al. 2003; Larson et al. 2006) showed an insufficient number of anchor markers. The *Leymus* EST-SSRs proved useful in filling the gap by mapping these *Leymus* ESTs, although this has been done only for linkage group 3 of *Leymus* specifically for this project. These *Leymus* ESTs were also useful in confirming the homoeology of LG3a and LG3b of *Leymus* and also showing that *Leymus* linkage group 3 is homologous to wheat linkage group 3 and rice

chromosome 1.

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Table 2-1: Query hits of *Leymus* ESTs with other genomes

	Barley_TC	Maize_TC	NCBI_Festuca	NCBI_Rice.aa	Rye TCs	Sorghum TCs	TAIR Arabidopsis.aa	Wheat TCs	nr.aa	Poaceae
Number of Queries	11281	11281	11281	11281	11281	11281	11281	11281	11281	11281
Number of Queries with Hits	9617	8994	7178	9671	4388	8477	8787	10223	9755	9487
Number of Queries with No Hits	1664	2287	4103	1610	6893	2804	2494	1058	1526	1794

Table 2-2: *Leymus* ESTs aligning to rice chromosome 1 and their location on *Leymus*.

SSR	Query_ID	Alignment (Tag)	HSP (Similarity)	Rice BAC	Physical position on rice	Rice Chrom.	BLASTn	Physical position on <i>Leymus</i>	SSR	Expected Amplicon in <i>Leymus</i>	Map Position	
											TTC1	TTC2
Ltc0195	BG01_2.2576.C2.	155	210.6	P0034C09	28811559 -	1	2.8e-63	28632970	(CGC)4	145	LG3a,	LG3a,
	Contig3435	(Rhizome)	(93)		28977377			-28633115			LG3b	LG3b
Ltc0207	BG01_2.4912.C1.	122	190.8	P0481E12	31584509 -	1	1.8e-28	31352290	(CGC)4	142	LG3a	LG3a
	Contig5845	(NA)	(95)		31740870			-31352405				
Ltc0209	BG01_2.1768.C1.	143	210.6	P0481E12	31584509 -	1	3.0e-33	31483669	(AGG)6	114	LG3b	LG3b
	Contig2511	(NA)	(94)		31740870			-31483802				
Ltc0210	BG01_2.2498.C1.	94	127.4	P0435B05	31902303 -	1	3.2e-65	31660892	(CCG)6	152	LG3a	-
	Contig3352	(Rhizome)	(93)		31971564			-31660979				
Ltc0211	BG01034B2D01.r1	140	188.8	OJ1414_E0	32067039 -	1	1.3e-36	31803616	(AAG)4	141	LG3b	LG3b
		(NA)	(93)	5	32160774			-31803746				
Ltc0214	BG01021A2C09.f1	195	252.3	OSJNBb00	32613923 -	1	7.4e-80	32374928	(CGA)5	158	-	LG3a
		(NA)	(92)	53G03	32703206			-32375110				
Ltc0218	BG01_2.1568.C1.	177	165.03	B1434D10	23934818 -	3	1.0e-44	32822722	(GCC)4	155	4Ns	4Ns
	Contig2243	(NA)	(85)		24050713			-32822889				
Ltc0222	BG01_2.2290.C1.	185	222.5	P0699H05	33998805 -	1	5.3e-61	33841098	(GCG)6	133	LG3a	LG3a
	Contig3115	(NA)	(90)		34125370			-33841273				
Ltc0240	BG01_2.1914.C1.	184	117.5	P0414E03	36824637 -	1	2.4e-16	36602331	(TG)7	110	LG3a	LG3a
	Contig2690	(NA)	(83)		36959813			-36602505				
Ltc0247	BG01030B2B06.f1	648	601.2	P0491F11	38154182 -	1	1.6e-102	37941148	(GAG)4	150	LG3b	LG3b
		(NA)	(87)		38264508			-37941762				
Ltc0248	BG01039B1A09.r1	50	69.88	B1027B04	38517882 -	1	1.6e-38	38275973	(AGC)7	158	LG3a	LG3a
		(Rhizome)	(93)		38683218			-38276019				
Ltc0252	BG01043A1B06.r1	54	61.95	P0026H03	5489466 -	2	7.5e-14	39066030	(GA)7	160	-	LG6a
		(NA)	(90)		5607092			-39066080				
Ltc0253	BG01_2.1398.C1.	124	107.5	P0035F12	39460876 -	1	8.1e-11	39289374	(ACGC)	115	-	LG3a
	Contig2035	(NA)	(86)		39611269			-39289491	4			
Ltc0256	BG01_2.1851.C1.	159	115.5	P0470A12	40026334 -	1	5.3e-45	39896948	(CCCTT)	143	Lg1a	Lg1a
	Contig2617	(Rhizome)	(84)		40167786			-39897097	)5			

Table 2-2 (continued)

SSR	Query_ID	Alignment (Tag)	HSP (Similarity)	Rice BAC	Physical position on rice	Rice Chrom.	BLASTn	Physical position on Leymus	SSR	Expected Amplicon in Leymus	Map Position	
											TTC1	TTC2
Ltc0257	BG01_2.3185.C1.	131	186.8	OSJNBa00	40257629 -	1	1.3e-33	40104375	(GAG)5	160	LG3b	LG3b
	Contig4102	(Rhizome)	(94)	34K07	40411155			-40104496				
Ltc0261	BG01_2.1331.C1.	320	301.8	P0026C12	40455601 -	1	3.9e-66	40331376	(CGG)6	155	LG3a	LG3a
	Contig1949	(NA)	(62)		40595978			-40331677				
Ltc0265	BG01_2.246.C1.C	545	511.9	P0482D04	40854476 -	1	6.5e-60	40699813	(AGCC	145	LG3a	LG3a
	ontig455	(Rhizome)	(87)		40999058			-40700330	A)4			
Ltc0268	BG01_2.4832.C1.	103	145.2	P0506E04	41199726 -	1	2.4e-36	40886499	(AGC)4	99	LG3b	LG3b
	Contig5768	(NA)	(93)		41262318			-40886595				
Ltc0270	BG01_2.4144.C1.	219	149.2	P0506E04	41199726 -	1	2.3e-40	41004473	(AGC)5	154	-	LG3a
	Contig5086	(NA)	(84)		41262318			-41004679				
Ltc0272	BG01_2.435.C1.C	60	73.84	P0504E02	41439800 -	1	2.7e-36	41212587	(GT)10	127	-	LG3b
	ontig733	(Rhizome)	(91)		41574903			-41212643				
Ltc0276	BG01_2.2719.C1.	364	349.4	B1147A04	42095419 -	1	5.1e-60	41891684	(TCG)4	208	LG3a	LG3a,
	Contig3595	(Rhizome)	(89)		42241167			-41892029				LG3b
Ltc0277	BG01013A1F01.r1	242	297.9	B1147A04	42095419 -	1	1.9e-107	41965847	(CGT)4	217	-	LG2b
		(NA)	(91)		42241167			-41966076				
Ltc0279	BG01021B1G12.r1	94	111.5	P0401G10	42582381 -	1	3.2e-25	42416705	(AG)8	151	LG3a	LG3a
		(Tiller)	(90)		42723237			-42416792				
Ltc0281	BG01_2.2073.C1.	131	155.1	P0483G10	42723238 -	1	6.7e-71	42487800	(GGC)5	158	LG3a	LG3a
	Contig2870	(NA)	(90)		42865873			-42487921				
Ltc0290	BG01_2.615.C1.C	215	173	P0436E04	173730 -	1	4.6e-51	183358	(CGG)5	150	LG3a	LG3a
	ontig990	(Rhizome)	(85)		300776			-183572				
Ltc0292	BG01007B1H03.f1	276	365.3	P0482C06	436799 -	1	2.6e-85	392495	(GAAAA	155	LG3b	LG3b
		(NA)	(91)		540380			-392770	)4			
Ltc0295	BG01_2.672.C2.C	85	113.5	OSJNBb00	659189 -	1	1.5e-25	623342	(TC)8	147	LG3a	-
	ontig1065	(Rhizome)	(91)	32H19	740492			-623426				
Ltc0299	BG01030B1H03.f1	74	83.75	P0698A04	1105711 -	1	1.9e-35	1076613	(AGC)4	137	LG3a	LG3a
		(NA)	(89)		1169567			-1076686				
Ltc0305	BG01_2.3879.C1.	170	163.1	P0409B08	1437631 -	1	2.5e-23	1547013	(TCA)5	109	LG3a	LG3a
	Contig4822	(Rhizome)	(87)		1566827			-1547182				
Ltc0306	BG01_2.2552.C1.	137	169	P0409B08	1437631 -	1	1.9e-38	1565038	(TA)7	160	LG3a	LG3a
	Contig3408	(Rhizome)	(90)		1566827			-1565174				

Table 2-2 (continued)

SSR	Query_ID	Alignment (Tag)	HSP (Similarity)	Rice BAC	Physical position on rice	Rice Chrom.	BLASTn	Physical position on Leymus	SSR	Expected Amplicon in Leymus	Map Position	
											TTC1	TTC2
Ltc0308	BG01_2.1412.C1.	92	103.6	P0480E02	2081895 -	1	1.5e-43	2082275	(CAG)4	127	-	LG3b
	Contig2055	(Tiller)	(89)		2218552			-2082366				
Ltc0309	BG01_2.4086.C1.	169	192.8	P0480E02	2081895 -	1	1.1e-30	2129378	(GGT)4	143	LG3a	LG3a
	Contig5029	(Rhizome)	(89)		2218552			-2129546				
Ltc0314	BG01027B1H01.r1	42	59.96	P0530H10	17102121 -	5	3.6e-26	3230370	(TTC)4	150	LG7b	-
		(Rhizome)	(92)		17218273			-3230411				
Ltc0315	BG01_2.2834.C1.	148	206.7	B1189A09	3200350 -	1	1.0e-94	3322304	(GGC)4	128	LG3b	LG3b
	Contig3716	(Rhizome)	(92)		3347934			-3322451				
Ltc0322	BG01_2.2434.C1.	223	291.9	OSJNBa00	3724618 -	1	2.2e-41	3808271	(GCG)5	110	LG3a	LG3a
	Contig3277	(Rhizome)	(91)		89K24 3878397			-3808493				
Ltc0323	BG01_2.143.C1.C	153	169	OSJNBa00	3724618 -	1	1.6e-66	3844387	(GGC)4	149	LG3b	LG3a, LG3b
	ontig291	(Tiller)	(88)		89K24 3878397			-3844539				
Ltc0329	BG01_2.5075.C1.	204	246.3	OSJNBa00	4878766 -	1	4.2e-79	4903570	(CGC)8	102	LG3b	LG3b
	Contig5991	(NA)	(90)		26L17 5041775			-4903773				
Ltc0334	BG01018A2E12.r1	138	131.3	P0665D10	6401809 -	1	6.0e-34	6416329	(GGC)4	160	LG3b	LG3b
		(Tiller)	(86)		6510374			-6416466				
Ltc0336	BG01_2.621.C1.C	52	71.86	P0483F08	6579030 -	1	3.2e-10	6583515	(GAC)4	87	LG7a	LG7a
	ontig998	(Tiller)	(92)		6666616			-6583566				
Ltc0341	BG01_2.1610.C1.	32	56	OSJNBa00	18464763 -	4	4.4e-06	7099728	(TGC)4	107	LG2b	LG2b
	Contig2299	(Rhizome)	(96)		72D21 18613036			-7099759				
Ltc0342	BG01_2.2776.C1.	201	192.8	P0596H10	29508778 -	6	1.7e-45	7276755	(CCG)4	88	LG7a	LG7b
	Contig3656	(Rhizome)	(87)		29666486			-7276955				
Ltc0345	BG01_2.924.C1.C	90	83.75	OSJNBa00	7617987 -	1	2.3e-34	7621141	(CTG)4	111	LG3b	LG3b
	ontig1412	(NA)	(86)		86P08 7732538			-7621230				
Ltc0351	BG01_2.3951.C1.	75	93.66	OSJNBb00	3421092 -	4	3.8e-44	9628288	(CTAT)5	149	LG6b	LG6b
	Contig4897	(Tiller)	(90)		76A11 3548510			-9628362				
Ltc0354	BG01_2.3859.C1.	828	847	B1153F04	12113700 -	1	1.4e-79	12048569	(GT)8	113	LG4Xm	LG4Xm
	Contig4802	(Rhizome)	(82)		12190836			-12049354				
Ltc0359	BG01_2.309.C3.C	54	61.95	OSJNBb00	431313 -	4	3.5e-27	12726498	(AT)20	157	LG6a	-
	ontig563	(Rhizome)	(90)		85F13 491496			-12726548				
Ltc0376	BG01_2.2202.C1.	84	75.82	P0520B06	18259563 -	1	5.4e-21	18068225	(CGC)4	117	LG2a	LG2a
	Contig3015	(Rhizome)	(87)		18353569			-18068302				

Table 2-2 (continued)

SSR	Query_ID	Alignment (Tag)	HSP (Similarity)	Rice BAC	Physical position on rice	Rice Chrom.	BLASTn	Physical position on Leymus	SSR	Expected Amplicon in Leymus	Map Position	
											TTC1	TTC2
Ltc0379	BG01013B1E12.f1	208 (NA)	198.7 (87)	B1147B04	19135755 - 19266682	1	3.2e-33	18923314 (GCA)4 -18923509	212		LG3a	-
Ltc0384	BG01_2.5314.C1. Contig6183	90 (NA)	103.6 (90)	B1144D11	21426366 - 21521135	1	4.8e-21	21243210 (GA)6 -21243293	118		LG3b	LG3b
Ltc0389	BG01_2.1480.C1. Contig2142	152 (Rhizome)	93.66 (83)	B1097D05	22852255 - 22959879	1	1.4e-40	22611170 (TC)10 -22611312	152		LG2b	LG6b
Ltc0393	BG01_2.1088.C1. Contig1626	188 (Rhizome)	141.2 (61)	OJ1115_D 04	28811559 - 29311629	5	1.1e-21	23893795 (CTA)4 -23893973	123		-	LG1a
Ltc0399	BG01_2.539.C1.C ontig889	244 (Rhizome)	262.2 (89)	P0046B10	24519755 - 24648769	1	3.6e-33	24358816 (GCA)4 -24359047	156		LG3b	LG3b
Ltc0401	BG01_2.3440.C1. Contig4367	157 (Rhizome)	127.4 (85)	P0686E09	24751587 - 24906778	1	3.1e-45	24650933 (CGC)4 -24651080	89		-	LG3a
Ltc0410	BG01_2.4457.C1. Contig5400	122 (NA)	174.9 (93)	P0434E03	18071324 - 18194501	8	9.7e-37	26761648 (AGC)4 -26761763	140		LG1a	LG1a
Ltc0413	BG01_2.4232.C1. Contig5175	418 (Tiller)	406.9 (87)	OSJNBb00 63G05	27560927 - 27735754	1	1.6e-81	27376278 (CT)16 -27376674	106		LG3b	LG3b
C4H0208	BG01_2.2568.C3. Contig3426	638 (NA)	597.18 (88)	OJ1529_G 03	35235423 - 35304861	1	1.3e-85	35035902- 35036506	208		-	LG3a

Table 2-3: Mapping ESTs in *Leymus* and homology of group 3 *Leymus* ESTs with wheat genome

SSR	Query_ID (Accession ID)	Annotation	Wheat EST (Wheat chromosome)	E-value	Expected amplicon	<i>Leymus</i> Linkage Group	Observed amplicon in <i>Leymus</i>		Observed amplicon in <i>P. juncea</i>
							TTC1	TTC2	
Ltc0195	BG01_2.2576.C2.Contig3435 (EG392537)	Putative Ser/Thr protein phosphatase [<i>Oryza sativa</i> (japonica cv-gp)]	-	-	145	3a, 3b	150, 140	150, 140	136
Ltc0207	BG01_2.4912.C1.Contig5845 (EG402550)	Unknown protein [<i>O. sativa</i> (japonica cv-gp)]	-	-	142	3a	137	137	-
Ltc0209	BG01_2.1768.C1.Contig2511 (EG383062)	P0481E12.40 [<i>O. sativa</i> (japonica cv-gp)]	-	-	114	3b	114	114	100-119
Ltc0210	BG01_2.2498.C1.Contig3352 (EG387468)	Putative 28 kDa heat and acid-stable phosphoprotein [<i>O. sativa</i> (japonica cv-gp)]	-	-	152	3a	116	-	-
Ltc0211	BG01034B2D01.r1 (EG394962)	Putative transcription activator RF2a [<i>O. sativa</i> (japonica cv-gp)]	-	-	141	3b	137	137	-
Ltc0214	BG01021A2C09.fl (EG387756)	Putative BSD protein [<i>O. sativa</i> (japonica cv-gp)]	BE423756_3A(L)	5.00E-14	158	3a	-	157	185
Ltc0222	BG01_2.2290.C1.Contig3115 (EG378341)	Ethylene-responsive element binding factor 3 [<i>O. sativa</i> (japonica cv-gp)]	-	-	133	3a	122	122	130-154
Ltc0240	BG01_2.1914.C1.Contig2690 (EG380493)	Unknown protein [<i>O. sativa</i> (japonica cv-gp)]	BF483477_3A(S)	1.00E-48	110	3a	103	103	100
Ltc0247	BG01030B2B06.fl (EG399441)	Putative amidophosphoribosyltransferase [<i>O. sativa</i> (japonica cv-gp)]	BE423756_3A(L)	5.00E-14	150	3b	149	149	130, 147
Ltc0248	BG01039B1A09.r1 (EG394324)	Glycogenin-like protein [<i>O. sativa</i> (japonica cv-gp)]	-	-	158	3a	158	158	155-177
Ltc0253	BG01_2.1398.C1.Contig2035 (EG399190)	Putative helix-loop-helix protein 1A [<i>O. sativa</i> (japonica cv-gp)]	BE471102_2ABD(L)	2.00E-51	115	3a	-	114	104, 157
Ltc0257	BG01_2.3185.C1.Contig4102 (EG390317)	P0413C03.22 [<i>O. sativa</i> (japonica cv-gp)]	-	-	160	3b	160	160	164-172
Ltc0261	BG01_2.1331.C1.Contig1949 (EG389877)	OJ1485_B09.11 [<i>O. sativa</i> (japonica cv-gp)]	-	-	155	3a	148	148	-
Ltc0265	BG01_2.246.C1.Contig455 (EG381616)	OSJNBa0093F16.12 [<i>O. sativa</i> (japonica cv-gp)]	BG313960_3ABD(L)	1.00E-70	145	3a	150	150	137-150
Ltc0268	BG01_2.4832.C1.Contig5768 (EG402572)	Unknown protein [<i>O. sativa</i> (japonica cv-gp)]	BE406368_5AD(S)	1.00E-79	99	3b	89	101	95-107
Ltc0270	BG01_2.4144.C1.Contig5086 (EG400140)	Copper chaperone (CCH)-related protein-like [<i>O. sativa</i> (japonica cultivar-group)]	BE498715_7DL	0	154	3a	-	166	-
Ltc0272	BG01_2.435.C1.Contig733 (EG380310)	Putative dermal glycoprotein precursor [<i>O. sativa</i> (japonica cv-gp)]	BF428535_3DL	0.088	127	3b	-	127	-
Ltc0276	BG01_2.2719.C1.Contig3595 (EG389964)	B1147A04.8 [<i>O. sativa</i> (japonica cv-gp)]	BG607163_3A(L)	3.00E-58	208	3a, 3b	206, -	199	-
Ltc0279	BG01021B1G12.r1 (EG388157)	Putative receptor-like protein kinase 1 [<i>O. sativa</i> (japonica cv-gp)]	-	-	151	3a	153	153	146
Ltc0281	BG01_2.2073.C1.Contig2870 (EG401420)	Membrane protein-like [<i>O. sativa</i> (japonica cv-gp)]	-	-	158	3a	160	160	156-167

Table 2-3 (continued)

SSR	Query ID (Accession ID)	Annotation	Wheat EST (Wheat chromosome)	E-value	Expected amplicon	Leymus Linkage Group	Observed amplicon in <i>Leymus</i>		Observed amplicon in <i>P. juncea</i>
							TTC1	TTC2	
Ltc0290	BG01_2.615.C1.Contig990 (EG375842)	Sterol-8, 7-isomerase [<i>Zea mays</i>]	BE517705 6BD(S)	1.00E- 21	150	3a	148	148	151
Ltc0292	BG01007B1H03.fl (EG396456)	Putative protein kinase [<i>O. sativa</i> (japonica cv-gp)]	BE423756 3A(L)	1.00E- 14	155	3b	148	153	146
Ltc0295	BG01_2.672.C2.Contig1065 (EG393720)	Unknown protein [<i>O. sativa</i> (japonica cv-gp)]	-	-	147	3a	150	-	-
Ltc0299	BG01030B1H03.fl (EG395508)	HGA1 [<i>Hordeum vulgare</i>]	BE423756 3A(L)	5.00E- 14	137	3a	261	256	-
Ltc0305	BG01_2.3879.C1.Contig4822 (EG389241)	Putative dehydration-induced myb-related-protein Cpm7 [<i>O. sativa</i> (japonica cv gp)]	-	-	109	3a	109	109	109
Ltc0306	BG01_2.2552.C1.Contig3408 (EG387074)	Putative nuclease I [<i>O. sativa</i> (japonica cv-gp)]	BE442599 3B(S)	0	160	3a	146	146	149, 149
Ltc0308	BG01_2.1412.C1.Contig2055 (EG376508)	Unknown protein [<i>O. sativa</i> (japonica cv-gp)]	BE443404 3ABD(S)	E-129	127	3b	-	147	-
Ltc0309	BG01_2.4086.C1.Contig5029 (EG397701)	Senescence induced receptor-like serine/threonine kinase [<i>O. sativa</i> (japonica cv-gp)]	-	-	143	3a	140	140	-
Ltc0315	BG01_2.2834.C1.Contig3716 (EG400073)	Binding / nucleic acid binding [<i>Arabidopsis thaliana</i>]	-	-	128	3b	121	121	125, 127
Ltc0322	BG01_2.2434.C1.Contig3277 (EG398526)	Putative TGACG-motif binding factor [<i>O. sativa</i> (japonica cv-gp)]	BG312702 3ABD(S)	4.00E- 30	110	3a	123	123	99
Ltc0323	BG01_2.143.C1.Contig291 (EG387769)	Biostress-resistance-related protein [<i>Triticum aestivum</i>]	BE442617 3B(S)	0	149	3a, 3b	-	256	284
Ltc0329	BG01_2.5075.C1.Contig5991 (EG394920)	Putative 6-phospho-l-fructokinase [<i>O. sativa</i> (japonica cv-gp)]	-	-	102	3b	84	84	87, 93
Ltc0334	BG01018A2E12.r1 (EG391079)	Putative 4-methyl-5 (B-hydroxyethyl)-thiazol mono-phosphate biosynthesis enzyme [<i>O. sativa</i> (japonica cv-gp)]	BE494030 4BD(L)	-	160	3b	239	239	231
Ltc0345	BG01_2.924.C1.Contig1412 (EG377941)	TPA: isoflavone reductase-like protein 5 [<i>Vitis vinifera</i>]	-	-	111	3b	111	111	-
Ltc0379	BG01013B1E12.fl (EG374429)	Putative DNA damage repair protein [<i>O. sativa</i> (japonica cv-gp)]	BF145935 7BD(L)	0.027	212	3a	-	211	-
Ltc0384	BG01_2.5314.C1.Contig6183 (EG394826)	Thioredoxin family Trp26-like protein [<i>O. sativa</i> (japonica cv-gp)]	-	-	118	3b	116	116	113
Ltc0399	BG01_2.539.C1.Contig889 (EG401602)	P0046B10.17 [<i>O. sativa</i> (japonica cv-gp)]	BF475141 7BD(S)	E-148	156	3b	149	149	148, 153
Ltc0401	BG01_2.3440.C1.Contig4367 (EG391370)	Unknown protein [<i>O. sativa</i> (japonica cv-gp)]	BM134371 3ABD(S)	0	89	3a	-	84	-
Ltc0413	BG01_2.4232.C1.Contig5175 (EG396571)	Putative GTP-binding protein [<i>O. sativa</i> (japonica cv-gp)]	BE606698 3A(L)	6.00E- 44	106	3b	106	106	-
C4H.208	BG01_2.2568.C3.Contig3426 (EG398949)	C4H cinnamate 4-hydroxylase CYP73 [<i>Citrus sinensis</i>]	BE443770 3ABD(L)	E-143	217	3a	-	208	-

Table2-4. EST-SSR profiles of wheat-*Leymus racemosus* addition lines

	Lines provided by Prof. H Tsujimoto (Kishii et al. 2004)												Lines provided by Dr. BS Gill (Qi et al. 1997)										CS		L. r		TC1	TC2	LiFO3																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
EST-SSR	21 ^u +1 ⁿ [A]*	21 ^u + 1 ⁿ [C]	21 ^u + 1 ⁿ [E]	21 ^u + 1 ⁿ [F]	[H]* 1 ⁿ [H]	[H]* 1 ⁿ [I]	21 ^u + 1 ⁿ [J]	21 ^u + 1 ⁿ [J]*	21 ^u + 1 ⁿ [K]	21 ^u + 1 ⁿ [L]	21 ^u + 1 ⁿ [n]	96- 1012	96- 1014	96- 1019	96- 1022	96- 1022 -5-1	96- 1027	*	Bulk	Bulk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2-4 (continued)

EST-SSR	Lines provided by Prof. H Tsujimoto (Kishii et al. 2004)											Lines provided by Dr. BS Gill (Qi et al. 1997)										
	21"+1" [A]*	21"+1" [C]	21"+1" [E]	21"+1" [F]	20"+1" [H]*	21"+1" [H]*	21"+1" [I]	21"+1" [J]*	21"+1" [K]	21"+1" [L]	21"+1" [n]#	96-1012	96-1014	96-1019	96-1022	96-1022-5-1	96-1027*	CS Bulk	<i>L. r</i> Bulk	TC1	TC2	LiFO3
Ltc290b	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-
Ltc292b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
Ltc295	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Ltc299	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-
Ltc305a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Ltc305b	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-
Ltc306	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ltc308	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Ltc309	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Ltc315	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+
Ltc322	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Ltc323	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Ltc329a	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ltc329b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Ltc334	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Ltc345	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Ltc379	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Ltc384	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Ltc399	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Ltc401	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Ltc413	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-

* Addition lines with *L. racemosus* chromosome 3L, # Addition lines with *L. racemosus* chromosome 3S, - polymorphic band, + polymorphic band
 CS is Chinese Spring; RWR is Russian wildrye (*Psathyrostachys juncea*); *L. r* is *Leymus racemosus*.

Table 2-5. EST-SSR profiles of wheat-*Thinopyrum* addition lines

EST primer	Observed size	Wheat- <i>Thinopyrum</i> Addition lines															
		E ^b	E ^e	1E ^b	2E ^b	3E ^b	4E ^b	5E ^b	6E ^b	7E ^b	1E ^e	2E ^e	3E ^e	4E ^e	5E ^e	6E ^e	7E ^e
Ltc195a	145	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ltc195b	147	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ltc247	120	-	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-
Ltc248a	186	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ltc248b	189	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ltc257a	168	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ltc257b	170	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ltc279a	467	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Ltc279b	475	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Ltc322a	101	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Ltc322b	102	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Ltc323	261	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-

E^b-*Thinopyrum bessarabicum*E^e-*Th. elongatum*1E^b - 7E^b *T. aestivum* lines with a pair of *Th. bessarabicum* chromosomes1E^e - 7E^e *T. aestivum* lines with a pair of *Th. elongatum* chromosomes

Fig.2-1. Comparison of genetic linkage groups for two full-sib *Leymus* populations, TTC1 and TTC2 derived from EST-SSRs (red). Molecular markers mapped in both TTC1 and TTC2 are indicated with line connections. Other SSR and STS anchor markers are identified by green text color. Text reference has been used to identify specific homoeologous chromosomes of wheat (ABD), barley (H), rye (R), and oat (C, F) linkage groups. L and S in parentheses represent long and short arms of their respective chromosomes. A bar on left indicates genetic recombination distances (cM).

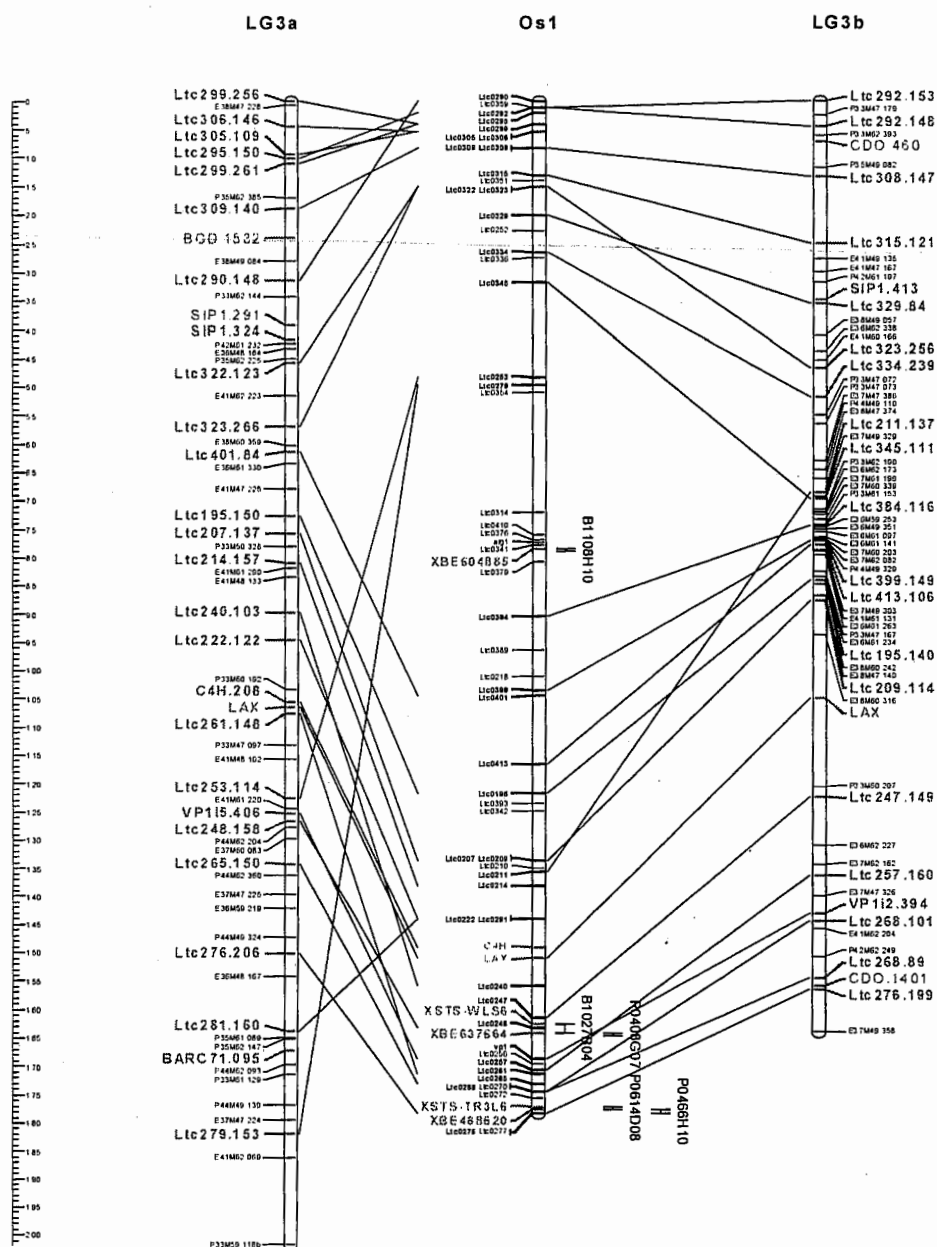


Fig.2-2. Comparison of EST-SSR (red) derived genetic linkage groups for two full-sib *Leymus* wildrye populations, TTC1 and TTC2 with *Oryza* chromosome 1. The updated linkage map includes homologous AFLP markers mapped in both TTC1 and TTC2 families (Wu et al. 2003). Homology with rice chromosome 1 is indicated with line connections. Bars on sides show the rice BACs, and pink text represents candidate genes. Other SSR and STS anchor markers are identified by green text color.

CHAPTER 3

FINE MAPPING *LEYMUS* WILD RYE GROWTH HABIT QTLs IN FOUR ADVANCED BACKCROSS FAMILIES

Abstract

Fine genetic mapping was conducted on four advanced backcross (BC2) populations of *Leymus* to validate and refine the location of previously identified QTLs on linkage group 3 of full-sib mapping families TTC1 and TTC2 derived from *L. triticoides* x *L. cinereus* hybrids backcrossed to the same *L. triticoides* tester. The QTLs in TTC1 and TTC2 were located in a 70-cM interval (130-200 cM) and a 65-cM interval (135-200 cM), respectively, in consensus linkage maps of TTC1, TTC2, and advanced backcross families. Three advanced backcross families (TTC1.168 x LtF03, TTC2.045 x LtF03, and TTC2.063 x LtF03) segregating for different regions of the putative growth habit QTL displayed QTLs for rhizome spread. One gene controlling growth habit trait is suspected to be present in a 30-cM interval between 135-165 cM regions of the consensus maps. This 30 cM QTL interval and nearby regions include 300 *Leymus* rhizome ESTs, based on alignments to rice physical maps. Shift of the QTL position to 100-130 cM in the TTC2.045 x Lt641 family points toward another gene controlling the trait. Advanced backcross families showed a significant QTL effect on LG3a with a negative effect of *L. cinereus* alleles. These results validate and refine the growth habit QTL on LG3 of *Leymus*.

Introduction

Perennial grasses provide an important source of forage for livestock and wildlife throughout the world. Perennial grasses are also cultivated for soil stabilization, weed management, turf, and dedicated bioenergy crops. These grasses show divergent adaptations in terms of growth habit, salinity tolerance, phenology, seed dormancy, and many other traits. Growth habit, the genetic tendency of a plant to grow in a certain shape and to attain a certain mature height and spread, is a highly variable and economically important trait contributing to resiliency, competitiveness, grazing tolerance, and other functionally important attributes of perennial grasses. Growth habit in perennial grasses is characterized by aggressive rhizomes, e.g., quack grass (*Elymus repens*) and Johnson grass (*Sorghum halepense*). New lateral branches of caespitose grasses grow from the crown of a mature plant, strictly upwards, often within the lower leaf sheath. Prostrate, more or less elongated, lateral branches of rhizomatous grasses grow partly or completely below the soil surface, and allow grasses to spread and form a sod that regrows each year by breaking out of a leaf sheath and growing approximately at the right angle to the leaf sheath. Rhizomes are important means of propagation and persistence of many grass species, and they enable young perennial plants to compete effectively in an already established plant community.

Leymus is a perennial relative of wheat (*Triticum*), barley (*Hordeum*), rye (*Secale*), and other annual and perennial Triticeae grasses. Worldwide distribution of 30 species of *Leymus* grasses ranges from Asia to Europe and North and South America (Asay and Jensen 1996) with remarkable variation in growth habit, stature, and adaptation to harsh, cold, dry, and saline environments. Being a wild relative of wheat

(tetraploid and hexaploid Triticeae species), *Leymus* has been used in wide-hybridization wheat breeding (Asay and Jensen 1996). *Triticum* and *Leymus* hybrids and wheat introgression lines containing *Leymus* chromosomes have been developed (Tsitsin 1965; Mujeeb-kazi et al. 1983; Plourde et al. 1992; Qi et al. 1997; Anamthawat-Jónsson et al. 1999; Ellneskog-Staam and Marker 2001). The main objective of these efforts was the transfer of i) alien genes for salinity tolerance, and ii) viral and fungal disease resistance from *Leymus* to wheat. More than half of the *Leymus* species are allotetraploid ($2n=4x=28$), but octoploid ($2n=8x=56$) and duodecaploid ($2n=12x=84$) variants may arise due to interspecific hybridization (Anamthawat-Jónsson and Bödvarsdóttir 2001) or autoduplication within species. Genome-specific markers have been used to distinguish several homoeologous linkage groups corresponding to the **Ns** and **Xm** genomes of *Leymus* (Wu et al. 2003). One diploid ancestor of allotetraploid *Leymus* has been identified as *Psathyrostachys*, contributing the **Ns** genome to *Leymus* (Zhang and Dvořák 1991; Wang and Jensen 1994; Wang et al. 1994; Anamthawat-Jónsson and Bödvarsdóttir 2001), while the other genome, **Xm**, remains unidentified (Wang and Jensen 1994; Wang et al. 1994; Sun et al. 1995). Löve (1984) speculated that the **Xm** genome originates from *Thinopyrum bessarabicum*, but chromosome pairing in *Thinopyrum* x *Leymus* hybrids has been ambiguous (Sun et al. 1995) or very low (Wang and Jensen 1994; Zhang and Dvořák 1991). Hybridization of *Leymus* DNA with repeated nucleotide sequences from other Triticeae species suggests that *Leymus* is a segmental autopolyploid, ($Ns_1Ns_1Ns_2Ns_2$), derived from two distinct *Psathyrostachys* species (Zhang and Dvořák 1991). Southern hybridization using total genomic DNA as a probe confirmed the *Leymus* genome to be either autopolyploid, having only **Ns**-genomic DNA, or segmental

allopolyploid (Anamthawat-Jónsson 2005).

These cool-season grasses display divergent adaptation and remarkable variation in terms of growth habit and architecture. *Leymus cinereus* and *L. triticoides* are closely related, but morphologically distinct, North American perennial range grasses. *Leymus cinereus* is the largest (up to 2 m tall), most abundant native *Leymus* species. It is a well adapted to a wide variety of habitats, ranging from deep well-drained soils from British Columbia to Saskatchewan to the north and California to Arizona and Colorado to the south. These large-statured *Leymus* wildryes may produce high biomass and valuable forage on many saline/alkaline sites where few other species are adapted. Cultivars of *L. cinereus* are commonly used in rangeland seed mixtures in western North America, but they have limited use in pastures or hay crops.

Creeping wildrye (*L. triticoides*), the second most common *Leymus* species in western North America, is shorter (0.3-0.7 m tall) but produces aggressive rhizomes. It is specifically adapted to poorly drained alkaline sites in the Great Basin and other regions of western North America. *Leymus triticoides* is cultivated as a saline biomass crop using vegetative propagules in California, but poor seed production limits widespread use of this species.

Most populations of *L. cinereus* and *L. triticoides* are allotetraploid, however octoploid forms of *L. cinereus* are typical in the Pacific Northwest. Both *L. cinereus* and *L. triticoides* are self-sterile outcrossing species that hybridize in nature (Jensen et al. 1990), although some interspecific hybrids are unable to produce seeds. Artificial hybrids of *L. cinereus*, *L. triticoides*, and other North American *Leymus* species display regular meiosis and stainable pollen (Stebbins and Walters 1949; Dewey 1972; Hole et al. 1999).

In general, caespitose and rhizomatous grasses dominate semi-arid and mesic grasslands, respectively (Sims et al. 1978). Where *L. cinereus* can grow from mesic and semi-arid communities, *L. triticoides* may be restricted to microsites by higher soil organic carbon and nitrogen concentration (Derner and Briske 2001). *Leymus triticoides* has been seen to inhabit riparian or wet zones and *L. cinereus* inhabiting dry adjacent uplands, restricted to seemingly different natural microhabitats (Larson et al. 2006). Conversely, these species have been observed to be growing in close proximity to each other at several distributed sites (Larson et al. 2006). *Leymus cinereus* and *L. triticoides* display differences in salinity tolerance, seed dormancy, seed color, seed shattering, mineral content, tillering, texture, and other characteristics.

These two species differ for the gravitropism, as *L. cinereus* is caespitose and *L. triticoides* is rhizomatous. Endodermal cells in shoots have been found to be gravity-perceiving, containing a large central vacuole influencing the sedimentation of amyloplasts in the endodermis (Kato et al. 2002). These contrasting characteristics of *L. cinereus* and *L. triticoides* make them a particularly useful genetic system to evaluate the inheritance of functionally important traits, particularly growth habit. Wu et al. (2003) constructed high-density molecular genetic linkage maps for two full-sib families TTC1 and TTC2, derived from two interspecific *L. triticoides* x *L. cinereus* F1 hybrids, TC1 and TC2, backcrossed to an *L. triticoides* parent. The F1 hybrids TC1 and TC2 are genetically different, being derived from naturally heterogeneous *L. cinereus* and *L. triticoides* seed accessions, Acc:636 from Alberta, and Acc:641 from Oregon respectively. Together the TTC1 and TTC2 populations provide a unique system to evaluate genetic control of growth habit and other traits in the perennial grasses.

Molecular genetic linkage maps have 1,583 AFLP markers and 67 anchor loci on 14 linkage groups, which correspond to the 14 chromosome pairs of allotetraploid *Leymus* (Wu et al. 2003; Larson et al. 2006). In addition, 48 CSU or KSU wheat SSR primer pairs (Yu et al. 2004) and 48 selected STS anchor markers, originally designed for wheat and barley (Mano et al. 1999; Taylor et al. 2001; Lem and Lallemand 2003), were tested (Larson et al. 2006) using methods described by Wu et al. (2003). A total of four growth habit/circumference quantitative trait loci (CIRC QTLs) have been detected on TTC1 and/or TTC2 families on LG3a, LG3b, LG5Xm, and LG6a, with *L. cinereus* alleles showing their negative effect on the QTLs. The strongest, and most consistent CIRC QTLs were located on homoeologous regions of LG3a and LG3b of both TTC1 and TTC2 that correspond to the distal region of Triticeae 3L (Larson et al. 2006). Presence of an anchor locus VP1 (viviparous) in both TTC1 and TTC2 families near to the QTL regions of LG3a and LG3b suggests that the QTLs are homoeologous. The transcription factor viviparous-1 (encoded by the VP1 gene) induces and maintains seed dormancy (McCarty et al. 1991) and has been mapped to orthologous loci in wheat, maize, and rice (Bailey et al. 1999). Rice chromosome 1 contains a putative transmembrane auxin efflux carrier and DnaJ-like genes (Sasaki et al. 2002) near the *Vp1* locus, originally identified in *Arabidopsis pin-forming* (Gälweiler et al. 1998) and *arg1* (altered response to gravity) (Sedbrook et al. 1999) mutants, respectively. Rhizome (Hu et al. 2003) and tiller angle (Li et al. 1999) QTLs also map to rice chromosome 1, but these rice loci were not located near the rice *Vp1* gene (Bailey et al. 1999; Sasaki et al. 2002). Recently, a tiller inhibition gene (*tin3*) has been located on long arm of chromosome 3A^m in *Triticum monococcum* (Kuraparthi et al. 2007).

Circumference (rhizome spread) measurements of F1 hybrids were significantly greater than *L. cinereus* Acc:636, but well below *L. triticoides* Acc:641, mid-parent, TTC1, and TTC2 averages, especially in the first year (Larson et al. 2006). Circumference of plant spread showed relatively strong broad-sense heritability in TTC1 and moderate heritability in TTC2 (Larson et al. 2006).

Advanced backcross (BC2) QTL analysis is a useful method for QTL detection and inheritance because this may fix the epistatic interactions between QTLs and other donor genes, leading to silencing of the measured QTL effects as compared to F2, F3, and BC1 generations (Pillen et al. 2003).

The aims of the present study were to develop advanced backcross populations of TTC1 and TTC2, and to use these segregating advanced backcross populations to develop consensus maps to validate and refine the location of growth habit QTLs in *Leymus* (Larson et al. 2006), and to compare the location of *Leymus* growth habit QTLs with gene sequences and other DNA markers mapped in wheat, barley, rice, and other Poaceae species.

Materials and methods

Plant materials and DNA isolation

Wu et al. (2003) developed two full-sib mapping populations, TTC1 and TTC2. The TTC1 and TTC2 full-sib mapping populations were derived from crosses of the TC1 and TC2 interspecific hybrids “backcrossed” to the same *L. triticoides* tester genotype. The TC1 and TC2 hybrids were derived from crosses of the heterogeneous *L. triticoides* Acc:641 and *L. cinereus* Acc:636 accessions. Selected genotypes from TTC1 and TTC2

families (Fig. 3-1) were further backcrossed to a rhizome donor *L. triticoides* T-tester to develop six different advanced backcross populations, namely 190-sib TTC1.168 x LtF03, 94-sib TTC2.045 x Lt641, 94-sib TTC2.111 x LtF03, 192-sib TTC2.002 x B2082, 94-sib TTC2.063 x LtF03, and 87-sib TTC2.005 x B2082 (Table 3-1). Marker-assisted selection was used to select putative QTL donor parents from the TTC1 and TTC2 BC1 populations using Graphical Genotyping Tools (GGT) software (Berloo 1999). Source clones of all these populations are currently being maintained in a greenhouse at the United States Department of Agriculture-Agriculture Research Services-Forage and Range Research Laboratory (Logan, UT). They were also planted in two clonally replicated blocks at the Utah Agricultural Experiment Station field site at Twin Canals, North Logan, Utah. Plant genomic DNA was obtained from the leaf samples of advanced backcross progenies and TTC1 and TTC2 hybrids using DNeasy® plant DNA isolation kits (Qiagen Inc., Valencia, CA).

Genetic maps

The respective full-sib TTC1 and TTC2 molecular genetic linkage maps described by Wu et al. (2003) and growth habit QTLs described by Larson et al. (2006) were used as a starting point to construct high-density molecular genetic linkage maps of advanced backcross populations. Briefly, molecular genetic linkage maps for advanced backcross families were developed using DNA markers (AFLPs, SSRs, STS, RFLPs) that were present in one or both hybrids (TC1, TC2), absent in the *L. triticoides* tester, and segregating among the full-sib progenies.

AFLP procedures

Reactions with AFLP markers were carried out as described by Vos et al. (1995) and Vuylsteke et al. (1999) using two different restriction enzyme combinations, *EcoRI*-*MseI*. The adapter sequences specific for those enzymes were prepared by standard synthesis of the following oligonucleotides:

EcoRI adapter: Forward, 5'-CTCGTAGACTGCGTACC-3';

Reverse, 3'-CATCTGACGCATGGTTAA-5';

MseI adapter: Forward, 5'-GACGATGAGTCCTGAG-3';

Reverse, 3'-GTAGTCACGTACGC-5'.

The first PCR amplification of restriction fragments with one selective nucleotide was performed using similar preparations of the following oligonucleotide sequences:

EcoRI (+1), 5'-GACTGCGTACCAATTCA-3'

MseI (+1), 5'-GATGAGTCCTGAGTAAC-3'

The second PCR amplification of restriction fragments was performed using the following combinations of oligonucleotide sequences with three selective nucleotides as

EcoRI (+3), 5'-GACTGCGTACCAATTCANN-3'

MseI (+3), 5'-GATGAGTCCTGAGTAACNN-3'

(Table 3-2). The 5' nucleotide of the *EcoRI* (+3) selective amplification primers was labeled with 6-carboxyfluorescein (6-FAM). Fluorescent-labeled DNA fragments were size-fractionated using an ABI Prism 3100 and 3730 genetic analyzer with 50-cm capillaries, POP-6 polymer, GS500 LIZ and rhodamine X (ROX)-labeled GS400 HD internal size standards according to manufacturer instructions (PE Applied Biosystems

Inc., Foster City, CA). The segregation of fluorescent AFLP, SSR, and STS-PCR markers was analyzed using a combination of Genescan software (PE Applied Biosystems) and Genographer version 1.5 software (Benham et al. 1999).

STS and SSR protocols

A volume of 25 μ L was used to carry out the PCR. Each reaction contained 1.0 U Promega *go-Taq* polymerase, 0.4 μ mol of each primer, 200 μ M dNTPs, 2.0 mM $MgCl_2$, 10 pmol fluorescent-labeled [R110] dCTP, 1X PCR buffer, and 50 to 100 ng of total genomic DNA. Cycling parameters generally consisted of 1.40 min at 94°C, followed by 35 cycles at 94°C for 20 sec, 50-60°C for 30 sec, and 72°C for 2 min, and a final extension step at 72°C for 7 min.

Genetic analysis and map construction in advanced backcross populations

Marker-assisted selection was used to select donor parents from TTC1 and TTC2 populations using graphical genotyping tools (GGT by Berloo 1999). The TTC1 and TTC2 families segregated for different regions of the putative growth habit QTL on LG3a. These genotypes can be used to compare the consistency of marker order and presence of CIRC QTLs on the previously identified regions of LG3 of TTC1 and TTC2 families. Genetic linkage maps for advanced backcross populations were constructed based on the segregation of DNA markers using the BC1 population model of JoinMap4 (van Ooijen 2006). In practice, this analysis was based on DNA fragments present in TC1 and/or TC2 hybrids, absent in the T-tester, and segregating in each advanced backcross progeny. The mapped DNA fragments in these linkage maps basically originated from *L.*

cinereus. These markers have a 1:1 expected segregation ratio (assuming disomic inheritance of monoallelic fragments). Only the markers present in the regions segregating for *L. cinereus* and *L. triticoides* alleles of group LG3 of QTL donor parents were tested on their respective populations to test the segregation QTL inheritance in the advanced backcross families.

Field Evaluations

Clones from each of the advanced backcross families were space-planted in a randomized complete block design with two replicates (blocks) per family at the Twin Canals Farm (North Logan, UT). Each block contained one clone each of the 190-sib TTC1.168 x LtF03, 94-sib TTC2.045 x Lt641, 94-sib TTC2.111 x LtF03, 192-sib TTC2.002 x B2082, and 94-sib TTC2.063 x LtF03, 87-sib TTC2.005 x B2082, and several parental clones. Individual clones were transplanted from soil containers (4-cm diameter) in the spring of 2003 to field plots with 2-meter row spacing and 2-meter spacing within rows (2-m centers). Plants were aligned among rows such that each plant had 4 equidistant neighboring plants, 2-m apart.

Rhizome proliferation was measured as plant circumference (CIRC) in late spring or early summer from 2004 to 2006. For caespitose plants, this was measured by stretching a tape ruler around the tussock at the soil surface. The CIRC of the plants with irregular sods, characteristic of the more rhizomatous plants, was approximated as the perimeter of a polygon, where the corners represent the outermost rhizome branches (i.e., the shortest distance around the outermost rhizomes) at the soil surface.

QTL data analysis

The QTL analyses were based on trait means from two clones from each of the advanced backcross populations segregating in 2004, 2005, and 2006. These QTL analyses were also performed on averages over all three years. Broad-sense heritabilities within years were obtained using SAS code for estimating heritability from lines evaluated in an RCB design in a single environment (Holland et al. 2003). The basic SAS code for estimating heritabilities is available at http://www4.ncsu.edu/~jholland/homepage_files/Page571.htm (verified 24 July 2006).

The QTL detection was performed using the MapQTL 5 package (van Ooijen 2004). The LG3a consensus map of TTC1 and TTC2 was used for the QTL analysis of the four advanced backcross progenies, 179-sib TTC1.168 x LtF03, 94-sib TTC2.045 x Lt641, 94-sib TTC2.111 x LtF03, and 94-sib TTC2.063 x LtF03. Genome-wide interval mapping (IM) (Lander and Botstein 1989; van Ooijen 1992) was performed in 1-cM increments to identify putative QTLs. Threshold LOD values of 1.5 and 2.1 were used throughout these QTL detection procedures as a close approximation for a genome-wide 5% and 1% significance levels, respectively, as determined from empirical thresholds based on permutation analyses with 1000 replications (Churchill and Doerge 1994). Genome-wide IM scans based on 2004, 2005, 2006, and average phenotypic values were performed for CIRC using MapChart version 2.1 (Voorrips 2002).

Results

A total of 190 sib TTC1.168 x LtF03, 94 sib TTC2.045 x Lt641, 94 sib TTC2.111 x LtF03, 192 sib TTC2.002 x B2082, 94 sib TTC2.063 x LtF03, and 87 sib TTC2.005 x

B2082 progenies were developed for each advanced backcross mapping population (Table 3-1). Marker analysis at initial stages showed that 11 individuals of population TTC1.168 x LtF03 and all the individuals of populations TTC2.002 x B2082 and TTC2.005 x B2082 were virtually identical to their respective T-tester. These genotypes were assumed to be resulted from the selfing of the female parent and were discarded from any further genotyping and analysis. Thus, finally we had four populations comprising a total of 179-sib TTC1.168 x LtF03, 94-sib TTC2.045 x Lt641, 94-sib TTC2.111 x LtF03, and 94-sib TTC2.063 x LtF03 progenies (Table 3-1).

Genetic linkage maps

A total of 17 AFLP primer combinations were tested in advanced backcross populations. Out of these, 16 segregating marker loci were scored on LG3a in TTC1.168 x LtF03, 28 in TTC2.045 x Lt641, 20 in TTC2.063 x LtF03, and 11 in TTC2.111 x LtF03 advanced backcross families (Table 3-2). Moreover, the previous analysis of marker scoring in TTC1 and TTC2 linkage groups was based on the separation of bands in ABI Prism 3100 Genetic Analyzer using rhodamine X (ROX)-labeled Gene Scan 400 HD internal size-standard. But for these populations, we used ABI 3730 Genetic Analyzer using 500 LIZ as an internal size-standard. Thus, the segregating bands were not located at the exact same locations as expected, but showed a size difference of 2-3 bp from the expected size. Thus, we located the correct marker by comparing the gels of advanced families with the gels that had been run on ABI 3100 Genetic Analyzer to test the amplification on the parental samples TC1, TC2, and T-tester. To maintain the consistency between studies, we used same marker positions as detected in TTC1 and

TTC2 families (Wu et al. 2003). For comparison and refinement of the QTL region, a single consensus map was developed for the BC1 mapping families TTC1, TTC2, and the advanced backcross families (BC2) using JoinMap 4 (van Ooijen 2006). This consensus map was used to conduct QTL analysis in each advanced backcross mapping family. The consensus map of LG3a of TTC1 and TTC2 and all advanced backcross-mapping families spanned 228 cM on the linkage map (Fig. 3-2).

Anchored PCR markers

An anchor marker, VP1, from wheat segregated in the linkage group LG3a of advanced backcross population TTC1.168 x LtF03 and TTC2.045 x Lt641, as expected based on the segregation patterns of their respective donor parents (Fig. 3-2).

A total of 16 new wheat and barley LG3 SSR and STS primer pairs were tested for amplification and polymorphism on TC1, TC2, and T-tester genotypes in addition to 67 anchor loci that had already been mapped in TTC1 and TTC2 progenies (Wu et al. 2003; Larson et al. 2006). One primer pair BARC71 (5'GCGCTTGTTCCCTCACCTGCTCATA3', and 5'GCGTATATTCTCTCGTCTTCTTGTTGGTT3') amplified PCR products that mapped near the VP1 locus on LG3a in TTC1 progeny. Marker BARC71 has been mapped on chromosome 3D of wheat. This marker when tested for amplification in the TTC1.168 x LtF03 population, mapped near the VP1 locus on LG3a of TTC1.168 x LtF03.

Growth habit QTLs and heritabilities

Growth habit QTLs located on LG3a and LG3b, identified in TTC1 and TTC2 families (Larson et al. 2006), were validated in the advanced backcross families. To maintain consistency between two studies, marker positions used were the same as detected in TTC1 and TTC2. Growth habit QTLs have been localized on LG3a in advanced backcross populations. Advanced backcross populations displayed increasing levels of rhizomatous spreading over three years (Table 3-3). The trait means of TTC1 and TTC2-derived advanced backcross progenies were greater than the trait means of their progenitors TTC1 and TTC2 as shown by Larson et al. (2006). Figure 3-2 shows and compares the presence of growth habit QTLs in TTC1, TTC2, and four advanced backcross progenies along with the segregating regions of other advanced backcross progenies. Based on these results, it may be surmised that at least one gene controlling growth habit is present in the 135-165 cM region (Table 3-4). As expected, in TTC2.063 x LtF03 and in TTC2.111 x LtF03, the QTL did not have any effect, which confirms the result that the QTL is present in the above 30-cM region. This 30-cM QTL interval and nearby regions include approximately 300 *Leymus* rhizome ESTs, based on alignments to rice physical maps (unpublished). Although the maximum QTL effect in TTC2.045 x Lt641 was shifted in between 100-130 cM interval with a maximum LOD of 3.90 in third years of experiments (2006).

Table 3-4 describes the summary of QTL effects detected using composite interval mapping model (CIM) scan of plant circumference in *Leymus* advanced backcross mapping families at a threshold LOD of ≥ 1.5 . This shows the year-wise CIRC QTL effects and average QTL effects across 3 years for all advanced backcross-mapping

families. The strongest QTL was located on population TTC2.063 x LtF03 with an LOD score of 4.08 in year 2004 ($R^2=0.204$), but the QTL effect was decreased over years, with an average LOD of 2.70. Although the QTL on TTC2.045 x Lt641 in year 2006 had a maximum LOD score of 3.90 ($R^2=0.187$) and an average LOD of 3.61 over three years. At a threshold LOD of 2.1, none of the QTLs were significant in TTC1.168 x LtF03, but at a threshold LOD of 1.5, the QTL was significant with an LOD value of 1.66 ($R^2=0.043$). None of the QTLs were significant in TTC2.111 x LtF03 at threshold values of 1.5 or 2.1. For third year (2004) data of LG3a of TTC1 and TTC2 progenies, the maximum LOD score was found to be 3.23 ($R^2=0.123$) and 3.71 ($R^2=0.101$), respectively, at a threshold LOD of 3.3 (Larson et al. 2006).

Circumference QTLs were detected in four advanced backcross populations. Except TTC2.111 x LtF03 and TTC2.063 x LtF03, all other populations showed a significant QTL effect on LG3a with the effect of *L. cinereus* alleles being negative. None of the QTLs were found to be significant on LG3b of any population. The strongest QTL was located on LG3a of TTC2.045 x Lt641. However, over the time the QTL effect decreased in TTC2.063 x LtF03 from 4.08 in 2004 to 1.50 in 2006 (Table 3-4).

Circumference of rhizome spread showed broad-sense heritabilities (h^2) in years 2004, 2005, and 2006 in all four advanced backcross populations. Heritability values increased over years 2004 to 2006 in all four advanced backcross populations (Table 3-5). Figure 3-3 compares the rhizome spread over three years for all four advanced backcross progenies along with the rhizome spread of parental genotypes. The plant circumference data was not available for B2-063 parental genotype. As expected, maximum variation in plant spread was observed in third year (2006).

Discussion

Tillers and rhizomes are important determinants of growth habit and plant architecture in grasses. Rhizomes are organs of fundamental importance to plant competitiveness, invasiveness and perenniality. This study was done to validate and refine the location of previously identified growth habit QTLs conferring rhizomatousness (Larson et al. 2006) in perennial *Leymus* grasses, to track the inheritance of BC1 growth habit QTLs through BC2 or advanced backcross populations, and to develop near isogenic lines (NILs).

Hacker (1983) performed genetic experiments concerning rhizomatous habit in the *Digitaria milaniana* (Rendle) Stapf (Poaceae) complex and reported that incompletely recessive genes controlled the rhizome development. In tall fescue (*Festuca arundinacea* Schreb.), rhizome expression is thought to be a quantitative trait with the number of rhizomes produced per plant controlled by additive genes (Whitehead 1986). In perennial rice (*Oryza longistaminata*), rhizomatous expression was found to be under the control of two dominant complementary genes (Hu et al. 2001), but Sacks et al. (2003) indicated the presence of rhizomes under the control of a single locus with large effect in a study conducted on an *O. sativa* x *O. longistaminata* population.

Leymus advanced backcross progenies were derived from different parental genotypes and showed different patterns of rhizome segregation on LG3a, suggesting that different alleles or allelic combinations may be controlling the rhizomatous expression among these populations. Although not all the markers present in the QTL region of donor parents were polymorphic in their respective advanced backcross progenies, this

may be because new recombination events changed the linkage patterns in advanced backcross populations.

Leymus cinereus Acc:636 and *L. triticoides* Acc:641 progenitor accessions displayed very different levels of rhizome spread, which became more pronounced each year. Also, spread of TTC1 and TTC2 F1 hybrids was significantly greater than *L. cinereus* Acc:636, although not above TTC1/TTC2 mid-parent means of, TTC1, and TTC2 (Larson et al. 2006). The increase in broad sense heritability (h^2) for circumference of rhizome spread over three years in all four advanced-backcross populations was expected because plant spreading is a cumulative trait (Larson et al. 2006).

Because of the presence of QTL effects near an anchor locus, VP1, on both LG3a and LG3b in both TTC1 and TTC2 families, Larson et al. (2006) suggested that growth habit is controlled by homoeologous copies of one gene. But in the advanced backcross, none of the CIRC QTL effects were found to be significant on LG3b. Disappearance of QTLs on LG3b in advanced generations may be due to actual interactions between QTL and the genetic background.

Throughout much of these linkage maps, relatively small irregularities in the LOD scan resulted from imperfect genotyping, missing data, and ambiguous marker orders. Future plans are to select recombinant lines from the TTC2.045 x Lt641 advanced backcross mapping family to more precisely detect, refine, and dissect the growth habit QTL in the third generation backcross (BC3) families. This TTC2.045 x Lt641 family is already established in clonally replicated field trials and will be used for genotyping and QTL evaluation. This growth-habit gene identification may be of importance in the development of perennial grain crops. No perennial species to date has been developed

that produce adequate grain harvest. There are several advantages of perennial grasses over annual grasses. If the genes(s) controlling growth habit are located and ultimately transferred to other grain crops such as wheat, sorghum etc., then improvement can be made for soil and water management and conservation and greater biomass and resource management capacity.

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Table 3-1: *Leymus* advanced backcross (BC2) populations

Population	Female	Male	Germinated Seeds	Advanced Progeny	Progeny size used for analysis
TTC1.168 x LtF03	LtF03	A1-168	258	190	179
TTC2.002 x B2082	B2082	B2-002	198	192	0
TTC2.005 x B2082	B2082	B2-005	97	87	0
TTC2.045 x Lt641	Lt641 (#524)	B1-045	154	94	94
TTC2.063 x LtF03	LtF03	B2-063	148	94	94
TTC2.111 x LtF03	LtF03	B1-111	131	94	94

Table 3-2 Summary of amplified fragment length polymorphisms (AFLPs) genetically mapped on LG3a in *Leymus* advanced backcross mapping populations TTC1.168 x LtF03, TTC2.045 x Lt641, TTC2.063 x LtF03 and TTC2.111 x LtF03, amplified by 17 *EcoRI-MseI* primer combinations with three selective nucleotides.

Primer combinations	Selective nucleotides	A1-168xLt-F03 LG 3a	B1-045xLt-641 LG 3a	B2-063xLt-F03 LG 3a	B1-111x Lt-F03 LG 3a
E36M48	E(ACC)/M(CAC)	2	2	1	0
E36M59	E(ACC)/M(CTA)	1	2	1	0
E36M61	E(ACC)/M(CTG)	0	3	3	0
E36M62	E(ACC)/M(CTT)	0	2	2	1
E37M47	E(ACG)/M(CAA)	2	3	3	1
E37M49	E(ACG)/M(CAG)	0	0	0	3
E37M60	E(ACG)/M(CTC)	3	1	0	0
E37M61	E(ACG)/M(CTG)	1	1	1	0
E37M62	E(ACG)/M(CTT)	0	0	0	0
E38M47	E(ACT)/M(CAA)	1	3	2	1
E38M60	E(ACT)/M(CTC)	0	2	1	0
E41M48	E(AGG)/M(CAC)	2	3	2	1
E41M49	E(AGG)/M(CAG)	0	0	1	1
E41M59	E(AGG)/M(CTA)	1	0	0	0
E41M60	E(AGG)/M(CTC)	1	1	0	0
E41M61	E(AGG)/M(CTG)	1	4	2	1
E41M62	E(AGG)/M(CTT)	1	1	1	2
EM total		16	28	20	11

Table 3-3 Trait means for circumference of plant spreading (CIRC) for advanced-backcross populations

Trait	Year	TTC1.168 x LtF03	TTC2.045 x Lt641	TTC2.111 x LtF03	TTC2.063 x LtF03
		(n=179*)	(n=94*)	(n=94*)	(n=94*)
CIRC	2004	113.80 ± 59.53	199.25 ± 101.06	154.79 ± 76.95	203.88 ± 105.28
	2005	456.24 ± 187.26	746.62 ± 176.54	669.46 ± 187.62	673.40 ± 228.61
	2006	629.35 ± 242.84	1013.36 ± 165.36	959.39 ± 225.70	890.66 ± 261.24
	Avg.	399.80 ± 159.46	653.08 ± 140.43	594.55 ± 153.21	589.31 ± 191.15

*Sample size based on means of two clones for each genotype (*n*)

Table 3-4 Summary of QTL effects detected using Composite Interval mapping model (CIM) scans of circumference of plant spreading (CIRC) in *Leymus* advanced-backcross mapping families. (NS - not significant)

Trait	BC2 Family	Linkage group (interval) ^a	2004 LOD (R ^{2b} , effect ^c)	2005 LOD (R ^{2b} , effect ^c)	2006 LOD (R ^{2b} , effect ^c)	Average LOD (R ^{2b} , effect ^c)
CIRC	A1-168	3a (139.9-142.6)	NS	NS	1.66 (4.3%, 102.256)	NS
	B1-045	3a (51.6-161.58)	3.55 (19.5%, -84.32)	2.58 (12.7%, -127.58)	3.90 (18.7%, -158.03)	3.61 (17.3%, -118.64)
	B1-111	NS	NS	NS	NS	NS
	B2-063	3a (67.39-88.71)	4.08 (20.4%, 94.71)	3.04 (16.1%, 182.75)	1.50 (8.4%, 150.56)	2.70 (14.4%, 144.41)

^a Positions (cM) where LOD ≥ 1.5.

^b Expressed as percent variance explained.

^c Mean of heterozygous genotype (*L. cinereus* and *L. triticoides* alleles) – mean of homozygous genotypes (two *L. triticoides* alleles) expressed in centimeters (CIRC).

Table 3-5 Heritability for circumference for three years (2004-2006)

Population	Year 2004	Year 2005	Year 2006
A1-168	0.27	0.48	0.54
B1-045	0.35	0.42	0.61
B1-111	0.22	0.48	0.62
B2-063	0.52	0.60	0.75

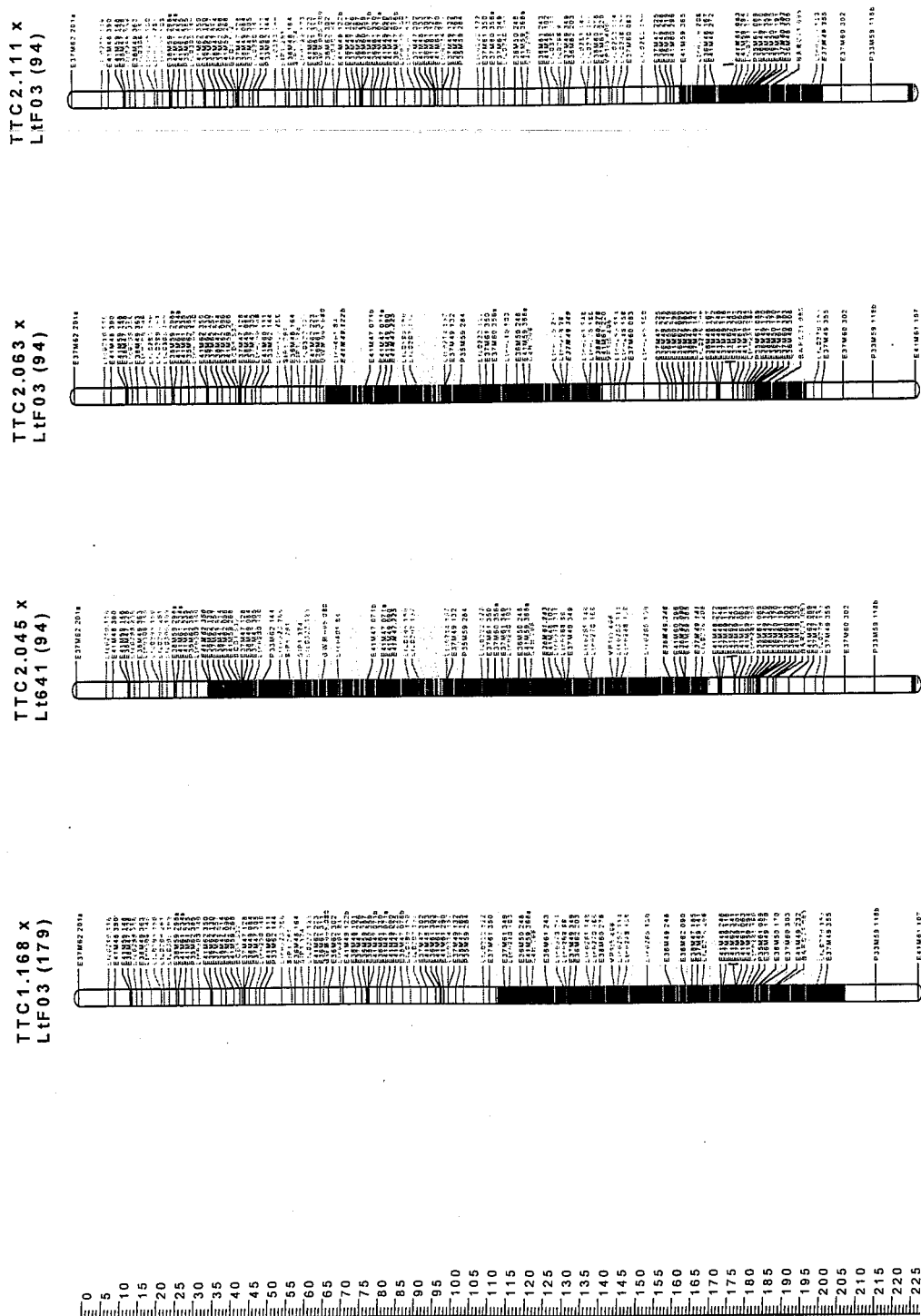
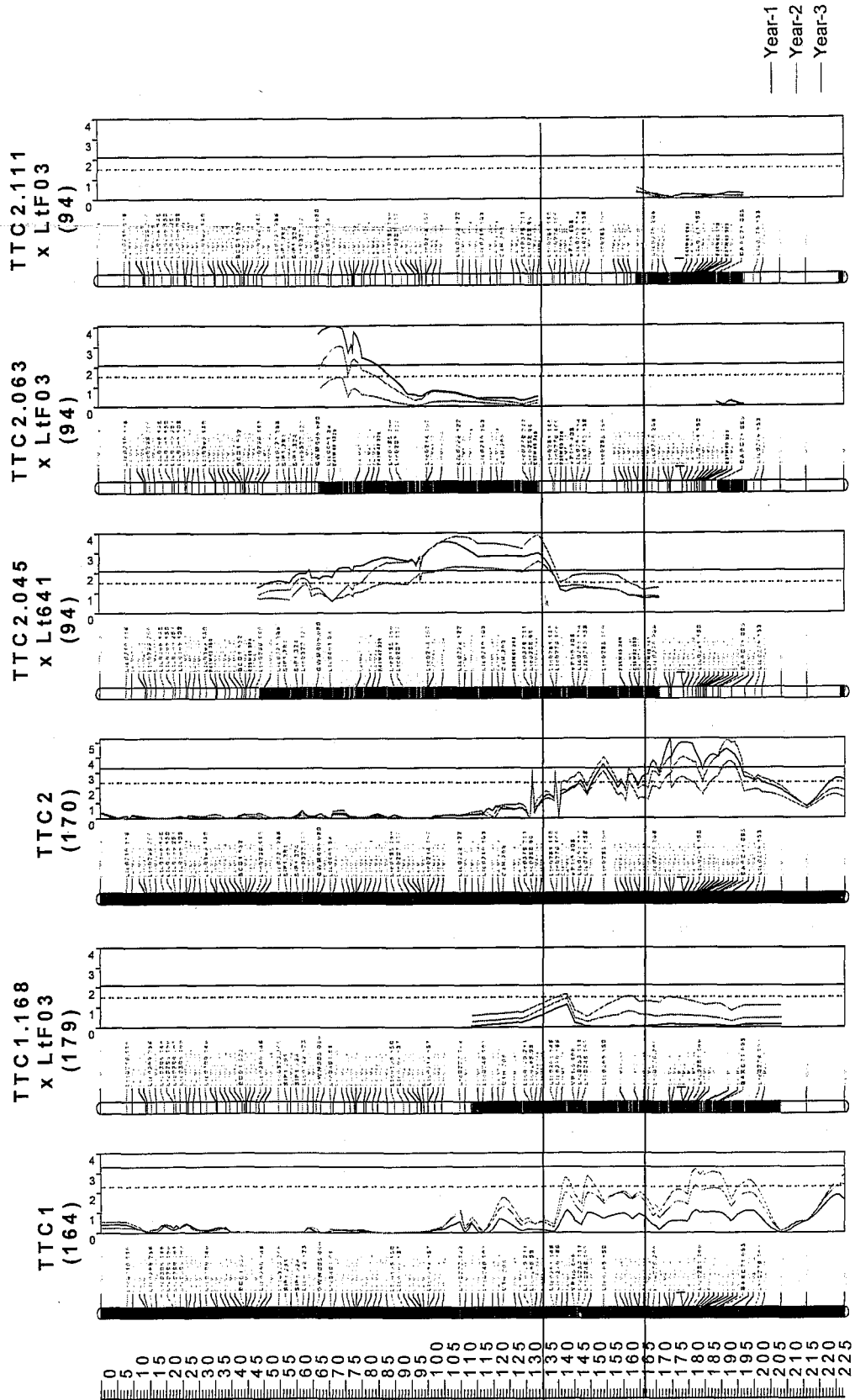


Fig 3-1: Recombinant BC1 lines as BC2 donor parents.

— Year-1
 — Year-2
 — Year-3

Fig. 3-2: LOD scans on linkage group *Leymus* LG3a; between the first TTC1 and TTC2 backcross families and four BC2 families. The approximate location of growth habit QTL effects (LOD 2.1), corresponding to a genome-wide $P \leq 0.01$ significance level, detected using a interval mapping are indicated in between two lines. + and – indicate presence or absence of QTL respectively in their respective population. Text in aqua and black color shows the segregating and non-segregating AFLP markers, respectively. Purple color shows the anchor markers and red shows the *Leymus* ESTs.



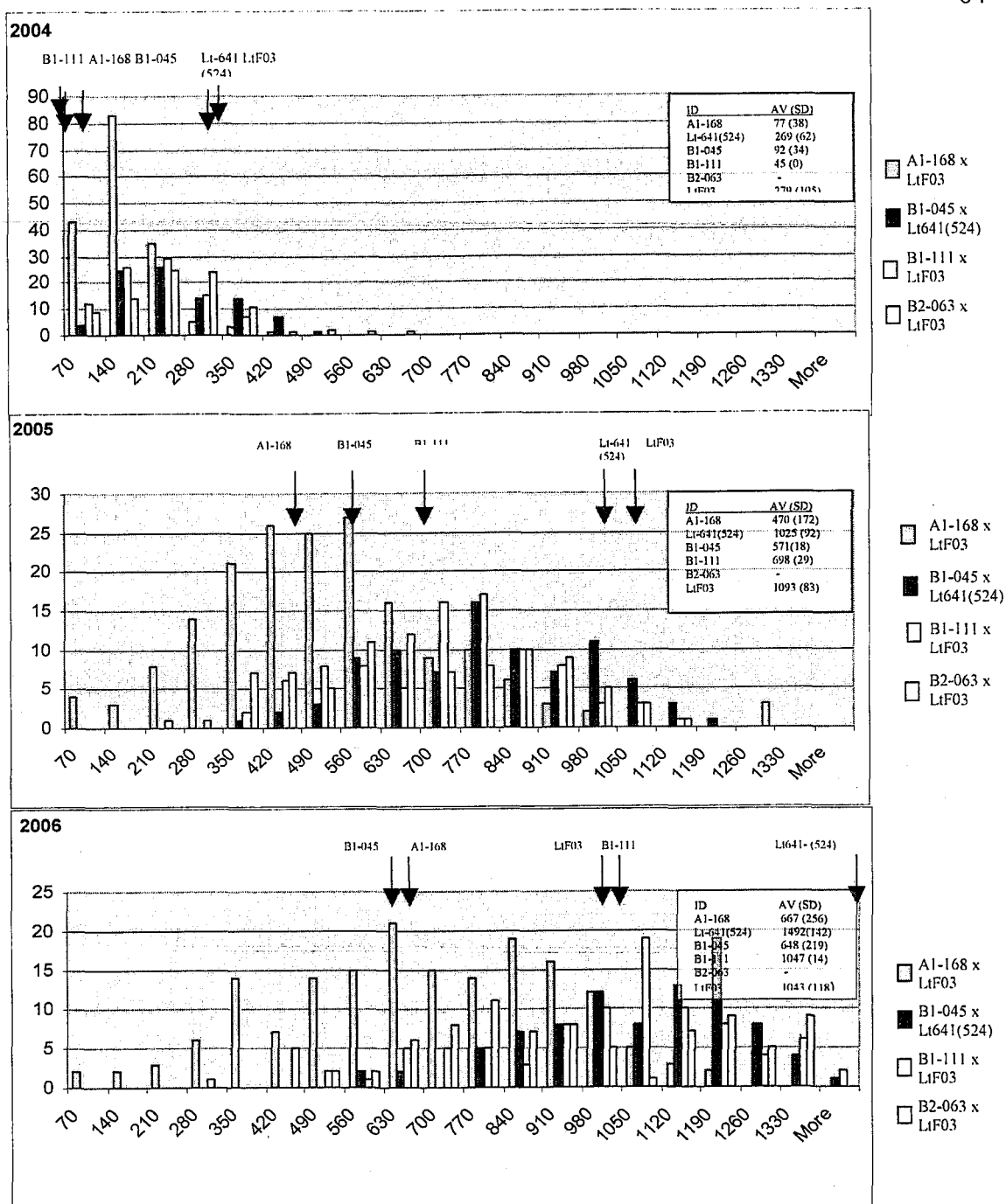


Fig. 3-3: Histograms for circumference (cm) of plant spreading in BC2 families, A1-168 x LtF03, B1-045 x Lt641 (524), B1-111 x LtF03, and B2-063 x LtF03 based on means of two clones, compared to the parental genotypes, A1-168, B1-111, B1-045, Lt-641 (524), and LtF03.

CHAPTER 4

GENE EXPRESSION POLYMORPHISMS AND ESTs ASSOCIATED WITH GRAVITROPIC RESPONSE OF SUBTERRANEAN BRANCH MERISTEMS AND GROWTH HABIT IN *LEYMUS* WILDRYES

ABSTRACT

Negatively orthogeotropic (NOGT) tiller and diageotropic (DGT) rhizome meristems develop from the same type of lateral axillary meristems and phytomer structure. Although subterranean NOGT and DGT buds appear similar, they display different responses to gravity and perhaps other cues governing branch angle and overall growth habit (GH). *Leymus* wildryes show remarkable variation in GH and include some of the largest native grasses in western North America. Previous studies detected GH QTLs on homoeologous regions of LG3a and LG3b controlling differences between caespitose *L. cinereus* and rhizomatous *L. triticoides* allotetraploids. Heterologous barley and wheat microarrays in conjunction with bulk segregate analysis were used to find gene expression polymorphisms associated with GH QTLs. Approximately 34% and 25% of the probe sets showed detectable signals on the barley and wheat arrays, respectively. Overall gene expression patterns of NOGT and DGT meristems were remarkably similar, consistent with the assertion that *Leymus* NOGT and DGT buds develop from homologous meristems. Only 28 and 27 genes on barley and wheat gene chips, respectively, showed more than two-fold differential expressions between NOGT and DGT tissues. One expression polymorphism genetically mapped in the *Leymus* LG3 rhizome QTL region.

INTRODUCTION

Growth habit (GH) is a functionally important, adaptive trait in perennial grasses. In general, grasses with caespitose and rhizomatous GH are adapted to xeric and mesic environments, respectively (Sims, Singh & Lauenroth 1978). Caespitose grasses form a compact tussock of upright aerial tiller stem branches, whereas sod-forming grasses typically spread via prostrate stolons or underground rhizome stem branches. The rhizomatous GH is also related to competitive ability and invasiveness of perennial grasses (Weaver 1963). Rhizomes provide protection from herbivory and trampling, storage tissues for vegetative propagation, and dispersal, which is problematic in perennial grass weeds such as quackgrass (*Elymus repens*) (Holm *et al.* 1977).

Despite differences in appearance, the aerial tiller and subterranean rhizome branches are homologous in that they develop from the same type of axillary meristem and eventually form the same basic phytomer organization and structure (Hyder 1974). Initially, the only obvious difference between the subterranean axillary meristems of caespitose and rhizomatous grass buds involves the direction of branch growth. The axillary meristems of caespitose grasses grow upwards (negative orthogeotropism), emerging within the leaf sheath, whereas the axillary meristems of rhizomatous grasses cut through the leaf sheath and grow outwards perpendicular to gravity (diageotropism). However, intermediate branch angles (plagiotropism) are also common. Rhizomes and tillers differentiate, at least temporarily, in that rhizomes may develop reduced scale-like leaves, roots at the nodes, and a sharp apical cap with lignified epidermal cell walls (Fahn 1982), whereas aerial tillers obviously display fully developed leaves and photosynthetic

capacity with exposure to light. Nevertheless, most rhizomes eventually emerge as fully functional aerial stems and retain the ability to flower.

Although plants are essentially sessile in nature, they show different types of movements in response to their environment. For example, the direction of growth in subterranean axillary meristems is primarily governed by gravity, but can be affected by light and perhaps other environmental cues (Hangarter 1997). It has been established that plants perceive gravity by specialized statocyte cells, particularly in the apical meristems of roots and shoots, which contain a special class of amyloplasts known as statoliths (Poff & Martin 1989). Stems generally show negative gravitropism mediated in part by increased cell expansion of the lower side of a horizontally placed stem in response to auxin, whereas roots generally show positive gravitropism and decreased cell expansion of the lower side of a horizontally placed root in response to auxin. Genetic studies suggest that different genes are involved in positive and negative gravitropism. For example, at least eight different loci have been shown to be involved in root gravitropism and six independent loci named SGR1 to SGR6 are related to shoot gravitropism in *Arabidopsis thaliana* (Fukaki, Fujisawa & Tasaka. 1996; Yamauchi *et al.* 1997; Ranjeva, Graziana & Mazars 1999).

The genus *Leymus* includes about 30 long-lived perennial grass species with remarkable variation in GH, stature, and adaptation to harsh cold, dry, and saline environments of Europe, Asia, and the Americas. Basin wildrye (*Leymus cinereus*) and several other large-statured *Leymus* species, including Altai wildrye (*L. angustus*) and mammoth wildrye (*L. racemosus*), have high biomass accumulation potential across a wide range of high-elevation or high-latitude growing environments of western North

America (Jefferson *et al.* 2002; Jensen *et al.* 2002; Lauriault, Kirsey & VanLeeuwen 2005), ideal for stockpiling forage or bioenergy feedstocks. *Leymus cinereus* is one of the largest native grasses (some ecotypes exceed 2 m tall) and the most abundant *Leymus* species in the Great Basin, Rocky Mountain, and Intermountain regions of western North America. However, caespitose *L. cinereus* is susceptible to damage by intense grazing of early season and fall regrowth. Once abundant on the floodplains of major rivers, alluvial gullies, and other watered areas with deep, well-drained soils in the Great Basin and Intermountain regions, *L. cinereus* has been eliminated from much of its former range due to grazing, harvesting, and cultivation of field crops. Cultivars of *L. cinereus* are commonly used in rangeland seed mixtures in western North America, but have limited use in pastures or hay crops. The second most common *Leymus* species in western North America is creeping wildrye (*L. triticoides*). *Leymus triticoides* is a shorter (0.3-0.7 m), but highly rhizomatous grass, specifically adapted to poorly drained alkaline sites in the Great Basin, California, and other regions of western North America. Creeping wildrye is cultivated using vegetative propagules as a saline biomass crop in California, but poor seed production limits widespread use of this species.

Two experimental TTC (*triticoides* X (*triticoides* X *cinereus*)) mapping families, TTC1 and TTC2, derived from interspecific hybrids of caespitose *L. cinereus* and rhizomatous *L. triticoides* have been developed for plant improvement and genetic investigations of functionally important traits in perennial forage grasses (Wu *et al.* 2003a; Hu *et al.* 2005; Larson *et al.* 2006). These interspecific hybrids are very robust plants exhibiting heterosis through increased plant height, large stems and leaves, prolific seed production, and improved seed germination from *L. cinereus* with vigorous

proliferation of tillers, rhizome production, regrowth potential, and plant resiliency from *L. triticoides*. In terms of applied breeding, breeding populations derived from interspecific hybrids of *L. cinereus* and *L. triticoides* show excellent potential for high biomass production, reduced susceptibility to grazing or harvest, and improved regrowth potential. The linkage maps include 67 cross-species anchor markers (i.e. markers mapped in other grass species) used to identify and compare the 14 linkage groups of allotetraploid *Leymus* ($2n=4x=28$) based on synteny of corresponding markers in closely related wheat (*Triticum* spp.), barley (*Hordeum vulgare*), and cereal rye (*Secale cereale*) Triticeae cereals (Wu *et al.* 2003a; Larson *et al.* 2006). Moreover, genome-specific markers have been used to distinguish several homoeologous linkage groups corresponding to the **Ns** and **Xm** genomes of *Leymus* (Wu *et al.* 2003a). The **Ns** genome originates from *Psathyrostachys* (Dewey 1984; Zhang & Dvořák 1991), whereas the **Xm** genome is considered unknown (Wang *et al.* 1994). Löve (1984) speculated that the other (**Xm**) genome originates from *Thinopyrum bessarabicum*, but chromosome pairing in *Thinopyrum* x *Leymus* hybrids has been ambiguous (Sun *et al.* 1995) or very low (Wang & Jensen 1994; Zhang & Dvořák 1991).

Significant GH QTLs were detected on LG3a, LG3b, and LG6a in the *Leymus* TTC1 family (Larson *et al.* 2006). Likewise, significant GH QTLs were detected on LG3a, LG3b, and LG5Xm in the *Leymus* TTC2 family. The LG3a and LG3b QTLs were evidently conserved between TTC1 and TTC2 families. Moreover, the LG3a and LG3b QTLs were located on homoeologous regions of the allotetraploid *Leymus* subgenomes (Larson *et al.* 2006). Although the TTC1 LG6a and TTC2 LG5Xm showed relatively large log likelihood ratio (LOD test statistics) effects in the third or last evaluation year,

the most consistent QTL effects over all three evaluation years were located on LG3a in both TTC1 and TTC2 families. Chromosome 3 is highly conserved within the Triticeae (Devos & Gale 2000), which includes LG3a and LG3b of *Leymus* (Wu *et al.* 2003a; Larson *et al.* 2006). Collinear from end to end with rice chromosome 1, Triticeae group 3 is also the most conserved of all chromosome groups when compared to rice (La Rota & Sorrells 2004). Rhizome and tiller angle QTLs also map to rice chromosome 1 (Hu *et al.* 2003; Li *et al.* 1999).

Gene expression is the fundamental mechanism that controls the developmental processes in a plant (Ji *et al.* 2004). Recent developments in techniques for large-scale analysis of gene expression, such as cDNA and oligonucleotide microarrays or gene chips, facilitate the search for genes associated with developmental variation (Aharoni *et al.* 2000; Girke *et al.*, 2000; Zhu *et al.* 2001). Microarrays can be used for comprehensive investigation of gene expression, providing important new insights into molecular mechanisms of biological processes (Brown & Botstein 1999; Lockhart & Winzeler 2000). Moreover, differences in mRNA levels of individual genes between genotypes are potentially used as an expression QTL (eQTL) to identify genotypic variation (Kirst *et al.* 2005; Xue *et al.* 2006). Although microarrays are a powerful method of transcriptome analysis, this technique is restricted to few model species, mainly due to lack of extensive sequence information of every species of interest. Manufacturing high-density microarrays is time-consuming and expensive, involving sequencing transcripts on a large scale from various tissues (Lipshutz *et al.* 1999). Ji *et al.* (2004) hypothesized that the sequence conservation between closely related species could be used for cross-species hybridizations, without having to design and construct microarrays for the many possible

species of interest. Based on the unique feature of the Affymetrix GeneChips®, where multiple probes represent each gene, it was hypothesized that sequence conservation within genera or families may be high enough to generate sufficient signals from some of the probes for expression analysis (Ji *et al.* 2004). This approach is still at the early stage of research development for the identification of genes with relatively small differences in expression levels between closely related genotypes from a segregating population. Recently, a number of studies of this kind have been reported in plants (Potokina *et al.* 2004; Lall *et al.* 2004; Kirst *et al.* 2004; Hazen *et al.* 2005).

Based on the assumption that NOGT and DGT subterranean meristems arise from homologous axillary buds, we speculate that overall gene expression profiles are predominantly similar between DGT and NOGT meristems during similar stages of early subterranean development. However, a relatively small number of genes may show mRNA expression polymorphisms specifically associated with branch angle differences in these same NOGT and DGT bud meristems. Thus, the objectives of this study were to compare overall gene expression profiles of *Leymus* DGT and NOGT meristems in a similar genetic background, identify gene expression polymorphisms specifically associated with the *Leymus* LG3a rhizome QTL by bulk segregate analysis, and identify other possible genes specifically involved in branch angle differences of otherwise similar DGT and NOGT meristems. At least some of these genes may be fundamentally important determinants of GH variation in grasses.

MATERIALS AND METHODS

Plant materials and RNA samples used for gene expression analysis

Full-sib mapping populations TTC1 and TTC2 were derived from one *L. triticoides* accession 641 plant (T-tester) pollinated by two different *L. triticoides* accession 641 $\times$ *L. cinereus* accession 636 F1 hybrids (TC1 and TC2). Accession 636 was received from the Agriculture Research Centre, Lethbridge, Alberta, Canada, and presumably originates from a natural population in Alberta or Saskatchewan. Accession 641 was collected from a natural population near Jamieson, OR, USA. The TTC1 and TTC2 families have very similar genetic backgrounds in that both families were derived from the same T-tester maternal parent genotype. Because more than two plants were used to derive the hybrid population from which the TC1 and TC2 genotypes were selected, it is not known for certain if they share any other relationship other than the fact that they were derived from crosses of the same two accessions. The 164-sib TTC1 and 170-sib TTC2 mapping populations, TC1 and TC2 hybrids, and T-tester clones are maintained by the United States Department of Agriculture- Agriculture Research Service, Forage and Range Research Laboratory (Logan, UT, USA).

Diageotropic and negatively orthogeotropic apical meristems, less than 2 cm long, of subterranean branches were harvested from greenhouse source clones of the TTC1 and TTC2 families during the spring of 2005 and 2006, weighed, and quickly placed in liquid nitrogen. NOGT branch meristem samples were selected from a total of six TTC1, and six TTC2 segregant progenies that carry the caespitose *L. cinereus* LG3a GH QTL alleles (Table 4-1) using genotypic data described by Wu *et al.* (2003a) and Larson *et al.* (2006).

Likewise, DGT meristems were selected from six TTC1 and six TTC2 clones, which carry the rhizomatous *L. triticooides* LG3a GH QTL allele (Table 4-1). Genotypic selection criteria required that progeny did not display any recombination events in the entire LG3a GH QTL region, based on graphical genotypes displayed using Graphical GenoTyping software (Berloo, 1999). Although many other TTC1 and TTC2 progeny met the genotypic selection criteria, the clones used in this experiment (Table 4-1) were the only clones that actually displayed DGT and NOGT subterranean branch meristems suitable for the intended experiments. Moreover, some different TTC1 and TTC2 progeny genotypes were used in 2005 and 2006 because of variance in the initiation and phenotype of subterranean branch meristems.

Subterranean branch meristems from different TTC1 and TTC2 clones were bulked (Table 4-1) so that approximately 100 mg of tissue was available for each RNA extraction. Total RNA was isolated using Qiagen RNeasy® plant mini kit (Qiagen, Valencia, CA). RNA quality was assessed by BIO-RAD automated electrophoresis station (Biocompare, Inc., San Francisco, CA). Total RNA was used to generate labeled, fragmented cRNA using One-Cycle Target Labeling and Control Reagents (Affymetrix, Inc., Santa Clara, CA) according to the manufacturer's protocol. Total RNA (5 µg), spiked with appropriately diluted Poly-A controls (1:10,000), was used in the synthesis of first and second-strand cDNA. Following second-strand synthesis, half of the purified cDNA (12 µL) was used in synthesis of biotin-labeled cRNA. Purification of labeling reaction typically yielded 45-55 µg labeled cRNA, as determined by spectrophotometric analysis of A₂₆₀. cRNA (20 µg) was fragmented in 5X fragmentation buffer (40 µL) by incubating at 94°C for 35 min. Quality of labeled cRNA and fragmented cRNA was

determined by gel electrophoresis and analyzed on an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA).

Microarray analysis

Microarray experiments were performed using one Affymetrix GeneChip Wheat Genome Array and one Affymetrix GeneChip® Barley Genome Array for each of the 12 samples (Table 4-1). The Affymetrix GeneChip® Wheat Genome Array contains 61,127 probe sets representing 55,052 transcripts, whereas the Affymetrix GeneChip Barley Genome Array has 22,840 probe sets. The Biotin-labeled, fragmented cRNA was hybridized to GeneChip® Wheat Genome Arrays (Affymetrix, Inc., Santa Clara, CA) for 16 hours according to the manufacturer's protocol. GeneChips were washed and stained with the fluidics script EukGE-WS2v5-450, using the Fluidics Station 450, and scanned using the GeneChip Scanner 3000, housed at the Center for Integrated BioSystems (Utah State University, Logan, UT). The total number of informative probe sets was determined by the number of signals present as determined by the Affymetrix GCOS software. Otherwise, data analysis of all gene expression arrays was conducted using ArrayAssist 3.3 software (Stratagene, La Jolla, CA). Probe-level analysis, normalization and summarization were performed by ArrayAssist's GC-RMA algorithm (Wu, LeBlanc & Irizarry 2003b). GC-RMA method of normalization provides increased accuracy by extending the model-based approach through the use of mismatch probes to generate final signal values. Significance analysis was conducted using *P*-value of 0.001 and two-fold differential expression.

***Leymus* EST library**

The *Leymus* EST library was constructed, normalized, sequenced, filtered, and assembled using the same methods described by Anderson *et al.* (2007) with the following modifications. Tissues were immediately flash-frozen in liquid nitrogen and stored at -80°C and ground to a fine powder in liquid nitrogen.

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) reagent following the manufacturers' protocol. Samples were purified using the RNA cleanup procedure with Qiagen Midi columns (Qiagen, Valencia, CA). The poly (A)⁺mRNA was converted to double-stranded cDNA using different NotI/oligo(dT)-tagged primers for two independent RNA samples as follows: [5'-

AACTGGAAGAATTCGCGGCCGCTCCGA(T)₁₈V-3'] for purple and green aerial regrowth (less than 10 cm above ground), from field evaluations of the *Leymus* TTC1 and TTC2 rhizomes (Larson *et al.* 2006), harvested under freezing cold temperatures about two hours after sunrise, and [5'-AACTGGAAGAATTCGCGGCCGCTCGCA(T)₁₈V-3'] for NOGT, DGT, and plagiotropic subterranean branch meristems of TTC1 and TTC2 plants sampled during March of 2005 (Table 4-1). Double-stranded cDNA samples ≥ 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 (cfu) before amplification was 3x10⁶, whereas the total number of clones with insert was 2x10⁶ 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.

The gene sequences of differentially expressed genes on wheat and barley chips were blasted to the *Leymus* EST database to find the *Leymus* homologs that were aligned to rice sequences using the Gramene database to identify the location of *Leymus* ESTs on the rice genome.

Validation of array results with qRT-PCR and *Leymus* ESTs

A subset of the differentially expressing genes selected based on their relatively high level of differential expression between the two groups in the gene-chip experiment was verified using quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). The nucleotide sequences for the differentially expressing genes on wheat and barley chips were extracted from wheat and barley consensus sequence files (www.Affymetrix.com). These sequences were blasted against the *Leymus* EST database (http://titan.biotec.uiuc.edu/cgi-bin/ESTWebsite/estima_start?seqSet=Leymus). The *Leymus* EST database sequences were used to design PCR primers specific to each EST using Primer3 Input (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

For qRT-PCR, the new tissue samples were recollected using the same method as for the microarrays, with the same plants used for microarray experiments in 2006. qRT-PCR was carried out in a two-step reaction in real time. Total RNA (2.0 µg) was reverse-transcribed from an oligo-dT primer using a First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD) in a 20 µL reaction at 37°C for 60 min followed by 70°C for 10 min. Reactions were diluted to 100 µL with 0.1 X TE, and stored at -20°C. First-strand cDNA (2 µL) was used as a template for PCR amplification of specific transcripts using

GoTaq DNA Polymerase (Promega, Madison, WI) in a 25- μ L reaction (1X reaction buffer, 25 mM $MgCl_2$, 0.2 μ M gene specific primers, 0.25 mM dNTP, 1.25 U Taq polymerase, 0.2X SYBR Green 1 (Molecular Probes, Inc., Eugene, OR)). Thermal cycling was carried out in DNA Engine Opticon2 System (MJ Research, Waltham, MA) for 36 cycles of 95°C for 15 sec, 55°C for 15 sec, 72°C for 75 sec. Opticon Monitor 2 version 2.02.24 software (MJ Research, Waltham, MA) was used to analyze the data. All measured samples were amplified in three replicates, and standard curves for each primer pair were established to quantify the samples. Standard curves had slopes of $y = -0.28$ to -0.44 and $r^2 = 0.829$ to 0.995 . The normalized values for each transcript were determined by dividing their average copy value by the average tubulin value. The specificity of PCR amplification was confirmed by the following criteria: (1) a single peak in the melting-curve analysis of real time PCR-amplified products; and (2) a single band as determined by agarose gel electrophoresis. The fold changes of expression values between NOGT and DGT subterranean meristems were compared for array and qRT-PCR experiments. For this, the average copy number value over six corresponding chips for NOGT and DGT tissues and the average copy number value over three bulks each of NOGT and DGT tissues for qRT-PCR were calculated and normalized by dividing the average values by the corresponding average tubulin expression (Table 4-4). Tubulin expression values from wheat (Ta.812.1.S1_x_at) and barley (Contig902_x_at) arrays were used to normalize array expression values. The tubulin PCR primers (5'ATCTCGATCCACATCGGCCA3' and 5'ATCACAGTGGGCTCAAGATC3') were designed from the *Leymus* EG385442 EST.

RESULTS

Analysis of GeneChip expression data

An average of 7,813 (34%) and 15,132 (25%) positive signals were detected by hybridization of the 12 *Leymus* subterranean branch meristem cRNA samples (Table 4-1) to the barley and wheat arrays, respectively. Thus, a total of 22,945 wheat or barley probe sets were interrogated. However, at least some of the wheat and barley probe sets were designed from homologous genes. Thus, the actual number of genes interrogated is greater than 15,132 but less than 22,945.

Twenty-seven wheat array probe sets detected significant ($P < 0.01$) differences in mRNA levels between the NOGT and DGT subterranean branch meristem samples, including 18 probe sets detecting greater expression in DGT samples and nine probe sets detecting greater expression in the NOGT samples (Table 4-2). Twenty-eight barley array probe sets detected significant ($P < 0.01$) differences in mRNA levels between the NOGT and DGT subterranean branch meristem samples, including eight probe sets detecting greater expression in DGT samples and 21 probe sets detecting greater expression in the NOGT samples (Table 4-3). Several genes were identified from both wheat and barley arrays including ATP synthase-like proteins (barley contig835_s_at and wheat Ta.2412.1.S1_at), putative glycine dehydrogenase (barley contig1483_at and wheat Ta.30795.1.S1_at), as well as 40S and 60S ribosomal subunits (Tables 4-2 and 4-3). Major genes identified as differentially expressing were those related to putative-auxin induced proteins, calcium-binding, calmodulin and calmodulin-like proteins, H^+ exchange proteins, chloroplast precursors and light harvesting proteins, MAP kinases and centromere/microtubule-binding proteins, and genes regulating glycine biosynthesis.

Other genes were related to nucleolar proteins such as fibrillarin, Nop56, and NopA64, and heat-shock proteins, in addition to some unknown genes or transcribed loci.

Identifying *Leymus* ESTs homologous to gene-expression polymorphisms

The GH QTLs in *Leymus* have been assigned to chromosome 3 (Larson *et al.* 2006), which is syntenic with rice chromosome 1. *Leymus* sequences, EG392086, EG390562, and EG383468 correspond to rice chromosome 1L, while EG375403 corresponds to rice chromosome 7L (Table 4-4). Three of these *Leymus* sequences were mapped on pre-existing linkage maps of *Leymus* (Wu *et al.* 2003a; Larson *et al.* 2006). Primers designed from EG392086 (5'GTGAATCGCAAGATCAGCAA3' and 5'AGCGTTAACGTTCTCGTCGT3') produced polymorphic PCR amplicons that mapped to LG5Ns. Primers designed from EG390562 (Table 4-4) produced PCR amplicons that contained an *AluI* restriction site polymorphism that mapped to LG3b. Primers designed from EG375403 (Table 4-4) produced polymorphic PCR amplicons that mapped to LG2a. EG383468 was not polymorphic and could not be mapped.

qRT-PCR analysis of differentially expressed genes

Eight differentially expressed genes showing high-fold differences between NOGT and DGT branch meristems were analyzed by qRT-PCR. Of the genes selected for validation by qRT-PCR, four genes showed higher expression in NOGT subterranean branch meristems and four genes showed higher expression in DGT meristems (Table 4-4). Probeset Ta.22762.2.S1_at (wheat array) had the smallest normalized NOGT/DGT

ratio of 6.67^{-1} and qRT-PCR resulted in a ratio of 11.11^{-1} . The largest normalized NOGT/DGT ratio was Contig3457_at (barley array) at 7.14 with a qRT-PCR ratio of 3.66. Quantification of transcript levels of all eight genes assayed by qRT-PCR resulted in ratios that supported the differential expression shown by the array data, although four of the probe sets (Ta.22762.2.S1_at, Ta.28292.1.S1_at, Contig2369_s_at, Contig3457_at) had about two-fold differences in array and qRT-PCR-derived expression ratios.

DISCUSSION

In this project, the usefulness of heterologous wheat and barley GeneChips to study gene expression of GH traits such as NOGT and DGT subterranean meristems in response to environmental factors such as gravity in grasses in the tribe Triticeae was demonstrated. Between 25 and 34% of probe sets on wheat and barley GeneChips were classified as “present” by Affymetrix GCOS software when hybridized with labeled *Leymus* cRNA. Given that hybridizing barley cRNA to barley chips resulted in 49% present calls (Mott, unpublished data), and hybridizing wheat cRNA to wheat chips resulted in 48% present calls (Mott and Wang, 2007), the results obtained using *Leymus* cRNA on these heterologous arrays seem reasonable. The results also suggest that *Leymus* genome has higher similarity to the barley genome than to the wheat genome. Somewhere between 15,132 and 22,945 probe sets were interrogated by the *Leymus* cRNA between the two arrays. Of those, only 55 (~0.29%) of the probe sets had greater than a two-fold difference in expression between NOGT and DGT meristems, demonstrating the remarkable similarity of the two structures.

Among the identified differentially expressing genes were those whose gene products have been previously implicated in plant gravitropism, such as putative auxin-induced proteins, Ta.12341.1.S1_at (Friml *et al.* 2002; Joo, Bae & Lee 2001), calcium-binding, calmodulin, and calmodulin-like proteins represented by Ta.27751.3.S1_at (Feldman & Gildow 1984; Björkman & Leopold, 1987; Lu & Feldman, 1997), MAP kinases, Ta.1130.1.S1_a_at, Ta.1130.2.S1_x_at, Ta.1130.3.S1_x_at (Clare *et al.* 2003; Hoshi *et al.* 1992; Baluska *et al.* 1996; Fisher & Shopfer 1998), and H⁺ exchange proteins, Contig835_s_at, Contig442_at (Wiesenseel & Meyer 1997; Scott & Allen 1999).

One differentially expressed gene, wheat Ta12341.1.S1_at, was related to a putative auxin-regulated protein and showed higher expression levels in DGT subterranean meristems with an NOGT/DGT expression ratio of 2.63⁻¹ (Table 4-2). Since auxin has been implicated in the inhibition of lateral bud growth (Chatfield *et al.* 2000), it is possible that auxin and some auxin-regulated proteins, such as Ta12341.1.S1_at, are involved in suppressing formation of NOGT or maintaining DGT. Since the asymmetric distribution of auxin at the elongation zone is considered to be the causative factor for asymmetric growth of curvature induced by gravity (McClure & Guilfoyle 1989; Parker and Briggs 1990; Friml *et al.* 2002), our results suggest that auxin may play an important role in determining what type of subterranean meristems develop, either NOGT or DGT. SAR (Suppressor of Auxin Resistance) DNA-binding protein and GBP16 (Gibberellin-Binding Proteins) have not been shown to be directly involved in plant gravitropism. However, it has been shown that auxin controls the growth of *Arabidopsis* roots through the modulation of the cellular response to phytohormone gibberellin (GA), which has

long been known to regulate shoot growth (Fu & Harberd 2003). Also, it was proposed that SAR1 is a regulator of cell growth and AXR1 (Auxin Resistant) acts to modify the activity of SAR1. AXR1 is likely to have a role in protein degradation and may promote the degradation of SAR1 in an auxin-dependent manner (Cernac *et al.* 1997). Both SAR-binding proteins and GBP16 are related to auxin action and their expression was found to be higher in NOGT meristems as opposed to auxin-induced genes, which were more expressed in DGT meristems. These may be the novel genes involved in apical bud differentiation to DGT or NOGT meristems. It may also suggest that a balanced action of auxin and GA is involved in determining NOGT growth.

Cdprk1, a gene coding for a calcium-dependent protein kinase (Ta.27751.3.S1_at), was expressed more in DGT meristems with an NOGT/DGT expression ratio of 4.76^{-1} . Ca^{2+} /calmodulin dependent protein kinases have been thought to be involved in light-dependent orthogravitropic response of roots in some cultivars of corn (Lu & Feldman 1997). High amounts of calmodulin associated with amyloplasts in statocytes (Allan & Trewavas 1985; Dauwalder, Roux & Hardison 1986) and expression of calmodulin-like genes usually known as touch genes are induced by mechanical stimulation (Jena, Reddy & Poovaiah 1989; Galaud, Lareyre & Boyer 1993). Lateral growth of DGT meristems and the mechanical hindrance of soil while growing through the ground may be responsible for the higher expression levels of *Cdprk1* in the DGT meristems. Ca^{2+} is also important for lateral distribution of auxin in maize roots (Young & Evans 1994). It was also hypothesized that the kinase is associated with the establishment of auxin gradients in corn roots via activation of proton pumps causing auxin redistribution (Young & Evans 1994).

Higher expression of MAP kinases (Ta.1130.1.S1_a_at, Ta.1130.2.S1_x_at, Ta.1130.3.S1_x_at) in the DGT tissues in the array experiments can be related to the findings of Clore *et al.* (2003), who stated the possibility that changes in MAPK activity in the gravistimulated maize pulvinus are a part of a signal cascade that may distinguish between minor perturbations in plant orientation and more significant and long-term changes, and may also help to determine the direction of bending. MAP kinases have also been shown to phosphorylate microtubule-associated proteins, thereby altering their ability to stabilize microtubules (Hoshi *et al.* 1992). Ta.9549.1.S1_x_at and Ta.9549.3.S1_x_at are related to putative centromere/microtubule-binding proteins and showed higher expression in NOGT meristems. The organization of microtubules is gravity-sensitive (Nick *et al.* 1990). Findings by Baluska *et al.* (1996) and Fisher & Shopfer (1998) established that the cortical microtubule might act as a strain gauge for amplification and stabilization of environmentally induced changes in direction of growth elongation.

Genes on both wheat and barley chips were found to be the associated with chloroplast precursors and light-harvesting proteins. This family of genes including lipoxygenases and esterase/lipase/thioesterase may be involved in plant gravitropism. The starch statolith hypothesis indicates that the starch-filled chloroplasts are gravisensors in shoots of plants (Björkman, 1988; Song *et al.* 1988; Kiss, Hertel & Sack 1989; Sack 1997). Some of these genes from this category were expressed more in NOGT meristems whereas others were expressed more in DGT meristems. Both gravity and light interact with each other to enhance or reduce the other's effectiveness (Correll & Kiss 2002) and they have common elements in their pathways (Watahiki *et al.* 1999). Thus, the

differences in the GH or response towards gravity of NOGT and DGT meristems may be due to the presence or absence of light during their growth because NOGT meristems are aerial and receive light whereas DGT meristems grow subterraneanly and show both positive and negative gravitropism. The position of meristems at the time of tissue collection might affect the gene expression in these meristems. The presence of the differential expression related to chlorophyll binding and light-harvesting proteins is also supported by the work of Robson & Smith (1996) and Poovaiah & Reddy (1993). They established that physical stimuli, such as phytochromes (photoreceptors), also interfere with some gravitropic responses and may play a crucial role in the expression of gravisensitivity.

Sobol *et al.* (2006) discovered the decrease in quantity of nucleolar proteins fibrillarin and NopA64 in roots of *Lepidium sativum* under altered/reduced gravity conditions. We found transcripts for these two pre-rRNA processing proteins, Nop56 (Ta.9201.3.S1_x_at) and fibrillarin (Contig3457_at, Contig3457_x_at), were highly expressed in NOGT meristems, suggesting the involvement of the nucleolous in the gravitropic signaling.

A putative glycine dehydrogenase gene (contig1483_at, Ta.30795.1.S1_at) had higher expression values in DGT branch meristems. A search for the conserved domains using the NCBI blast (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) revealed that Ta.28357.1.A1_s_at, which was also expressed higher in DGT branch meristems, was related to glycine cleavage system aminomethyltransferases and the glycine cleavage T-protein C-terminal barrel domain. Kamada, Higashitani & Ishioka (2005) found that expression levels of genes related to the cytoskeleton, such as glycine

hydroxymethyltransferase, changed in response to gravitational signals. Finding three different genes involved in glycine biosynthesis with higher expression in DGT branch meristems suggests that glycine may play a role in gravitropism.

The role of heat-shock proteins HSC70-11, Hsp60p (Contig6096_at, Ta.6964.1.S1_at, respectively) in gravitropism has not been elucidated yet, but HSC70-11 was found to be differentially expressing during the transcription profiling of the early gravitropic response in *Arabidopsis* (Moseyko *et al.* 2002).

Genes functioning as transcription factors may indirectly affect the GH QTLs. This might explain why only one out of three expression polymorphisms (EG390562) that aligned to rice chromosome 1 (Table 4-4) mapped on LG3b of *Leymus*. However, we cannot exclude the possibility that EG390562 is also on LG3a, as both LG3a and LG3b show the presence of the GH QTL and are suggested to be homoeologous due to the presence of VP1 loci occurring near the QTL region on both groups (Larson *et al.* 2006). Also, the progeny-selection criterion for the gene-expression experiments was no recombination events in the LG3a GH QTL region, which may explain why EG390562 did not map on LG3a. Additionally, wheat linkage group 5 is the least conserved among all the chromosomes, with genes scattered across all 12 rice chromosomes; however, it shows some regions of homology to rice chromosomes 3, 9, and 12 (Sorrells *et al.* 2003). This may explain the expression polymorphism aligning to rice 1L on *Leymus* 5Ns. Rice chromosome 9 also contains a major QTL (*Ta*) for tiller angle (Li *et al.* 1999). Alignment of a gene expression polymorphism on rice chromosome 7L and its mapping on *Leymus* LG2a can be supported by the findings of Sorrells *et al.* (2003) that rice chromosome 7 shows 62% homology with short arm of wheat chromosome 2.

Many of the differentially expressing genes we found in this study were involved directly or indirectly in gravitropism pathways. This supports the interpretation that differences in GH of NOGT and DGT meristems can be due to their responses towards earth's gravitational force, achieved probably through the auxin-signaling pathway. Continued efforts to assess differential expression in these meristems and fine genetic mapping and positional cloning should advance the discovery of genes related to GH.

SUPPLEMENTARY MATERIALS

Array data files, including text files of normalized expression data for each probe set on both wheat and barley genome arrays, have been submitted to ArrayExpress at the European Bioinformatics Institute (www.ebi.ac.uk) and assigned accession number E-MEXP-1265.

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Table 4-1. Description of *Leymus* clones used for comparison of gene expression in diageotropic (DGT) and negatively orthogeotropic (NOGT) subterranean branch meristems.

<i>GeneChip ID</i>	<i>Year</i>	<i>LG3a GH-QTL genotype</i>	<i>Branch meristem phenotype¹</i>	<i>Leymus clones²</i>
SL01	2005	T / C	NOGT	A092, A171*, B2062
SL02	2005	T / C	NOGT	A141*, B1056*
SL03	2005	T / C	NOGT	A133*, B1083*, B1062
SL04	2005	T / T	DGT	A053, A086*, B1078
SL05	2005	T / T	DGT	A106*, A183, B1085*
SL06	2005	T / T	DGT	A072*, B2014*, B2037
SL07-PK7	2006	T / C	NOGT	A133*, B1083*
SL08-PK8	2006	T / C	NOGT	A187, B1108, B2009
SL09-PK9	2006	T / C	NOGT	A141*, A122, A171*, B1056*
SL10-PK10	2006	T / T	DGT	A086*, B2014*, B2083
SL11-PK11	2006	T / T	DGT	A106*, A072*, B1085*
SL12-PK12	2006	T / T	DGT	A119, B2044

* Clones used in both 2005 and 2006 experiments.

¹ Selected based on genotype of rhizomatous *L. triticoides* (T) or upright *L. cinereus* (C) LG3a growth habit QTL (GH-QTL) alleles.

² A and B clones are from TTC1 and TTC2 mapping families, respectively.

Table 4-2. Description of Wheat Genome Array probe sets that detected different gene expression levels between negatively orthogeotropic (NOGT) and diageotropic (DGT) subterranean branch meristems of *Leymus (triticoides X (triticoides X cinereus))* progeny selected for upright *L. cinereus* or rhizomatous *L. triticoides* LG3a growth habit QTL alleles (see Table 4-1).

<i>Probe Set ID</i>	<i>Probe set description</i>	<i>Expression level NOGT branch meristem¹</i>	<i>Expression level DGT branch meristem¹</i>	<i>Expression ratio NOGT / DGT</i>	<i>Differential expression P-value</i>
Ta.636.1.S1_s_at	Weakly similar to NP_566086.1 expressed protein [A. thaliana]	31	359	11.1 ⁻¹	0.00061*
Ta.22762.2.S1_at	Strongly similar to XP_477140.1 putative mRNA binding protein precursor [O.sativa (japonica cv-gp.)]	11	106	9.09 ⁻¹	0.00032
Ta.2431.1.S1_x_at*	Strongly similar to XP_462957.1 Putative hydrolase [O.sativa]	22	158	7.14 ⁻¹	0.00031
Ta.20878.1.S1_at	Moderately similar to XP_479480.1 putative ultraviolet-B-repressible protein [O.sativa (japonica cv-gp.)]	11	72	6.67 ⁻¹	0.00026
Ta.27751.3.S1_at	Cdpl1 protein	68	321	4.76 ⁻¹	0.00074
Ta.641.1.S1_at	Weakly similar to XP_507201.1 Predicted P0426E02.15-2 gene product [O.sativa (japonica cv-gp.)]	16	71	4.55 ⁻¹	0.00014
Ta.2412.1.S1_at	Moderately similar to NP_194953.1 ATP synthase family [A. thaliana]	354	1519	4.35 ⁻¹	0.00034
Ta.18087.1.S1_at	Transcribed locus	18	70	3.85 ⁻¹	0.00032
Ta.28292.1.S1_at	Moderately similar to NP_918616.1 OSJNBa0094H06.16 [O.sativa (japonica cv-gp.)]	115	397	3.45 ⁻¹	0.00099
Ta.485.1.A1_at	Strongly similar to XP_464447.1 putative Lipxygenase 2.3, chloroplast precursor [O. sativa (japonica cv-gp.)]	107	353	3.33 ⁻¹	0.00099
TaAffx.33924.1.S1_at	Strongly similar to XP_483046.1 putative CfxY protein [O.sativa (japonica cv-gp.)]	21	70	3.33 ⁻¹	0.00087
Ta.2431.1.S1_at*	Strongly similar to XP_462957.1 Putative hydrolase [O.sativa]	37	118	3.23 ⁻¹	0.00069
Ta.7338.1.A1_at	Moderately similar to NP_919061.1 putative N5'-nucleotidase [O.sativa (japonica cv-gp.)]	79	249	3.13 ⁻¹	0.00006
Ta.3366.1.S1_at	Clone wlm4.pk0022.h2:fis, full insert mRNA sequence	109	325	3.03 ⁻¹	0.00062

Table 4-2 (continued)

<i>Probe Set ID</i>	<i>Probe set description</i>	<i>Expression level NOGT branch meristem¹</i>	<i>Expression level DGT branch meristem¹</i>	<i>Expression ratio NOGT / DGT</i>	<i>Differential expression P-value</i>
Ta.12341.1.S1_at	Moderately similar to XP_483757.1 putative auxin-regulated protein [O. sativa (japonica cv-gp.)]	240	632	2.63 ⁻¹	0.00078
Ta.28357.1.A1_s_at	Transcribed locus	633	1602	2.50 ⁻¹	0.00014
Ta.30795.1.S1_at	Strongly similar to NP_916596.1 putative glycine dehydrogenase [O.sativa (japonica cv-gp.)]	267	601	2.27 ⁻¹	0.00096
Ta.3581.1.S1_x_at	Strongly similar to NP_909865.1 photosystem-1 F subunit precursor [O.sativa (japonica cv-gp.)]	90	190	2.13 ⁻¹	0.00087
Ta.27377.1.S1_x_at	Ubiquitin/ribosomal fusion protein	8287	3880	2.14	0.00078
Ta.9201.3.S1_x_at	Moderately similar to NP_176007.1 nucleolar protein Nop56, putative [A. thaliana]	67	29	2.32	0.00882
Ta.8431.1.S1_at	Strongly similar to XP_469398.1 silencing gp. B protein [O.sativa (japonica cv-gp.)]	361	144	2.51	0.00006
Ta.28703.2.S1_x_at*	Strongly similar to XP_472792.1 OSJNBb0048E02.13 [O.sativa (japonica cv-gp.)]	1239	479	2.58	0.00083
Ta.28703.2.S1_at*	Strongly similar to XP_472792.1 OSJNBb0048E02.13 [O.sativa (japonica cv-gp.)]	1163	448	2.60	0.00067
Ta.6315.1.S1_a_at	Strongly similar to XP_469984.1 putative RNA helicase [O.sativa (japonica cv-gp.)]	306	98	3.12	0.00086
Ta.912.1.S1_at	Moderately similar to NP_192960.1 esterase/lipase/thioesterase family protein [A. thaliana]	9653	3091	3.12	0.00068
Ta.9549.3.S1_x_at*	Strongly similar to XP_479248.1 putative centromere/microtubule binding protein [O.sativa (japonica cv-gp.)]	373	95	3.92	0.00085
Ta.9549.1.S1_x_at*	Strongly similar to XP_479248.1 putative centromere/microtubule binding protein [O.sativa (japonica cv-gp.)]	908	160	5.69	0.00011

*Significant differences detected by different probe sets designed from same sequence.

¹ Expression levels shown are the average from six GeneChips.

Table 4-3. Description of Barley Genome Array probe sets that detected different gene expression levels between negatively orthogeotropic (NOGT) and diageotropic (DGT) subterranean branch meristems of *Leymus* (*triticoide*s X (*triticoide*s X *cinereus*)) progeny selected for upright *L. cinereus* or rhizomatous *L. triticoide*s LG3a growth habit QTL alleles (see Table 4-1).

<i>Probe Set ID</i>	<i>Probe set description</i>	<i>Expression level NOGT branch meristem¹</i>	<i>Expression level DGT branch meristem¹</i>	<i>Expression ratio NOGT / DGT</i>	<i>P-value</i>
Contig1523_at	Chlorophyll a/b-binding protein CP24 precursor [<i>Vigna radiata</i>]	8	57	7.14 ⁻¹	0.00074*
Contig835_s_at	H ⁺ -transporting ATP synthase-like protein [i]	18	90	5.00 ⁻¹	0.00053
Contig2083_s_at	Photosystem I reaction centre subunit III, chloroplast precursor	21	103	4.76 ⁻¹	0.00044
Contig2985_s_at	(AY039003) Mg-chelatase subunit XANTHA-F [<i>H. vulgare</i>] [<i>H. vulgare</i> subsp. <i>vulgare</i>]	22	69	3.13 ⁻¹	0.00054
Contig25506_at	None	46	138	3.03 ⁻¹	0.00065
HO04H12S_s_at	None	57	171	3.03 ⁻¹	0.00075
Contig6148_at	50S ribosomal protein L24, chloroplast precursor; protein id: At5g54600.1, supported by cDNA: 27973[<i>A. thaliana</i>]	55	160	2.86 ⁻¹	0.00045
Contig1483_at	Glycine dehydrogenase (decarboxylating) (EC 1.4.4.2) [imported] - <i>H. sp.</i> x <i>Triticum sp.</i>	1896	3959	2.08 ⁻¹	0.00034
Contig3268_at	Beta 5 subunit of 20S proteasome [<i>O. sativa</i> (japonica cv.-gp.)]	581	285	2.04	0.00031
Contig1810_at	40S ribosomal protein S9-like; protein id: At5g39850.1, supported by cDNA: 41408. [<i>A. thaliana</i>]	1801	858	2.10	0.00017
HVSMEg0010B23r2_at	7e-32 putative ribosomal protein; protein id: At3g23390.1, supported by cDNA: 13557, gi_17065557	277	132	2.11	0.00009
Contig1343_at	Putative ribosomal protein L7; protein id: At2g01250.1, supported by cDNA: 13298, gi_13877566	2619	1171	2.24	0.00083
Contig1139_s_at	Putative 60S ribosomal protein [<i>Sorghum bicolor</i>]	1102	490	2.25	0.00076
Contig1819_s_at	Unnamed protein product [<i>O. sativa</i> (japonica cv.-gp.)]	781	344	2.27	0.00069
Contig3176_at	Probable DNA-binding protein GBP16 - rice gb AAB80919.1 [<i>O. sativa</i>]	550	237	2.32	0.00057
Contig2290_s_at	60S ribosomal protein L31 gb AAF42953.1 AF237624_1	1524	651	2.34	0.00039

Table 4-3 (continued)

<i>Probe Set ID</i>	<i>Probe set description</i>	<i>Expression level NOGT branch meristem¹</i>	<i>Expression level DGT branch meristem¹</i>	<i>Expression ratio NOGT / DGT</i>	<i>P-value</i>
Contig1962_at	80S ribosomal protein L31 [<i>Perilla frutescens</i>] Putative 40S ribosomal protein S26 [<i>O. sativa</i> (japonica cultivar-group)]	2251	959	2.35	0.00015
Contig2369_s_at	ESTs AU077873 (S1878), D40121 (S1878), Similar to <i>A. thaliana</i> 60S	645	267	2.42	0.00047
Contig1248_s_at	60S Ribosomal protein L6 (YL16-LIKE) pir S28586 ribosomal protein ML16, cytosolic - common ice plant	464	190	2.44	0.00021
Contig1809_at	Acidic ribosomal protein P2a-2 [<i>Zea mays</i>]	2852	1141	2.50	0.00075
Contig1950_at	40S ribosomal protein S12 gb AAD39838.1 AF067732_1 ribosomal protein S12 [<i>H. vulgare</i>]	1333	514	2.59	0.00076
Contig2468_at	Putative 40S ribosomal protein S24 [<i>O. sativa</i> (japonica cultivar-group)]	1899	717	2.65	0.00021
Contig6096_at	DnaK-type molecular chaperone HSC70-11	343	126	2.72	0.00090
Contig3821_s_at	SAR DNA binding protein [<i>O. sativa</i>]	101	34	3.01	0.00064
HA08n04r_s_at	5e-36 60S Ribosomal protein L34 pir S48027 ribosomal protein L34, cytosolic - common tobacco	121	38	3.21	0.00056
Contig11344_s_at	Putative protein; protein id: At4g25340.1, supported by cDNA: gi_15982871 [<i>A. thaliana</i>]	134	41	3.24	0.00074
Contig3457_x_at	Probable fibrillarin [<i>Picea mariana</i>]	534	59	8.99	0.00026
Contig3457_at	Probable fibrillarin [<i>P. mariana</i>]	471	51	9.32	0.00020

*Significant differences detected by different probe sets designed from same sequence.

¹ Expression levels shown are the average from six GeneChips.

Table 4-4. Validation of gene expression polymorphisms detected using wheat or barley genome arrays (Tables 4-2 and 4-3) based on expression levels of homologous *Leymus* ESTs detected by qRT-PCR

Microarray probe set	Array level (SD) NOGT ¹	Array level (SD) DGT ²	Array ratio NOGT / DGT	<i>Leymus</i> EST (rice genome alignment)	Primers Designed from <i>Leymus</i> EST	qRT-PCR level (SD) NOGT ³	qRT-PCR level (SD) DGT	qRT-PCR ratio NOGT / DGT
Wheat	0.08	0.55	6.87 ⁻¹	EG386159	GGGATTTACCTTCCCACTGA	1.29	21.12	11.11 ⁻¹
Ta.22762.2.S1_at	(0.002)	(0.36)		(7S)	TTCTGTGCGAAGAACCCTCCT	(1.04)	(0.51)	
Wheat	1.28	5.42	4.17 ⁻¹	EG392086	GCAGCTTGACAGAGACAAGG	0.14	1.21	9.09 ⁻¹
Ta.28292.1.S1_at	(0.29)	(3.28)		(1L)	CTTGGTTGGCGTTTGAGAT	(0.23)	(0.72)	
Barley	0.006	0.01	1.66 ⁻¹	EG383468	TTCTTCCGTTTCATCATTTTCG	0.73	1.07	1.47 ⁻¹
HO04H12S_s_at	(0.002)	(0.006)		(1L)	GTGGTAGTCACCTGGCAAGA	(0.47)	(0.67)	
Wheat	2.66	6.13	2.33 ⁻¹	EG390562	ATCCTGCAAGTGCTGCTATG	0.34	0.69	2.04 ⁻¹
Ta.30795.1.S1_at	(0.73)	(2.83)		(1L)	TCTCATCAATGCCTTCTTCG	(0.22)	(0.45)	
Wheat	91.61	44.21	2.07	EG382389	GGGAAGACCATCACCTAGA	0.52	0.30	1.74
Ta.27377.1.S1_x_at	(0.92)	(0.39)		(4L)	GAGTCCACCCTCCATCTTGT	(0.14)	(0.03)	
Wheat	1.92	0.86	2.23	EG375403	GTCAACACATAACCGCAGGAC	2.56	1.24	2.06
Ta.28703.2.S1_at	(0.07)	(0.18)		(7L)	TGCAAGCAGCTTCTGGTACT	(0.48)	(0.19)	
Barley	0.08	0.03	2.66	EG376293	GCCCTACCTCTGGACCTCTT	2.49	0.40	6.26
Contig2369_s_at	(0.01)	(0.01)		(6S)	CATACCCTCAAGCGACCTGT	(0.71)	(0.37)	
Barley	0.05	0.007	7.14	EG387031	GAGAAGTTTCGTCCACAAGCA	4.53	1.24	3.66
Contig3457_at	(0.02)	(0.004)		(5S)	CCCACCTTCTCGATGTTCTT	(0.35)	(0.82)	

¹ Negatively orthogeotropic (NOGT) subterranean branch meristems of the *Leymus* TTC1 and TTC2 progeny selected for upright *L. cinereus* LG3a growth habit QTL alleles (see Table 4-1).

² Diageotropic (DGT) subterranean branch meristems of the *Leymus* TTC1 and TTC2 progeny selected for rhizomatous *L. triticoides* LG3a growth habit QTL alleles (see Table 4-1).

³ Array and qRT-PCR values shown are average gene specific expression values normalized to the average tubulin expression.

CHAPTER 5

SUMMARY AND CONCLUSIONS

One-third of the planet's arable land has been lost to soil erosion in recent decades, and the pace of this degradation will increase as the limits of our food-production capacity are stretched. In addition to soil erosion, increasing demands of limited water resources, various environmental pressures, and increased public awareness of the importance of preserving natural resources demand a responsible preservation of these lands. Range and pasture lands in the United States provide a source of water for residential, agricultural, and industrial needs, forage and habitat for wildlife and domestic livestock, and recreational areas for a variety of uses. The intrinsic values of rangelands, their soil, water, and vegetation, must be preserved. Disturbances caused by wildfire, weed invasion, climatic changes, and human impact, create a need for the development of new germplasm specifically adapted to semiarid rangelands. Rangelands also provide vegetation for livestock and wildlife and are particularly crucial where extreme seasonal and annual variations in temperatures and precipitation are common. In many areas, invasive weeds threaten the integrity of critical rangeland functions where they interact with frequent wildfires and severe droughts to create troublesome management problems. Consequently, there is a tremendous need to repair and stabilize these landscapes. The adaptive mechanisms and traits such as growth habit associated with invasive weed dominance must be understood if range and pasture land improvements are to be achieved in a timely manner.

Growth habit is a highly variable, ecologically, and functionally important trait in perennial grasses. Genes controlling growth habit are the primary targets of plant domestication and breeding. One of the important prospects of identification of genes related to growth habit is to develop multipurpose forage and bioenergy feedstocks adapted to the unique growing environments in North America. Although this complex trait is controlled by a multitude of genetic and environmental factors, Wu et al. (2003) and Larson et al. (2006) used high-density molecular genetic linkage maps and clonally replicated field evaluations during a three-year period to identify and characterize chromosome regions controlling major differences between *L. cinereus* or basin wildrye (a tall conspicuous bunchgrass) and *L. triticoides* or creeping wildrye (a relatively short and strongly rhizomatous grass). To support this accomplishment, we developed four advanced backcross populations of *Leymus* to precisely locate specific gene(s) or QTL(s) controlling key attributes of plant architecture in perennial grasses, and we compared these results to genetic maps for related traits in other Poaceae (grass or cereal) species. These efforts may in turn facilitate the breeding and management of many important forage, turf, and weedy perennial grass species.

Thus, we genetically mapped regions on *Leymus* chromosome LG3a that contain functionally important genes controlling adaptive differences among divergent grasses and cereals. These genes may also serve as important candidates for gene cloning efforts for the growth habit trait.

Additional resources from EST libraries from the *L. triticoides* x *L. cinereus* hybrids were also incorporated to facilitate gene discovery research in *Leymus* wildryes. These *Leymus* EST libraries have been generated and 15,000 sequences made available to

the public for the *L. cinereus* x *L. triticoides* hybrid. *Leymus* ESTs represent rhizome and tiller-induced genes and represent a valuable source of gene sequences for marker development, gene discovery, and grass relationships.

In addition to genetic mapping and QTL analysis to locate the genes controlling the growth habit trait, comparisons of overall gene expression profiles of negatively orthogeotropic (NOGT) and diageotropic (DGT) subterranean branch meristems in a similar genetic backgrounds were performed. Gene expression polymorphisms specifically associated with the *Leymus* LG3a rhizome QTL by bulk segregant analysis, and other possible genes specifically involved in branch angle differences of otherwise similar NOGT and DGT meristems were identified. These genes may be fundamentally important determinants of growth habit variation in grasses, as many of the differentially expressed genes we found in this study were involved directly or indirectly in gravitropism pathways.

No perennial species to date have been developed that produce adequate grain harvests. Yet perennial plant communities store more carbon, maintain better soil and water quality, and manage nutrients more conservatively than do annual plant communities, and they have greater biomass and resource management capacity. These advantages of perennial grasses can be used to transfer genes controlling perennial growth habit to other grain crops such as wheat, sorghum, etc. Thus, the discovery of the gene controlling the growth habit trait may also be useful in the future development of perennial grain crops.

CURRICULUM VITAE

PARMINDER KAUR

EDUCATION

- Ph.D. (Plant Science) Utah State University, Logan, UT, USA
Dissertation: DNA markers and gene expression polymorphisms associated with growth habit QTLs in *Leymus* wildryes
Dec 2007 GPA: 3.73
- M. S. (Plant Breeding) Punjab Agricultural University, Ludhiana, Punjab, India
Thesis: Resynthesis of *Brassica juncea* L. (Coss) as an alternate source for genetic diversity.
Aug 2003 GPA: 7.92 / 10.00
- B. S. (Agriculture) Hons. Punjab Agricultural University, Ludhiana, Punjab, India
Aug 2001 GPA: 7.76 / 10.00

RESEARCH EXPERIENCE

- Research associate, USDA-ARS, Forage and Range Research Laboratory, Logan, UT, 2004-2007.
- Research assistant, Department of Genetics and Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India, 2003-2004.

AWARDS AND HONORS

- Research assistantship throughout the Ph.D. program
- Dale W. and Adele C. Young scholarship (2007)
- USU graduate student senate travel award (2006)
- Honor role for fall semester (2005)

PRESENTATIONS

Poster presentations at conferences

- **Kaur P**, Mott IW, Bushman BS, Larson SR (2007) Comparison of gene expression in *Leymus* tiller and rhizome meristems using heterologous Affymetrix wheat and barley gene chips. In Plant and Animal Genomes XV, Abstracts, San Diego, CA.
- **Kaur P**, Mott IW, Bushman BS, Larson SR (2005) Comparison of gene expression in tiller and rhizome meristems of perennial *Leymus* wildryes using Affymetrix GeneChips. In Agronomy Abstracts, Madison, WI.

- **Kaur P**, Larson SR (2005) Fine genetic mapping of rhizome QTLs in perennial *Leymus* wildryes. In Agronomy Abstracts, Madison, WI.
- **Kaur P**, Banga SK (2003) Resynthesis of *Brassica Juncea* (L.) Coss as an alternate source for genetic diversity. In Proceedings International Congress on Emerging Trends in Plant Chromosome Research, Abstracts, Patiala, India.

Departmental graduate seminar presentations (PSB 7970)

- Plant gravitropism: An overview (2007)
- DNA markers and gene expression polymorphisms associated with growth habit QTLs in *Leymus* wildryes (2006)

PROFESSIONAL SKILLS

Plant breeding

- Theoretical and practical knowledge of breeding crop plants for biotic stresses and breeding crop plants for qualitative and quantitative traits.

Tissue culture and Cytogenetics

- Practical experience in embryo-rescue, anther culture, microscope culture, ovule culture, somatic cell culture, shoot multiplication, plant regeneration and genetic transformation of crop plants, Practical experience in cytogenetics

Molecular Biology

- In-depth experience in working with PCR based molecular markers, especially RAPDs, SSRs, EST-SSRs, RACE, qPCR
- Genomic and plasmid DNA isolation and purification from different organisms.
- Gel blot and PAGE analysis of DNA molecules, gene linkage and sequence analysis.
- Practical experience with microarrays

Computer proficiency

- ArrayAssist software, Genographer, Map QTL, Join Map, Mapmaker, NTSYS, Sequencher.

PUBLICATIONS

Kaur P, Banga SK (2003) Resynthesis of *Brassica Juncea* (L.) Coss as an alternate source for genetic diversity. In Proceedings International Congress on Emerging Trends in Plant Chromosome Research, Abstracts, Patiala, India.

Kaur P, Mott IW, Bushman BS, Larson SR (2005) Comparison of gene expression in tiller and rhizome meristems of perennial *Leymus* wildryes using Affymetrix GeneChips. *In* Agronomy Abstracts, Madison, WI.

Kaur P, Larson SR (2005) Fine genetic mapping of rhizome QTLs in perennial *Leymus* wildryes. *In* Agronomy Abstracts, Madison, WI.

Kaur P, Mott IW, Bushman BS, Larson SR (2007) Comparison of gene expression in *Leymus* tiller and rhizome meristems using heterologous Affymetrix wheat and barley gene chips. *In* Plant and Animal Genomes XV, Abstracts, San Diego, CA.

Singh K, Ghai M, Garg M, Chhuneja P, **Kaur P**, Schnurbusch T, Keller B and Dhaliwal HS (2007) An integrated molecular linkage map of diploid wheat using a *Triticum boeoticum* x *T. monococcum* RIL population. *Theor Appl Genet* 115: 310-312.

Kaur P, Mott IW, Larson SR, Bushman BS, Hernandez AG, Kim WR, Liu L, and Mikel MA Gene expression polymorphisms and ESTs associated with gravitropic response of subterranean branch meristems and growth habit in *Leymus* wildryes (Submitted).